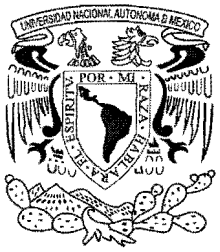

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Redes genéticas y diferenciación celular en tejidos epidérmicos de *Arabidopsis Thaliana*

T E S I S

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P R E S E N T A

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Resumen

Uno de las principales cuestiones por resolver en biología del desarrollo es el origen de la información necesaria para que ocurran los procesos del desarrollo, que constan de los procesos de diferenciación celular y morfogénesis. En particular, interesa entender la manera en que se genera la información posicional que subyace la determinación de tipo celular en plantas y animales. Por otra parte, aunque en años recientes se han caracterizado algunas propiedades potencialmente genéricas de los sistemas de desarrollo (modularidad, robustez, capacidad de evolucionar, etc.), aún es necesario entener cómo surgen, evolucionan y se relacionan entre sí dichas propiedades. En este trabajo se abordan estas cuestiones desde un enfoque de modelación de red de regulación genética y se toma como sistema modelo al de determinación de tipo celular en la epidermis de *Arabidopsis thaliana*. Éste es uno de los sistemas vegetales mejor descritos genética, celular y anatómicamente. Como parte de este trabajo se recabó la evidencia experimental reportada en la literatura y se integró en modelos dinámicos de redes discretas. Dichos modelos permitieron postular una explicación al origen de la información posicional asociada a la determinación y arreglo de las células epidérmicas, descubriendo un módulo funcional que es suficiente para la generación de los patrones de expresión genética observados en sistema de estudio. El análisis teórico del sistema permitió también mostrar que la definición clásica de información posicional es, aunque útil, insuficiente para entender sistemas de desarrollo en que el contexto celular (posición relativa) es generado dinámicamente por factores intra y extracelulares. Por otra parte, los modelos analizados dieron lugar a predicciones verificables experimentalmente, algunas de las cuales han sido confirmadas. El análisis de estos modelos contribuyó también a entender el origen de la robustez en el sistema de estudio y en las redes de regulación genética en general. Esto llevó a vislumbrar el papel de la redundancia dinámica en estas redes y a plantear nuevas preguntas respecto a la evolución de los sistemas de desarrollo.

Abstract

One of the main issues in current developmental biology is the understanding of positional information required for cell differentiation and morphogenesis to take place. In particular, it is central to understand how positional information underlying cell-fate determination is generated in different plant and animal systems. Also, although some potentially generic properties are begining to be uncovered and understood in gene regulatory networks (modularity, robustness, evolvability, etc.), it is still necessary to comprenhend the way these properties emerge, evolve and relate to each other. This study adopts a modeling approach and explores such questions by using as a model system one of the best characterized developmental plant systems: that of cell-fate determination and patterning in the epidermis of *Arabidopsis thaliana*. As part of this work, a vast amount of experimental evidence was gathered and integrated into dynamic models of discrete gene networks. Such models enabled explaning the origin of the positional information underlying cell-fate determination and patterning, uncovering a functional module that is both necessary and suffiecient for pattern generation in the epidermis of *Arabidopsis thaliana*. Moreover, the theoretical analysis of the system under study showed that the classic definition of positional informatios is, although useful, insufficient to study developemental systems in which cell context (relative position) is dynamically generated by intra and extracellular factors. These models also yielded predictions that are experimentally testable, some of which have already been confirmed. Finally, analizing the proposed models contributed to understanding the origin of robustness in the system under study, as well as in gene regulatory networks in general. This helped to further comprehend the role of dynamic redundancy in such networks, opening new questions regarding the evolution of developmental systems.

Índice general

Capítulo 1

Introducción

1.1. Estudios teóricos en biología evolutiva y del desarrollo

La biología del desarrollo es una ciencia de procesos, de cambio. Estudia la transformación de cigotos unicelulares en embriones y luego en organismos adultos de una gran complejidad estructural y functional (Gilbert 2006). Además, en el caso de las plantas, estudia también el surgimiento y modificación de órganos y estructuras (hojas, flores, semillas, etc.) a lo largo de toda la vida del organismo. Contrario a lo que ha planteado el llamado preformacionismo en sus diferentes versiones (Oyama 2000, Lewontin 2000) — incluyendo áreas de la genómica — el desarrollo de los seres vivos no está programado *a priori* ni consiste en el desenvolvimiento o decodificación de formas preexistentes, sino en el surgimiento mismo de las formas y de las heterogeneidades que caracterizan a los organismos (Waddington 1957, Oyama 2000). Así, una de las preguntas centrales para la biología del desarrollo es la de cómo surgen estas formas y patrones a partir de interacciones entre factores hereditarios, físicoquímicos y ambientales, entre otros. En el intento de responder esta pregunta, la biología del desarrollo de nuestros días integra areas como genética, biología molecular, fisiología, ecología, matemática y las recién llegadas genómica y proteómica.

El estudio de la biología del desarrollo es fundamental para el entendimiento de la embriología humana y el concomitante diseño de terapias y aplicaciones clínicas, por lo que las posibles aplicaciones de la biología del desarrollo, que son muchas y de gran alcance, la han llevado a ocupar un papel central en la

biología contemporánea. Además, se ha enfatizado su estudio e importancia debido a su estrecha relación con la biología evolutiva. Esta relación tiene su base en que únicamente entendiendo los procesos que dan lugar a las formas vivas durante el desarrollo será posible entender el origen, conservación y diversificación de los fenotipos, es decir, la evolución de los seres vivos. El papel clave del desarrollo en la evolución no se refleja en la llamada nueva síntesis, que en los años treinta y cuarenta integró la propuesta darwiniana de selección natural con la genética clásica y de poblaciones, pero que dejó fuera una larga tradición en embriología y anatomía comparada. Sin embargo, empieza a vislumbrase otra síntesis, una en que se integran la evolución y el desarrollo. Esta síntesis es comúnmente conocida como *evo-devo*, por evolución y desarrollo en inglés (Müller 2007, Sommer 2009).

Algunos de los conceptos que dirigen la investigación actual en el área de evo-devo son aquéllos relacionados con las propiedades organizativas de los sistemas de desarrollo. Estas propiedades se refieren a características sistémicas de la dinámica o estructura de los procesos de desarrollo y algunas de las más estudiadas hasta ahora son modularidad, robustez, plasticidad y capacidad de evolucionar [1].

La modularidad consiste en la organización de un sistema en subunidades semiautónomas y se observa frecuentemente en los seres vivos, tanto en aspectos morfológicos, como metabólicos o de regulación genética. Esta caraterística permite que se modifiquen partes del organismo sin que se afecte al resto y permite también que se reusen módulos funcionales en distintas etapas del desarrollo o partes del organismo (Wagner 2005, Müller 2007). La robustez es la capacidad de un sistema de mantener a lo largo de la evolución alguna función o característica en presencia de perturbaciones (genéticas, ambientales, estocásticas, etc.), mientras que la plasticidad consiste en la capacidad de un sistema de ajustarse para responder a cambios o señales ambientales. En particular, la plasticidad fenotípica permite a un organismo modificar su fenotipo ante condiciones externas cambiantes. Finalmente, aunque existen distintas definiciones, la capacidad de evolucionar consiste en la capacidad de un organismo de generar variabilidad e innovaciones en la escala evolutiva, mismas que pueden estar sujetas a selección natural (Wagner 2005, Draghi & Wagner 2009, Wagner 2009). Es interesante mencionar que estas propiedades parecen estar relacionadas entre sí. Por ejemplo, la modularidad puede conferir robustez (Kitano 2004, Hintze & Adami 2008) o la robustez y la capacidad de evolucionar

[1] En ocasiones se hace referencia a la capacidad de evolucionar como *evolvabilidad*

pueden depender una de otra y aparecer juntas durante la evolución de los seres vivos (Wagner 2008).

Algunos de estos conceptos pueden rastrearse a las propuestas teóricas de Conrad Hal Waddington, quien desarrolló un marco teórico para estudiar el desarrollo como un sistema dinámico (Waddington 1957, Slack 2002). Entre los conceptos que desarrolló y que actualmente se han retomado para orientar muchos de los estudios en biología del desarrollo son los de canalización (asociado a robustez), plasticidad y paisaje epigenético. El paisaje epigenético en particular es una representación que ha permitido enmarcar preguntas sobre la dinámica del desarrollo. Consiste en un paisaje cuya topología está determinada por interacciones epigenéticas [2] involucradas en el desarrollo; los valles de este paisaje corresponden a los estados o secuencias estables o canalizadas del desarrollo. Recientemente, esta representación ha comenzado a aterrizarse para algunos casos particulares (ver por ejemplo Apéndice G), lo que ha permitido empezar a explorar aspectos de la dinámica global del desarrollo que habían permanecido poco estudiados.[3]

Como ya se mencionó, la biología del desarrollo estudia procesos que surgen de interacciones entre diversos elementos, tanto genéticos como no genéticos. Estas interacciones son típicamente no aditivas y ocurren en un amplio intervalo de escalas espaciales y temporales. Dado que los modelos matemáticos permiten integrar de forma sistemática diversos y numerosos datos experimentales, así como estudiar la dinámica colectiva de elementos genéticos y no genéticos en diferentes escalas de tiempo y espacio, este tipo de modelo se ha vuelto sumamente importante en el estudio de los procesos del desarrollo.

Históricamente, la elaboración de modelos y trabajos teóricos en biología

[2]Debido al uso reciente del término epigénesis para hacer referencia a al conjunto de modificaciones heredables que no dependen de la secuencia de DNA (e.g. metilación de la cromatina), es importante mencionar que, en este trabajo, el término *epigenético* hace referencias a los procesos e interacciones requeridas para que ocurra un proceso del desarrollo no programado, por lo que puede hacer referencia a procesos de regulación genética, comunicación celular, mecanismos y restricciones físico-químicas, etc.

[3]La metáfora del paisaje en el contexto adaptativo, así como otras metáforas comúnmente usadas en biología, ha sido criticada, entre otras cosas, por su ambigüedad (Olson & Arroyo-Santos 2009, Kaplan 2008). En contraste, la metáfora del paisaje epigenético parece estar definiéndose cada vez más claramente en términos de los nuevos descubrimientos en las áreas de desarrollo, genética molecular y epigenética (e.g. Huang *et al.* 2005, Apéndice G). Esto, sin embargo, no la pone a salvo de los *peligros* asociados al uso de metáforas: la reificación y su uso inconsciente. Citando a N. Wiener (en Lewontin 2000), *el precio de las metáforas es la eterna vigilancia.*

del desarrollo ha permitido referir cuestiones tales como el origen de patrones y formas en los organismos (e.g. Turing 1952, Meinhardt 1982), el origen de la robustez del desarrollo ante perturbaciones (Waddington 1957), la caracterización de regularidades dinámicas o estructurales en los procesos del desarrollo (Waddington 1957, Wagner 2005, Salazar-Ciudad *et al.* 2000, Newman *et al.* 2006) y las fuentes de variabilidad o plasticidad en las formas de los seres vivos (Ancel 1999, West-Eberhard 2005, Lande 2009), entre otras. Si bien el entendimiento de estas cuestiones ha avanzado con el desarrollo de modelos matemáticos y, más recientemente, también computacionales, han surgido nuevas preguntas y aún quedan muchas por resolver. Actualmente existe una gran cantidad de información molecular, anatómica, morfológica y genómica disponible y se han desarrollado también nuevos acercamientos teóricos y matemáticos a los sistemas biológicos, lo que ha dado lugar a nuevos enfoques teóricos y de modelación, planteando nuevas líneas de trabajo en el área de evo-devo (Müller 2007).

1.2. Desarrollo en plantas: la epidermis de *Arabidopsis thaliana* como sistema modelo

Las plantas y los animales son organismos multicelulares con tipos celulares, órganos y estructuras diferenciados. Pese a que las plantas y los animales presentan procesos y estructuras similares durante su desarrollo, existen diferencias importantes en el desarrollo de plantas y animales, debido, en gran parte, a que la multicelularidad aparentemente se originó de forma independiente en estos dos linajes (Meyerowitz 2002). En contraste con lo que ocurre en animales, las plantas se desarrollan continuamente a partir de cúmulos de células indeferenciadas (meristemos), dando lugar a ramas, raíces, hojas, flores y frutos durante toda su vida y no sólo durante la etapa embrionaria. Por otra parte, los animales presentan migración celular durante desarrollo, mientras que las paredes celulares características de las plantas impiden la migración celular. Además, las plantas son organismos que, probablemente debido a su naturaleza sésil, exhiben una considerable plasticidad fenotípica. El estudio del desarrollo en plantas ofrece, entonces, la posibilidad de entender aspectos como la plasticidad o la indeterminación de desarrollo, pero también de llevar a cabo un auténtico estudio comparativo que contribuya a entender el origen de las diferentes formas de multicelularidad y a identificar aspectos genéricos

del desarrollo de organismos multicelulares (Meyerowitz 2002).

Uno de los problemas centrales en el estudio del desarrollo consiste en entender cómo se genera la información necesaria para que ocurra la determinación de distintos tipos celulares a partir de interacciones genéticas y epigenéticas. En el caso del desarrollo vegetal, se ha sugerido que la determinación del tipo celular depende en gran medida de la posición de las células respecto a otras células (Scheres 2001). Así, en plantas, la pregunta sobre determinación de identidad celular está muy relacionada con la cuestión de cómo surgen los patrones espaciales, por ejemplo, de expresión genética o de concentración de hormonas, que constituyen información posicional.

Aquí es importante mencionar que la información posicional no es un campo fijo o preestablecido, sino que emerge durante el desarrollo en un proceso en el que intervienen diversos factores moleculares y celulares (Jaeger & Reinitz 2006). De hecho, como se ilustrará más adelante en este trabajo, la información posicional puede ser generada en interacción con las mismas células que están por diferenciarse. En otras palabras, este tipo de información no precede ni regula unidireccionalmente la determinación de indentidades celulares, sino que se genera y mantiene dinámicamente durante el proceso mismo de determinación del tipo celular.

Arabidopsis thaliana es una planta modelo cuyo desarrollo ha sido estudiado meticulosamente desde diferentes enfoques (Bowman 1994, Walbot 2000). En particular, uno de los sistemas ahora clásicos en el estudio de la determinación de tipo celular es el de epidermis de raíz y hoja de *Arabidopsis thaliana*. Dicho sistema ha sido ampliamente estudiado debido a que la epidermis— la capa celular más superficial — de raíz y hoja presenta dos tipos celulares, con pelo y sin pelo, que son visibles a simple vista y cuyo desarrollo puede ser modificado experimentalmente sin que las plantas mueran. Estos dos tipos de células epidérmicas están arreglados en patrones espaciales no triviales, a saber, los tricomas o pelos de la hoja están alejados unos de otros y presentan una disposición espaciada, mientras que los tricoblastos, que dan lugar a los pelos radicales, se hallan organizados en bandas paralelas al eje de crecimiento de la raíz (Apéndice D y referencias ahí citadas).

En este sistema, la información posicional que subyace la formación de los arreglos espaciales de células con tricomas y pelos radicales está dada principalmente por patrones espaciotemporales de expresión genética. Sin embargo, estos patrones no están fijos ni son externos a las células epidérmicas que se diferencian, por lo que el problema de la formación de patrones epidérmicos

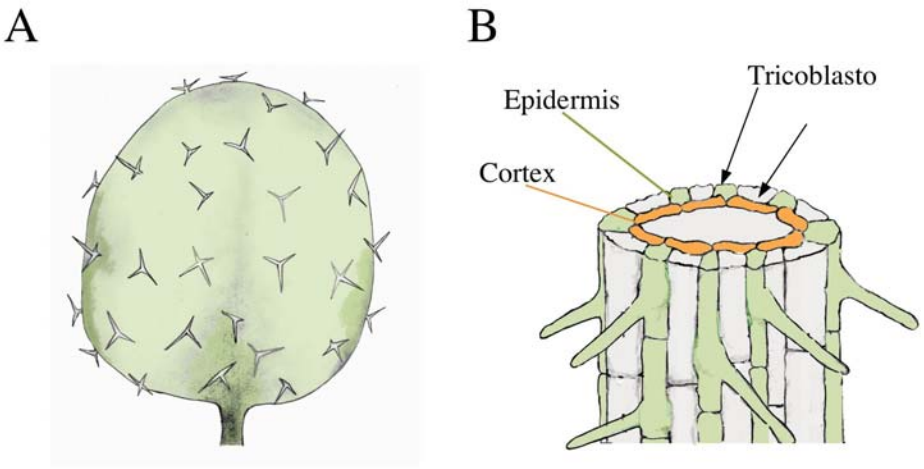


Figura 1.1: La epidermis de hoja y raíz de *Arabidopsis thaliana* es un sistema modelo para el estudio de diferenciación celular en plantas. A la izquierda se muestra el arreglo espaciado de los pelos de la hoja y a la derecha, el patrón bandeado de los pelos radicales. La posición de las células radicales está correlacionada con la posición de las células de la epidermis respecto las del córtex.

tiene que ver, en gran parte, con el origen y mantenimiento de dichos patrones de expresión genética como parte del proceso mismo de determinación del tipo celular en epidermis.

Este sistema presenta un par de características relevantes respecto al origen de la información posicional. Por un lado, se ha documentado experimentalmente que algunas de las proteínas codificadas por elementos de la red de regulación genética asociada a la determinación de pelos y no pelos se mueven a través de orificios en la pared celular llamados plasmodesmos (Wada *et al.* 2002, Bernhardt *et al.* 2005, Kwak *et al.* 2005). Mediante este movimiento, que hasta donde se sabe es pasivo, éstas porteínas afectan a las redes de regulación genética de las células epiérmicas vecinas, dando lugar a un sistema de redes acopladas. Por otro lado, se ha encontrado que los tricoblastos se forman únicamente en bandas de células ubicadas sobre la unión de células de la capa celular subyacente (córtex), lo que, aunado a detallados experimentos de biología molecular (Dolan 1996, Kwak & Schiefelbein 2007), muestra la actividad de una señal posicional proveniente del córtex de la raíz que sesga la posición de los pelos radicales.

Si bien los aspectos moleculares, y genéticos en particular, del sistema de determinación celular en la epidermis de *Arabidopsis thaliana* han sido bastante bien caracterizados (Apéndice D), quedan aún preguntas abiertas respecto a la manera en que la interacción de los elementos moleculares, así como las interacciones epigenéticas, dan lugar a la información posicional que subyace la determinación y arreglo de los distintos tipos celulares. Más específicamente, es importante saber si la red de regulación genética caracterizada hasta ahora es necesaria y suficiente para la generación de los patrones epidérmicos, así como cuál es el efecto de la comunicación entre células de la epidermis y el de la señal proveniente del córtex en la formación de patrones. El objetivo principal de esta tesis es abordar dichas preguntas mediante la elaboración y análisis de modelos dinámicos, tales como los modelos de redes genéticas que se describirán en el siguiente capítulo. Así, la hipótesis central de este trabajo es que las redes de regulación genética y los procesos de comunicación celular documentados experimentalmente hasta ahora en el sistema de epidermis de raíz y hoja son suficientes para la determinación de pelos y no pelos y para la formación de los patrones de tricomas y tricoblastos.

Otro de los objetivos de este trabajo es hacer uso de los modelos mencionados para analizar propiedades organizativas de las redes de regulación asociadas a estos sistemas y apuntar hacia aspectos dinámicos y estructurales

que sean potencialmente comunes a los sistemas de determinación de tipo celular y de generación de información posicional de plantas y animales. Con ello se pretende también tener información para discutir hipótesis relativas a la evolución del desarrollo (e.g. Waddington 1953, 1957, Newman & Bhat 2009, Salazar-Ciudad *et al.* 2000) y, por lo tanto, de las formas vivas.

Capítulo 2

Origen de la información posicional en epidermis de *Arabidopsis thaliana*

Marco Polo describe un puente, piedra por piedra.
— ¿Pero cuál es la piedra que sostiene el puente?
— pregunta Kublai Kan.
— El puente no está sostenido por esta o aquella piedra — respone
Marco—, sino por la línea del arco que ellas forman.
Kublai permanece silencioso, reflexionando. Después añade:
—¿Por qué me hablas de las piedras? Es sólo el arco que me importa.
Polo responde: — Sin piedras no hay arco.

Las Ciudades Invisibles, Italo Calvino

El sistema de determinación del tipo celular en la epidermis de *Arabidopsis thaliana* plantea, pese a su relativa sencillez, preguntas fundamentales respecto al origen y naturaleza de la información posicional, la determinación y arreglo espacial de tipos celulares y del desarrollo en general. Con el fin de abordar estas preguntas desde un enfoque de modelación matemática se desarrollaron modelos dinámicos para la determinación de tipo celular en raíz y hoja de la epidermis de *Arabidopsis thaliana*. Es importante mencionar que la gran cantidad de experimentos detallados que han sido reportados para estos sistemas (caracterización de patrones de expresión, de fenotipos de líneas mutantes, de interacciones entre proteínas, entre otros) ha permitido basar dichos modelos en evidencia experimental (Apéndices A, B y D). La información experimen-

tal mencionada se refiere principalmente a las relaciones de regulación entre un conjunto de genes involucrados en la determinación y arreglo espacial de tricomas y tricoblastos.

2.1. De relaciones de regulación genética a modelos de redes

Dado que la información posicional que subyace la determinación de pelos y no pelos en el sistema de estudio parece estar dada por patrones de expresión genética, se propone que la determinación y arreglo de los tipos celulares epidérmicos resultan en parte de las relaciones de activación e inhibición que los genes involucrados mantienen entre sí. Estas relaciones de regulación entre genes pueden ser integradas en modelos dinámicos conocidos como modelos de redes de regulación genética (RRG de aquí en adelante); estudiar las propiedades dinámicas y estructurales de estas redes puede ayudar a comprender cómo las interacciones genéticas, en conjunción con otros factores, contribuyen a la determinación de los destinos celulares pelo y no pelo y a la determinación de su arreglo espacial en la epidermis de *Arabidopsis thaliana*.

Es importante mencionar que, aunque los genes constituyen parte importante de un sistema ontogenético, existen factores no-genéticos que son igualmente importantes y cuya modificación puede también generar cambios fenotípicos. Sin embargo, la complejidad del sistema y el *sesgo genético* en la información experimental disponible nos impide estudiar explcitamente todos estos elementos. Modelar una red de genes es una simplificación fuerte, pero en este punto resulta sumamente útil para el entendimiento e integración de los datos disponibles actualmente.

Los modelos de RRG constituyen un tipo de modelo matemático que captura la lógica de la regulación genética y que permite hacer estudios cualitativos del comportamiento de conjuntos de genes que interactúan entre sí en el tiempo y el espacio. Así mismo, estos modelos abren la posibilidad de entender y evaluar las consecuencias, no siempre intuitivas, de las interacciones entre genes (frecuentemente no lineales).

En los modelos de RRG los nodos corresponden a genes unidos por aristas o conexiones que representan las relaciones de regulación (activación o inhibición) entre ellos. En los organismos, estas relaciones de regulación están mediadas por numerosos procesos que involucran a enzimas, RNA, proteínas,

y muchos otros elementos moleculares. Aunque, en principio, estos procesos pueden ser integrados en modelos de RRG, los modelos de RRG que se plantean aquí los omiten y se enfocan básicamente en la lógica de regulación entre genes.

En 1969, Stuart Kauffman (Kauffman 1969) propuso un tipo de modelo de RRG que ha sido sumamente útil en el estudio cualitativo de la actividad colectiva de genes. En este modelo, conocido como de redes Booleanas, los nodos que representan genes pueden tomar sólo dos estados, 0 ó 1, que corresponden a que el gen esté inactivo (0) o activo (1). De esta manera, en las redes de Kauffman los genes corresponden a N variables cuyo estado en el tiempo $t+1$ depende de una función del tipo:

$$g_{n(t+1)} = F_n(g_{n1(t)}, g_{n2(t)}, ..., g_{nk(t)})$$

En esta ecuación, $g_{n1(t)}, g_{n2(t)}, ..., g_{nk(t)}$ son los reguladores del gen g y F_n es una función conocida como regla lógica. Las reglas lógicas integran la información disponible sobre la regulación de los genes (ver ejemplos de especificación de estas reglas en Espinosa-Soto *et al.* 2004 y Apńdices B y J). Dados los elementos g_n y funciones F_n que describen a una RRG Booleana es posible estudiar cómo los componentes de la red cambian sus configuraciones de activación y entonces puede seguirse la dinámica de los estados de activación genética, siendo un estado un vector de ceros y unos. Una de las características más importantes de este tipo de redes es la existencia de estados estacionarios de expresión genética. Estos estados de activación de los componentes de la red se conocen también como atractores y dado que son estados auto-sostenidos, una vez que la red llega a ellos se mantiene ahí para siempre. Kauffman propuso que estos estados estacionarios correspondían a los diferentes tipos celulares (Kauffman 1969).

Si bien los modelos de RRG propuestos por Kauffman son relativamente simples, se ha mostrado que pueden reproducir los aspectos cualitativos más relevantes de la dinámica de regulación genética. En particular, se ha encontrado que modelos de este tipo pueden explicar la formación de diferentes tipos celulares en distintas etapas del desarrollo de plantas y animales (Mendoza & Alvarez-Buylla 2000, Albert & Othmer 2003, Espinosa-Soto *et al.* 2004, Huang *et al.* 2005). Una descripción más detallada de este tipo de modelos y su comparación con otros enfoques de modelación está disponible en los apéndices E, H e I.

Siguiendo la noción de paisaje epigenético inicialemente planteada por C. H.

Waddington (Waddington 1957), algunos autores han representado la dinámica multidimensional de las RRG como un paisaje con crestas y valles. En este paisaje, cada punto corresponde a configuraciones de activación de los genes que conforman la red, los puntos más bajos del paisaje constituyen atractores y las cuencas o valles corresponden al conjunto de configuraciones que llegan a cada uno de los atractores. Es importante mencionar que, siguiendo la metáfora del paisaje epigenético, la topografía de dicho paisaje es generada por los componentes y reglas lógicas que definen a la RRGs. Así, el sistema estudiado puede verse como un balín que, al ser colocado en algún punto del paisaje, 'rueda' durante el procesos de desarrollo hasta alcanzar un atractor. Conforme se han ido acumulando los datos moleculares y fisiológicos, está siendo posible plantear modelos de las redes genéticas y de los paisajes epigenéticos asociados a ellas y con ello se está avanzando el entendimiento de rasgos distribuidos y emergentes de los procesos del desarrollo (ver ejemplo en Apéndice G).

La metáfora del paisaje epigenético planteada por Waddington (Waddington 1957) ha sido criticada por West-Eberhard (West-Eberhard 2003) principalmente porque en esta metáfora el paisaje que determina la dinámica del desarrollo está dada únicamente por interacciones entre genes. Si bien Waddington mencionó que cierta trayectoria del desarrollo podía ser modificada por factores ambientales, no consideró que este tipo de factores contribuyeran a la determinación de la topología del paisaje mismo. En este paisaje, dice West-Eberhard, las influencias ambientales son vistas como desviaciones de lo dictado por los genes. West-Eberhard argumenta que, en todo caso, los valles y crestas de dichos paisajes también son modificados y moldeados por aspectos ambientas y que son, además, cambiantes incluso a lo largo de la vida de un organismo.

Dada la evidencia experimental disponible ahora, y el reciente énfasis en el estudio de la plasticidad fenótipica y su relación con la evolución (West-Eberhard 2003, Garland & Kelly 2006, West-Eberhard 2005, Lande 2009), probablemente pronto será factible desarrollar modelos de paisajes epigenéticos que consideren en el efecto de fatores ambientales, entre otros, en la topología (dinámica) del paisaje.

2.2. Una red RRG mínima equivalente a un sistema activador-inhibidor

En una primera etapa de este trabajo se realizó una revisión de los datos experimentales disponibles hasta el 2006, mismos que se integraron en un par de modelos continuos de RRG, uno para la epidermis de hoja de *Arabidopsis thaliana* y otro para la de raíz. Dichos modelos se simularon computacionalmente y se encontró que, pese a algunas diferencias en las reglas lógicas, mismas que correspondían a diferencias en la regulación genética, ambos sistemas presentaban una dinámica global equivalente. Esto es, ambos sistemas, el de hoja y el de raíz, se caracterizaban por tener dos atractores que correspondían, respectivamente, a los estados de activación genética documentados para las células de pelos y de no pelos. Más aún, las cuencas de atracción asociadas a cada atractor (i.e. el conjunto de condiciones iniciales que llegan a cada atractor) resultaron ser muy similares en ambos sistemas (Figura 1 del Apéndice A).

Los resultados del análisis de las RRGs simuladas para raíz y hoja mostraron que ambas redes exhibían una dinámica global equivalente: las dos convergieron únicamente un a par de atractores, mismos que corresponden a las configuraciones de expresión genética de los tipos celulares identificados como pelo y no pelo. Además, considerando también el movimiento de proteínas que afecta la expresión de RRG en células vecinas, la dinámica de ambos sistemas resultó ser comparable con la de un sistema de inhibición lateral en el que céulas inicialmente iguales interctúan entre sí y adoptan destinos celulares distintos (Apéndice B). De hecho, Pesch y Hülskapm (Pesch & Hülskamp 2004) plantearon que el sistema de determinación celular en la epidermis de hoja podía reducirse a un sistema de inhibición lateral bien caracterizado y conocido como *sistema activador-inhibidor*.

El sistema activador-inhibidor (Meinhardt 1982, Meinhardt & Gierer 2000) es un tipo de sistema de reacción-difusión que explica la formación de patrones espaciales, tales como puntos o bandas, a partir de la reacción y difusión de un par de sustancias llamadas morfógenos (Turing 1952). En el sistema activador-inhibidor en particular, se parte de un par de morfógenos: uno de ellos (activador) promueve su propia actividad y la del otro, mientras que el otro reprime al primero (inhibidor). Este sistema se caracteriza también porque el inhibidor ejerce una acción lateral de largo alcance, mientras que al activador ejerce una acción local. Si bien la determinación de células con y sin pelos en la epidermis

de *Arabidopsis thaliana* no depende únicamente de un par de morfógenos, se ha sugerido que las RRGs asociadas a este sistema actúan, colectivamente, como un sistema activador-inhibidor (Pesch & Hülskamp 2004). De hecho, la RRG de determinación de tipo celular que se estudia se caracteriza por tener un subconjunto de elementos que actúan conjuntamente como un activador y otro subconjunto que tiene el papel de inhibidor y que tiene una acción de largo alcance (Schnittger *et al.* 1999, Wada *et al.* 2002, Kwak *et al.* 2005).

Pesch y Hülskamp (Pesch & Hülskamp 2004) presentaron una revisión meticulosa de las características que un sistema activador-inhibidor debe exhibir, así como de las que se había encontrado entonces en el sistema de determinación de tipo celular en epidermis. Una de las interacciones características del activador-inhibidor es, como se mencionó antes, la capacidad de autorregulación positiva del activador. Aunque hay evidencia indirecta apuntando a que esto de hecho ocurre en el sistema de epidermis, esta interacción no ha sido claramente identificada en las redes de regulación genética que aquí se estudian. Como se discutirá más adelante, los modelos dinámicos que se han desarrollado para este sistema (Savage *et al.* 2008, Apéndices A, B y D) han permitido evaluar y discutir la importancia de esta interacción y probar *in silico* si es necesaria para que se generen los patrones de pelos espaciados y bandeados.

2.2.1. Resultados del análisis de la red reducida a un sistema activador-inibidor

Una vez que se analizaron las dos redes de regulación genética y se encontró que eran dinámicamente equivalentes entre sí, y que se mostró que ambas se comportaban como un sistema de tipo activador-inhibidor, se redujeron las RRGs documentadas para epidermis de hoja y raíz a un modelo de red mínima compuesta únicamente por dos elementos: un activador de acción local y un inhibidor con efectos de largo alcance. Este modelo fue elaborado mediante el uso de ecuaciones diferenciales parciales acopladas (Apéndice A) que permitió abordar una de las preguntas que resultaba de interés para la biología del desarrollo: si un mismo sistema de regulación genética puede dar lugar a patrones espaciales distintos al encontrarse en diferentes contextos celulares, pero sin cambios en los valores de parámetros. Con el fin de responder esta pregunta, se realizaron simulaciones computaciones en que la misma red mínima se encuentra en un contexto tipo hoja (sin señales provenientes de otras capas

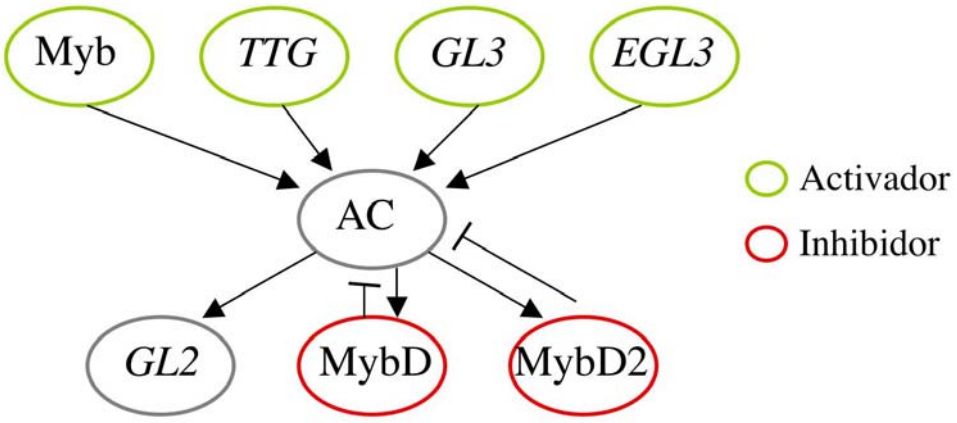


Figura 2.1: Representación gráfica de la red que fue reducida a un sistema genérico del tipo activador-inhibidor

celulares y con células que en promedio tienen el mismo ancho que largo) y en un contexto tipo raíz (con señales como las provenientes del córtex y células rectangulares). Se encontró que, dada esta red y los contextos celulares contrastantes, es posible obtener los patrones bandeados y espaciados de pelos. De hecho, un análisis de bifurcación realizado en colaboración con el Dr. José Díaz reveló que los patrones bandeados observados en raíz difícilmente podrían aparecer variando los parámetros del modelo y sin la intervención de una señal posicional (Apéndice A).[1]

Estos resultados sugieren que existen módulos de regulación genética que pueden ser reusados en distintas regiones y momentos del desarrollo de los organismos y que, al formar parte de distintos contextos celulares, pueden dar lugar a patrones muy diversos (von Dassow & Munro 1999), aún cuando los valores de parámetros asociados a la cinética de regulación permanezcan constantes. Además, sugieren que los elementos de RRGs pueden actuar colectivamente como un sistema tipo activador-inhibidor y que los morfógenos de Turing pueden, de hecho, ser colecciones de genes interactuando de forma compleja.

[1]El análisis del sistema activador-inhibidor es también parte de la tesis doctoral de Carlos Espinosa Soto y los resultados se presentan en el artículo de Benítez *et al.* 2007.

Diversos autores han planteado el papel de aspectos genéricos tales como efectos físico-químicos en el surgimiento de patrones y formas a lo largo del desarrollo (Goodwin 2001, Newman *et al.* 2006, Newman & Bhat 2009). El modelo de red mínima permitió referir también preguntas relacionadas con estos planteamientos, en particular, con el papel de la geometría celular en el patrón de pelos de la epidermis de raíz. Para ello se simuló la dinámica de la red mínima en dos condiciones, una en la que las células de la raíz se suponen rectangulares, como de hecho son, y otro en que se suponen células cuadradas. Los resultados mostraron que aunque un patrón que recuerda a bandas puede surgir con células cuadradas, las bandas se vuelven mucho más estables cuando se consideran células rectangulares, sugiriendo que la geometría celular puede reforzar o contribuir a la estabilización de patrones espaciales, en particular el de células radicales con pelos.

El modelo de red mínima que se comporta como un sistema activador-inhibidor produjo patrones espaciales que son cualitativamente iguales a los patrones de pelos obsevados en hoja y raíz, permitiendo probar el papel de módulos de regulación y del contexto y geometría celulares en la formación de dichos patrones. Sin embargo, esta red reducida no reproduce todos los aspectos del sistema de estudio. Por ejemplo, el modelo de red mínima se utilizó para probar diversas hipótesis respecto al gen blanco de la señal del córtex y los resultados de este análsis (Resultados y Tabla en Apéndice A) apuntaron a que la señal del córtex debía impactar a la red de regulación genética regulando positivamente a los elementos activadores en las bandas en que se forman los tricomas. Sin embargo, esta predicción fue refutada por experimentos que demostraron que el efecto de la señal era sobre uno de los activadores, pero negativo. Además, la red reducida no consideraba muchas de las interacciones documentadas, mismas que podrían ser importantes para la robustez y riqueza dinámica del sistema global, y suponía un espacio continuo no celularizado. Por ello, se desarrolló un modelo que considera espacio celularizado y RRGs tan completas como es posible definir con los datos experimentales disponibles. Este modelo se presenta en la siguiente sección.

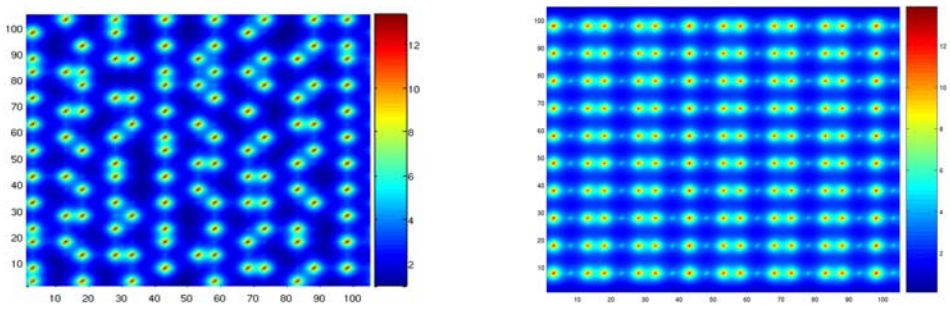


Figura 2.2: Patrones característicos resultantes de dinámicas de reacción-difusión. El rojo representa alta concentración del complejo activador, mientras que el azul representa baja concentración del mismo. A la izquierda se muestra el patrón que representa al de los tricomas de una hoja y el de la derecha el patrón bandeado de los tricoblastos.

2.3. Un sistema de RRG acopladas en un dominio celularizado

Si bien el modelo de red mínima similar a un sistema activador-inhibidor permitió avanzar en el entendimiento del sistema de estudio, dicho modelo deja fuera aspectos relevantes de la determinación de tipo celular en epidermis de *Arabidopsis thaliana*. Por ello, se elaboró un modelo con los siguientes componentes: un dominio bidimensional formado por celdas que representan células de la epidermis, una red de regulación genética detallada representada en cada celda [2] del dominio, comunicación intercelular entre células vecinas y una señal asociada a la unión de células del cortex en el caso de raíz. A continuación se describe cada elemento de este modelo, pero puede encontrarse una descripción más detallada en el Apéndice B.

El dominio espacial está representado como una rejilla cuadrada de 20×20 células que corresponde a una sección cuadrada de la epidermis de hoja y a una sección cilíndrica de la epidermis de raíz. En el caso de hoja, se definieron condiciones de frontera de flujo cero en todos los lados del cuadrado, mien-

[2]Las celdas del dominio espacial son representaciones simplificadas de células de la epidermis, sin embargo, de aquí en adelante se hablará indistintamente de celdas o células del dominio.

tras que en el de raíz se definieron condiciones periódicas para los dos bordes verticales y de flujo cero para los horizontales, simulando un cilindro.

En cada caso, epidermis de hoja y de raíz, se definió una red de regulación genética generada a partir de los datos experimentales reportados en la literatura hasta junio del 2007 (revisión actualizada en Apéndice D). La evidencia experimental, parte de la cual no estaba disponible cuando se propuso el modelo de red mínima descrito en la sección anterior, muestra que ambas redes, la de hoja y la de raíz, comparten ciertos genes e interacciones, pero también que tienen algunos elementos específicos de cada red, por lo que en este caso se mantuvieron como redes distintas. La arquitectura asociadas a cada una de estas redes puede verse en la figura 2.3 (detalles en Apéndice B).

La comunicación celular se modeló mediante una versión discreta de difusión y representa el movimiento pasivo de proteínas a través de plasmodesmos que comunican a células vecinas. Para cada una de las dos redes, se ha descrito un conjunto distinto de elementos de la red que tienen un efecto no autónomo, es decir, que afectan a células vecinas. Así, en cada caso, los elementos móviles son distintos. Al implementarse la comunicación celular en el modelo, se tiene un sistema de redes acopladas, ya que los elementos móviles modifican el estado de ciertos elementos de la red en células vecinas. Por ello, llamamos a este modelo un modelo de *meta-red* (figura 2.3).

Finalmente, en el caso del modelo de epidermis de raíz, se modeló la señal posicional proveniente del córtex, cuya identidad y efecto habían sido ya bien descritos para cuando se elaboró el modelo de meta-red. Esta señal se intodujo como un regulador negativo de *WER*, uno de los genes de la red de raíz, en las células epidérmicas ubicadas sobre la unión de dos células del córtex.

En las simulaciones, los modelos de meta-red se iniciaron con estados de activación de los genes dados aleatoriamente, posteriormente se realizaron iteraciones en que, alternadamente, se actualizaba el estado de los genes de cada célula aplicando las reglas lógicas y se difundían los elementos móviles del sistema (figura 2.4).

2.3.1. Resultados del modelos de redes acopladas

Una vez especificados los modelos de meta-red para epidermis de hoja y de raíz, se realizaron simulaciones con el fin de investigar si los elementos considerados en estos modelos eran suficientes para dar lugar a dos estados estacionarios de expresión genética en las células (correspondientes a células

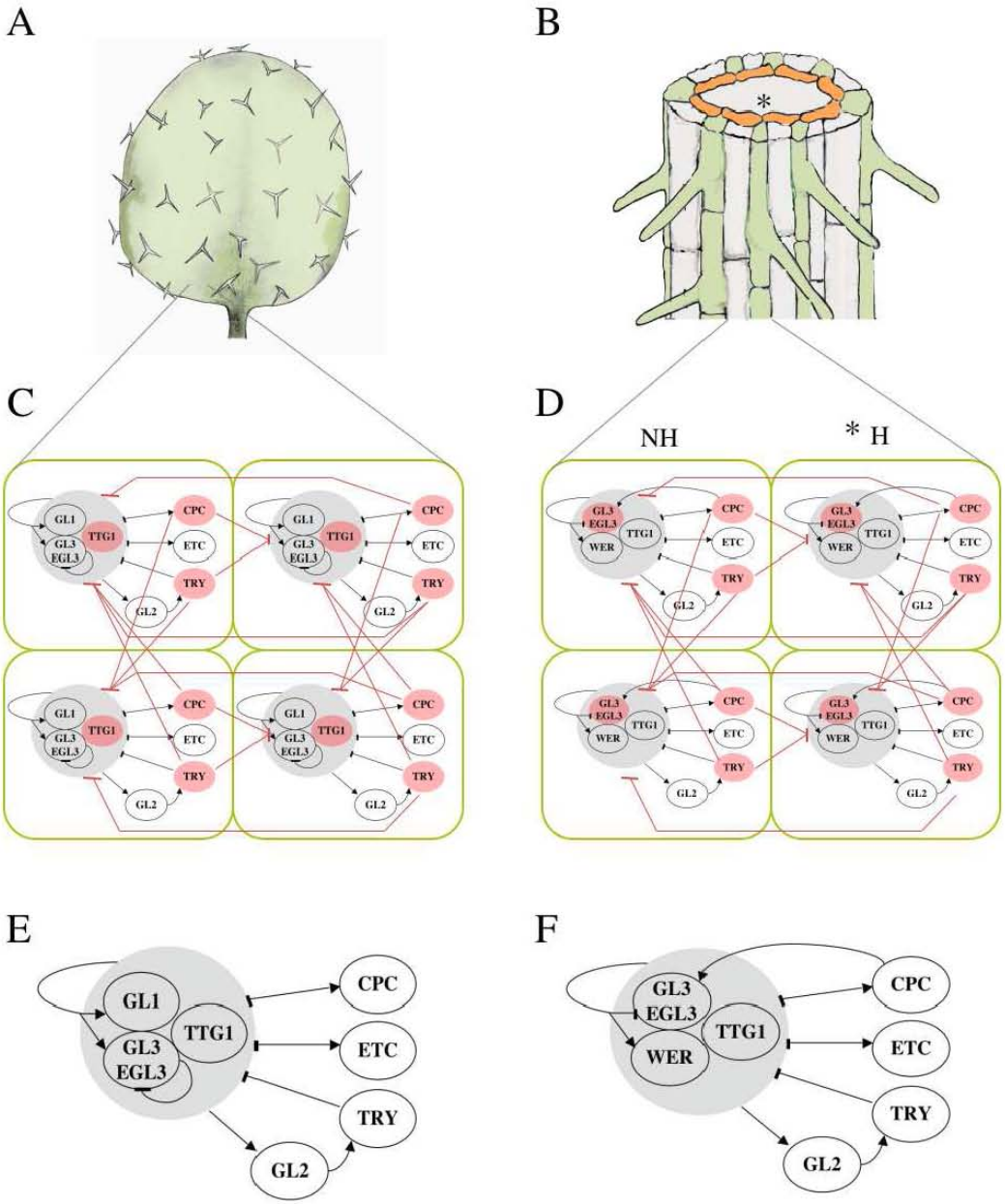


Figura 2.3: Representación gráfica del modelo de redes acopladas. (A) Patrón de pelos en la hoja y (B) patrón de pelos en la raíz; el asterisco indica la posición en la que surgen los tricoblastos. (C y D) Cada una de las celdas contiene una RRG y éstas están acopladas a través del moviemiento de ciertas proteínas. (E y F) Detalle de redes de regulación genética asociadas a las determinación de tipo celular en la epidermis de hoja y raíz, respectivamente.

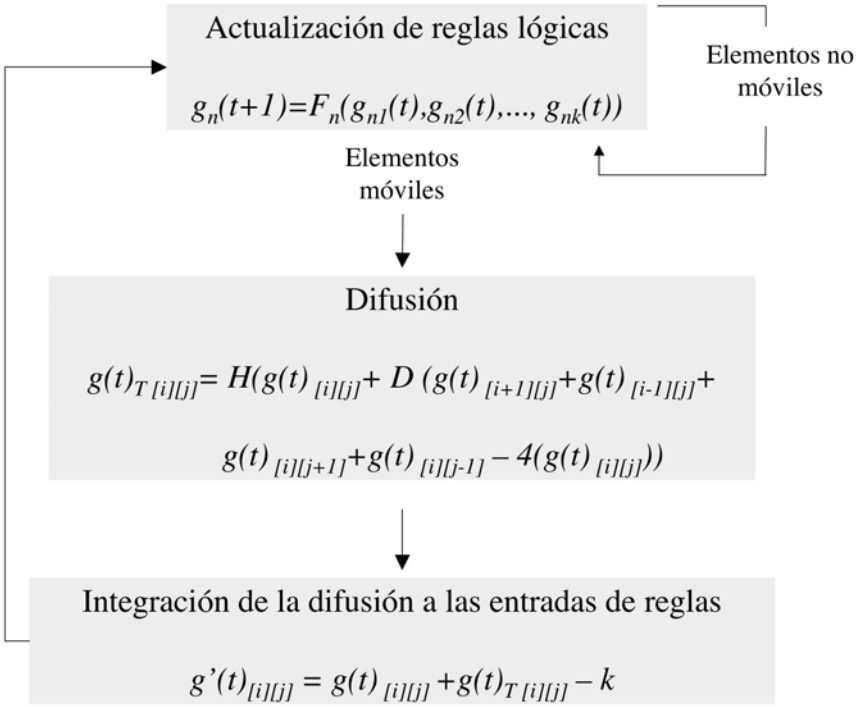


Figura 2.4: Diagrama de pasos seguidos en la simulación del modelo de redes acopladas.

de pelos y no pelos) y a un arreglo de células similar al que se observa en epidermis de hoja y de raíz.

Las simulaciones mostraron que ambos modelos eran capaces de reproducir cualitativamente el arreglo espacial de las células con pelos y sin pelos observado en cada tipo de epidermis. El modelo de raíz generó sólo dos configuraciones genéticas estacionarias que coinciden con los perfiles de expresión genética reportados para tricoblastos y atricoblastos y, más aún, generó arreglos bandeados de estos dos tipos de células. Por su parte, el modelo del sistema de hoja generó configuraciones celulares estacionarias equivalentes a tricomas y células de cementación (no pelos) arreglados en patrones como los observados en la hoja. Ambos patrones se mantuvieron constantes ante variaciones en el tamaño del dominio espacial y en el parámetro asociado a la tasa de difusión de elementos móviles y, en el caso del sistema de raíz, en la intensidad de la señal correspondiente a la señal del córtex.

En ambos casos se llevó a cabo una evaluación cuantitativa de la similitud entre los patrones obervados y los generados por el modelo. En el caso de raíz se midió la proporción de células correspondientes a tricoblastos y atricoblastos que se encontraban en una posición equivocada. Ésta es una medida de error que se utiliza comúnmente en los análisis experimentales de este sistema, por lo que fue posible compararla con datos empíricos. Se encontró que el porcentaje de células ectópicas es comparable (mismo orden de magnitud) en el sistema real y el simulado. Para el caso de la hoja se obtuvo una medida del espaciamiento entre células correspondientes a tricomas que también se ha reportando para hojas reales, y se encontró que esta medida efectivamente reflejaba un patrón espaciado de los tricomas.

Con el fin de validar los modelos de determinación y arreglo de tipo celular en ambos sistemas, se simularon los mutantes que han sido reportados experimentalmente y se compararon los patrones generados por las simulaciones de mutantes con los fenotipos reportados en cada caso. Los mutantes simulados reprodujeron cualitativamente los mutantes sencillos, dobles y triples que se probaron (figuras 2.5 y 2.6, discusión en Apéndice B), lo que sugiere que el modelo reproduce adecuadamente la dinámica de determinación de tipo celular en cada caso y constituye un módulo funcional que es suficiente para que este proceso del desarrollo se lleve a cabo.

Es importante mencionar que para las RRGs asociadas a raíz y a hoja se supuso que ciertos elementos, a saber *GLABRA1* (*GL1*, específico de hoja) y *WERWOLF* (*WER*, específico de raíz), presentan una asa de autorregulación

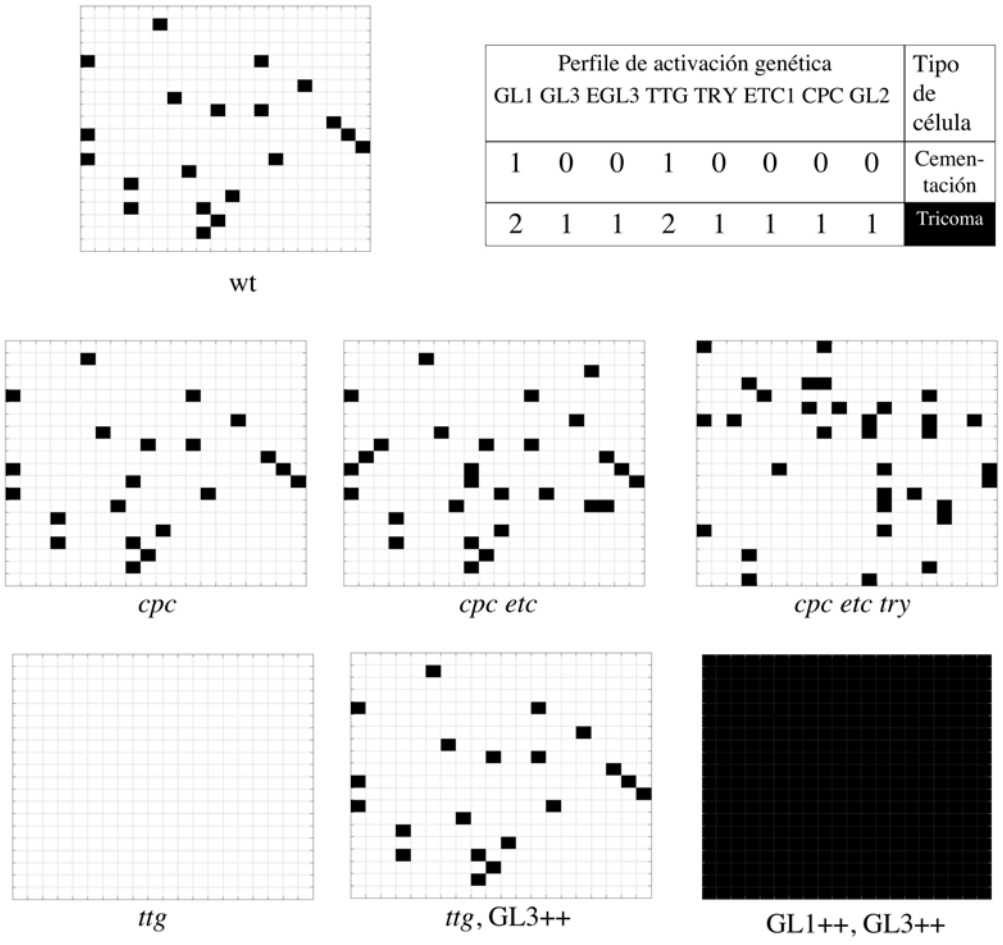
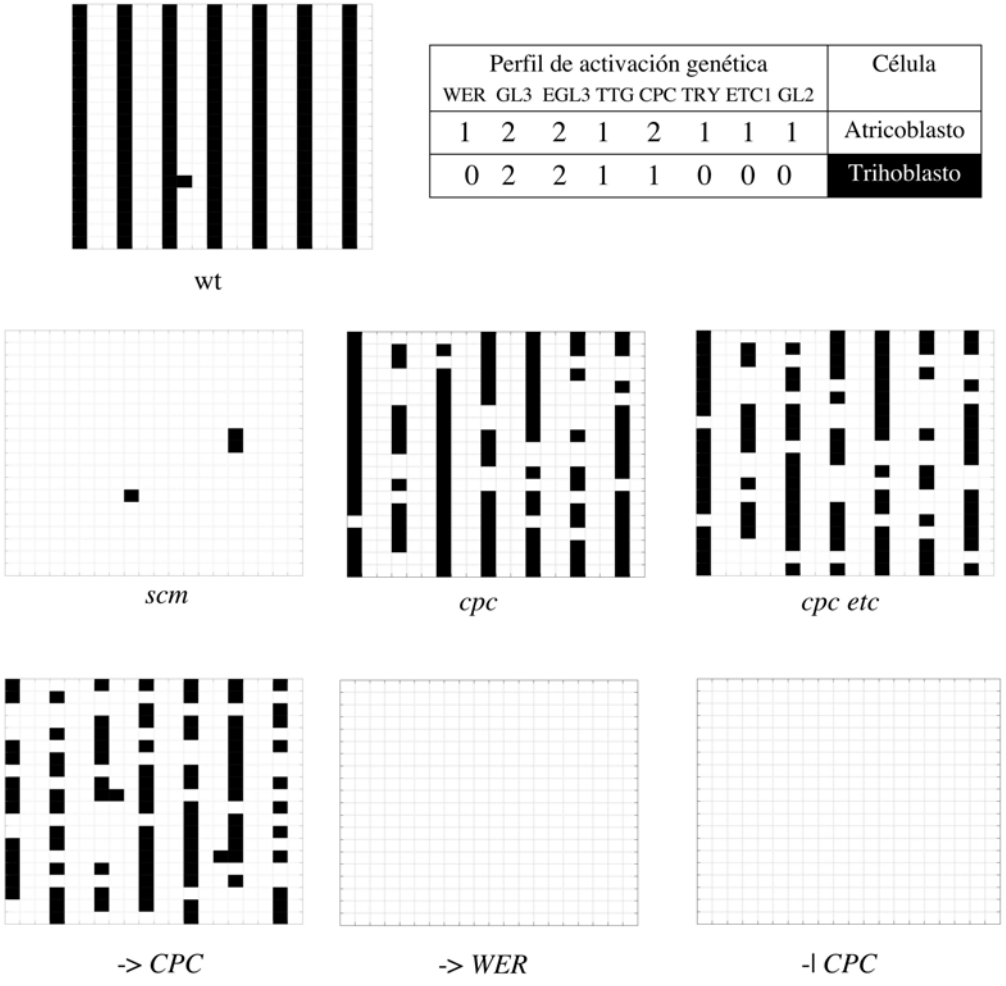


Figura 2.5: Patrones resultantes de la simulación del sistema de hoja (wt) y de las líneas mutantes del mismo.



Perfil de activación genética								Célula
WER	GL3	EGL3	TTG	CPC	TRY	ETC1	GL2	
1	2	2	1	2	1	1	1	Atricoblasto
0	2	2	1	1	0	0	0	Trihoblasto

Figura 2.6: Patrones resultantes de la simulación del sistema de raíz (wt) y de las líneas mutantes del mismo.

positiva, ya sea directa o indirecta. Si bien cuando se adoptó esta suposición no había datos que la apoyaran directamente, sí se contaba con evidencia indirecta que sugería que esto ocurre. Por ejemplo, ambos genes se encuentran activos en el mismo sitio que algunos de sus inhibidores. Uno de los resultados derivados del análisis de mutantes consiste en que se observó que la autorregulación positiva de *WER* y *GL1* es importante para la reproducción de los patrones observados en todos los mutantes.

Algunos autores han propuesto mecanismos que no requieren de la autoactivación de estos genes, en particular de *WER* y presentan resultados teóricos y experimentales que apoyan su propuesta (Savage *et al.* 2008). Sin embargo, esta evidencia es, como se discute con detalle en el Apéndice B, insuficiente para probar su afirmación. De hecho, ahora hay evidencia experimental de una autorregulación positiva de *WER* a través de otro elemento del sistema de raíz, *SCRAMBLED (SCM)* (Kwak & Schiefelbein 2008). La relación del mecanismo que se propone en esta tesis con otros mecanismos propuestos para la formación de patrones de pelos en *Arabidopsis thaliana* se discute en el siguiente capítulo.

Los resultados descritos arriba alentaron el uso de estos modelos para probar hipótesis y generar nuevas predicciones. A continuación se presenta uno de los análisis diseñados en esta dirección y en el siguiente capítulo de discuten otros análisis de este tipo.

Desde el trabajo con la red mínima referido en el capítulo anterior, se propuso que la geometría celular tiene un efecto estabilizador del patrón espacial que caracteriza la disposición de tricoblastos en la raíz de *Arabidopsis thaliana*. Esta hipótesis se puso a prueba mediante el uso del modelo de meta-red del sistema de epidermis de raíz. Para ello, se simuló una diferencia en la tasa de difusión de los elementos móviles en diferentes direcciones, haciendo la tasa de difusión en la dirección horizontal mayor que aquélla de la dirección vertical (correspondiente al eje apico-basal de la planta). Este cambio en las tasas de difusión vertical y horizontal corresponde al hecho de que las células de la epidermis de raíz se alargan en la dirección apico-basal durante su desarrollo, haciendo que sus núcleos, donde se transcriben los elementos de la red, se encuentren más alejados entre sí en la dirección vertical que en la horizontal. Como resultado de estas simulaciones se encontró que efectivamente una mayor tasa de difusión en el eje apico-basal genera patrones con menos errores, es decir, una menor proporción de células correspondientes a tricoblastos o atricoblastos ectópicos (Figura 5 del Apéndice B).

Estos resultados muestran que un mecanismo que genere esta diferencia en las tasas de difusión vertical y horizontal efectivamente estabiliza el patrón bandeado que se observa en epidermis de raíz. En este trabajo se propone que tal mecanismo está dado por el alargamiento de las células en una dirección.

Del análisis de los modelos de meta-red para epidermis de raíz y hoja puede entonces concluirse que los elementos que conforman el modelo (la RRG documentada experimentalmente, el dominio espacial celularizado, la comunicaión celular de corto alcance y la señal del córtex en raíz) conforman un sistema epigenético que es suficiente para la determinación de los tipos celulares con y sin pelo, así como para su disposición espaciada y bandeada. Además, puede concluírse que la información posicional necesaria para la determinación y arreglo espacial de células con pelos y no pelos se genera durante el proceso mismo de diferenciación celular y con la intervención de las células que han de diferenciarse.

Por ello, se concluye que la deficinición tradicional de información posicional (Wolpert 1996), en la que la ésta se entiende como fija e independiente de las células que se diferencian, es una definición limitada e insuficiente para el estudio de sistemas del desarrollo. A su vez, la noción de información posicional suele contraponerse a la de diferenciación por linaje celular. Sin embargo, los resultados aquí presentados invitan a revisar algunas de las dicotomías que comúnmente se manejan en biología del desarrollo, tales como innato-adquirido o bien, posición-linaje, teniendo en mente que la diferenciación celular es un proceso en el que los factores intra y extracelulares se retroalimentan y que las células mismas participan en su diferenciación. Por otra parte, se mostró que la formación de patrones de tricomas y tricoblastos no depende de valores de parámetros, sino, al parecer, de la dinámica colectiva de una población de células con RRGs acopladas. Esto resuena con la noción de morfogénesis que plantea S. Oyama en su libro, The Ontogeny of Information (Oyama 2000):

> Form emerges in successive interactions. Far from being imposed on matter by some agent, it is a function of the reactivity of matter at many hierarchical levels, and of the responsiveness of those interactions to each other.

Capítulo 3

Propiedades organizativas del sistema de determinación de tipo celular en epidermis de *Arabidopsis thaliana*

Como se mencionó en la introducción, algunos de los conceptos centrales en el área de evo-devo son aquéllos relacionados con las propiedades organizativas de los sistemas de desarrollo, es decir, con propiedades como la modularidad, plasticidad y robustez. Éstas se refieren a características sistémicas, dinámicas o estructurales, de los procesos de desarrollo y su estudio es imprescindible para el entendimiento de la evolución del desarrollo en organimos muticelulares. Esto se debe a que el entendimiento de estas propiedades y el estudio de su origen permitirá comprender mejor cuáles son los cambios genéticos, ambientales o de otros tipo que pueden originar nuevas formas, cuáles son factores que determinan el que un proceso pueda repetirse de forma estereotipada en distintas condiciones, cuáles son los procesos genéricos que se repiten en distintos linajes de seres vivos y cómo éstos dan cuenta de formas y estructuras recurrentes en las formas vivas, etcétera. En este capítulo se discute el papel de ciertas propiedades organizativas en el desarrollo y se plantean las preguntas relacionadas con estas propiedades que han sido referidas mediante el uso del modelo de meta-red. Finalmente, se presentan los resultados de análisis de robustez, modularidad, plasticidad y redundancia del modelo de meta-red, mismos que se discuten en el marco de evo-devo. Los detalles de este capítulo pueden consultarse en los Apéndices B y C.

3.1. Modularidad

La modularidad es una propiedad que parece común a los más diversos sistemas biológicos (Wagner 2005, Wagner *et al.* 2007), ya sea en aspectos morfológicos, fisiológicos, etológicos o genéticos. Consiste en que un sistema biológico puede dividirse en subconjuntos con algún grado de autonomía, ya sea funcional, morfológica o de otro tipo. Se ha planteado que la modularidad es importante para el mantenimiento de un organismo o sistema biológico, pues la organización modular evita que el daño o alteración de un módulo afecte a otras partes del sistema. Por otra parte, se ha propuesto que los módulos pueden ser reusados o combinados de distintas formas a lo largo de los procesos evolutivos, dando lugar a sistemas que son robustos ante daños o alteraciones y también flexibles (von Dassow & Munro 1999). Algunas de las preguntas abiertas respecto a la modularidad en sistemas de desarrollo, y en sistemas biológicos en general, están relacionadas con el origen de esta propiedad. Más específicamente, pese a que se han propuesto algunos mecanismos que podrían subyacer la generación de modularidad en redes complejas (Kashtan & Alon 2005), aún no se sabe cómo surge durante la evolución de redes de regulación genética o de los seres vivos, ni si es una consecuencia inevitable de su evolución o si es una propiedad que pueda resultar de la acción de la de selección positiva.

El estudio de la determinación de tipo celular en epidermis de raíz y hoja de *Arabidopsis thaliana* ha permitido descubrir conjuntos de interacciones genéticas intra e intercelulares que se postulan como módulos funcionales asociados a la generación de dos perfiles de expresión genética estables (pelo y no pelo) y al surgimiento de arreglos espaciales de estos dos tipos de células.

Los modelos de meta-red descritos antes parecen efectivamente constituir módulos semiautónomos suficientes para generar dos tipos celulares organizados en distintos patrones espaciales. Es interesante mencionar que ambos módulos comparten la mayoría de los genes que los conforman y que los que son diferentes entre raíz y hoja pertenecen a las mismas familias multigénicas. Más aún, pese a que tienen diferencias, ambos módulos parecen también compartir motivos de regulación del tipo activador-inhibidor. Esto sugiere que los módulos o meta-redes de raíz y hoja tienen un origen evolutivo común.

3.2. Plasticidad

Si bien los módulos descritos en este trabajo son suficientes para la determinación y arreglo espacial del tipo celular, es importante mencionar que ambos están relacionados con otros módulos y vías de señalamiento. Así, estos módulos no se manifiestan en células de la endodermis en la raíz o del parénquima en la hoja pues están regulados por factores de crecimiento, hormonas u otros factores específicos de la epidermis. De igual manera, estos módulos afectan a módulos que tienen que ver con la diferenciación de las células de pelos y no pelos y que median la transformación de una célula asignada a cierto destino celular a una célula con las carcterísticas de dicho destino celular (e.g. vacuolas grandes, proyecciones de la membrana, etc.).

El módulo de determinación de tipo celular de hecho actúa como integrador de señales ambientales que regulan la producción y distribución de hormonas, mismas que, a su vez, actúan como *entradas* de las meta-redes que se han descrito en este trabajo. De esta forma, estas redes están asociadas a la generación de fenotipos distintos en distintos momentos del desarrollo y ante distintas condiciones ambientales, es decir, la repuesta plástica de la *Arabidopsis thaliana*.

Como parte del desarrollo plástico, la regulación de estas meta-redes a través de hormonas y factores ambientales puede generar mayor o menor densidad de pelos, así como cambios en su distribución espacial. Los tricomas, por ejemplo, se distribuyen de manera diferencial en las distintas estructuras y órganos de la planta, e inclusive en las diferentes regiones de una misma estructura [1].

Entre las hormonas que afectan al módulo de la epidermis de hoja, se ha identificado a las giberelinas (GA) como inductores constitutivos y necesarios de tricomas en la parte aérea de *Arabidopsis thaliana* (Telfer *et al.* 1997). Perazza y colaboradores (Perazza *et al.* 1998) mostraron que las GA tienen un efecto positivo sobre la expresión de *GL1*, uno de los componentes de la meta-red asociada a determinación de tipo celular en epidermis de hoja. Sin embargo, en principio existen muchas maneras de actuar sobre la red transcripcional de manera que se aumente la densidad de tricomas y la expresión de *GL1*. El papel de *GL1* como promotor de tricomas se dedujo principalmente

[1] Éstos suelen ser mucho más abundantes en la cara adaxial que en la abaxial y su densidad y distribución en cada cara puede cambiar durante el desarrollo vegetal (Tsukaya & Horiguchi 2005)

a partir del fenotipo *calvo* de mutantes con pérdida de función y de los tricomas ectópicos (por ejemplo, en pétalos) en experimentos de sobreexpresión de *GL1*. No obstante, la sobreexpresión de *GL1* no produce un aumento claro en la densidad de tricomas foliares (Larkin *et al.* 1994) y hasta ahora no hay evidencia de que las moléculas que median la vía de GA se unan al promotor o a alguna otra región de *GL1*.

Por ello, y como una primera aproximación al estudio de la plasticidad en este sistema, se probó de manera sistemática las diferentes maneras en que las vías de señalamiento pueden estar afectando a la red de regulación transcripcional. Se simuló de manera dinámica el conjunto de posibles interacciones sencillas, i.e., sobre un sólo gen, que podrían mediar el efecto de las GA sobre la meta-red y se compararon los resultados obtenidos para cada posible interacción con los fenotipos reportados para inhibición o sobreexpresión de GA.

Los resultados de estas simulaciones muestran que el efecto positivo de la señal sobre los genes de la familia *bHLH* y negativo sobre *TRY* son los únicos que dan lugar a un aumento en la densidad de tricomas (Apéndice B). Estos resultados apuntan a que las GA pueden estar afectando a la red de regulación genética no vía *GL1*, sino vía los genes *bHLH* o *TRY*, lo que da lugar a una prediccón precisa y que puede ponerse a prueba experimentalmente. De hecho, un par de grupos han reportado que el blanco de diversas vías hormonales, entre las que se encuentra la de GA, es *GL3* o *EGL3* (los genes *bHLH*) (Maes *et al.* 2008, Yoshida *et al.* 2009), lo que confirma la predicción derivada de las simulaciones.

Existen aún numerosas preguntas abiertas respecto al origen de la plasticidad del desarrollo en plantas, así como al papel de la plasticidad en la diversificación de los organismos durante la evolución (Waddington 1953, Baldwin 1896, West-Eberhard 2003). Sin embargo, como se muestra en esta sección, la caracterización y modelación dinámica de los módulos de desarrollo descritos experimentalmente puede ayudar a entender cómo éstos integran las señales del mismo organismo y del ambiente, mediando la repuesta plástica. Además, este tipo de estudios promete contribuir al entendimiento de cómo el desarrollo plástico puede estar relacionado con fenómenos como la asimilación genética (Waddington 1953) y el origen de novedades evolutivas.

3.3. Robustez y redundancia dinámica

Los procesos que caracterizan el desarrollo de plantas y animales ocurren de manera relativamente determinada aún cuando los organismos se desarrollan en medios muy distintos o están sujetos a diversos tipos de fluctuaciones y cambios ambientales. Incluso ciertos aspectos del desarrollo están conservados en linajes muy distantes. Por ello, se dice que el desarrollo es un proceso robusto ante diversos tipos de perturbaciones. La robustez ante perturbaciones es entonces una propiedad importante de los sistemas de desarrollo y, más aún, parece estar asociada a otras propiedades organizativas, tales como la capacidad de evolucionar de un sistema (Wagner 2005). En particular, en esta sección se presentan los resultados de análisis de robustez para las meta-redes que son objeto de estudio en este trabajo, así como los resultados de un análisis orientado a estudiar la relación entre redundancia y robustez del sistema (detalles en Apéndice C).

3.3.1. Análisis de robustez

Con el fin de evaluar la robustez de los módulos de determinación y del arreglo de tipo celular en epidermis de *Arabidopsis thaliana* ante distintos tipos de perturbaciones, se llevaron a cabo las pruebas que se describen en los siguientes párrafos.

Para el sistema de epidermis de hoja, se modificaron las condiciones de frontera haciéndolas periódicas y se verificó que, para rejillas de 20×20 celdas, estas condiciones no afectan la determinación y distribución espacial de tricomas. También se variaron sistemáticamente los valores de los parámetros de difusión de elementos móbiles de la meta-red y se encontró que el patrón de espaciamiento típico de los tricomas se mantiene para un amplio intervalo de valores (Apéndice B). Así mismo, el sistema de hoja resultó ser sumamente robusto a alteraciones puntuales en las reglas lógicas. Las reglas lógicas se representan en tablas en las que la última columna es la "salida" de las reglas. Estas salidas se modificaron sistemáticamente de una en una y se encontró que el 88 % de las alteraciones sencillas de las reglas no afecta la naturaleza de los atractores obtenidos ni la disposición espacial de los tipos celulares.

Se realizaron análisis similares para el caso del sistema de raíz y se encontró que también este sistema es sumamente robusto ante cambios en valores de parámetros, condiciones de frontera y alteraciones puntuales de las reglas lógicas. Juntos, estos resultados muestran que el comportamiento de las meta-redes

no depende tanto de valores precisos de parámetros o de reglas particulares como de la estructura global del sistema.

3.3.2. La redundancia dinámica confiere robustez a las meta-redes

Los esfuerzos de modelación de sistemas de desarrollo, en conjunción con numerosos trabajos experimentales, están descubriendo algunas propiedades de las red de regulación genética que se proponen como propiedades organizativas potencialmente genéricas (e.g. von Dassow & Odell 2002, Espinosa-Soto *et al.* 2004, Jönsson *et al.* 2006). En particular, se ha propuesto a la redundancia dinámica al interior de redes, es decir, al acoplamiento coherente de sub-redes, como una de propiedad común a diversos sistemas biológicos (Kwon & Cho 2008). Con el fin de distinguir entre redundancia de elementos idénticos en un sistema y redundancia funcional mediada por elementos diferentes, se maneja también el término *degeneración* (Tononi *et al.* 1999) para hacer referencia a lo que en este trabajo se denomina redundancia dinámica.

Durante los últimos años se han propuesto, además de los modelos de meta-redes presentados en este trabajo, diferentes modelos dinámicos para la determinación y arreglo de tipos celulares en la epidermis de hoja y raíz de *Arabidopsis thaliana* (Benítez *et al.* 2008, Bouyer *et al.* 2008, Digiuni *et al.* 2008, Savage *et al.* 2008, Kwak & Schiefelbein 2009, figura 3.1). Si bien estos modelos tienen diferentes enfoques, métodos y alcances, colectivamente proveen de una visión amplia y rica de los sistemas de estudio. De hecho, cada uno de estos modelos propone un mecanismo de determinación y arreglo de células epidérmicas basado en un subconjunto de las interacciones que han sido consideradas en los modelos de meta-red. Es importante mencionar que cada uno de estos mecanismos no excluyentes es, en principio, suficiente para la determinación de células con y sin pelo. Esto sugiere que los sistemas de hoja y raíz contienen sub-módulos funcionales que son redundates entre sí (Benítez *et al.* 2008, Schiefelbein *et al.* 2009) y que, probablemente, confieren robustez al sistema completo.

Aunque el efecto de la redundancia genética ha sido bastante estudiado en el contexto de RRGs, el papel de la redundancia de motivos de regulación o sub-redes se ha mantenido poco explorado, sobre todo en sistemas documentados experimentalmente. Entonces, con el fin de investigar si efectivamente los sistemas estudiados presentan redundancia dinámica y si, de presentarla, ésta

confiere robustez al sistema global, se utilizó la metodología desarrollada para la simulación de redes acopladas y se simularon de forma independiente todos los mecanismos de formación de patrones celulares propuestos hasta ahora. Las simulaciones se llevaron a cabo de acuerdo con los métodos establecidos para la modelación espaciotemporal de las meta-redes (Apéndice B).

Tras llevar a cabo la modelación de los distintos mecanismos haciendo uso de la misma plataforma de simulación, se encontró que cada uno de ellos es suficiente para la determinación de dos tipos celulares y para la generación de un arreglo espaciado o bandeado de estos tipos, según sea el caso (Apéndice C), lo que indica que efectivamente los sistemas de determinación y arreglo de células de la epidermis exhiben redundancia dinámica.

Posteriormente se propusieron dos estimaciones de robustez, una que refleja la robustez de los sistemas ante mutaciones sencillas de pérdida de función, y otra que refleja su robustez ante perturbaciones estocásticas. Tras evaluar en estos términos la robustez de cada uno de los mecanismos propuestos y de compararla con la de todos los mecanimos acoplados, se encontró que los mecanismos acoplados son significativamente más robustos que cada uno de los mecanismos aislados. Esto confirma la hipótesis de que la redundacia dinámica confiere robustez a los sistemas completos de determinación y arreglo de tipos celulares en la epidermis de *Arabidopsis thaliana* (Apéndice C).

Estos análisis permiten plantear una relación entre modularidad dinámica, redundancia y robustez que valdrá la pena estudiar en otros casos particulares documentados experimentalmente. Además abren numerosas preguntas respecto al origen de esta relación, tales como si la redundancia y modularidad dinámicas son producto de la manera en que los sistemas de desarrollo y, en particular las RRG se conforman y cambian, o bien, si son seleccionadas positivamente en caso de que los patrones que generan sean adaptativos.

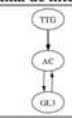
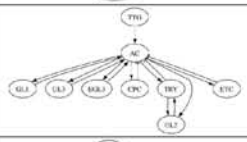
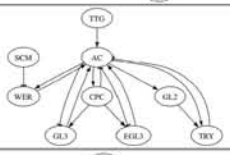
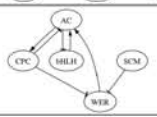
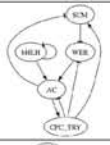
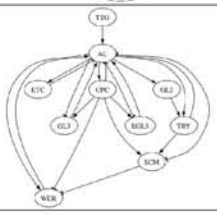
	Módulo	Diagrama de interacciones	Suficiente	Necesario
Epidermis de hoja (TTG, TRY y CPC pueden moverse entre células)	Mecanismo de agotamiento de TTG (Bouyer et al., 2008)		Si (Asume que el AC activa a *GL3* y *GL2* independientemente de *GL1*)	No (La sobreexpresión de *GL3* recupera la pérdida de fución de *ttg*)
	Inhibición por competencia (Digiuni et al., 2008)		Si (Asume que TTG está presente en todas las células)	No (Los sistemas del tipos A-I también exhiben el patrón)
	Meta-GRN (Benítez et al., 2008); también es el sistema de módulos acoplados.		Si (Asume que *GL1* es autoactivado directa o indirectamente)	No (El sistema de inhibición por competencia también exhibe el partón)
Epidermis de raíz (bHLH, TRY y CPC se mueven entre células; en todos los casos SCM inhibe a *WER* en la posición H)	Modelo con autoactivación de *WER* (Benítez et al., 2008)		Si (Asume que *WER* se auroactiva directa o indirectamente)	No (Los otros módulos pueden dar lugar al patrón)
	Mecanismo de mantenimiento mútuo (Savage et al., 2008)		Si (Asumes que *WER* y *GL3* tienen una expresión basal constante)	No (Los otros módulos pueden dar lugar al patrón)
	Sistema de acumulación de SCM (Schiefelbein and Kwak 2008)		Si (Asumes que *WER* y *GL3* tienen una expresión basal constante)	No (Los otros módulos pueden dar lugar al patrón)
	Sistema de módulos acoplados		Yes (Incorpora las suposiciones de cada módulo)	No (Los módulos independientes pueden dar lugar al patrón)

Figura 3.1: Módulos que han sido propuesto como responsables de la formación de patrones celulares en epidermis de raíz y hoja de *Arabidopsis thaliana*.

Capítulo 4

Discusión y conclusiones

Como resultado de este trabajo de tesis se obtuvieron dos modelos de sistemas de redes acopladas, basados en evidencia experimental, que han permitido proponer una explicación dinámica al origen de la información posicional en la epidermis de hoja y raíz de *Arabidopsis thaliana*. Estos sistemas integran los genes y relaciones de regulación genética reportados como relevantes en la literatura y, más aún, los integra en un modelo en que las redes de regulación genética de cada célula se comunican con las redes de células vecinas. Los modelos fueron validados mediante la realización de pruebas en las que se reprodujeron los arreglos de pelos y no pelos descritos para distintos tipos de mutantes de la red. Por otra parte, se generaron distintas predicciones, algunas de las cuales han sido verificadas experimentalmente por otros laboratorios. Los resultados de este trabajo sugieren que la información posicional necesaria para la determinación de tipo celular en epidermis emerge durante el proceso mismo de determinación de tipo celular, a partir de relaciones de regulación genética y comunicación intercelular. De esta forma, los patrones de expresión celular que constituyen la información posicional son robustos ante diferentes tipos de perturbaciones y pueden regenerarse.

Pese a que el sistema de determinación de tipo celular en epidermis en *Arabidopsis thaliana* es un sistema relativamente sencillo y aparentemente no fundamental para la supervivencia de las plantas en el laboratorio, es uno de los sistemas de determinación de destino celular mejor descritos y su estudio ha permitido avanzar en el entendimiento de los procesos celulares asociados a la diferenciación, así como apuntar propiedades potencialmente genéricas de las RRGs.

Los módulos funcionales que aquí se reportan son muy útiles como base para

integrar nuevos datos relacionados con el sistema particular, así como para el análisis y comparación de procesos del desarrollo, calibración de nuevos métodos de inferencia de redes, entre otras cosas. En particular, serán sumamente útil para integrar los datos que se generen en relación al papel de las hormonas vegetales en el desarrollo, el desarrollo plástico y la determinación de estomas, proceso que parece estar relacionado con la determinación y arreglo células con y sin pelos epidérmicos (Serna 2005 y referencias ahí citadas).

Por una parte, se encontró que los elementos de las redes, de forma colectiva, actúan de manera similar a un sistema del tipo activador-inhibidor. Este resultado sugiere que los morfógenos propuestos por Turing podrían tener su origen en colecciones de moléculas o procesos celulares y no necesariamente corresponder a una sola sustancia. Debido a que el sistema activador-inhibidor parace formar parte de otras redes asociadas a procesos del desarrollo (e.g. Newman & Bhat 2007), es posible que los sistemas de activador-inhibidor sean *temas* en la evolución del desarrollo, temas que además podrían haber sido fundamentales para el origen de la diversidad en plantas y animales (Newman & Bhat 2009).

Por otra parte, se mostró que las redes aquí estudiadas están formadas por subredes que, en principio, son suficientes por separado para la determinación de los tipos celulares y la distribución espacial de pelos. Luego, se mostró que dichas subredes actúan de forma redundante y que en conjunto incrementan la robustez del sistema de determinación y arreglo de pelos en epidermis. Estos resultados indican que la redundancia dinámica es uno de los mecanismos de canalización (*sensu* Waddington 1957) del desarrollo y que el estudio de esta propiedad, inexplorada hasta ahora, será importante en el entendimiento de la evolución de las RRGs y del desarrollo.

4.1. Perspectivas

Una de las preguntas interesantes que pueden abordarse ahora que se cuenta con una caracterización dinámica y estructural del módulo funcional asociado a la determinación y arreglo de tipos celulares en epidermis, es la de si dicho módulo se halla conservado en otros linajes de plantas y, en caso de estarlo, si cambios en este módulo (por ejemplo en la secuencia de expresión de genes) pueden explicar los diferentes arreglos de pelos observados en otras plantas. Otra de las preguntas que se derivan de esta tesis es la de cómo se han integrado durante la evolución los submódulos que conforman las RRGs estudiadas. En

particular, podrá ponerse a prueba la hipótesis de Newman y Baht (Newman & Bhat 2009), a saber, que los módulos más antiguos son aquéllos que subyacen la diversificación de los fenotipos y que articulan mecanismos genéricos del tipo reacción difusión. Además, será interesante estudiar si la selección natural ha tenido un papel importante en la evolución de este módulo funcional y en los genes que conforman las redes estudiadas y, con ello, en la evolución del arreglo celular de la epidermis. Este tipo de preguntas podría referirse en un inicio con un enfoque comparativo y podría complementarse con simulaciones de distintos tipos y, finalmente, con experimentos.

La idea de que ambos módulos tienen un origen común y de que a lo largo de la evolución se han reusado en distintas estructuras de la planta está también sustentada en el hecho de que el hipocotilo (región de transición entre la parte aérea de la planta y su raíz) presenta estomas en un patrón bandeado que parece estar regulado por algunos de los genes involucrados en la determinación y arreglo de pelos en hoja y raíz (Serna 2005). Este sistema "intermedio" podría contener elementos de los dos módulos y su mejor entendimiento ayudaría a comprender el origen evolutivo y la regulación durante el desarrollo de los módulos estudiados en raíz y hoja.

Con el fin de estudiar la evolución de los procesos del desarrollo, será interesante hacer uso de técnicas de biología molecular y de bioinformática para investigar si los módulos aquí estudiados están conservados en otras plantas y si pueden, al estar embebidos en distintos contextos celulares, dar lugar a la diversidad de patrones de pelos que se observan en plantas terrestres o si en otras plantas están asociados a procesos del desarrollo distintos. También, en caso de que los módulos no estén conservados, será interesante estudiar si la determinación y arreglo de pelos y no pelos en otras plantas se lleva a cabo por módulos dinámica o estructuralmente equivalentes a los que aquí se estudian.

En una revisión, Müller (Müller 2007) plantea que algunas de las preguntas fundamentales en el área de evo-devo están relacionadas con el entendimiento del origen y evolución del *repertorio* del desarrollo, el origen de las novedades fenotípicas y la relación entre condiciones ambientales y ecológicas con el desarrollo de plantas y animales. Tanto el trabajo que aquí se presenta, como los estudios a los que da pie, abordan algunas de estas cuestiones y proponen nuevas ideas y métodos respecto al origen de la información posicional, al origen de temas básicos en el repertorio del desarrollo (e.g. el sistema activador-inhibidor), a la relación de redes con el ambiente a través de hormonas, el origen de las novedades (distintos tipos de arreglos celulares) y mediante el

estudio de módulos redundantes y la evolución de la robustez a la evolución de los procesos y repertorio del desarrollo.

Apéndice A

Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in Arabidopsis root and leaf epidermis: a dynamic model.

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Original Article

Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in *Arabidopsis* root and leaf epidermis: a dynamic model

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ABSTRACT In *Arabidopsis thaliana,* leaf and root epidermis hairs exhibit contrasting spatial arrangements even though the genetic networks regulating their respective cell-fate determination have very similar structures and components. We integrated available experimental data for leaf and root hair patterning in dynamic network models which may be reduced to activator-inhibitor models. This integration yielded expected results for these kinds of dynamic models, including striped and dotted cell patterns which are characteristic of root and leaf epidermis, respectively. However, these formal tools have led us to novel insights on current data and to put forward precise hypotheses which can be addressed experimentally. In particular, despite subtle differences in the root and leaf networks, these have equivalent dynamical behaviors. Our simulations also suggest that only when a biasing signal positively affects an activator in the network, the system recovers striped cellular patterns similar to those of root epidermis. We also postulate that cell shape may affect pattern stability in the root. Our results thus support the idea that in this and other cases, contrasting spatial cell patterns and other evolutionary morphogenetic novelties originate from conserved genetic network modules subject to divergent contextual traits.

KEY WORDS: *gene network, activator-inhibitor, hair patterning*

Introduction

In multicellular organisms cell types are often arranged in a non-random manner, resulting in spatial patterns that may have important functional roles. Cell identity can be acquired and maintained by at least two non-excluding mechanisms, one involving cell-lineage and the other, positional information. The latter seems to be the predominant mechanism in plants (Scheres, 2001). In positional information dependent systems cells attain their identity through mediation of signals (chemical or other) that are not homogenously distributed throughout the organism. Under such circumstances, a pattern is likely to result from the interplay between gene regulatory networks and positional information, that includes contextual traits such as initial conditions, cell arrangement and domain size, geometry and growth (Goodwin, 2001).

Root and leaf epidermal cell patterning in *Arabidopsis thaliana* (*Arabidopsis* hereafter) provides a simple system to explore the processes by which gene regulatory circuits, coupled to the action of contextual conditions, determine spatial cell arrangement in biological systems. In addition, this system has been the subject of careful genetic studies and there is a vast amount of experimental evidence that can be integrated in a dynamic gene network model (see reviews in: Pesch and Hülskamp, 2004; Serna, 2005; Dolan, 2006 and references therein). Leaf epidermis bears hairs (trichomes) interspersed across the leaf surface. These structures tend to appear away from each other (Larkin *et al.*, 1996) resulting in dotted patterns with no clusters (Fig. 1A). *Arabidopsis* root epidermis has two cell types, hair and non-hair

Abbreviations used in this paper: AC, activator complex; bHLH, basic helix-loop-helix; CPC, CAPRICE; EGL3, ENHANCER OF GLABRA3; GL3, GLABRA3; H, hair (cell); NH, non-hair (cell); SCM, SCRAMBLED; TTG, TRANSPARENT TESTA GLABRA; TRY, TRIPTYCHON; WER, WEREWOLF.

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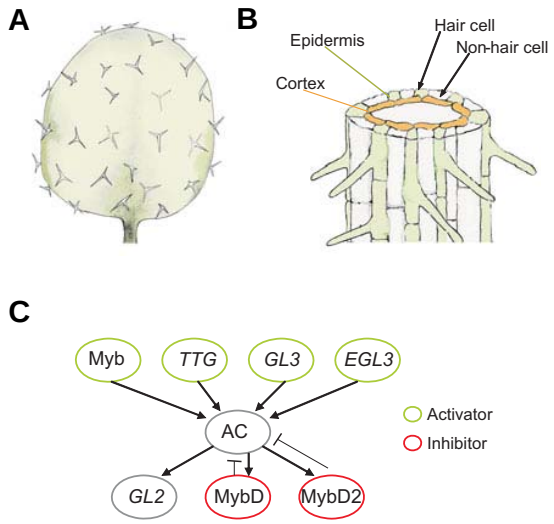

Fig. 1. **Networks regulating trichome and root hair patterns in *Arabidopsis*. (A)** *Sparsely dotted trichome pattern in a wild type* Arabidopsis *leaf.* **(B)** *Striped trichoblast pattern in* Arabidopsis *roots. Root hairs develop on epidermal cells that contact two cortical cells.* **(C)** *Genes that are not shared between these two networks belong to the same gene families. Myb corresponds to* GL1 *in leaves and to* WER *in roots, while MybD corresponds to* TRY *in leaves and to* CPC *in roots and MybD2 stands for* CPC *and* TRY *in leaves and roots, respectively.* CPC *and* TRY *have a partially redundant inhibitory effect on the Activator Complex (AC), these are known as the inhibitory elements of the network. The AC is composed of the activator genes TTG, GL3, EGL3 and GL1 or WER. Drawing (A) based on photograph from Larkin* et al. *(1996).*

cells that develop from trichoblasts and atrichoblasts, respectively. In all Brassicaceae species, including *Arabidopsis*, the root exhibits an arrangement of alternating cell files composed by either trichoblasts or atrichoblasts (Fig. 1B; Galway *et al.*, 1994; Dolan, 1996).

Despite the different spatial cell patterns found in leaf and root epidermis, the gene networks that underlie cell type subspecification in roots and leaves are very similar (Fig. 1C). Some genes are involved in both the root and leaf systems (Galway *et al.*, 1994; Di Cristina *et al.*, 1996; Payne *et al.*, 2000; Bernhardt *et al.*, 2003) and few are not (Oppenheimer *et al.*, 1991; Lee and Schiefelbein, 1999). However, for each privative gene of one of these systems there is a gene belonging to the same gene family that is privative of the other one. Moreover, interactions among the genes seem to be preserved in both cases (Pesch and Hülskamp, 2004), despite subtle differences (Zhang *et al.*, 2003; Bernhardt *et al.*, 2005; Kurata *et al.*, 2005). Therefore, networks with very similar architectures and molecular components yield strikingly different spatial cell arrangements in leaf and root epidermis (Fig. 1).

The contrasting patterns could be due to differences in the kinetic functions of the networks in each epidermal system (Meinhardt, 1982; Murray,1989). However, experimental data suggest that this is not the case because, at least for two key network components, wild type spatial cell patterns are recovered in transgenic lines that bear constructs with promoters swapped between leaf and root genes (Lee and Schiefelbein, 2001). An alternative explanation would imply that different contextual traits providing positional cues in the tissues are responsible for the distinct spatial patterns of cell types in leaves and roots.

In *Arabidopsis* roots, only epidermal cells that overlie two cortical cells become trichoblasts (Fig. 1B; Galway *et al.*, 1994), suggesting the presence of a positional signal that biases root epidermal cell fate. Since root cells mainly divide in one direction, all descendants of a root initial are arranged in a row along the baso-apical axis (Meyerowitz, 1997), giving rise to a constant spatial relation among distinct root cell layers. This does not happen in leaves. Such contextual differences may be responsible for the contrasting spatial cell patterns of root and leaf epidermis.

Lee and Schiefelbein (2001) have suggested that genetic networks underlying hair and non-hair determination and arrangement are equivalent in both epidermal systems. However, although these investigators have proven that the coding regions of two genes (*WEREWOLF*, *WER* and *GLABRA1*, *GL1*) are functionally equivalent, the different promoters could still be responsible for different transcription rates that could in turn be critical for attaining the two patterns. It has also been proposed that these networks can be understood as an activator-inhibitor dynamical system, a type of Turing reaction-diffusion system (Pesch and Hülskamp, 2004). Yet, this has not been formally tested.

In this article we first show that despite slight differences, the root and leaf networks are dynamically equivalent and then we explicitly test if, assuming such genetic networks with equal kinetics and regulatory interactions, different contextual traits are sufficient or necessary to yield contrasting spatial cell patterns resembling those observed in leaf and root epidermis. To achieve this, we developed network models grounded on experimental data for gene interactions during epidermal cell fate and then built a single generic network for either of both systems (Fig. 1C).

We found that the gene regulatory network (Fig. 1C) exhibits the same qualitative behavior as a simplified discrete system in which only one activator and one inhibitor are considered. Therefore, we reduced the network model to a type of reaction-diffusion system. Here we report that this continuous system, under different contexts is sufficient to recover the two spatial cellular patterns and that the root striped cellular pattern is only recovered when a positional signal affects the system in a specific manner. Moreover, we were able to reproduce and explain empirical results and provide novel predictions on gene interactions that may be tested experimentally. It would not be feasible to achieve these goals without a dynamical model that integrates the available empirical data.

Experimental Evidence

TRANSPARENT TESTA GLABRA (TTG) and bHLH proteins (GLABRA3, GL3 and ENHANCER OF GLABRA 3, EGL3) form functional multimers with Myb proteins GLABRA1 (GL1) (Larkin *et al.*, 1997; Payne *et al.*, 2000) and WEREWOLF (WER) (Lee and Schiefelbein, 1999; Bernhardt *et al.*, 2003, 2005) in leaf and root, respectively. Hence we refer to the TTG, GL3, EGL3 and GL1 protein complex as the leaf activator and to the TTG, GL3, EGL3 and WER protein complex as the root activator. Both activators promote transcription of *GLABRA2* (*GL2*, Di Cristina *et al.*, 1996;

Hung *et al.*, 1998; Szymanski *et al.*, 1998; Lee and Schiefelbein, 1999; Payne *et al.*, 2000; Wada *et al.*, 2002; Costa and Dolan, 2003; Bernhardt *et al.*, 2003, 2005;), whose expression determines leaf trichome cell-fate (Rerie *et al.*, 1994; Szymanski *et al.*, 1998) and root atrichoblast identity (Di Cristina *et al.*, 1996). Therefore, the leaf and root activator complexes, in which *GL1* and *WER*, respectively, take part (Larkin *et al.*, 1993; Lee and Schiefelbein, 1999), determine trichome cell identity in the leaf and atrichoblast fate in the root.

TRIPTYCHON (TRY) and *CAPRICE (CPC)* are the main repressors of the leaf and root activators, correspondingly (Schnittger *et al.*, 1999; Lee and Schiefelbein, 2002), although both have partially redundant roles in both root and leaf epidermal systems. *ENHANCER OF TRIPTYCHON AND CAPRICE 1* (*ETC1*) has also been proven to play an important role in inhibiting both activator complexes (Kirik *et al.*, 2004). Experimental evidence shows that *CPC* is upregulated by the root activator (Lee and Schiefelbein, 1999, 2002; Wada *et al.*, 2002; Bernhardt *et al.*, 2003; Costa and Dolan, 2003; Ryu *et al.*, 2005). On the other hand, the highest level of *TRY* expression occurs in the cells with maximum *GL1* and *GL3* expression levels (Larkin *et al.*, 1993; Schellmann *et al.*, 2002; Zhang *et al.*, 2003), suggesting that the leaf activator may also activate this inhibitor.

There is no experimental evidence for *GL1* or *WER* self-activation (Pesch and Hülskamp, 2004). However, *WER* and *CPC* have highest levels of expression in atrichoblasts (Lee and Schiefelbein, 1999; Wada *et al.*, 2002), while *GL1* and *TRY* peaks of expression occur in trichome cells (Larkin *et al.*, 1993; Schellmann *et al.*, 2002). As *TRY* and *CPC* repress *GL1* and *WER* expression, respectively, either direct or indirect self-activation of the activators is needed to overcome the negative regulation of the inhibitors. This should be tested experimentally.

GL3 and *EGL3* take part in the activator complexes, promoting non-hair cell fate in root and trichome fate in leaf (Payne *et al.*, 2000; Bernhardt *et al.*, 2003; Zhang *et al.*, 2003). In yeast-two hybrid, the GL3 and EGL3 proteins interact with CPC (Zhang *et al.*, 2003). Despite being included in the activator complexes, a recent work (Bernhardt *et al.*, 2005) has demonstrated that *GL3* and *EGL3* are not primarily expressed in non-hair root cells. Surprisingly, they are expressed in the hair cells and move towards those where the other activating elements are mainly expressed. Bernhardt *et al.* (2005) proposed that *GL3* and *EGL3* are activated by the inhibitor *CPC* and are repressed by the activator complex. In contrast to these results, in leaves *GL3* expression occurs in trichome cells, where *GL1* maximum expression and hence maximum levels of leaf activator activity, are found.

Although the above data indicate that root and leaf network topologies are slightly different, it seems that the dynamical behavior of both networks remains qualitatively the same because: i) since other elements *(e.g. WER)* of the activator complex are present only in non-hair cells and are a limiting factor for the formation of the activator complex, the concentration of such complex is always higher in non-hair cells, ii) diffusion tends to homogenize concentration of free GL3 or EGL3 proteins, iii) CPC,

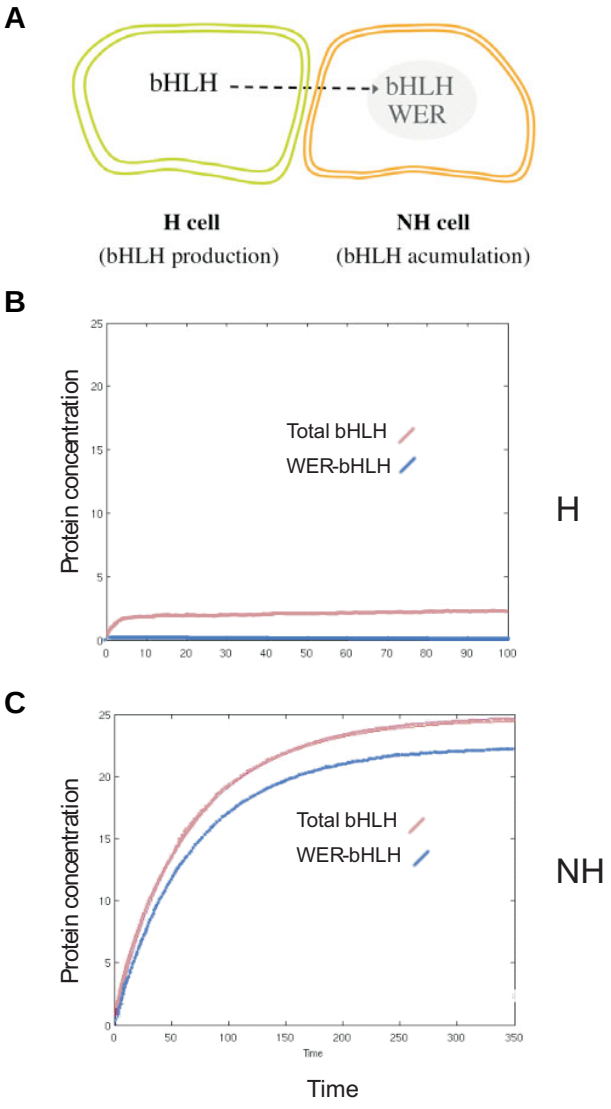

Fig. 2. Data on bHLH expression and movement (Bernhardt *et al.*, 2005) are consistent with Werewolf (WER) and bHLH proteins being mainly located in the same type of cells. (A) *Schematic of a two-cell system.* WER *represses the production of bHLH proteins, such as GL3 and EGL3, which tend to diffuse from high to low concentrations and can move to neighboring cells. As bHLH proteins interact with WER in a bHLH/WER complex, the concentration of free bHLH decreases in non-hair (NH) cells; consequently, bHLH produced in neighboring hair (H) cells keeps moving towards cells where* WER *is expressed. Since the activator complex seems to negatively regulate the expression of* GL3 *and* EGL3 *(Bernhardt* et al., *2005), no bHLH protein is actually produced in cells where* WER *is present. Free* GL3 *dynamics were modeled according to* $\frac{\partial G}{\partial t} = D\nabla^2 G + \frac{k_1}{C + k_1} - k_2 G - k_3 WG + k_4 C$ *while WER dynamics respond to* $\frac{\partial W}{\partial t} = f(x) - k_5 W + k_4 C - k_3 WG$ *and the rate of change in the concentration of the GL3-WER complex is given by* $\frac{\partial C}{\partial t} = k_3 WG - k_4 C$ *where D* = 0:35, k_1 = 0:3, k_2 = 0:22, k_3 = 0:6, k_4 = 0:15, k_5 = 0.2 *and f(x) equals 0.5 in NH cells and 0 in H cells.* **(B,C)** *In a continuous system in which the WER/bHLH complex is being formed, the total amount of bHLH protein (free and attached to WER) present in NH cells is higher than that present in H cells.*

which interacts with GL3 and EGL3, is expressed in hairless cells and moves to hair cells, exhibiting an homogenous or even atricoblast-biased concentration in root epidermal cells (Wada *et al.*, 2002; Kurata *et al.*, 2005); finally, iv) the total concentration of the GL3 or EGL3 protein is given by free protein plus that attached to the activator complex; then the concentration of total bHLH protein is always higher in the cells where both WER and TTG are present in comparison to cells where these components of the activator complex are not expressed. This final result is equivalent to that given by the leaf network where *GL1* and *GL3* or *EGL3* are mainly expressed in the same cell type in leaf epidermis, which could be due to *GL3/EGL3* activation by *GL1*. Simulation results (Fig. 2) support and illustrate this claim.

Results

The discrete cell-autonomous network resembles the reaction term of an activator-inhibitor system

Based on experimental evidence summarized above a gene network model was proposed. The network nodes represent genes and protein complexes involved in epidermal cell type determination and the edges stand for interactions among them (see Fig. 1 and Methods for a more detailed description of this model). Such interactions were formalized in logical rules that enabled a dynamic analysis of the network.

We considered all possible initial conditions in the network and found only two steady states representing self-sustained gene activity profiles (see Appendix).

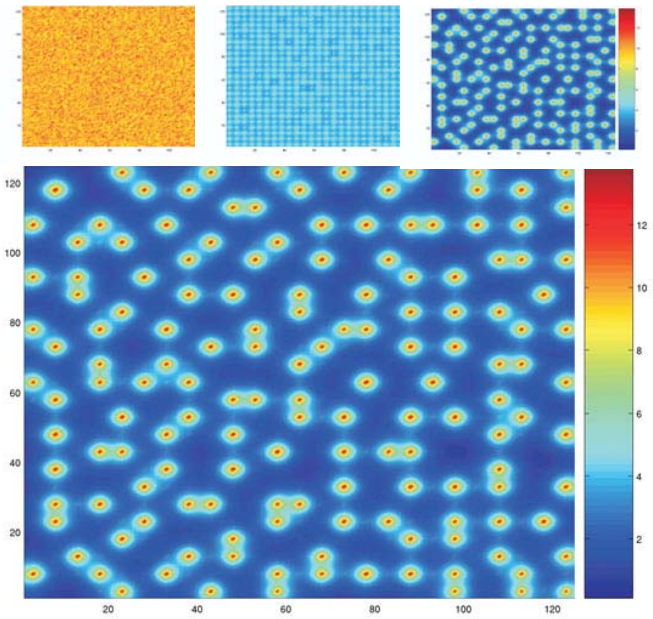

Fig. 3. **Activator:inhibitor ratio for the leaf system at different time steps.** *Attainment of stable patterns for the leaf system. Nuclei are arranged in a regular lattice. Time periods for all small panels from left to right: t=0, t=10 and t=100. The large panel corresponds to t=2000. Parameter values for all simulations are:* k_1=0.3; k_2=0.3; k_3=0.4; k_4=0.75. *The initial diffusion rates are:* D_a=0.15; D_i=1.65. *Red represents high values of the activator:inhibitor concentration ratio.*

Kauffman suggested that steady states, or attractors, of gene networks correspond to cell types (Kauffman, 1969, 1996) and this proposal has been explored with the use of some well documented discrete gene networks grounded on experimental data (Mendoza and Alvarez-Buylla, 1998, 2000; Mendoza *et al.*, 1999; Albert and Othmer, 2003; Espinosa-Soto *et al.*, 2004). Congruent with this hypothesis, the configuration of the nodes' states for the two attractors matches the expression profiles of the two cell types in *Arabidopsis* epidermis. In one of these attractors the inhibitors, but also the activator complex and therefore *GL2*, are not active while in the other, they all are (see Appendix). The configuration with *GL2* 'off' corresponds to non-trichome cells in the leaf and to trichoblasts in the root.

We introduced a reduced network system that maintains the relevant traits of the complete network dynamics. The reduced network has only two elements: an activator that positively regulates an inhibitor and itself and the inhibitor, which in turn, negatively regulates the activator. The activator stands for the activator complex (integrating the activity of *WER* or *GL1*, *GL3*, *EGL3* and *TTG)* and the inhibitor represents the joint action of *TRY* and *CPC*. The dynamical analysis of this system led to similar results as those obtained for the whole network: it has only two attractors with gene activation configurations matching those observed in either of the two cell types.

In addition, the comparison of the states diagram of the discrete reduced system and the well-known phase plane associated to the reactive terms of a typical activator-inhibitor continuous system, strongly suggests that these are qualitatively equivalent. They both have two steady states (network system) or equilibrium points (continuous activator-inhibitor system) and exhibit comparable trajectories (see Appendix). Hence, the reduction of the complete network to an activator-inhibitor system is justified. By doing this we propose that the cell-autonomous gene network can be compared to the reactive terms of a continuous activator-inhibitor system, while the spatially explicit terms of the latter may account for positional biases and cell-cell communication via the inhibitors movement. We intend to represent a system in which positional information and cell communication affect the initial conditions of dynamic gene networks that ultimately attain a steady state corresponding to a cell type.

A leaf activator-inhibitor system recovers spatial patterns that resemble those observed for trichomes

Turing (1952) proposed systems in which reactants with different diffusion coefficients can produce spatial patterns *de novo* and maintain them. Activator-inhibitor systems are a particular case of reaction-diffusion (Turing) systems and are based on local self-activation and lateral inhibition (Meinhardt, 1982; Nijhout, 2003). Given the results obtained with the discrete system, we introduced a spatially explicit model of this kind. In it two interactors are considered: the activator and the inhibitor, which correspond, in the discrete system, to the AC node (WER/GL1, TTG, GL3, EGL3) and to the partially redundant action of *CPC* and *TRY*, respectively. The activator promotes its own synthesis and that of the inhibitor. The inhibitor, in turn, represses the synthesis of the activator complex (see equations and a more detailed description of this model in Methods).

Even though it is known that activator-inhibitor systems can produce qualitatively different patterns when subject to different

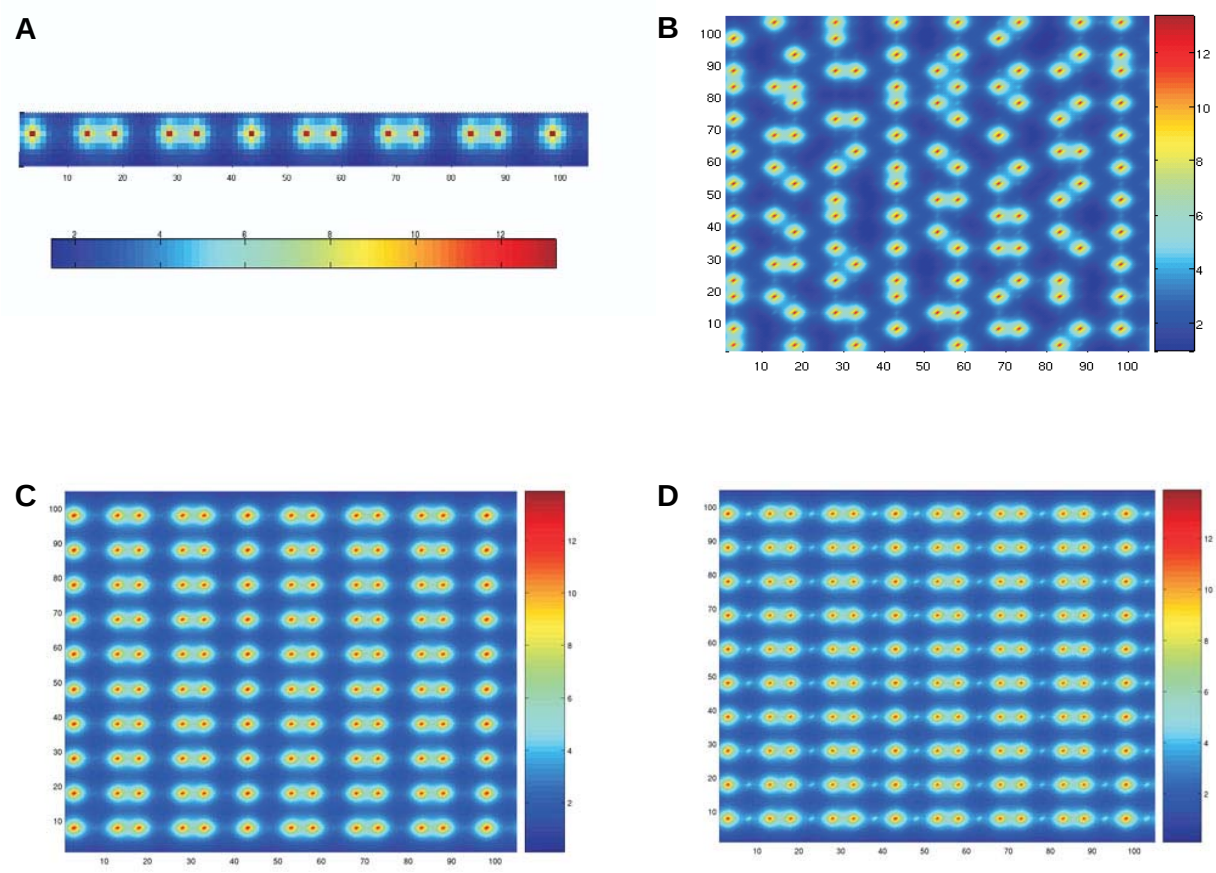

Fig. 4. Patterns for the root system. *(See Equations 3 and 4 in the Methods section).* **(A)** *Stable pattern generated in a one-dimensional ring of cells* **(B)** *Spatially unstable root-like patterns observed for the two-dimensional root system (Equations 3 and 4 in the Methods section) considering squared cells. Notice simulated hair cell bands are recognizable but somewhat unaligned.* **(C)** *Here, nearest neighbor of a nucleus on the x axis is closer to it than its nearest neighbor on the y axis and hence little variation is observed in the activator:inhibitor ratio within the same column.* **(D)** *The 35S:WER root system also yields striped patterns. W=0.4 stands for the contribution of WEREWOLF protein due to the 35S promoter (see Equation 5 in the Methods section). In all cases, time stable patterns at t=2000 are shown, parameter values for all simulations as in Fig. 3 and f(x)=*0.06 *and is included in the system as in Equation 3 (Methods section). Red represents high values of the activator:inhibitor concentration ratio.*

parameter values, we address here if an activator-inhibitor system can produce patterns similar to those observed in leaf and root epidermis with the same parameter values but under contrasting contextual conditions.

For the leaf system, we considered a squared spatial domain and zero-flux boundary conditions, representing a leaf's section. The system (equations *(1)* and *(2)* in Methods) was initialized with random conditions for the concentration of the leaf activator and a constant value for that of the inhibitor, therefore, the activator:inhibitor ratio could never be undefined. We picked parameter values in the Turing space and as expected for systems of this kind, the simulations for the leaf equations recovered dotted patterns. Figure 3 shows a color-coded graph of the steady ratio of leaf activator over leaf inhibitor throughout the spatial domain.

The root model recovers Arabidopsis*-like root-hair patterns and predicts the way the* scrambled *gene affects the network*

In our model, contextual differences between the two epidermal systems are considered. The most important one concerns the fact that only root epidermal cells that overlie two cortical cells develop as trichoblasts, whereas the rest develop as atrichoblasts (Galway *et al.*, 1994). This suggests the existence of a signal from the cortex that biases epidermal cell identity. The signal from the cortex seems to be constant throughout the process of epidermal cell differentiation because when occasional longitudinal anticlinal divisions occur in root epidermal cells, daughter cells attain their identity according to their position relative to cortical cells (Berger *et al.*, 1998). This kind of signal has not been found in the leaf epidermis. Hence, we introduced an additional constant term into one of the root system's equations, representing positional cues produced by the signal from the cortex cells (equations 3 and 4 in Methods). The signal has been shown to partially depend on the gene *SCRAMBLED (SCM)*, which encodes a membrane kinase receptor protein (Kwak *et al.*, 2005). In principle, this signal could act either positively or negatively on the inhibitor or the activator. We tested all four possibilities (see Fig. 5 and Discussion) of which, within a wide range of signal values (0.06 to 0.30), only the one assuming a positive regulation over the activator yielded the expected striped spatial pattern typical of *Arabidopsis*

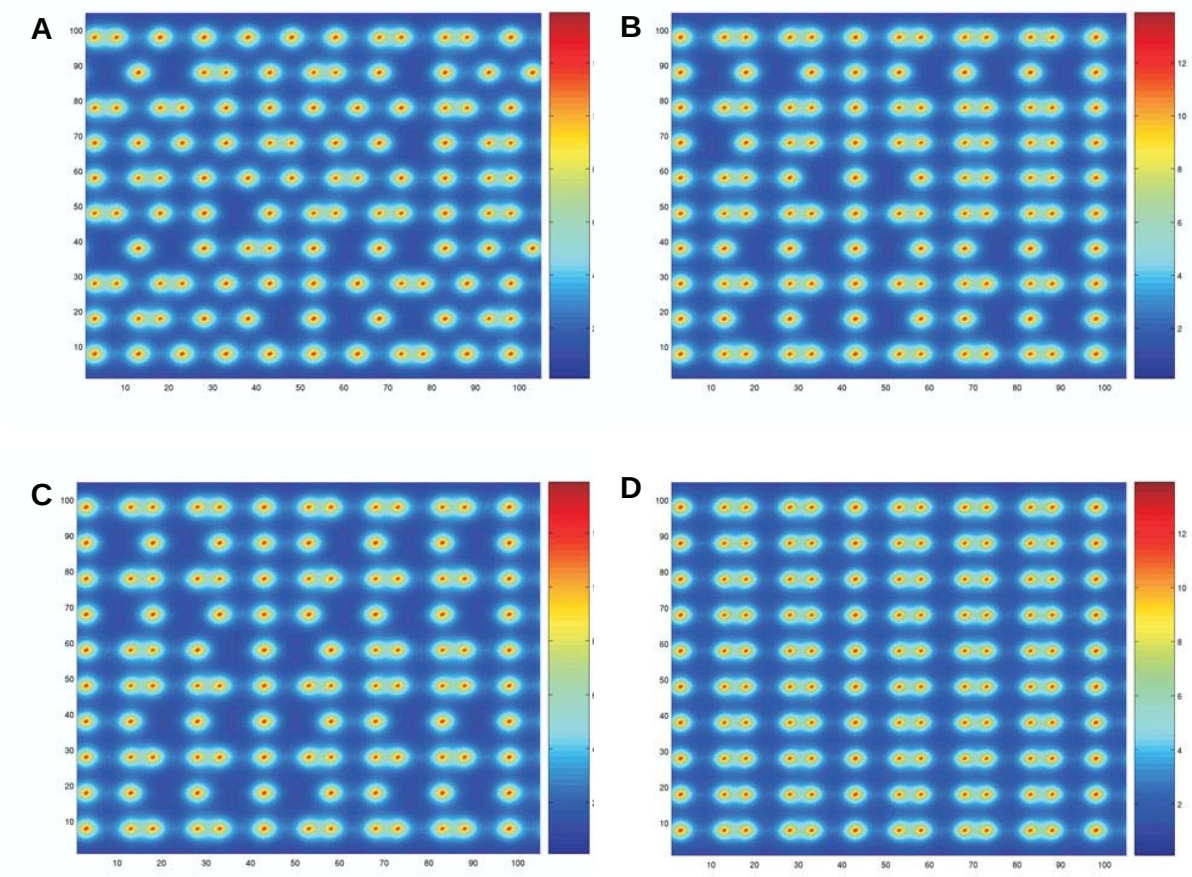

Fig. 5. Root-like patterns in the model with elongated cells are spatially stable only when the signal corresponding to that going from cortical to epidermal cells is assumed to positively regulate the activator (D). *The root pattern is not recovered when the signal* **(A)** *represses the inhibitor,* **(B)***represses the activator or* **(C)***activates the inhibitor. Initial conditions for each simulation were random and the parameters were the same as in Fig. 3. Red represents high values of the activator:inhibitor concentration ratio.*

root epidermis. Therefore, we present the results for this particular type of signal (equations 3 and 4 in Methods).

When considering squared root cells in a two-dimensional domain, the resulting patterns present some bands that resemble observed hair cell stripes; however, the concentration maxima are not aligned as clearly as in real roots (Fig. 4B). Therefore the obtained patterns can be described as spatially unstable root patterns. There are a number of factors that could yield the formation of almost perfect cell type bands in actual roots. For instance, the intensity of the cortex signal, the effect of unknown genetic elements or interactions in the network or the curvature of the domain.

Another possibility is that cell geometry affects the final two-dimensional patterns. Cell elongation, which actually takes place in the elongation zone basal to the root meristem, may affect final pattern formation by increasing the distance among nuclei in the root axis direction. Thus, the cortical signal might prevail on the activator-inhibitor mechanism in this direction. This does not undermine the importance of the activator-inhibitor mechanism; it still would be relevant for stripe generation because of it's laterally inhibitory effect on one direction, the one in which nuclei are closer.

We considered a squared domain and zero-flux boundary conditions on two of the borders and periodic boundary conditions on the other two, simulating a cylinder. This was also initialized with random and constant conditions for the concentration of the activator and inhibitor, respectively. If root cells are considered squared, the patterns obtained resemble a striped pattern, yet such patterns are not as clear as those found in real roots (Fig. 4B).

Then, we explicitly test if once the pattern is formed in one dimension, cell elongation can be responsible for pattern spatial stabilization in the two-dimensional domain. To this end, we introduced a two-dimensional root model as the one described by equations *(3)* and *(4)* (Methods), but with cells elongated in the baso-apical direction (its nearest neighbor along the *x*-axis is closer than its nearest neighbor along the *y*-axis), similar to those observed in the differentiation root zone. Although root patterning begins before cells are elongated, we postulate that the final striped arrangement in the two-dimensional domain may be stabilized during cell elongation.

Once the root system was specified with elongated cells, we tested all four possible kinds of positional signal that might represent the *SCM*-dependent cue. Given a relatively weak signal intensity (0.06), the clear pattern observed in roots is only recovered when such signal acts as a positive regulator of the root

activator (Fig. 5). The way in which *SCM* affects epidermal cell identity was pointed out as one of the key issues that remain to be clarified in order to understand pattern formation in root epidermis (Dolan, 2006). This issue has been explored experimentally, yet empirical data do not allow to discern the necessary and sufficient conditions in particular concerning the magnitude and type of signal, that may recover the observed patterns (Kwak *et al*. 2005). The type of dynamic system presented here, enable predictions that cannot be based solely on experimental data and thus provide novel dynamical insights on this problem of pattern formation (see Discussion).

Since the root pattern does not arise in a sheet of undifferentiated cells but in a ring of small cells, we also performed simulations of the system described in equations *(3)* and *(4)* in a one-dimensional domain with periodic boundary conditions, simulating a ring of cells. We found patterns like those observed in rings of root cells (Fig. 4A). Arrangements obtained for two-dimensional systems (equations 3 and 4 in Methods) with squared and rectangular cells can be described as repetitions of the ring patterns, although the system that includes elongated cells exhibits clearer patterns, suggesting that cell elongation or some other stabilizing factor with a similar effect might underlie the emergence of aligned cell type bands.

These results for the root system show that the gene networks responsible for hair patterning in leaves and roots may be equivalent and still able to yield different spatial patterns due to differences in the cellular contexts in which they are embedded.

In order to validate the model we tested if it recovers observed patterns when gene expression is altered. *Arabidopsis* plants constitutively over-expressing *WER (35S:WER)* in wild type background show wild type trichoblast and *GL2* expression patterns, while *wer* loss of function homozygous mutants that overexpress *WER* present ectopic *GL2* expression (Lee and Schiefelbein, 2002). Available experimental data does not provide a clear explanation of how the *WER* endogenous copy can buffer this increase in *WER* expression. To address this issue and further test the model we simulated this experiment by assuming a constitutive production of the activator in the root system and by modifying the root activator (equation 5 in Methods). As it happens in *35S:WER* plants, columns in which the ratio of activator:inhibitor is higher are still formed, reinforcing the compatibility of our model with experimental data (Fig. 4D). This result suggests that *35S:WER*-dependent inhibitor upregulation preserves the activator:inhibitor ratio. Under this scenario, the pattern is recovered because of spatial differences in endogenous *WER* expression.

It is noteworthy that both the leaf and root patterns are robust to strong noisy perturbations (see Appendix). This suggests that the system is able to buffer developmental noise and canalizes the patterns to those expected in a deterministic system. The system's parameters may be associated to genetic modifications affecting production, diffusion or degradation rates of the molecules involved. By randomly varying parameter values, we found that the patterns obtained do not depend on particular or rare combinations of parameters. Both systems are considerably robust to alterations in parameter values, although the root system with elongated cells is more sensitive than the leaf one (see Appendix).

Interestingly, analyses presented in the Appendix also support that it is not likely that a system like the one presented in *(1)* and *(2)* (Methods), without a biasing signal, is able to render striped root-like patterns.

Discussion

Development of morphological traits depends not only on complex interactions among an organism's molecular constituents but also on specific properties of the domain where morphogenesis occurs. The complexity of such processes and the overwhelming amount of data that is rapidly accumulating is demanding the use of formal dynamical models that integrate available information. These models provide a solid base to understand biological developmental systems, as they suggest hypotheses that can be addressed experimentally, provide tools to interpret counterintuitive experimental data and enable the performance of vast computer explorations on parameter values or initial conditions that can then be tested *in vivo*.

Trichoblast and trichome pattern formation in *Arabidopsis* has been attributed to an activator-inhibitor mechanism (Pesch and Hülskamp, 2004). Here we provide a dynamic model that supports this. All relevant properties required for such a mechanism are preserved in our discrete network model that integrates most of the genes known to participate in these processes and in a reduced version of it. The reduced network model is dynamically very similar to the well-mixed case (no diffusion) of a typical activator-inhibitor system. It is important to stress that the steady states of the discrete network systems are compared to the equilibrium points of the reactive terms of the activator-inhibitor continuous system, suggesting that attractors or steady states of gene networks correspond to different cell types and that contextual traits (including positional information) affect the initial conditions and trajectories that lead to such attractors.

Experimental data had already suggested that equivalent networks could underlie leaf and root epidermal cell-fate determination (Lee and Schiefelbein, 2001). However, recent evidence suggested differences in the regulation of *GL3* and *EGL3* (Bernhardt *et al*., 2005). We have shown here that despite these dissimilarities, the root and leaf genetic networks behave qualitatively the same (Fig. 2).

Indeed, the model grounded on experimental data that we put forward has enabled us to formally show that contextual traits are sufficient to yield the contrasting Arabidopsis leaf and root epidermal cell patterns, when equivalent networks are assumed. These contextual conditions might be necessary for stabilizing such patterns. The contextual traits considered were the relative position of epidermal cells with respect to cortex cells - which in the root epidermis determine a biasing signal - and cell shape, that was assumed to be rectangular in the root case, but squared in the leaf system. Cell shape is only one of the possible traits that could stabilize the striped pattern of root epidermis, however our simulations support that cell elongation or a mechanism with a similar effect on the system may indeed contribute to spatially stabilize the two-dimensional patterns.

Further experimental data and computational analyses are needed to test if the gene networks underlying trichome and root-hair cell determination are really equivalent. For example, genes that are not active in both networks may have different activation functions or behave differently in terms of transport. If the two

networks were not equivalent, quantitative parameter differences could be responsible for the attainment of the divergent spatial cellular patterns. However, the stability analysis presented here (see Appendix) suggest that this is not the case, given that the structure of the equations that govern the gene network dynamics is conserved in both systems. Since such analysis leads to the conclusion that for this particular system, stripes are very unlikely to form without a biasing signal, our results suggest that the positional cue is necessary to recover the root spatial pattern.

In this work, we provide new hypotheses that can be addressed experimentally. The first one concerns the nature of the signal associated to *SCM*. This outstanding issue for the study of pattern formation in epidermis remains unclear (Dolan, 2006). Kwak *et al.* (2005) suggested that the positional cue mediated by the membrane receptor SCM could act on *WER*. Still, their results are equally compatible with SCM acting on *CPC*: In *scm* mutants the same researchers found altered patterns of expression of *CPC*, *GL2* and *WER* and suggested that since *WER* regulates the expression of *GL2* and *CPC*, the signal could act exclusively on *WER*. However, *CPC* also regulates the expression of *GL2* (Wada *et al.,* 2002, 1997; Costa and Dolan, 2003) and *WER* (Lee and Schiefelbein, 2002), so their data does not discard the possibility that the signal acts exclusively on *CPC*.

The signal associated to the position of epidermal cells relative to cortex cells could act either on the inhibitor or the activator, either favoring or limiting their expression. Interestingly our simulations strongly suggest that assuming equal and relatively small signal intensities, such biasing signal necessarily acts positively on *WER* or any other component of the activator complex in order to recover the striped pattern of root-hair cells. Since the signal was modeled explicitly in the four cases by including the *f(x)* term with different signs in both the activator and inhibitor equations, the striped pattern can not be an artifact derived from the inclusion of *f(x)*. The model may be modified in order to test alternative hypotheses concerning the nature of the biasing signal and could guide future experiments.

Experimental evidence also supports that the biasing signal in the root acts positively on the activator complex. Plants that overexpress *WER* but preserve the wild type copy of this gene exhibit normal root-hair and *GL2* expression patterns. This does not happen in transgenic plants over-expressing *WER* with non-functional endogenous *WER* (Lee and Schiefelbein, 2002), showing that in order to attain normal trichoblast patterns the functional wild type *WER* promoter is required. Also, Bünning found that in radish epidermal cells developing independently from other tissues bear root hairs (reviewed in Dolan *et al.,* 1994). These epidermal cells would be deprived from molecular signals coming from the cortex and attain the same cell fate that *Arabidopsis* root cells have when there is no activator.

The second hypothesis we put forward states that root cell elongation along the baso-apical axis stabilizes the root striped pattern. This hypothesis could be tested by crossing a *GL2:GUS* or GFP marker line to mutants or pharmacologically treated plants that have altered cell shape (see for example Fagard *et al.,* 2000; Le *et al.,* 2001; Ramirez-Parra *et al.,* 2004; Campanoni and Nick, 2005).

Given that most kinetic parameter estimates are not known, we reduced the root-hair and trichome systems to an analogous system with only two elements. However, we did so based on qualitative equivalence of key dynamical traits of the gene network and the discrete version of the activator-inhibitor system. It should also be noticed that the reduction accurately summarizes most available experimental evidence (see Methods). However, a few factors were left out, such as genes that seem to be upstream from the ones in these networks, for instance *REDUCED TRICHOME NUMBER (RTN;* Larkin *et al.,* 1996*)* and *GLABROUS INFLORESCENCE STEMS* (Gan *et al.,* 2006).

The plant hormones auxin and ethylene also participate in the development of Arabidopsis root hairs (Dolan *et al.,* 1994; Masucci and Schiefelbein, 1996), but they act either downstream or in parallel of the genes included in this study (Masucci and Schiefelbein, 1996; Mendoza and Alvarez-Buylla, 2000) and consequently do not affect the behavior of the networks we worked with, further supporting their dismissal for the specific aims of this contribution. Nevertheless, future models that incorporate all of these factors will be very useful for predicting the relative importance of each gene or molecule in response to different signaling pathways in root-hair and trichome pattern formation and evolution.

Epidermal cell type determination network constitutes a small regulatory module and in this work we address the particular issue of how such gene network and contextual traits give rise to cell type patterns. However, the study of this specific and well documented system provides results that can be discussed in terms of evolution of morphogenesis.

As previously discussed, the root and leaf networks seem to be dynamically equivalent. This finding, along with the fact that an activator-inhibitor mechanism coupled to contextual traits recovers characteristic features of cell patterning, supports the claim that few generic and relatively simple mechanisms could be responsible for generating and maintaining heterogeneities during morphogenesis (Turing, 1952; Meinhardt, 1982).

In order to understand how gene networks take part in the generation of robust cellular patterns it will certainly be useful to characterize pattern-generating regulatory modules in well documented gene networks and point them as potentially generic patterning mechanisms. Congruent with the proposal stated by Pesch and Hülskamp (2004), we identify an activator-inhibitor system that arises from the collective behavior of a gene network and point to this mechanism as a potentially generic one. We emphasize that simple conserved mechanisms, such as the activator-inhibitor, may rely not only on couples of elusive morphogenes, but also on the interactions among several genes.

Our simulations also suggest that the networks underlying root and leaf epidermis cell patterning are robust modules that regulate cell-fate determination in different cellular contexts. Such contexts may have evolved yielding the contrasting spatial patterns found in roots and leaves. If we extrapolate this example to thinking about evolution of pattern formation, we may postulate that equivalent networks might render different patterns due to the evolution of dissimilarities in contextual traits between structures and organisms. This suggests that the alteration of genes associated to key contextual traits could underlie the origin of novel and diverse patterns, even if gene

regulatory networks of cell fate determination remain qualitatively the same, thereby constituting developmental and evolutionary robust modules (von Dassow *et al.,* 2000). Further analyses are required to test how frequent context-dependent evolution could have been in the history of life.

Methods

Discrete gene regulatory network dynamics

Grounded on the experimental data summarized in the Introduction, we defined a network in which most nodes correspond to genes and one corresponds to the 'Activator Complex' (AC) node and represents a protein complex constituted by the products of *WER* or *GL1, GLABRA3 (GL3), ENHANCER OF GLABRA3 (EGL3)* and *TRANSPARENT TESTA GLABRA (TTG).* Edges stand for regulatory interactions between nodes (activation or repression; Fig. 1C). Since this system is restrained to the cell-autonomous ambit, space and protein movement are neglected. In order to enable different activity thresholds when there is experimental information suggesting their existence (Thomas, 1991), some nodes – *GL1, WER, CAPRICE (CPC), TRIPTYCHON (TRY), GL3* and AC– can attain three states (0 corresponding to no expression, 1 mild expression and 2 high expression), whereas the activity of two nodes *(TTG* and *EGL3)* is defined by two states (0 no expression, 1 expression).

The experimentally documented regulatory interactions among the nodes were formalized as logical rules (see example in Appendix). Given these, it is possible to follow the dynamics of the network for any initial configuration of the nodes expression states and find if the network attains steady states representing self-sustained gene activity profiles, as those thought to characterize cell-types (Kauffman, 1969, 1996). For simplicity, we used synchronic updating of the nodes' states.

In order to compare trajectories in the discrete system with the vector field of the reaction terms in a typical activator-inhibitor system, we also performed simulations using a reduced system where only two nodes were kept, one representing the activator complex and the other the inhibitor complex.

The activator-inhibitor system

We introduced a continuous activator-inhibitor system in which an activator element, representing the activator complex, positively regulates itself and an inhibitor element, that represents the joint action of inhibitory genes, *CPC* and *TRY*. The inhibitor negatively regulates the activator. These interactions are compatible with the available empirical information summarized in the introduction.

For an activator-inhibitor system to create a pattern it is necessary that the inhibitor moves along a wider spatial range than the activator (Meinhardt, 1982). Experimental evidence suggests that this is the case for both systems, since the activators seem to act locally, while the inhibitors do not. In cpc loss of function mutants the cells that overlie two cortical cells develop as atrichoblasts, instead of trichoblasts as it occurs in wild type plants (Wada *et al.,* 1997). Besides, *CPC* mRNA is localized only in epidermal cells located on top of a single cortical cell, but the CPC protein is found throughout the root epidermis as assayed by green fluorescent protein, strongly suggesting that the CPC protein moves among cells (Wada *et al.,* 2002). Moreover, the *CPC* coding sequence bears specific motifs that allow it to move (Kurata *et al.,* 2005). Even though the *TRY* sequence does not have these motifs (Kurata *et al.,* 2005), it is expressed in trichome cells, but cells that normally do not develop trichomes will develop them when TRY is absent, suggesting that this gene function is also non-cell autonomous (Schnittger *et al.,* 1999). Diffusion is the simplest mechanism of protein movement and it is an important type of intercellular communication during plant development (Kim and Zambryski, 2005). Indeed, the CPC and TRY proteins are much smaller than components of the activator complex (Oppenheimer *et al.,* 1991; Wada *et al.,* 1997; Lee and Schiefelbein, 1999; Schellmann *et al.,* 2002), further suggesting that CPC and TRY may move through plasmodesmata.

Genetic regulation can take place at many levels. For simplicity, we neglected translation, in other words, we assumed transcription (mRNA synthesis) to be the rate-limiting step for a protein to exert its function. Although proteins are synthesized outside the nucleus, transcription regulation requires proteins to bind the DNA and the concentration of the modeled proteins' is evaluated by the promoter, in the nucleus. Hence, we considered that gene activation does not occur throughout the entire spatial domain but only in discrete small areas (N). We arranged these in a regular lattice throughout the whole space. As usual, degradation rate was assumed proportional to concentration. Therefore, we considered the following equations for the rate of activator and inhibitor concentration, respectively:

$$\frac{\partial a_{x,y}}{\partial t} = \begin{cases} D_a \nabla^2 a_{x,y} - k_1 a_{x,y} + k_2 \dfrac{{a_{x,y}}^2}{i_{x,y}} & \text{if } (x,y) \in N \\ D_a \nabla^2 a_{x,y} - k_1 a_{x,y} & \text{if } (x,y) \notin N \end{cases} \quad (1)$$

$$\frac{\partial i_{x,y}}{\partial t} = \begin{cases} D_i \nabla^2 i_{x,y} - k_3 i_{x,y} + k_4 {a_{x,y}}^2 & \text{if } (x,y) \in N \\ D_i \nabla^2 i_{x,y} - k_3 i_{x,y} & \text{if } (x,y) \notin N \end{cases} \quad (2)$$

where *t* represents time, *x* and *y* the spatial coordinates, a the concentration of the activator, i that of the inhibitor and ∇a and ∇i are the laplacians for the activator and inhibitor, respectively, that define diffusive movement of the molecules. The strength of such movement is characterized by the diffusion coefficients *Da* and *Di*, for the activator and the inhibitor respectively (with $Da << Di$). k_1 and k_3 are degradation constants for the activator and inhibitor, respectively and k_2 and k_4 are their corresponding production constants.

For the root system, we introduced a bias representing the signal associated to *SCM*. We tried positive or negative signals either over the activator or the inhibitor complexes. We found that of the four possibilities, given a relatively small signal (0.06 to 0.30), only a positive signal over the activator complex was able to recover the observed root-like pattern. Therefore, given the specification of the activator-inhibitor dynamic system presented here, such signal is thus necessary and sufficient to recover the observed pattern and we show here the equations for this case:

$$\frac{\partial a_{x,y}}{\partial t} = \begin{cases} D_a \nabla^2 a_{x,y} - k_1 a_{x,y} + k_2 \dfrac{{a_{x,y}}^2}{i_{x,y}} + f(x) & \text{if } (x,y) \in N \\ D_a \nabla^2 a_{x,y} - k_1 a_{x,y} & \text{if } (x,y) \notin N \end{cases} \quad (3)$$

where *f(x)* represents the positional cues coming from the cortex. *f(x)* acquires the value of a small positive constant if *x'* is equal to one value of an a priori defined set that corresponds to the nucleus, otherwise *f(x)* equals zero. The equation for the root inhibitor is the same as that for the leaf inhibitor and all common terms are as in the leaf equations above:

$$\frac{\partial i_{x,y}}{\partial t} = \begin{cases} D_i \nabla^2 i_{x,y} - k_3 i_{x,y} + k_4 {a_{x,y}}^2 & \text{if } (x,y) \in N \\ D_i \nabla^2 i_{x,y} - k_3 i_{x,y} & \text{if } (x,y) \notin N \end{cases} \quad (4)$$

Non-linear terms are needed in the reaction part of the reaction-diffusion equations to attain pattern formation (Meinhardt, 1982; Murray, 1989; Meinhardt and Gierer, 2000). In fact, there is experimental evidence for the inclusion of quadratic terms. It seems that *TTG* lacks activation domains, unlike *GL3* (Payne *et al.,* 2000), *WER* (Lee and Schiefelbein, 1999) and *GL1* (Oppenheimer *et al.,* 1991). Moreover, *GL3* activity apparently depends on *WER* as the hairy phenotype on roots

over-expressing *GL3* is suppressed when *WER* is non-functional (Bernhardt *et al.,* 2003), while *GL3* function does not depend on *TTG* (Payne *et al.,* 2000; Bernhardt *et al.,* 2003; Zhang *et al.,* 2003). This data supports that in order to form functional multimers, at least functional GL3 (or a redundant protein, EGL3) and either GL1 or WER must be present According to this and to the mass-action law the quadratic nature of the activation terms $k_2 \frac{a_{x,y}^{\ 2}}{i_{x,y}}$ and $k_4 a_{x,y}^{\ 2}$ follows.

To discard that differences in boundary conditions were the cause of differences in pattern we also modeled the root system using zero-flux boundary conditions in all borders (data not shown); the patterns were identical to those discussed above.

Equation 3 was modified to simulate constituve expression or *WER*, yielding

$$\frac{\partial a_{x,y}}{\partial t} = \begin{cases} D_a \nabla^2 a_{x,y} - k_1 a_{x,y} + k_2 \frac{a_{x,y}^{\ 2}}{i_{x,y}} + f(x) + W & \text{if } (x,y) \in N \\ D_a \nabla^2 a_{x,y} - k_1 a_{x,y} & \text{if } (x,y) \notin N \end{cases} \quad (5)$$

where *W* represents the constant production rate of WER protein simulating its production in *35S:WER* transgenic plants.

In order to address if the patterns attained were sensitive to stochastic transient perturbations in the concentration of the molecules involved in the system, we repeated the simulations adding a stochastic term in equations (1) and (3) for both the leaf and root systems. Given that diverse patterns may arise when varying parameter values in Turing systems, we also tested deviations in single parameter values in order to test if results depend on a specific combination of parameter values (see Appendix). Equations were solved numerically using Euler's method (Burden and Faires, 1997) with a timestep equal to 0.01 or 0.001. Both timestep values resulted in equivalent patterns. Programs were written in C++ and are available upon request. Results were graphed using MATLAB.

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Note added in proof

A paper published while the proofs of this manuscript were being prepared, provides evidence that the gene *SCRAMBLED (SCM)* encodes a very important component of the biasing signal and that this inhibits the transcription of *WER*, which is a component of the activator complex (Kwak & Schiefelbein, 2006, *Dev. Biol.* In press). Our work suggests that, given relatively low biasing signal intensities (up to 0.3), root-like patterns arise only when the biasing signal acts positively on the activator. Hence, our prediction seems to conflict with the experimental evidence provided by Kwak & Schiefelbein (2006). However, Kwak & Schiefelbein (2006) also show that in the absence of *SCM*, the spatial root pattern of alternate atrichoblast and trichoblast columns is not lost completely. This suggests that there are additional components of the biasing signal which act through an *SCM*-independent pathway, and that the biasing signal is more complex than we had thought according to previous data. This is also supported by additional simulations (see Table 1 below, added during proof stage) which show that, given the assumptions of our model, root-like patterns are not recovered when the signal acts exclusively by repressing the activator, even with a 100-fold increase in signal intensity. We are thus extending our model to enable explorations of the necessity and sufficiency of different types of biasing signals. Such analyses are only possible with a dynamic model as the one presented here.

TABLE 1

Sign	Target	0.06	0.3	0.6	6.0
+	Activator	Y	Y	Y	Y
+	Inhibitor	N	N	N	N
- (degradation)	Inhibitor	N	N	Y	Y
- (less production)	Inhibitor	N	N	N	N
- (degradation)	Activator	N	N	N	N
- (less production)	Activator	N	N	N	N

Y, simulations in which the root-like pattern was recovered; N, simulations in which the root-like pattern was not recovered. All other parameters kept the same. We considered two kinds of negative signals; degradation of target proteins/mRNA or decreased production of target.

References

ALBERT, R. and OTHMER, H. (2003). The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in Drosophila melanogaster. *J. Theor. Biol.* 223: 1–18.

BERGER, F., HUNG, C., DOLAN, L. and SCHIEFELBEIN, J. (1998). Control of cell division in the root epidermis of Arabidopsis thaliana. *Dev. Biol.* 194: 235–245.

BERNHARDT, C., LEE, M., GONZALEZ, A., ZHANG, F., LLOYD, A. and SCHIEFELBEIN, J. (2003). The bHLH genes GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) specify epidermal cell fate in the Arabidopsis root. *Development* 130: 6431–6439.

BERNHARDT, C., ZHAO, M., GONZALEZ, A., LLOYD, A. and SCHIEFELBEIN, J. (2005). The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis. *Development* 132: 291–298.

BURDEN, R. and FAIRES, J. (1997). Numerical Analysis. Brooks/Cole Publishing Co., Pacific Grove, CA, 6th edition.

CAMPANIONI, P. and NICK, P. (2005). Auxin-dependent cell division and cell elongation. 1-naphtaleneacetic acid and 2,4-Dichlorophenoxyacetic acid activate different pathways. *Plant Physiol.* 137: 939–948.

COSTA, S. and DOLAN, L. (2003). Epidermal patterning genes are active during embryogenesis in Arabidopsis. *Development* 130: 2893–2901.

DI CRISTINA, M., SESSA, G., DOLAN, L., LINSTEAD, P., BAIMA, S., RUBERTI, I. and MORELLI, G. (1996). The Arabidopsis Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development. *Plant J.,* 10: 393–402.

DOLAN, L. (1996). Pattern in the root epidermis: an interplay of diffusible signals and cellular geometry. *Ann. Bot.* 77: 547–553.

DOLAN, L., DUCKET, C., GRIERSON, C., LINSTEAD, P., SCHNEIDER, K., LAWSON, E., DEAN, C., POETHING, S. and ROBERTS, K. (1994). Clonal relationships and cell patterning in the root epidermis of Arabidopsis. *Development* 120: 2465–2474.

DOLAN, L. (2006). Positional information and mobile transcriptional regulators determine cell pattern in the Arabidopsis root epidermis. *J Exp Bot.* 57: 51-54.

ESPINOSA-SOTO, C., PADILLA-LONGORIA, P. and ALVAREZ-BUYLLA, E. (2004). A Gene Regulatory Network Model for Cell-Fate Determination during Arabidopsis thaliana Flower Development That Is Robust and Recovers Experimental Gene Expression Profiles. *Plant Cell* 16: 2923–2939.

FAGARD, M., DESNOS, T., DESPREZ, T., GOUBET, F., REFREGIER, G., MOUILLE, G., MCCANN, M., RAYON, C., VERNHETTES, S. and HOFTE, H. (2000). PROCUSTE1 encodes a cellulose synthase required for normal cell elongation specifically in roots and dark-grown hypocotyls of Arabidopsis. *Plant Cell* 12: 2409–2423.

GALWAY, M., MASUCCI, J., LLOYD, A., WALBOT, V., DAVIS, R. and SCHIEFELBEIN, J. (1994). The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root. *Dev. Biol.* 166: 740–754.

GAN, Y., R. KUMIMOTO, C. LIU, O. RATCLIFFE, H. YU and P. BROUN. (2006). GLABROUS INFLORESCENCE STEMS Modulates the Regulation by Gibber-

ellins of Epidermal Differentiation and Shoot Maturation in Arabidopsis. *Plant Cell* 18: 1383–1395.

GOODWIN, B. (2001). How the leopard changed its spots. The evolution of complexity. Princeton Science Library, U.S.A., 2nd edition.

HUNG, C., LIN, Y., ZHANG, M., POLLOCK, S., MARKS, M. and SCHIEFELBEIN, J. (1998). A common position-dependent mechanism controls cell-type patterning and GLABRA2 regulation in the root and hypocotyl epidermis of Arabidopsis. *Plant Physiol.* 117: 73–84.

KAUFFMAN, S. (1969). Metabolic stability and epigenesis in randomly constructed genetic nets. *J. Theor. Biol.* 22: 437–467.

KAUFFMAN, S. (1996). At home in the Universe: The search for laws of complexity. Penguin books, England.

KIM, I. and ZAMBRYSKI, P. (2005). Cell-to-cell communication via plasmodesmata during Arabidopsis embryogenesis. *Curr. Opin. Plant Biol.* 8: 593–599.

KIRIK, V., SIMON, M., HUELSKAMP, M. and SCHIEFELBEIN, J. (2004). The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis. *Dev. Biol.* 268: 506–513.

KURATA, T., ISHIDA, T., KAWABATA-AWAI, C., NOGUCHI, M., HATTORI, S., SANO, R., NAGASAKA, R., TOMINAGA, R., KOSHINO-KIMURA, Y., KATO, *et al.* (2005). Cell-to-cell movement of the CAPRICE protein in Arabidopsis root epidermal cell differentiation. *Development* 132: 5387–5398.

KWAK, S.-H., SHEN, R. and SCHIEFELBEIN, J. (2005). Positional signaling mediated by a receptor-like kinase in Arabidopsis. *Science* 307: 1111–1113.

LARKIN, J., MARKS, M., NADEAU, J. and SACK, F. (1997). Epidermal cell fate and patterning in leaves. *Plant Cell* 9: 1109–1120.

LARKIN, J., OPPENHEIMER, D., POLLOCK, S. and MARKS, M. (1993). Arabidopsis GLABROUS1 gene requires downstream sequences for function. *Plant Cell* 5: 1739–1748.

LARKIN, J., YOUNG, N., PRIGGE, M. and MARKS, M. (1996). The control of trichome spacing and number in Arabidopsis. *Development* 122: 997–1005.

LE, J., VANDENBUSSCHE, F., VAN DER STRAETEN, D. and VERBELEN, J.-P. (2001). In the early response of Arabidopsis roots to ethylene cell elongation is upand down-regulated and uncoupled from differentiation. *Plant Physiol.* 125: 519–522.

LEE, M. and SCHIEFELBEIN, J. (1999). WEREWOLF, a MYB-related protein in Arabidopsis, is a position-dependent regulator of epidermal cell patterning. *Cell*, 99: 473–483.

LEE, M. and SCHIEFELBEIN, J. (2001). Developmentally distinct MYB genes encode functionally equivalent proteins in Arabidopsis. *Development* 128: 1539–1546.

LEE, M. and SCHIEFELBEIN, J. (2002). Cell Pattern in the Arabidopsis Root Epidermis Determined by Lateral Inhibition with Feedback. *Plant Cell* 14: 611–618.

MASUCCI, J. and SCHIEFELBEIN, J. (1996). Hormones act downstream of TTG and GL2 to promote root hair outgrowth during epidermis development in the Arabidopsis root. *Plant Cell* 8: 1505–17.

MEINHARDT, H. (1982). Models of biological pattern formation. Academic Press, London.

MEINHARDT, H. and GIERER, A. (2000). Pattern formation by local selfactivation and lateral inhibition. *BioEssays* 22: 753–760.

MENDOZA, L. and ALVAREZ-BUYLLA, E. (1998). Dynamics of the genetic regulatory network of Arabidopsis thaliana flower morphogenesis. *J. Theor. Biol.* 193: 307–319.

MENDOZA, L. and ALVAREZ-BUYLLA, E. (2000). Genetic regulation of root hair development in Arabidopsis thaliana: a network model. *J. Theor. Biol.* 204: 311–326.

MENDOZA, L., THIEFFRY, D. and ALVAREZ-BUYLLA, E. (1999). Genetic control of flower morphogenesis in Arabidopsis thaliana: a logical analysis. *Bioinformatics* 15: 593–606.

MEYEROWITZ, E. (1997). Genetic control of cell division patterns in developing plants. *Cell*, 88: 299–308.

MURRAY, J. (1989). Mathematical Biology. Springer, Berlin.

NIJHOUT, H. (2003). Gradients, diffusion and genes in pattern formation. In MULLER, G. and NEWMAN, S., editors, Origination of organismal form. Beyond the gene in developmental and evolutionary biology, pp. 165–182. MIT press, Cambridge, Massachusetts.

OPPENHEIMER, D., HERMAN, P., SIVAKUMARAN, S., ESCH, J. and MARKS, M. (1991). A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. *Cell* 67: 483–493.

PAYNE, T., ZHANG, F. and LLOYD, A. (2000). GL3 encodes a bHLH protein that regulates trichome development in Arabidopsis through interaction with GL1 and TTG1. *Genetics* 156: 1349–1362.

PESCH, M. and HÜLSKAMP, M. (2004). Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation. *Curr. Opin. Genet. Devel.* 14: 422–427.

RAMIREZ-PARRA, E., LOPEZ-MATAS, M., FRUNDT, C. and GUTIERREZ, C. (2004). Role of an atypical E2F transcription factor in the control of Arabidopsis cell growth and differentiation. *Plant Cell* 16: 2350–2363.

RERIE, W., FELDMAN, K. and MARKS, M. (1994). The GLABRA2 gene encodes a homeodomain protein required for normal trichome development in Arabidopsis. *Genes Dev.* 8: 1388–1399.

RYU, K., KANG, Y., PARK, Y., HWANG, I., SCHIEFELBEIN, J. and LEE, M. (2005). The WEREWOLF MYB protein directly regulates CAPRICE transcription during cell fate specification in the Arabidopsis root epidermis. *Development* 132: 4765–4775.

SCHELLMANN, S., SCHNITTGER, A., KIRIK, V., WADA, T., OKADA, K., BEERMANN, A., THUMFAHRT, J., JURGENS, G. and HÜLSKAMP, M. (2002). TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. *EMBO J.* 21: 5036–5046.

SCHERES, B. (2001). Plant cell identity. The role of position and lineage. *Plant Physiol.* 125: 112–114.

SCHNITTGER, A., FOLKERS, U., SCHWAB, B., JURGENS, G. and HÜLSKAMP, M. (1999). Generation of a spacing pattern: the role of TRIPTYCHON in trichome patterning in Arabidopsis. *Plant Cell* 11: 1105–1116.

SERNA, L. (2005). Epidermal cell patterning and differentiation throughout the apical-basal axis of the seedling. *J. Exp. Bot.* 56: 1983–1989.

SZYMANSKI, D., JILK, R., POLLOCK, S. and MARKS, M. (1998). Control of GL2 expression in Arabidopsis leaves and trichomes. *Development* 125: 1161–1171.

THOMAS, R. (1991). Regulatory networks seen as asynchronous automata: A logical description. *J. Theor. Biol.* 153: 1–23.

TURING, A. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 237: 37–72.

VON DASSOW, G., MEIR, E., MUNRO, E. and ODELL, G. (2000). The segment polarity network is a robust developmental module. *Nature* 406: 188–193.

WADA, T., KURATA, T., TOMINAGA, R., KOSHINO-KIMURA, Y., TACHIBANA, T., GOTO, K., MARKS, M., SHIMURA, Y. and OKADA, K. (2002). Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation. *Development* 129: 5409–5419.

WADA, T., TACHIBANA, T., SHIMURA, Y. and OKADA, K. (1997). Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, CPC. *Science* 277: 1113–1116.

ZHANG, F., GONZALEZ, A., ZHAO, M., PAYNE, C. and LLOYD, A. (2003). A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. *Development* 130: 4859–4869.

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Appendix

Analysis of the number and profiles of the steady states for both the complete network and the reduced system.

TABLE S1

STATE OF THE ACTIVATION COMPLEX AS A DEPENDENT VARIABLE OF THE ACTIVATION STATE OF *WER* (IN ROOT) OR *GL1* (IN LEAF), *TTG*, *GL3* AND *EGL3*.

Activator genes				
WER/GL1	**TTG**	**GL3**	**EGL3**	**Activator complex**
0	X	X	X	0
1,2	0	0,1	X	0
1,2	1	0	0	0
1,2	0	2	X	1
1,2	1	0	1	1
1,2	1	1	X	1
1,2	1	2	X	2

A

TABLE S2

Initial condition			Steady state		
***CPC* in root *TRY* in leaf**	***TRY* in root *CPC* in leaf**	**Activator complex**	***CPC* in root *TRY* in leaf**	***TRY* in root *CPC* in leaf**	**Activator complex**
0	X	1	1	1	1
1	0,1	1	1	1	1
X	X	0	0	0	0
2	X	1	0	0	0
X	X	2	0	0	0
1	2	1	0	0	0

B

Initial condition		Steady state	
Activator complex	**Inhibitor complex**	**Activator complex**	**Inhibitor complex**
1	1	1	1
0	X	0	0
1	2	0	0
1	0	0	0
2	X	0	0

Steady states for the complete network (A) and for the reduced network of an activator and an inhibitor (B). In both cases, the initial conditions from which each steady state is attained are listed. The *GL2* "on" state corresponds with the activator complex being in a state different from 0.

The reduced network is dynamically equivalent to the reactive terms of a classic activator-inhibitor system (see Fig. S1).

We compare both the equilibrium points for the well-mixed case (no diffusion) and the reduced network stationary states. In a typical activator-inhibitor system with no sources, there are two equilibria: the origin, which is unstable and another one, asymptotically stable (see for instance the standard reference by Murray (1989) on mathematical biology).

For fixed time intervals, a well known theorem (continuous dependence with respect to parameters and initial conditions) guarantees that a discretization can be made as accurate as possible. However, this is not the case when long times are involved. In our case, the scenario cannot be avoided, since the nontrivial equilibrium point is asymptotically stable and therefore acts as a global attractor. However, from the phase portrait, we can observe that for moderately long times and initial conditions not near this nontrivial equilibrium, the behavior of a system with a limit cycle and the behavior of the system with a stable spiral are very similar. In other words, when approximating a continuous dynamical system by a discrete one, there can be several possibilities, depending on the time-scale at which the system is to be approximated.

Another important issue here is that, if no limit cycle is introduced and one tries to reason in the opposite direction, that is, to recover the continuous dynamical system from the given discrete approximation, an spurious equilibrium point is introduced as implied by the Poincaré-Bendixon theorem.

Solving the simplified system is a useful approach that is further justified because:

1. If after solving the simplified system meaningful dynamics are recovered, this serves as *a posteriori* justification of the fact that the reduction is valid.
2. Provided that the genes in the activator complex play a similar role (directly or indirectly), the reactive functions in a full model (including all of them) become functions depending, besides the inhibitor, on only one of the activator genes (the other two being functionally related). Therefore, the three reaction diffusion equations corresponding to each of the activator genes in the activator complex can be added to obtain an single equation for an “effective” activator. In other words, given that the three activator genes are highly correlated, the equation for any of them, or as just explained, an effective equation for the three can be used.

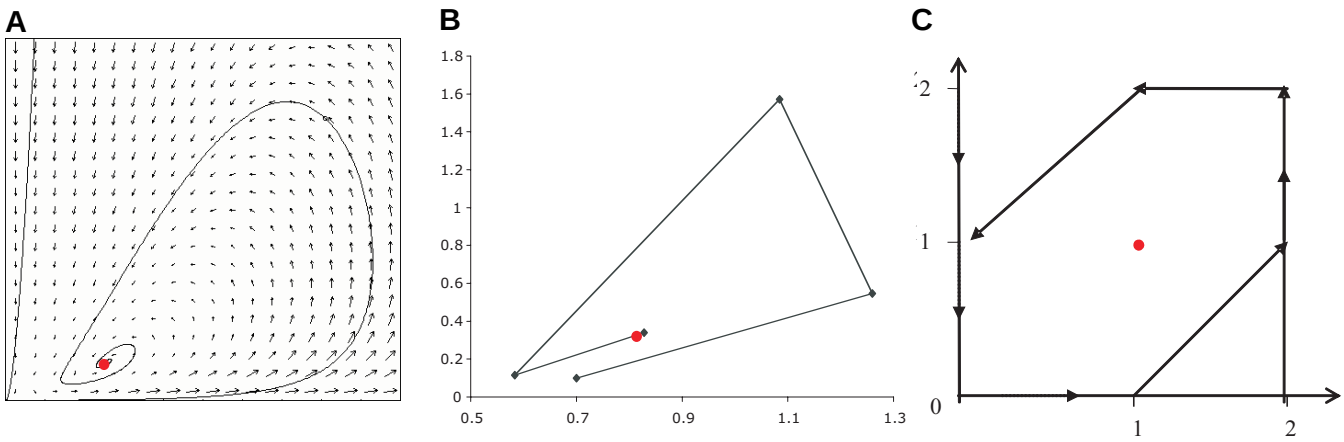

Fig. S1. *Trajectories for the discrete reduced system* **(C)** *are qualitatively equivalent to those of the activator-inhibitor system* **(A)** *and to those obtained for a discretized activator-inhibitor system* **(B)**.

Analysis of robustness of the model in the presence of transient perturbations and changes in the parameter values

In order to test the robustness of the system in the presence of transient perturbations, we introduced a stochastic variable into equations 1 and 3 from the main text. The simulations were repeated for different intervals of this variable and in both cases, patterns equivalent to those obtained in the deterministic system were obtained for relatively strong noisy perturbations (0.3).
We also addressed the question of whether our results depended on a specific combination of the parameter values. We found that this was not the case by picking random values of each parameter (see Table S3). The system's parameters may be associated to production, diffusion or degradation rates of the molecules involved. As shown in table S3, we found that the patterns obtained do not depend on particular or rare combinations of parameters. Both systems are considerably robust to alterations in parameter values, although the root system with elongated cells is more sensitive than the leaf one, especially regarding inhibitor's diffusion. However, it is not surprising that the root system with elongated cells is sensitive to the increase in this parameter since cell elongation mainly affects the communication among nuclei in the baso-apical direction. The leaf system is considerably more robust to parameter changes (results not shown).

TABLE S3

	PARAMETER (P)				
	Inhibitor's diffusion coefficient	**Activator's production**	**Inhibitor's production**	**Activator's degradation**	**Inhibitor's degradation**
Successful values (%) in [P/2, P]	28	100	4	72	4
Successful values (%) in [P, 2P]	0	36	96	4	2

The root system is robust when confronted with variations in the parameters. Each parameter (D_i, k_1, k_2, k_3 and k_4 in equations 3 and 4) was varied randomly on a one-by-one basis, with a uniform distribution in the intervals [P/2, P] and [P, 2P] [N=25 for each interval], where P is the parameter value for which simulations are shown in this investigation [Activator diffusion rate (D_a) = 0.15; inhibitor diffusion rate (D_i) = 1.65; activator degradation rate (k_1) = 0.3; activator production constant (k_2) = 0.3; inhibitor degradation rate (k_3) = 0.4; inhibitor production constant (k_4) = 0.75]. The table presents the percentage of values for which the patterns resembling those in *Arabidopsis* are maintained. Parameters for the leaf system are more robust than those for the root system in the face of changes (results not shown).

Stability analysis I: reactive part of the model for the nuclear interactions

From the original set of equations (1) and (2) of the main text,

$$\frac{\partial a}{\partial t} = -k_1 a + k_2 \frac{a^2}{i} + D_a \nabla^2 a$$
$$\frac{\partial i}{\partial t} = -k_3 i + k_4 a^2 + D_i \nabla^2 i \qquad \textbf{(S1)}$$

we propose the following reassignment of variables in order to obtain an adimensional set of equations from the original system (S1):

$$\tau = \frac{D_i}{L^2} t$$
$$a_o a = A$$
$$i_o i = I$$
$$u = \frac{x}{L} \qquad \textbf{(S2)}$$
$$v = \frac{y}{L}$$

This set leads to

$$\frac{\partial A}{\partial \tau} = \Psi\left(-\alpha A + \beta \frac{A^2}{I}\right) + \varepsilon\left[\frac{\partial^2 A}{\partial u^2} + \frac{\partial^2 A}{\partial v^2}\right]$$
$$\frac{\partial I}{\partial \tau} = \Psi\left(-\gamma I + A^2\right) + \left[\frac{\partial^2 I}{\partial u^2} + \frac{\partial^2 I}{\partial v^2}\right] \qquad \textbf{(S3)}$$

where

$$\Psi = \frac{L^2 k_2}{D_i}$$

$$\alpha = \frac{k_1}{k_2}$$

$$\beta = \frac{i_o}{a_o}$$

$$\gamma = \frac{k_3}{k_2}$$

$$\frac{k_4 i_o}{k_2 a_o^2} = 1$$

$$\varepsilon = \frac{D_a}{D_i} \qquad \textbf{(S4)}$$

The steady state obtained from (S3) without the diffusive part is:

$$A^o = \frac{\beta\gamma}{\alpha} \qquad I^o = \frac{\beta^2\gamma}{\alpha^2} \qquad \textbf{(S5)}$$

When the system (S3) is linearized in the neighborhood of the steady state (S5), assuming small perturbations δA and"δI from this value, the Jacobian matrix of the perturbed system is:

$$\mathbf{J} = \Psi \begin{pmatrix} \alpha & -\dfrac{\alpha^2}{\beta} \\ \dfrac{2\beta\gamma}{\alpha} & -\gamma \end{pmatrix} \qquad \textbf{(S6)}$$

Using (S6), we can write the linearized reactive system as:

$$\frac{\partial}{\partial\tau}\begin{bmatrix} \delta A \\ \delta I \end{bmatrix} = \mathrm{J}\begin{bmatrix} \delta A \\ \delta I \end{bmatrix} \qquad \textbf{(S7)}$$

With the characteristic equation

$$\lambda^2 + \Psi(\gamma - \alpha)\lambda + \Psi^2\alpha\gamma = 0 \qquad \textbf{(S8)}$$

the corresponding eigenvalues can be computed from:

$$\lambda = \Psi\left[\frac{(\alpha-\gamma)}{2} \pm \frac{\sqrt{(\alpha-\gamma)^2 - 4\alpha\gamma}}{2}\right] \qquad \textbf{(S9)}$$

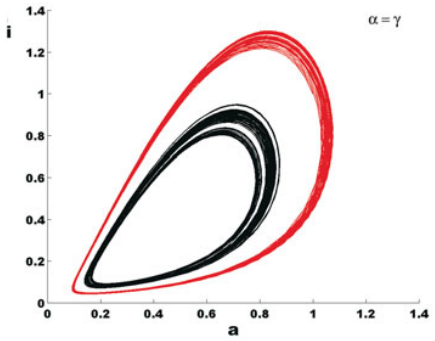

Fig. S2. *In this figure:* $k_1 = k_3 = 0.4$, *with* $k_2 = 0.3$ *and* $k_4 = 0.75$ *from the original system of equations (S1), which corresponds to* $\alpha = \gamma = 1.33$ *in system (S3). The set of equations (S1) were numerically integrated using the Runge-Kutta 45 method, without any consideration being given to the diffusive part.* $\Psi = 1$ *in both cycles. For the red curve, the initial conditions were* i = 1 *and* a = 1*; for the black curve, the initial conditions were* i = 0.8 *and* a = 0.5.

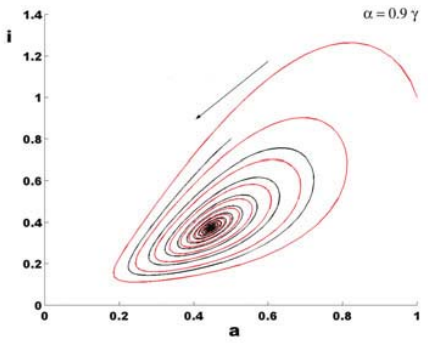

Fig. S3. *Here,* $k_1 = 0.3591$, $k_2 = 0.3$ $k_3 = 0.4$, $k_4 = 0.75$, *which corresponds to* $\alpha = 0.9\gamma$ *and* $\Psi = 1$. *For the red curve, the initial conditions were* i = 1 *and* a = 1 *and for the black curve, the initial conditions were* i = 0.8 *and* a = 0.5.

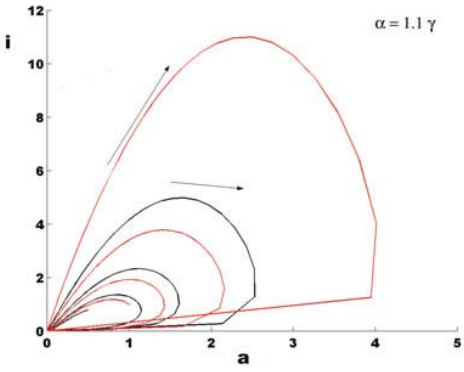

Fig. S4. *In this figure,* $k_1 = 0.4389$, $k_2 = 0.3$ $k_3 = 0.4$, $k_4 = 0.75$, *which correspond to* $\alpha = 1.1\gamma$ *and* $\Psi = 1$. *For the red curve, the initial conditions were* i = 1 *and* a = 1 *and for the black curve, the initial conditions were* i = 0.8 *and* a = 0.5.

If we take γ as the bifurcation parameter, we can characterize the temporal behavior of the system (S3) from the behavior of the eigenvalues computed from equation (S9). In this case, we have the following dynamical features of the system:
1) If $\alpha = \gamma$, then (S9) has two pure imaginary conjugated roots $\lambda = \pm \Psi\gamma$ i. In this case the steady state point becomes a center and the system evolves into a cycle with angle velocity $\omega = \Psi\gamma$.
2) If $\alpha < \gamma$ and the discriminant part of (S9) is such that $\alpha^2 - 6\alpha\gamma + \gamma^2 < 0$, then (S8) has two conjugated complex roots with Re(λ) < 0. This is true for all values $\alpha \in \left(\left(3-2\sqrt{2}\right)\gamma, \gamma\right)$. In this case the system evolves to a stable steady state through a spiral trajectory.
3) If $\alpha > \gamma$ and the discriminant part of (S9) is such that $\alpha^2 - 6\alpha\gamma + \gamma^2 < 0$, then (S8) has two conjugated complex roots with Re(λ) > 0. This is true for all values $\alpha \in \left(\gamma, \left(3+2\sqrt{2}\right)\gamma\right)$. In this case, the system escapes from the unstable steady state through a spiral trajectory.
4) If $\alpha < \gamma$ and the discriminant part of (S9) is such that $\alpha^2 - 6\alpha\gamma + \gamma^2 > 0$, then (S8) has two negative real roots. This is true for all values $\alpha \in \left(-\infty, \left(3-2\sqrt{2}\right)\gamma\right)$. In this case, the steady state is stable.
5) If $\alpha > \gamma$ and the discriminant part of (S9) is such that $\alpha^2 - 6\alpha\gamma + \gamma^2 > 0$, then (S8) has two positive real roots. This is true for all values $\alpha \in \left(\left(3+2\sqrt{2}\right)\gamma, \infty\right)$. In this case, the steady state is unstable.

In Fig. S5, we present the bifurcation diagram corresponding to equation (S8). The bifurcation parameter is γ and we use the linear relation $\alpha = b\gamma$, where b takes the corresponding value indicated in the abscissa axis of the figure.

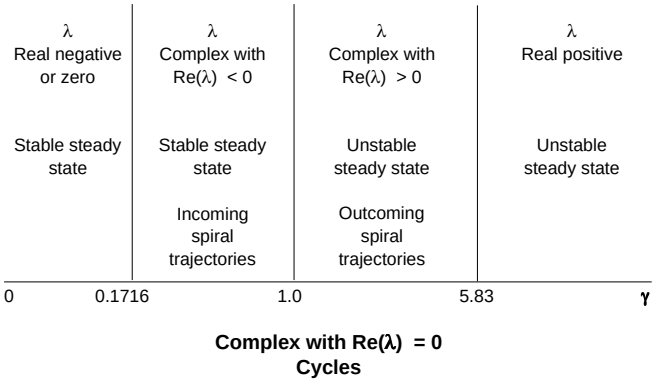

Fig. S5. *Bifurcation diagram for the reactive part of the model.*

Stability analysis II: diffusive part of the model for the nuclear interactions

If the system (S3) is now linearized around the steady state (S5), then in considering the diffusive component, we obtain the vector expression:

$$\frac{d}{d\tau}\delta\mathbf{w} = \mathbf{J}\delta\mathbf{w} + \mathrm{n}\nabla^2\delta\mathbf{w} \tag{S10}$$

where:

$$\delta\mathbf{w} = \begin{bmatrix}\delta A \\ \delta I\end{bmatrix} \qquad \mathrm{n} = \begin{pmatrix}\varepsilon & 0 \\ 0 & 1\end{pmatrix} \tag{S11}$$

If we now define the operator: $\mathbf{H} = \mathbf{J} + \mathrm{n}\nabla^2$, then we have the following linear problem with boundary conditions:

$$\frac{\partial}{\partial\tau}\delta\mathbf{w} = \mathbf{H}\delta\mathbf{w} \quad \text{subject to } \left(\mathbf{n}\cdot\nabla\right)\delta\mathbf{w} = \mathbf{0} \text{ at the boundary} \tag{S12}$$

We now can propose as the solution to this linear problem the vectorial function given by

$$\delta\mathbf{w} = \mathbf{C}_{\mathbf{l},\mathbf{m}} e^{\lambda\tau}\, cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right) \tag{S13}$$

which leads to

$$\mathbf{C}_{l,m} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right)\lambda e^{\lambda\tau} = \mathbf{J}\mathbf{C}_{l,m} e^{\lambda\tau} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right) - \mathrm{n}\mathbf{C}_{l,m} e^{\lambda\tau}\left[\frac{l^2\pi^2}{p^2} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right) + \frac{m^2\pi^2}{q^2} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right)\right] \tag{S14}$$

and

$$\mathbf{C}_{l,m} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right)\lambda - \mathbf{J}\mathbf{C}_{l,m} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right) = -\mathrm{n}\mathbf{C}_{l,m}\left[\frac{l^2\pi^2}{p^2} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right) + \frac{m^2\pi^2}{q^2} cos\left(\frac{l\pi u}{p}\right) cos\left(\frac{m\pi v}{q}\right)\right] \tag{S15}$$

Finally,

$$\lambda\mathbf{I} - \mathbf{J} = -\mathrm{n}\left[\frac{l^2\pi^2}{p^2} + \frac{m^2\pi^2}{q^2}\right] \tag{S16}$$

where

$$\kappa^2 = \pi^2\left(\frac{l^2}{p^2} + \frac{m^2}{q^2}\right) \tag{S17}$$

and

$$\mathbf{J} - \lambda\mathbf{I} - \mathrm{n}\kappa^2 = 0 \cdot \quad \textbf{(S18)}$$

Thus:

$$\delta\mathbf{w}(u,v,\tau) = \sum_j \sum_l \sum_m \mathbf{C}_{l,m} e^{\lambda_j(\kappa^2)\tau} \cos\left(\frac{l\pi u}{p}\right) \cos\left(\frac{m\pi v}{q}\right) \quad \textbf{(S19)}$$

The characteristic equation of the non-diffusive part of the linearized system has two eigenvalues, thus the solution to equation (S12), subject to the given boundary conditions, indicates that j = 1, 2; l = 0, ±1, ±2, ±3,…; m = 0, ±1, ±2, ±3,…, with $p = q$ = 1 and that the system wave number is given by (S17).
Based on (S12) to (S19), we can give the secular equation of the linearized system as:

$$det\left(\mathbf{J} - \mathrm{n}\kappa^2 - \lambda\mathbf{I}\right) = 0 \quad \textbf{(S20)}$$

Taking the following values from the main text:

k_1 = 0.3, k_2 = 0.3, k_3 = 0.4, k_4 = 0.75, L = 1.5; D_i = 1.65; D_a = 0.15,

we get: ψ = 0.41, α = 1, γ = 1.333, ε = 0.09

Substituting these values into equation (S20), we find that there is no set of values of κ^2 for which there are eigenvalues with a real positive part. Thus, the system is stable and the probability of pattern formation is very small near the steady state.

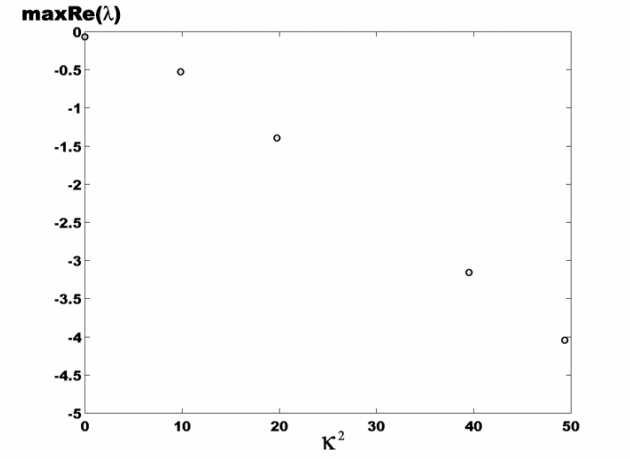

Fig. S6. *In the absence of a biasing signal, all eigenvalues of equation (S18) have a negative real part, indicating that the steady state is stable against any perturbation and that spatial patterns may not arise for any value of* κ^2 *with* L = 1.5

However, if the size of the system is increased to L = 10, which leads to Ψ = 18.18, there is a set of κ^2 values for which there are eigenvalues with a real positive part. The maximum value is obtained when κ^2 ~ 80 (see Fig. S7).

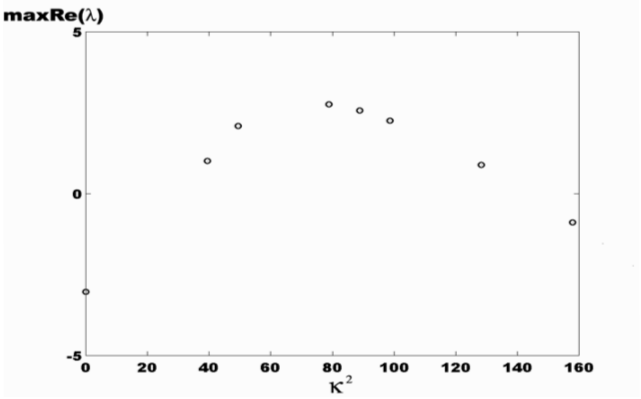

Fig. S7. *Real parts of the eigenvalues of equation (S18) as a function of the wave number* κ^2 *for the cells in the absence of a biasing signal and for* L = 10.

This value of κ^2 corresponds to the mode l = 2 and m = 2. The expected type of spatial pattern for this value of κ^2 is of the form:

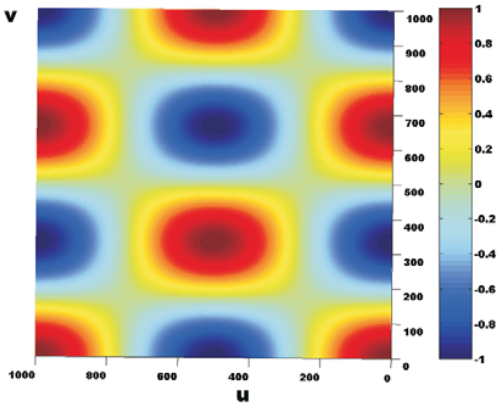

Fig. S8. *Expected dissipative spatial pattern expected for the maximum positive real part of the eigenvalue corresponding to* κ^2 ~ 80. *In this case, the modes are l = 2 and m = 2.*

As we increase L, the value of ψ increases, fixing the values of k_2 and D_i. When Ψ is ~100, which corresponds to a size of ~29, the maximum real positive part of the eigenvalues of equation (S18) is obtained when κ^2 = 404, which corresponds to the mode numbers: l = 5 and m = 4. The spatial pattern expected in this case is:

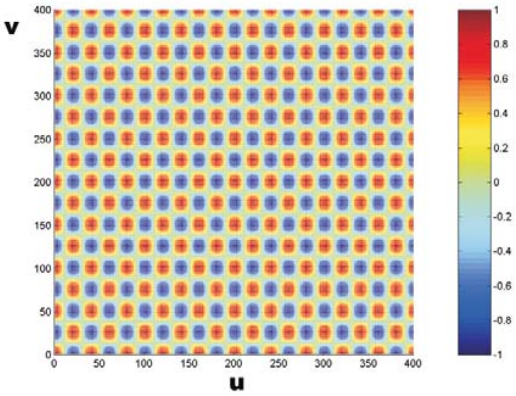

Fig. S9. *Expected dissipative spatial pattern for the maximum positive real part of the eigenvalue corresponding to* $\kappa^2 \sim 404$. *In this case, the modes are* l=*5 and* m=*4.* L=*29.*

CONCLUSION

From this stability analysis we may conclude that it is quite unlikely that banded root-like patterns arise in the absence of a biasing signal similar to the one coming from the cortex cells towards the root epidermis. Therefore, such a biasing signal *f(x)* seems to be not only sufficient but also necessary for the formation of the striped pattern of hair and non-hair cells in the root epidermis.

Apéndice B

Interlinked nonlinear subnetworks underlie the formation of robust cellular patterns in Arabidopsis epidermis

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Research article

Interlinked nonlinear subnetworks underlie the formation of robust cellular patterns in *Arabidopsis* epidermis: a dynamic spatial model

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Abstract

Background: Dynamical models are instrumental for exploring the way information required to generate robust developmental patterns arises from complex interactions among genetic and non-genetic factors. We address this fundamental issue of developmental biology studying the leaf and root epidermis of *Arabidopsis*. We propose an experimentally-grounded model of gene regulatory networks (GRNs) that are coupled by protein diffusion and comprise a meta-GRN implemented on cellularised domains.

Results: Steady states of the meta-GRN model correspond to gene expression profiles typical of hair and non-hair epidermal cells. The simulations also render spatial patterns that match the cellular arrangements observed in root and leaf epidermis. As in actual plants, such patterns are robust in the face of diverse perturbations. We validated the model by checking that it also reproduced the patterns of reported mutants. The meta-GRN model shows that interlinked sub-networks contribute redundantly to the formation of robust hair patterns and permits to advance novel and testable predictions regarding the effect of cell shape, signalling pathways and additional gene interactions affecting spatial cell-patterning.

Conclusion: The spatial meta-GRN model integrates available experimental data and contributes to further understanding of the *Arabidopsis* epidermal system. It also provides a systems biology framework to explore the interplay among sub-networks of a GRN, cell-to-cell communication, cell shape and domain traits, which could help understanding of general aspects of patterning processes. For instance, our model suggests that the information needed for cell fate determination emerges from dynamic processes that depend upon molecular components inside and outside differentiating cells, suggesting that the classical distinction of lineage *versus* positional cell differentiation may be instrumental but rather artificial. It also suggests that interlinkage of nonlinear and redundant sub-networks in larger networks is important for pattern robustness. Pursuing dynamic analyses of larger (genomic) coupled networks is still not possible. A repertoire of well-characterised regulatory modules, like the one presented here, will, however, help to uncover general principles of the patterning-associated networks, as well as the peculiarities that originate diversity.

Background

Complex interactions among diverse elements underlie the appearance of robust cell patterns during development. Understanding how the topology and dynamics of these interactions are related to phenotypical traits represents one of the most important challenges in systems biology, and is necessary to build a more general theory of development and evolution [1].

Cell-type determination and patterning are key developmental processes. Two overall modes of cell-type determination have been distinguished. The first is the lineage-based mode, which depends on factors transmitted from progenitor to daughter cells. The second one depends on positional information in the context of differentiating cells [2]. The definition of these two types of cell fate determination is instrumental for studying and intervening in developmental systems, especially if one focuses on a particular spatio-temporal interval. The model we present here, however, suggests that the emergence of information needed for cell fate determination results from a dynamic process that depends upon molecular components that are both inside and outside cells. In other words, such information is dynamically generated by the interaction among molecular components within the undetermined cells and those in their context.

Since the generation and maintenance of most cellular patterns may depend on the interplay among gradients, cellular communication, environmental signals and lineage-related mechanisms, the intrinsic *vs.* extrinsic distinction that could be identified with lineage *vs.* positional modes of fate determination is sometimes blurred (see more examples of this claim in [3]). Models that consider gene regulatory networks (GRNs), coupled by cell communication and subject to environmental signals, are thus helpful for an understanding of the emergence of the information needed for cell patterning. Such models will be useful in evaluating to what extent positional or lineage-related mechanisms are distinguishable, independent of undetermined cells, and necessary or sufficient for cell determination and patterning in a particular system.

Dynamical GRN models have been fruitfully used to study cell-fate determination [4-7]. In such models, the steady gene activation states (attractors) to which the dynamic system converges correspond to the multigene configurations that characterise different cell types [8]. A discrete approach to modelling the dynamics of such genetic networks was first introduced by Kauffman [8] in order to describe qualitatively the concerted action of several genes during cell differentiation. This approach has been proven to capture the logic of regulatory interactions key to cell-type specification in several biological systems (e.g. [5-9]). Nevertheless, data availability has limited most GRN models to intracellular behaviour, and therefore cellular communication and other important features of pattern formation have been omitted from most models. On the other hand, mesoscopic models that have considered the latter features [10-12] do not usually incorporate complex intracellular networks that regulate cell-type determination.

The epidermis of the model plant *Arabidopsis thaliana* is one of the most thoroughly studied cell-type determination and patterning systems. Such experimental efforts have enabled the postulation of GRNs for cell-fate determination in this system (see recent reviews in [13-15]). Moreover, relatively simple models may capture relevant aspects of this system as it may be accurately represented in a two-dimensional domain, and because *Arabidopsis* epidermal cells attain their fate in a fixed domain before they elongate. Actually, some theoretical studies on this system have been published [16-20]. Of these studies, only one [20] has considered the spatio-temporal dynamics of cell-fate determination by explicitly modelling GRNs, yet it focused on leaf patterning and was mainly aimed at presenting a useful modelling platform rather than studying a particular patterning mechanism.

We put forward a spatio-temporal model of coupled GRNs that integrates updated experimental evidence for cell-fate determination in *Arabidopsis* epidermis and incorporates cellular communication, domain characteristics (size and boundary conditions) and cell shape. We intend to provide a better understanding of how the dynamics of genetic interactions within a cell, in conjunction with cell-to-cell communication, give rise to robust spatial patterns of gene expression and, concomitantly, to cell type determination and arrangement.

Arabidopsis leaf epidermis bears hairs (trichomes) that tend to appear away from each other (Figure 1A; [21]), while root epidermis exhibits trichoblast (root hair precursors) and atrichoblasts arranged in bands of a single cell type (Figure 1B; [22,23]). Despite the contrasting spatial patterns, epidermal cell type determination in root and leaf appears to be associated with similar GRNs (Figures 1E and 1F). Furthermore, although root and leaf GRNs exhibit some differences, they seem to be qualitatively equivalent in dynamical terms [18,24].

The GRNs associated with epidermal cell type determination have been thought to behave as a so-called activator-inhibitor system [25,26] and some theoretical work has been done in this direction [17-19]. In a previous paper, we simplified these GRNs to an activator-inhibitor system, so that we could explore the role of cell contextual or positional traits in cellular spatial patterns [18]. It is often the case, however, that the structure of the GRN confers

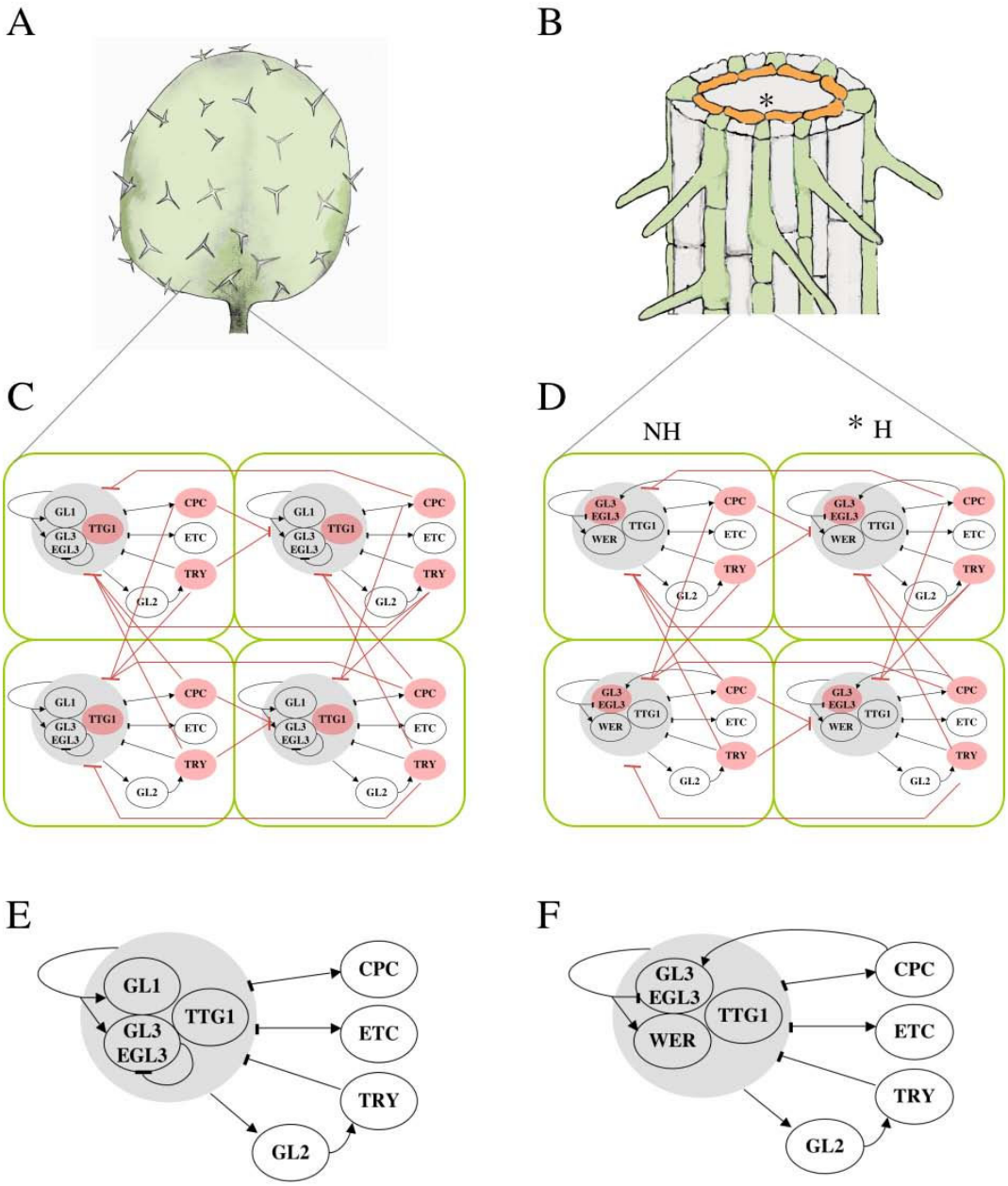


Figure 1

Cellular patterns and meta-gene regulatory network models for leaf and root *Arabidopsis* epidermis. Spaced-out pattern of trichome distribution in the leaf of *Arabidopsis thaliana* (A). Root-hairs (green) are arranged in bands that overlie the junction of two cortex cells (yellow) (B). Coupled gene regulatory network (GRN) model for cell type determination in leaf epidermis (C). GRN underlying cell-fate determination in root epidermis (D). (E) and (F) represent the GRN for leaf and root epidermis, respectively. In both networks, nodes correspond to genes, arrows stand for positive regulatory interactions and flat-end edges stand for negative ones. Red nodes represent elements that are able to move among cells and couple the GRN into meta-GRN. Red lines stand for intercellular interactions established by mobile elements. Asterisks in (B) and (D) indicate the H position where the cortex-related signal is acting.

important dynamic properties on the system and that these properties are not recovered by simplified versions of the system [27]. For example, coupled GRNs in cellularised spatial domains do not necessarily recover the attractors of single-cell GRNs. Moreover, some recent results indicate that the processes giving rise to the epidermal patterns may be richer than an activator-inhibitor system, as different nonlinear sub-networks of the GRN may generate or reinforce such patterns (e.g. [28]). A meta-GRN model that encompasses these sub-networks is required to explore whether coupled GRNs in cellularised spatial domains recover the expected and robust gene activation configurations, and to study the role of sub-network redundancy in pattern formation.

Here we have modelled the meta-GRN by integrating the updated experimental data (Figures 1C–1F) into models that explicitly consider GRNs coupled via cell-to-cell communication in a cellularised domain. Interestingly, the attractors for the single cell GRN [18] were qualitatively preserved in the meta-GRN and the simulated spatial patterns of steady states corresponding to hair and non-hair cells matched those observed in actual *Arabidopsis* wild-type plants. The proposed model therefore accounts for the formation of cellular patterns from initially homogeneous domains containing cells with the same GRN. The model presented here is also useful for exploring the stability of such patterns in the face of environmental and developmental perturbations.

We validated the model by simulating mutant networks and comparing our results with reported gene expression profiles of mutant phenotypes. Then we used the model to postulate novel and testable predictions regarding additional regulatory interactions, the effect of cell shape on patterning and the link between the simulated GRN and the Gibberelic acid signalling pathway. Our results suggest that the proposed GRN constitutes the core of a complex regulatory module associated with *Arabidopsis* epidermal cell type-determination and patterning. Finally, this study contributes to uncovering both generic and specific aspects of GRN coupling mechanisms and patterning processes in biological systems.

Methods

The gene regulatory network model we postulate here is grounded on available experimental data up to June 2008. This evidence is summarised in the following paragraphs. In both root and leaf epidermis, TRANSPARENT TESTA GLABRA 1 (TTG1), bHLH proteins, GLABRA3 (GL3) and ENHANCER OF GLABRA 3 (EGL3), and Myb proteins, GLABRA1 (GL1) and WEREWOLF (WER) in leaf and root, respectively, form a complex that positively regulates the transcription of *GLABRA2* (*GL2*). In turn, the expression of *GL2* determines leaf trichome cell-fate and root atrichoblast identity. *TRIPTYCHON* (*TRY*), *CAPRICE* (*CPC*) and *ENHANCER OF TRIPTYCHON AND CAPRICE 1, 2 and 3* (*ETC1*, 2 and *3*) repress the leaf and root activators in partially redundant ways. All of these inhibitors are upregulated, directly or indirectly, by the root and leaf activators. Interestingly, *GL2* positively regulates *TRY* in both root and leaf epidermis [29,30].

WER and *GL1* are assumed to be directly or indirectly upregulated by themselves. *TTG1* is expressed in all the epidermal cells during hair and non-hair determination and the TTG1 protein moves through plasmodesmata among neighbouring cells in the leaf epidermis, accumulating in trichomes [29,30]. The fact that TTG1 moves among leaf epidermal cells and binds GL3 in the formation of a protein complex has recently led Bouyer and collaborators [28] to postulate a *TTG1*-trapping/depletion mechanism that could explain the accumulation of TTG1 in cells with GL3 (trichomes) and could suffice for trichome pattern formation. This mechanism is discussed later in the context of other experimental data and our results.

Despite being part of the activator complexes, *GL3* and *EGL3* are primarily expressed in root hair cells and their proteins move towards cells in which the other components of the complex are present. *GL3* and *EGL3* also seem to be activated by the inhibitor *CPC* and repressed by the root activator complex [31,32]. In leaves, *GL3* expression occurs in trichome cells, where *GL1* maximum expression is reported. Transcription of *GL3* seems to be downregulated by itself and the localisation of the GL3 protein within the cell nucleus is regulated by *TTG1* and *GL1* [31]. Experimental evidence also shows that *TRY* acts non-cell autonomously in leaves and that the CPC protein moves through plasmodesmata among cells in both leaf and root epidermis [33,34]. In the root, *SCRAMBLED* (*SCM*) seems to be a crucial component of a positional signal downregulating *WER* in root-hair bands and therefore biasing the cellular pattern [35,36].

In the proposed GRN model, elements or nodes of the network correspond to genes. The *ETC* node summarises the partially redundant activity of the three enhancers of *TRY* and *CPC*. Edges of the network stand for regulatory interactions between nodes (activation or repression; Figures 1E and 1F). Nodes representing genes for which there is enough information can attain three different states (0, no expression; 1, mild expression; 2, strong expression). In the leaf system, only *ETC* can either be expressed, 1, or not expressed, 0, and only *ETC* and *TRY* are binary in the root system. For simplicity, the state of all nodes was updated synchronically. In single-cell GRNs, the updating scheme does not seem to affect attractors when these are fixed-point type [37]. Since the attractors of our GRN

model are indeed fixed-point ones, it could be the case that asynchronicity does not affect them, yet this issue has not been explored in coupled GRN models.

In the GRN model, each node's state depends on that of other nodes, its regulators. The level of expression of a given gene is represented by a discrete variable g (0, 1 or 2) and it depends on the level of expression of other components of the network, g_1, g_2, ..., g_N. The state of every gene g therefore changes according to:

$$g_n(t+1) = F_n(g_{n1}(t), g_{n2}(t), ..., g_{nk}(t)). \quad (1)$$

In this equation g_{n1}, g_{n2}, ..., g_{nk} are the regulators of gene g_n, and F_n is a discrete function known as logical rule (logical rules were grounded on available experimental data and are graphically represented in Figures 1E, 1F, [see Additional file 1]). Given the logical rules, it is possible to follow the dynamics of the network for any initial configuration of the nodes expression states. Since the GRN is simulated as discrete, all possible combinations of gene activation states may be explored. One of the most important dynamical traits of a GRN is the existence of attractors. Starting out from an initial state, the reiterated application of equation (1) generates dynamics in which genes go through transient states until the whole network enters into a stationary or periodic profile of multi-gene expression. Such stationary or periodic expression profiles constitute the GRNs' attractors, which correspond to gene expression profiles characteristic of particular cell types.

In order to couple the GRNs in a compartmentalised (cellular) domain, we considered a discrete lattice of $n \times n$ elements, in which each element (i, j) represents a cell with a GRN. As in real organisms, all cells bear the same GRN. Modelled lattices are a simplified representation of leaf or root epidermal sections. Cells in the lattice have exactly four neighbours and there is no difference in permeability between them.

According to experimental data, some proteins codified by elements of the network move to neighbouring cells and affect gene expression in a non-cell-autonomous fashion (TRY, CPC and TTG1 in the leaf epidermis and CPC, GL3 and EGL3 in the root epidermis), giving rise to a network of coupled networks (herein *meta-GRN*). Although empirical evidence supporting cell-to-cell motion rather than aplopastic transport only exists for CPC and TTG1, all available data are congruent with the assumption that all mobile elements of the GRN move in a cell-to-cell manner. In the spatial model we therefore allowed for certain elements to move among neighbouring cells (Figures 1C, 1D and 2) following the equation:

$$g(t)_{T\,[i]\,[j]} = H(g(t)_{[i]\,[j]} + D(g(t)_{[i+1]\,[j]} + g(t)_{[i-1]\,[j]} + g(t)_{[i]\,[j+1]} + g(t)_{[i]\,[j-1]} - 4(g(t)_{[i]\,[j]})), \quad (2)$$

where $g(t)_{T\,[i]\,[j]}$ is the total amount of protein g in cell (i, j). D is a continuous variable that determines the proportion of g that can move from any cell to neighbouring ones and is correlated to the diffusion rate of g. H is a step function that converts the continuous values corresponding to the amount of g diffused into each cell into a discrete variable that may attain values of 0, 1 or 2. In order to simulate the effect on diffusion of protein attachment to a protein complex, our simulations consider that the mobile elements of the network diffuse with a lower rate from inside to outside when the other protein complex components are inside the same cell. So, for instance, when there is GL3 in a cell, the term $-4(g(t)_{[i]\,[j-1]})$ in the diffusion equation of TTG1 is removed or decreased (see also [28]). More generally, we defined a term $-A(g(t)_{[i]\,[j]})$, in which de value of A depends on the presence of trapping proteins in a cell (i, j) at time t ($A = 4$ when no trapping proteins are present and $A < 4$ when these are present).

D was systematically varied from 0.0 to 0.25 with a step size of 0.01, enabling the generation of parameter spaces in which we plotted the average number of ectopic trichoblasts and atrichoblasts for every combination of parameter values. Four types of 'trapping' diffusion terms were also tested ($-A(g(t)_{[i]\,[j]})$, with A = 0,1,2,3; see Results). In the leaf system, boundary conditions were simulated as zero-flux, while in the root system, two borders were identified and two were kept at zero-flux, simulating a root-like cylinder. In the root model, the positional signal associated with *SCM* was simulated as a constant downregulation of *WER* every three cell files (two NH files between H files) [36].

Then, $g(t)_{T\,[i]\,[j]}$ was considered in order to estimate the $g(t)_{[i]\,[j]}$ value, which was then used to update the logical rules, according to the equation below:

$$g'(t)_{[i]\,[j]} = g(t)_{[i]\,[j]} + g(t)_{T\,[i]\,[j]} - k, \quad (3)$$

where k stands as a degradation constant. $g'(t)$ was taken as an input to evaluate the logical rules and obtain $g(t+1)_{[i]\,[j]}$.

In brief, each cell's GRN is initialised with a random gene activation profile. Then, three steps are sequentially repeated until the whole lattice reaches a steady state (diagrammatic representation in Figure 2): (1) application of logical rules, (2) diffusion of mobile elements and (3) integration of diffused protein into the GRN inputs for the next updating step according to the logical rules.

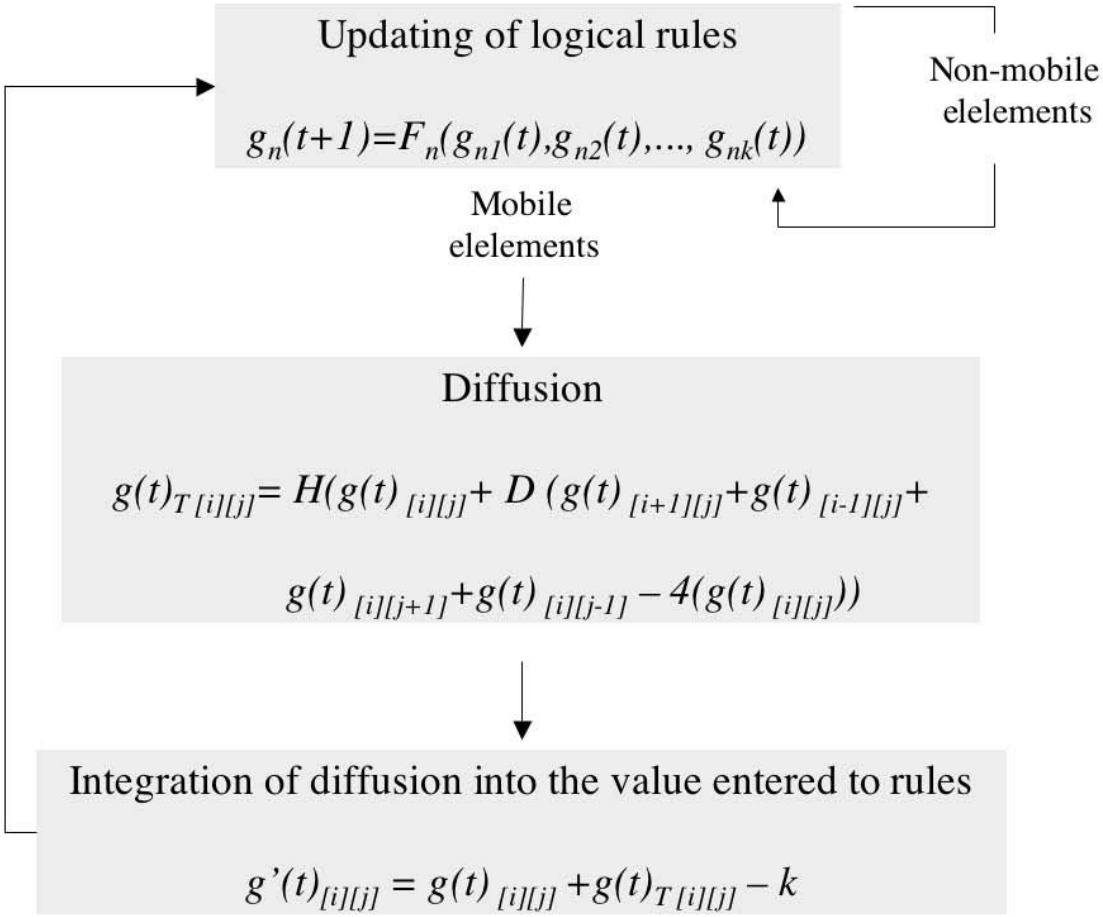


Figure 2
Diagrammatic representation of the model structure. In every time-step, the nodes' states are updated according to logical rules [see Additional file 1], then the mobile elements are allowed to diffuse and, finally, diffusion is considered to recalculate the states of mobile elements. These new values are entered into the logical rules in the next iteration. The state of non-mobile elements is only determined by the logical rules applied every time-step.

In order to test the model, we also simulated networks that correspond to reported mutants. The loss and gain of function simulations were done by fixing the expression value of the altered gene to *0* or *2*, respectively. The random seed was the same for both wild-type and mutant simulations so that results were comparable.

It should be noted that there are several dozens of genes involved in hair determination, patterning and differentiation in *Arabidopsis* [38]. We have, however, incorporated in the GRN models only the elements that seem to be responsible for the decision: hair *vs.* non-hair. Changes in expression states of these elements do indeed yield changes on epidermal cell identity. In contrast, most of the genes that were not included seem to act downstream of the GRNs modelled here and may play important roles in morphogenesis once cells are committed to a particular fate. A few other genes that are not considered seem to act upstream of the GRN postulated here, probably linking the GRNs with signalling pathways or tissue-specific factors. Our simulations of both wild-type and mutant networks indeed suggest that the GRNs modelled here incorporate the necessary components to render the robust gene activation profiles characteristic of epidermal hair and non-hair cells in both roots and leaves, and thus uncover the core of a regulatory module that regulates epidermal cell patterning in *Arabidopsis*.

Graphics were elaborated in MATLAB and programs in C (codes available upon request).

Results

Gene expression profiles and wild-type spatial patterns are robustly recovered by the leaf GRN model

The spatial model of the leaf system (Figure 1C) was initialised by random assignment of activation states (0, 1 or 2) to the nodes in the GRNs. In this case, TRY, CPC and TTG1 were allowed to move among cells according to experimental data (see previous section). After a few iterations, the GRNs in all cells in the lattice reached one of two attractors. One of these attractors exhibits a GRN activation profile that matches the characteristic profile of trichome precursor cells (black cells in Figure 2A), in which the expression of all network elements peaks. The other attractor matches the gene activation profile reported for pavement or non-trichome cells, in which the networks' elements are less expressed than in trichomes or not expressed at all (white cells in Figure 2A). This result indicates that the GRN structure, interaction rules and protein diffusion functions considered in the leaf GRN model are sufficient to generate the two, and no more, stable expression profiles that mimic those reported for leaf hair and non-hair precursor cells in *Arabidopsis*.

Simulated spatial domains show a dotted pattern similar to that of trichomes in actual leaves. Moreover, the simulated trichome distribution was also significantly more spaced out than expected in a random spatial distribution, as is the case in actual leaves. This was found by calculation of the *R*-value ([39,21]: $R = 1$ in a random distribution; $R > 1$ in a pattern with elements more spaced out than in a random case; and $R < 1$ in a clustered distribution). *Arabidopsis* trichomes of the Col ecotype have an $R = 1.40$ and the clustering probability (C) is around 0.08 [21], while the typical values we found in our simulations are $1.2 < R < 1.40$ and $C < 0.02$ (Table 1). Since our model does not consider growth or proliferation, it is important to note that the *R*-values estimated in real leaves were measured before growth and proliferation affected trichome distribution [21] and are overall comparable to those obtained in our simulations.

Table 1: The leaf meta-GRN recovers the spaced-out pattern characteristic of leaf trichomes.

	<R>	<C>
Random	1	0.5
Clustered	< 1	1
Spaced-out	> 1	0
WT leaf	1.4	0.08
Simulation	1.3	0

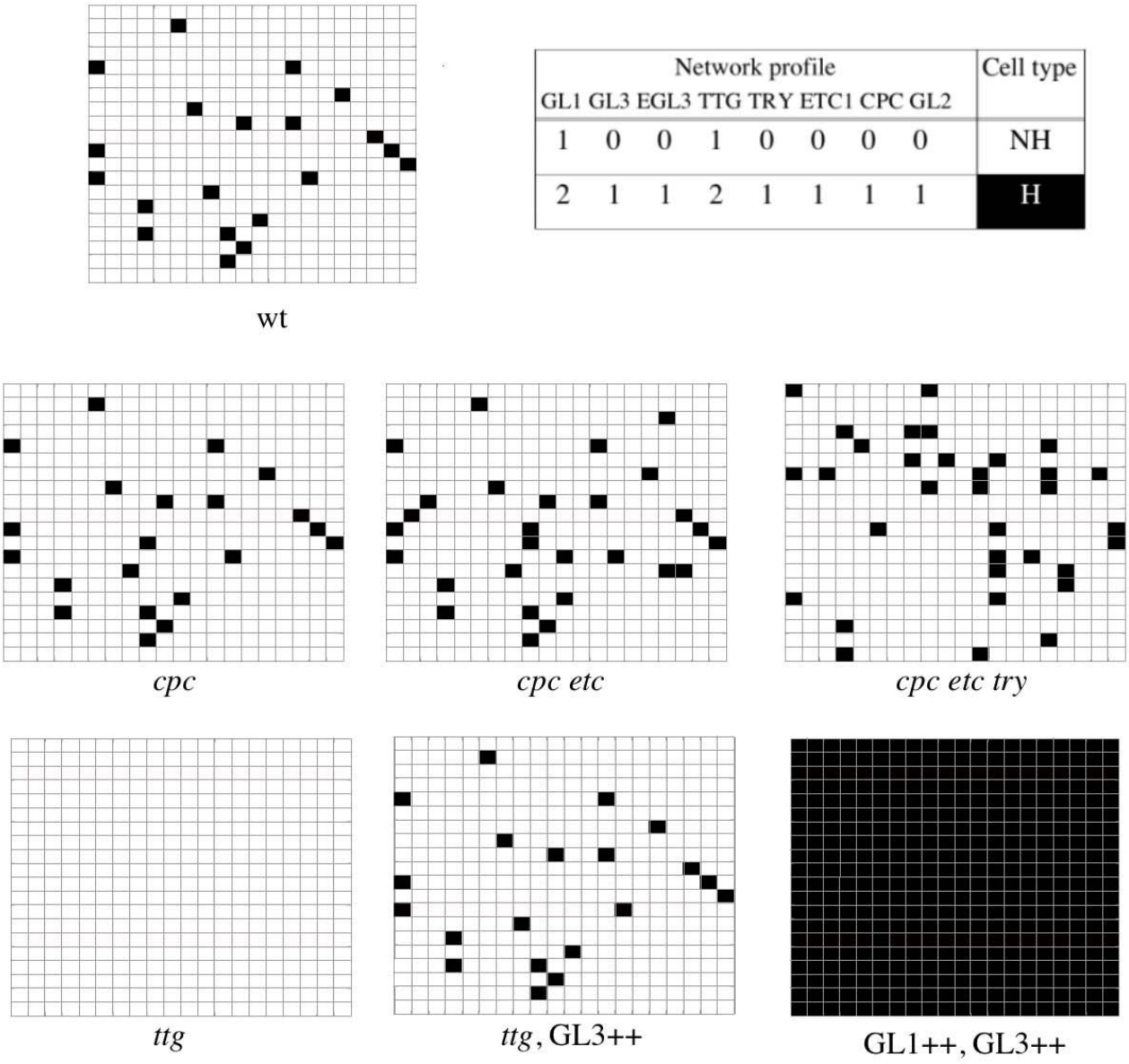
Measures for matrices with different 'hair' arrangements are presented. *R* indicates how clustered or spaced-out the arrangement is [21], while *C* indicates the clustering probability. Simulations of the leaf meta-GRN model were carried out with $D_{CPC} = 0.08$, $D_{TRR} = 0.08$, $D_{TTG} = 0.06$.

We then asked if the simulated pattern depended on gene interaction details, particular domain characteristics (boundary conditions and size), or specific diffusion parameters, or if it depended on the overall coupled GRNs system. We found that the simulated leaf pattern was robust to changes in boundary conditions. Diffusion parameters D_{TRY}, D_{CPC} and D_{TTG1} were also systematically varied and the simulated trichome pattern ($R > 1$, $C \approx 0$) was not altered significantly for a wide range of parameter values. The pattern was maintained for $0.01 < D_{TRY/CPC} < 0.26$, independently of the D_{TTG1} value. Interestingly, this result indicates that, although the *TTG1*-trapping/depletion mechanism suffices to generate a trichome-like pattern [28], in the context of the whole GRN it may not be a necessary mechanism. This is also suggested by experiments showing that plants overexpressing *GL3* are able to recover a wild-type phenotype in a *ttg1* mutant background [40]. The interplay among different potential patterning sub-networks is discussed in more detail below. As mentioned above, diffusion of mobile proteins from a cell [*i*] [*j*] to neighbouring cells was restrained when other interacting proteins were present in the [*i*] [*j*] cell. This was done by taking the term $-4(g(t)_{[i]\,[j]})$ in equation (1) as $A(g(t)_{[i]\,[j]})$ with $A < 4$. We varied such a term by taking $A = 0, 1, \ldots, 4$ and no differences in the simulated wild-type patterns were observed.

Interestingly, the spaced-out pattern was also found to be robust to point alterations of the gene interaction functions. We tested the latter by modifying the output of all logical rules, one at a time. We found that 88% of the alterations yielded the same attractors and trichome-like spatial pattern as the original model. Together these results suggest that the overall GRN topology and the coupling mechanisms, rather than detailed aspects of the network kinetics or specific parameter values, underlie the emergence and stability of the cell patterns in *Arabidopsis* leaf epidermis.

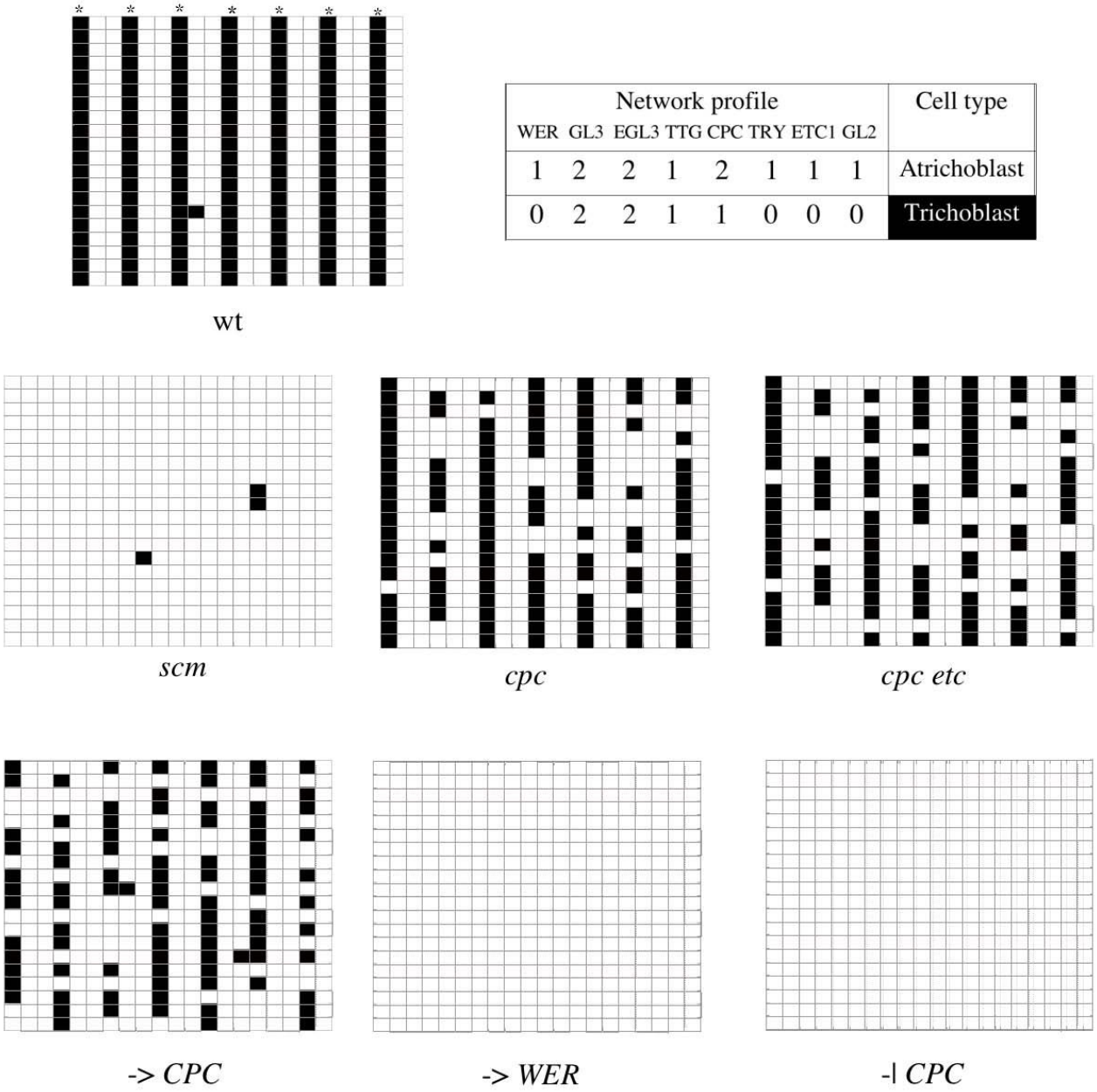
The root GRN model robustly recovers the profiles and patterns characteristic of the wild-type root epidermis

In the root meta-GRN model, CPC and bHLH proteins (GL3 and EGL3) were allowed to diffuse, as suggested by experimental data (see Methods). In this case, the cortical signal associated with *SCM* was simulated every two cell files as a downregulating input on *WER*. GRNs were also randomly initialised and two network attractors were found. In the first (white cells in Figure 3), *WER*, *TTG*, *CPC*, *TRY* and *ETC* are expressed, and owing to diffusion GL3 and EGL3 proteins are also present. This profile corresponds to that reported for cells committed to the non-hair fate (atrichoblasts). The other attractor matches the characteristic profile of cells that will bear root-hairs (trichoblasts), as *GL3*, *EGL3* and *TTG* are expressed in it and the CPC protein is present.



Network profile								Cell type
GL1	GL3	EGL3	TTG	TRY	ETC1	CPC	GL2	
1	0	0	1	0	0	0	0	NH
2	1	1	2	1	1	1	1	H

Figure 3
The model renders cellular patterns similar to those observed in the leaf epidermis. The simulated cellular patterns for wild-type (wt) and mutant networks are consistent with the patterns reported in the literature. Black squares correspond to trichomes and white ones to pavement cells (non-hair cells). Captions under the matrixes indicate the simulated genotype that gave rise to each of them (++ stands for overexpression, while lower case italics indicate loss of function). The table shows that the network profiles typical of hair and non-hair cells are recovered by the meta-GRN model. These simulations were all performed in 20 × 20 matrices with parameter values $D_{CPC} = 0.05$, $D_{TRR} = 0.05$, $D_{TTG} = 0.03$.



Network profile								Cell type
WER	GL3	EGL3	TTG	CPC	TRY	ETC1	GL2	
1	2	2	1	2	1	1	1	Atrichoblast
0	2	2	1	1	0	0	0	Trichoblast

Figure 4

The model renders cellular patterns similar to those observed in the root epidermis. Cellular patterns obtained from simulations of wild-type (wt) and mutant networks are consistent with the patterns reported in the literature. Black squares correspond to trichoblasts and white ones to atrichoblasts. Captions under the matrixes indicate the simulated genotype that gave rise to each of them (lower case italics indicate loss of function, -> indicates the simulation of a positive upstream signal, while -| stands for a negative one). Asterisks indicate the hair (H) position. The table shows that the network profiles characteristic of hair and non-hair cells are recovered by the coupled GRN model (B). These simulations were all performed in 20 × 20 matrices with the following parameter values: D_{CPC} = 0.01, D_{GL3} = 0.01, D_{EGL3} = 0.01.

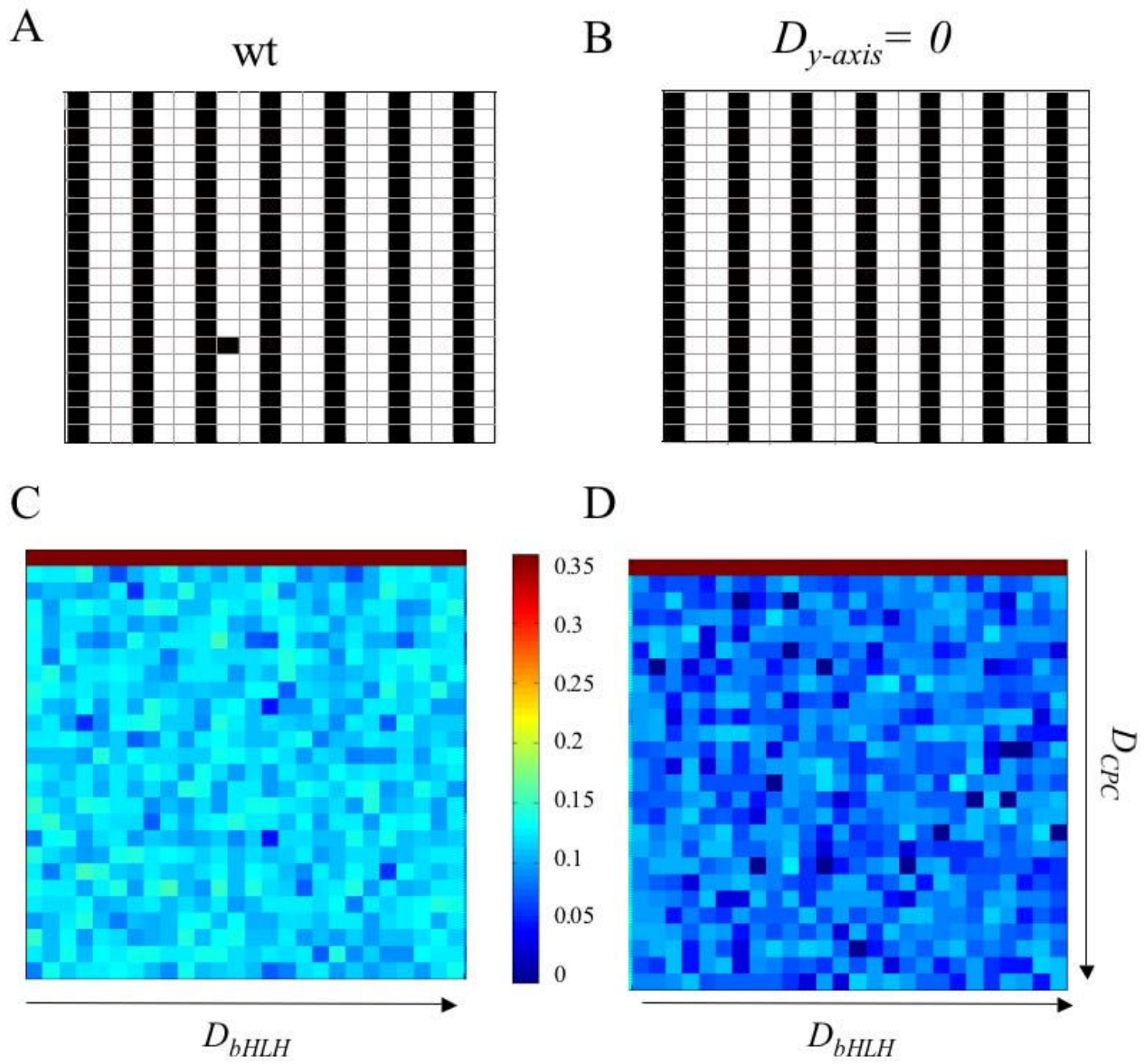


Figure 5

Spatial root-like pattern is stabilised by differential diffusion in the *x* and *y* axes. The pattern generated by the coupled GRN model gives rise to a striped pattern with some 'errors' that would correspond to ectopic hairs (A). A similar pattern but with fewer or no errors is obtained when diffusion rate in the *x* axis is larger than that in the *y* axis ($D_{y\text{-}axis} = 0$) and the same random seed is taken (B). The parameter spaces for each case are presented below their typical cell arrangements (C), (D). The colour scale indicates the logarithm of the average number of ectopic cell-types for every combination of parameters. Note that, overall, the parameter space obtained for differential diffusion exhibits fewer ectopic trichoblasts.

The simulated spatial cellular pattern of hair and non-hair young cells is very similar to that observed in *Arabidopsis* roots. It is characterised by bands of trichoblasts (black cells in Figure 4) in the H position, where the positional cue is simulated, and bands of atrichoblasts (white cells in Figure 3) in the NH position. This pattern is recovered within a wide range of parameter values (Figure 5). In most of the parameter space generated by varying the diffusion values D_{CPC} and D_{bHLH} from 0 to 0.25 (step size 0.1), the percentage of errors (cells in the NH position without activator complex) is approximately 0.16% (Figure 5). Such positional errors would correspond to ectopic hairs in the root and are smaller than those reported in actual wild-type *Arabidopsis* roots (Col) [41].

The root-like spatial pattern is also robust to different kinds of perturbations. It is resilient to the intensity of the *SCM* signal, measured as a probability value P_s, and is also robust to alterations in the output of logical rules: in 20% of the tested changes the number of ectopic cell-types increases, yet the root-like pattern is still clearly discernible. Only 0.06% of the changes modify the nature of attractors or drastically disorganise the spatial arrangement. All simulations shown were performed in a 20 × 20 (rows × columns) lattice. As for the leaf system, variations of the $-A(g(t)_{[i][j]})$ term do not affect the wild-type pattern.

Twenty is a fair approximate of the number of epidermal cells in a transversal root section. Given, however, that the pattern may arise in rings of cells, we also tested lattice sizes from 1 × 20 to 40 × 20 and recovered the same results. This suggests that, for a wide range of domain sizes, the root pattern is not sensitive to changes in the number of cells nor to the root section where epidermal cells attain their fate.

Simulation of mutant GRNs renders spatial patterns that match those observed in actual mutants

In order to validate further the meta-GRN models, we simulated mutations in the GRN and compared the resulting patterns with the reported phenotypes. Mutations were modelled by fixing the state of a node at 0 (loss of function) or 2 (gain of function) throughout the simulations. Overall, the simulated mutants qualitatively resembled those reported in the literature.

We illustrate these simulations with some examples (Figures 3 and 4; [see Additional file 1]) and discuss a few cases in which quantitative differences between simulated and observed patterns were encountered. For the leaf case (Figure 3), the *ttg1* loss of function simulated mutant gives rise to a homogeneous pattern of no *GL2* expression that corresponds to a hairless leaf. The simulated *cpc* mutant gives rise to more trichomes than observed in the wild type, but not to more trichome clusters. This is consistent with the available experimental evidence but the increase in hair number that we recover (around 5% more trichomes than in wild type) is smaller that that observed in real *cpc* mutants. As in real plants, the simulated single *etc* mutant does not affect trichome number or distribution, but the *cpc* phenotype is enhanced in the double *cpc etc* simulated mutant. Also compatible with experiments, in the triple *cpc etc try* simulated mutant, the trichome number and clustering probability greatly increase in comparison with the wild-type simulated leaf section. The recovery of a wild-type arrangement in *ttg* simulated mutants overexpressing *GL3* was also reproduced by our model.

There are two mutant simulations that partially differ from reported leaf phenotypes. The double *cpc etc* simulated mutant gives rise to few clusters, yet not as many as the *try* simulated mutant, while in the actual *cpc etc* mutant trichome clusters are extremely rare. This discrepancy could be because of the fact that in our model all *ETC*s (*ETC1*, 2 and 3) were summarised in one node. On the other hand, simulated overexpression of *GL1* and *GL3* genes generates a totally hairy domain that resembles the extremely hairy epidermis of the double overexpression line. A few non-hair cells are still observed, however, in real plants overexpressing *GL1* and *GL3*. This could be explained if in real plants the overexpression of *GL1* and/or *GL3* is not as effective as assumed in the model.

The root meta-GRN patterns of simulated mutants are also very similar to those observed in plants (Figure 4). For instance, the simulated mutant for *cpc* loss of function displays a pattern that matches the increase of ectopic atrichoblasts observed in these mutants. The *scm* simulation gives rise, as in real roots, to a disorganised pattern of trichoblasts and atrichoblasts. The simulated *wer* loss of function shows a hairy phenotype similar to that observed in actual *wer* mutants. Finally, this model also recovers that *etc* enhances *cpc* mutant phenotypes. The leaf and root mutant simulations show that the meta-GRN model is able overall to recover qualitative aspects of the system behaviour and that, in this context, may be helpful for an understanding of pattern formation and providing novel qualitative predictions.

Novel predictions: the role of cell shape, GL1, GL3 *and* WER *upregulation and GRN interactions with the gibberelic acid pathway and cortex-associated signals*

The model we present reproduces documented gene activity configurations in wild-type and mutant plants and also constitutes a powerful predictive tool regarding cellular and genetic mechanisms that may affect the *Arabidopsis* epidermal system.

Cell growth and the root hair pattern

In a previous work [18], we postulated that elongation of root epidermal cells could stabilise the early striped pattern by increasing the distance between neighbouring nuclei in the baso-apical direction of the root, thus giving rise to an effective increase of the diffusion rate along this axis. We used the meta-GRN model to simulate a higher diffusion rate on the baso-apical direction than in the radial one. Interestingly, such bias makes the spatial pattern more stable and reduces the average number of ectopic cell-types for the whole parameter space (Figure 5). These new results therefore support the idea that either cell elongation or differential diffusion rates between the longitudinal and radial axes in the root epidermis contribute to stabilising the observed cellular spatial configuration.

Trichome patterning and the gibberellin signalling pathway

Density and distribution of root and leaf hair cells change during development and are affected by diverse internal and external signals. Some of these signals are hormones that may affect the GRN modelled here [16,42,43]. We explore the interaction of the leaf GRN and the Gibberelic acid (GA) hormone signalling pathway. Leaf trichome density is positively regulated by GA, among other hormones. Recently, Gan and collaborators [43] found that some transcription factors (*GIS*, *GIS2* and *ZFP8*) act as GA receptors and act upstream of some elements of the GRN modelled here, biasing cell fate towards trichomes. Indeed, GA receptors upregulate *GL1*, although it is still unknown whether transcription factors like *GIS* act directly on *GL1* and if such upregulation would suffice to make the leaves hairy.

Several interactions of the GRN could in principle cause *GL1* upregulation and, at the same time, yield a denser trichome pattern. We simulated GA up and downregulation for all the possible targets within the GRN. We did this by fixing a constant minimum expression of each tested target to 1 (upregulation) or by fixing the node's state to 0 (downregulation) with a probability P_{GA} (equal for all cells) in all cells in the lattice. For simplicity, we assumed that GA is homogeneously distributed in the modelled spatial domain and that the signalling pathway regulates only one of the network's components at a time. We also assume that this hormone does not cause an overexpression effect on its target (i.e. it does not fix the expression state to 2). This last assumption is based on the fact that overexpression of the network components often leads to drastic phenotypes that are not observed in wild-type *Arabidopsis* development and during which GA signalling is present. Results in Table 2 were generated by simulation of two particular P_{GA} values (1 and 0.01), each one representing one of two broad ranges of values that render qualitatively different results.

The simulation results suggest that an increase in *GL3* and *EGL3* or a decrease in *TRY* or *CPC* expression leads to a higher density of trichomes and, therefore, to a higher *GL1* expression levels (Table 2). Both the complete and partial repression of *TRY*, however, yield a higher clustering probability (Table 2). Given that (i) GA partially underlies the differences in trichome density exhibited by certain regions of the plant throughout development, even under normal growth conditions [43,44] and that (ii) clusters are very rare in wild-type plants, it is unlikely that the GA pathway acts directly on *TRY*. In contrast, *bHLH* genes and *CPC* are likely targets of the GA pathway. Although the trichome number increase for *CPC* downregulation is small, it seems that the meta-GRN model underestimates the increase in hair numbers observed in the *cpc* mutant (see previous section in Results) and therefore *CPC* is also a good candidate for GA regulation.

Table 2: Simulation of possible interactions of the GA pathway and the GRN.

	Trichome number	Trichomes adjacent to another one
wt	19	0
bHLH*(+)	**44**	**2**
bHLH (+)	400	400
GL1 (+)	19	0
TTG1 (+)	19	0
GL2 (+)	0	0
GL2 (+)*	11	0
CPC (-)*	19	0
***CPC*(-)**	**20**	**0**
TRY (-)	28	8
ETC (-)	19	0

Only a positive interaction on *bHLH* genes or a negative one on *CPC* (rows in grey) produces an increase in trichome density and maintains the spaced-out pattern. The sign of interaction is in parentheses. Asterisks indicate an interaction probability $P_{GA} < 1$, while no asterisk means that this probability was equal to one. The two cases $P_{GA} = 1$ and $P_{GA} < 1$ are only shown when their results are different. These simulations were performed in 20 × 20 matrices with the following parameter values: $D_{CPC} = 0.05$, $D_{TRR} = 0.05$, $D_{TTG} = 0.03$.

Possible cortex-related signals in the root system

We modelled the signal associated with *SCM* as a negative regulation on *WER*, as suggested by recent experimental data [36]. Nevertheless, this may not be the only positional cue biasing the root GRN and it does not affect cell-type patterning in hypocotyls [36], which seems to share the root GRN and also exhibits a striped cell-type arrangement. It is likely that there are additional *SCM*-independent signals and our model may be used to predict the nature of such signals. We made some predictions concerning the possible targets of such signals among GRN nodes.

We simulated several possible targets of both positive and negative putative signals. We thus simulated: upregulation of *WER* in the NH position, upregulation of *CPC* in the H position and downregulation of *CPC* in the NH position. In our model, the negative regulation of *SCM* over *WER* is the one that best recovers the observed root-like striped arrangement (wt in Figure 4). This was the signal used throughout all the simulations mentioned in previous sections, as it is the only one empirically documented. Upregulation of *CPC*, however, also gives rise to a striped pattern (-> *CPC* in Figure 4), although a significantly less stable pattern than the one recovered with the negative signal on *WER*. Negative regulation of *CPC* (-> *CPC* in Figure 4), and upregulation of WER (-> *WER* in Figure 4) render, on the other hand, uniform patterns that are very different from the observed banded ones. Our model therefore suggests that if there were additional signals associated with the root striped cellular patterns, these should act positively on *CPC*.

GL3, WER *and* GL1 *positive regulation*

GL3 expression peaks in the cells committed to the trichome identity in leaf epidermis. This suggests that it could be upregulated by the activator complex or by members of such a complex. Yet it was recently shown that its transcriptional regulation is *GL1*-independent [31], although *GL1* and *TTG1* seem to regulate the location of the GL3 protein within the cell. Since *CPC* upregulates *GL3* in the root epidermis, and given that its expression peaks coincide with that of *GL3*, *CPC* could be responsible for *GL3* activation. We therefore used our model to test whether the simulated trichome pattern remained the same if *CPC* was assumed to activate *GL3*. Our simulations suggest that the upregulation of *GL3* by *CPC* would cause a drastic increase in trichome density and a clustering probability close to one, contrasting with observed patterns. This result suggests that, if *CPC* upregulated the transcription if *GL3* in leaf epidermis, another unknown factor would have to restrain *GL3* expression to trichomes.

As mentioned above, we performed a systematic exploration of the effect of alterations in the logical rules. Interestingly, our simulations suggest that the self-activation of *WER* and *GL1* is necessary to recover all the wild type and mutant patterns. This prediction is consistent with the fact that these two genes peaks of expression are in the same cells where their inhibitors are also expressed at the highest levels.

Discussion

We put forward an experimentally-grounded dynamical model that integrates experimental evidence and helps us to study the concerted action of multiple molecular components during *Arabidopsis* hair pattern formation. This constitutes one of the best experimental systems to address questions concerning cell differentiation and spatio-temporal arrangement of cell types. This study uncovered a regulatory module that is sufficient to recover the cell types and spatial configurations characteristic of *Arabidopsis* root and leaf epidermis. The model of coupled GRNs in a cellularised spatial domain was used to provide predictions regarding the effect of unknown interactions, signalling mechanisms acting on the GRNs and cell shape on such epidermal patterning.

Besides the insights of our meta-GRN model concerning the particular cell patterning system under consideration, it makes a general contribution to a fundamental issue in developmental biology: the origin of the information needed for the emergence of cell-type patterns from homogeneous domains. In contrast with single-cell models previously published, we achieved this by coupling single-cell GRNs by explicitly incorporating cell-to-cell communication in a cellularised spatial domain. This enabled us to evaluate whether the observed gene activation profiles are recovered in the meta-GRN.

In the case considered here, the information needed for cell-type arrangement consists of heterogeneous patterns of gene expression and is determined by complex interactions among multiple intra- and extra-cellular factors. Our model therefore recovers the *de novo* formation of positional information from such complex GRNs and suggests that the appearance of this information is very robust. It does not depend on particular initial conditions or domain size, and is resilient in the face of single perturbations in the logical rules, as well as changes in the protein diffusion rates. Robustness has been documented as well for single-cell GRN models [5,6].

As mentioned above, our model also enabled testable predictions on the behaviour of the particular system under study. The first prediction states that cell elongation, or other processes that could alter or bias diffusion rates in the x and y axes of the root (for example, differential transport rate), have a relevant role in stabilising the spatial pattern generated early in root development. This predic-

tion points at a precise way in which cellular morphological traits may affect cellular patterning and could be tested by crossing a GL2:GUS or GFP marker line plants with altered cell shapes (e.g. [45,46]) and search for alterations in the spatial pattern of this genetic marker of epidermal cell type.

Another prediction concerns the action of GA signalling on the GRN, suggesting that this hormone signalling pathway acts on *CPC* or *bHLH* genes. This prediction is consistent with recent experiments with *bHLH* inducible overexpression lines [47] that suggest that an increase in *bHLH* expression suffices to produce more trichomes. Both *bHLH* and *CPC* actually have GA response motives (PLACE, http://www.dna.affrc.go.jp/PLACE/), but this does not imply that they respond directly to GA. According to our prediction, mutagenesis of the GA response motives should cause trichome density alterations in response to GA treatments. As data on other signal transduction pathways accumulate, the framework put forward here could be useful for an understanding of how diverse environmental cues are integrated by the regulatory module during epidermal cell-fate determination. This would contribute to a better comprehension of the plastic developmental responses of plant and animals to environmental cues.

The GRN modelled here is sufficient to recover the gene activation profiles and the hair spatial patterns typical of *wt* and mutants' root and leaf epidermis. Our results therefore support the idea that developmental processes, such as cell-type determination and patterning, depend on regulatory modules, which are semi-autonomous with respect to the rest of the genome [48]. According to this and other studies (e.g. [6]) the outputs of these modules (i.e. attractors that correspond to the possible alternative multigene expression profiles) are highly robust in the face of diverse perturbations, suggesting that these may act as patterning modules for a wide range of parameter values in kinetic functions of the network. Moreover, it seems that these modules may have been co-opted during evolution to regulate the decision underlying cell-fate determination of different structures in diverse tissues/organs (e.g. root, hypocotyl and leaf). Indeed, the latter could be the case in plant species with contrasting cellular patterns, in which local traits, such as cell size and shape, positional cues or domain geometry, may affect the type of pattern, even if the regulatory modules have been largely conserved during evolution.

The results presented here also support the idea that the coupled-GRNs system modelled here includes smaller coupled sub-networks that could themselves constitute mechanisms that contribute to the formation or maintenance of cellular patterns. In the leaf system, these mechanisms are: (i) the regulatory and protein-protein interactions between GL3 and TTG1 that give rise to the TTG1-trapping/depletion mechanism postulated by Bouyer and collaborators [28], (ii) the so-called activator-inhibitor system conformed by the activators of GL2 and its inhibitors [17,18], and (iii) the feedback loop established between TRY and GL2 [29,30]. Although these sub-networks or motifs could be important or even sufficient for the generation and maintenance of the epidermal cellular patterns, independently of the rest of the GRN, it is likely that they all act redundantly in the generation of such patterns in a robust manner. The latter seems to be the case as, for instance, our results suggest that TTG1 diffusion is not necessary for patterning when the whole GRN is present. Experimental data also show that wild-type cell configurations are observed in *ttg1* mutants when *GL3* is overexpressed [40]. Since these mechanisms are not independent of each other, the meta-GRN model encompassing them all in conjunction with cell-cell communication is useful for studying the spatio-temporal patterning. The interlinkage of redundant sub-networks found in this system supports the hypothesis that interconnection among feed-back loops or small nonlinear systems confers robustness to biological networks and may constitute an important trait in network evolution [49].

Conclusion

The model and results presented enable us to conclude that cell-type determination and patterning in leaf and root epidermis of *Arabidopsis* are regulated by a complex GRN that encompasses redundant sub-networks. Since all these sub-networks involve cell-to-cell communication, the spatial meta-GRN model is central to studying how these interact with each other, giving rise to the information needed for cellular patterning. Unlike the importance of gene redundancy, the relevance and implications of sub-network redundancy remain largely unexplored. This work is one of the first contributions regarding this issue.

We could also conclude that the meta-GRN qualitatively reproduces pattern formation in the system under study. This is why this model could be used to state precise predictions, namely, that the GA pathway acts on *CPC* or the *bHLH* genes, that *GL1* and *WER* are self-upregulated, that root cell shape has a stabilizing role on the banded pattern and that new cortex-related biasing signals may upregulate *CPC*.

Future developments of the meta-GRN model proposed here should allow for cell proliferation or continuous gradients in pattern formation. Nevertheless, the model presented here constitutes a starting-point from which to integrate future experimental evidence of the intracellular GRNs themselves (e.g. epigenetic regulation), as well as

diverse cellular processes. As further meta-GRN models are postulated and validated for diverse biological systems, we will be able to evaluate which types of intracellular GRNs, regulatory motifs or sub-networks, as well as coupling mechanisms, underlie patterning and morphogenesis in living organisms.

As exemplified by the GRN studied here, development depends upon non-additive interactions occurring in a wide range of spatial and temporal scales. Accomplishing the paramount task of understanding how robust patterns arise during development may therefore be facilitated by computational or mathematical models that bridge the divide between dynamics of interactions at the microscopic level and the origin and evolution of morphological qualities. Indeed, models like the one put forward here constitute useful tools for integrating experimental work, hinting at new experiments and providing novel insights into the complex interactions that underlie patterning processes and, therefore, into developmental and evolutionary biology.

Authors' contributions

MB participated in the study design, specified the GRN model, wrote the computer programs and carried out the simulations, participated in the discussion of results and contributed to writing the manuscript. CES participated in the study and computer program design, as well as in the discussion of results. PPL participated in the study design, the mathematical formulation of the model, and in the discussion of results. EAB established the project and coordinated this study, participated in the study design, in the analysis of results and in writing the manuscript. All authors read and approved the final manuscript.

Note added in proof

In the root meta-GRN model we assume that *WER* activates itself. This interaction is not essential for the formation of wild-type pattern, but it is required for the formation of the pattern observed in the absence of *SCM*. While this paper was under review, Savage and collaborators (2008) [50] put forward a mathematical model and a patterning mechanism that do not involve *WER*'s self-activation. These authors also provide new data regarding *WER* regulation that are consistent with their proposal. However, the data does not reject the possibility that *WER* self-activates, as they are also consistent, for instance, with the combined activity of a self-activating loop and another input activating *WER*. According to available empirical data, both the model presented here and that proposed by Savage and collaborators (2008) [50] appear to be plausible. Further tests could help find if a mechanism involving *WER*'s self-activation, another mechanism, or the coupled activity of more than one mechanism are important for cell-type arrangement in *Arabidopsis* root epidermis. Interestingly, the existence of more than one patterning mechanisms would emphasize the importance of sub-network redundancy in developmental networks.

Additional material

Additional file 1

This file contains the thorough topology and updating GRN functions.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1752-0509-2-98-S1.doc]

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References

1. Dover G: *Dear Mr. Darwin: Letters on the Evolution of Life and Human Nature* University of California Press; 2006.
2. Scheres B: **Plant cell identity. The role of position and lineage.** *Plant Physiol* 2001, **125:**112-114.
3. Dinneny JR, Benfey PH: **Plant stem cell niches: standing the test of time.** *Cell* 2008, **132:**553-557.
4. von Dassow G, Meir E, Munro E, Odell G: **The segment polarity network is a robust developmental module.** *Nature* 2000, **406:**188-193.
5. Albert R, Othmer H: **The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in Drosophila melanogaster.** *J Theor Biol* 2003, **223:**1-18.
6. Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla E: **A Gene Regulatory Network Model for Cell-Fate Determination during Arabidopsis thaliana Flower Development that is Robust and Recovers Experimental Gene Expression Profiles.** *Plant Cell* 2004, **16:**2923-2939.
7. Almaas E: **Biological impacts and context of network theory.** *J Exp Biol* 2007, **210:**1548-1558.
8. Kauffman S: **Metabolic stability and epigenesis in randomly constructed genetic nets.** *J Theor Biol* 1969, **22:**437-467.
9. Huang S, Ingber DE: **A non-genetic basis for cancer progression and metastasis: self-organizing attractors in cellregulatory networks.** *Breast Dis* 2006, **26:**27-54.
10. Ingolia NT: **Topology and robustness in the Drosophila segment polarity network.** *PloS Biol* 2004, **2:**e123.
11. Jönsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E: **An auxin-driven polarized transport model for phyllotaxis.** *Proc Natl Acad Sci USA* 2006, **103:**1633-1638.
12. Grieneisen VA, Xu J, Maree AF, Hogeweg P, Scheres S: **Auxin transport is sufficient to generate a maximum and gradient guiding root growth.** *Nature* 2007, **449:**1008-1013.
13. Guimil S, Dunand C: **Cell growth and differentiation in Arabidopsis epidermal cells.** *J Exp Bot* 2001, **58:**3829-3840.

14. Schellmann S, Hülskamp M, Uhrig J: **Epidermal pattern formation in the root and shoot of Arabidopsis.** *Biochem Soc Trans* 2007, **35:**146-148.
15. Ishida T, Kurata T, Okada K, Wada T: **A Genetic Regulatory Network in the Development of Trichomes and Root Hairs.** *Annu Rev Plant Biol* 2008, **59:**365-86.
16. Mendoza L, Alvarez-Buylla E: **Genetic regulation of root hair development in Arabidopsis thaliana: a network model.** *J Theor Biol* 2000, **4:**311-326.
17. Pesch M, Hulskamp M: **Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation.** *Curr Opin Genet Devel* 2004, **14:**422-427.
18. Benítez M, Espinosa-Soto C, Padilla-Longoria P, Díaz J, Alvarez-Buylla ER: **Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in Arabidopsis root and leaf epidermis: a dynamic model.** *Int J Dev Biol* 2007, **51:**139-155.
19. Savage N, Schmidt W: **From priming to plasticity: the changing fate of rhizodermic cells.** *Bioessays* 2008, **30:**75-81.
20. Dupuy L, Mackenzie J, Rudge T, Haseloff J: **A system for modelling cell-cell interactions during plant morphogenesis.** *Ann Bot (Lond)* 2008, **101:**1255-1265.
21. Larkin J, Young N, Prigge M, Marks M: **The control of trichome spacing and number in Arabidopsis.** *Development* 1996, **122:**997-1005.
22. Galway M, Masucci J, Lloyd A, Walbot V, Davis R, Schiefelbein J: **The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root.** *Dev Biol* 1994, **166:**740-754.
23. Dolan L: **Pattern in the root epidermis: an interplay of diffusible signals and cellular geometry.** *Ann Bot* 1996, **77:**547-553.
24. Lee M, Schiefelbein J: **Developmentally distinct MYB genes encode functionally equivalent proteins in Arabidopsis.** *Development* 2001, **128(9):**1539-1546.
25. Meinhardt H, Gierer A: **Pattern formation by local self-activation and lateral inhibition.** *BioEssays* 2000, **22:**753-760.
26. Schellmann S, Schnittger A, Kirik V, Wada T, Okada K, Beermann A, Thumfahrt J, Jurgens G, Hulskamp M: **TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis.** *EMBO J* 2002, **21:**5036-5046.
27. Hogeweg P: **Evolving mechanisms of morphogenesis: on the interplay between differential adhesion and cell differentiation.** *J Theor Biol* 2000, **203:**317-333.
28. Bouyer D, Geier F, Kragler F, Schnittger A, Pesch M, Wester K, Balkunde R, Timmer J, Fleck C, Hülskamp M: **Two-dimensional patterning by a trapping/depletion mechanism: the role of TTG1 and GL3 in Arabidopsis trichome formation.** *PLoS Biol* 2008, **6:**e141.
29. Srinivas BP: **Understanding the function of the Arabidopsis GLABRA2 gene in trichome patterning, morphogenesis and differentiation.** In *PhD thesis* Faculty of Mathematics and Natural Sciences, University of Cologne, Germany; 2004.
30. Simon M, Lee MM, Lin Y, Gish L, Schiefelbein J: **Distinct and overlapping roles of single-repeat MYB genes in root epidermal patterning.** *Dev Biol* 2007, **311:**566-578.
31. Zhao M, Morohashi K, Hatlestad G, Grotewold E, Lloyd A: **The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci.** *Development* 2008, **135:**1991-1999.
32. Bernhardt C, Zhao M, Gonzalez A, Lloyd A, Schiefelbein J: **The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis.** *Development* 2005, **132:**291-298.
33. Wada T, Kurata T, Tominaga R, Koshino-Kimura Y, Tachibana T, Goto K, Marks M, Shimura Y, Okada K: **Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation.** *Development* 2002, **129:**5409-5419.
34. Kurata T, Ishida T, Kawabata-Awai C, Noguchi M, Hattori S, Sano R, Nagasaka R, Tominaga R, Koshino-Kimura Y, Kato T, Sato S, Tabata S, Okada K, Wada T: **Cell-to-cell movement of the CAPRICE protein in Arabidopsis root epidermal cell differentiation.** *Development* 2005, **132:**5387-5398.
35. Kwak SH, Shen R, Schiefelbein J: **Positional signaling mediated by a receptor-like kinase in Arabidopsis.** *Science* 2005, **307:**1111-1113.
36. Kwak SH, Schiefelbein J: **The role of SCRAMBLED receptor-kinase in patterning the Arabidopsis root epidermis.** *Dev Biol* 2007, **302:**118-131.
37. Gershenson C: **Updating Schemes in Random Boolean Networks: Do They Really Matter?** In *Artificial Life IX, Proceedings of the Ninth International Conference on the Simulation and Synthesis of Living Systems* Edited by: Pollack J, Bedau M, Husbands P, Ikegami T. Watson RA: MIT Press; 2004:238-243.
38. Schellmann S, Hülskamp M: **Epidermal differentiation: trichomes in Arabidopsis as a model system.** *Int J Dev Biol* 2005, **49:**579-584.
39. Clark PJ, Evans FC: **Distance to nearest neighbor as a measure of spatial relationships in populations.** *Ecology* 1954, **35:**445-453.
40. Zhang F, Gonzalez A, Zhao M, Payne CT, Lloyd A: **A network of redundant bHLH protein functions in all TTG1 dependent pathways of Arabidopsis.** *Development* 2003, **130:**4859-4869.
41. Lee M, Schiefelbein J: **Cell Pattern in the Arabidopsis Root Epidermis Determined by Lateral Inhibition with Feedback.** *Plant Cell* 2002, **14:**611-618.
42. Gan Y, Kumimoto R, Liu C, Ratcliffe O, Yu H, Broun P: **GLABROUS INFLORESCENCE STEMS Modulates the Regulation by Gibberellins of Epidermal Differentiation and Shoot Maturation in Arabidopsis.** *Plant Cell* 2006, **18:**1383-1395.
43. Gan Y, Liu C, Yu H, Broun P: **Integration of cytokinin and gibberellin signalling by Arabidopsistranscription factors GIS, ZFP8 and GIS2 in the regulation of epidermal cell fate.** *Development* 2007, **134:**2073-2081.
44. Tsukaya H: **Leaf development.** *The Arabidopsis Book* [http://www.aspb.org/publications/arabidopsis/]. The American Society of Plant Biologists, Rockville, MD
45. Ramirez-Parra E, Lopez-Matas M, Frundt C, Gutierrez C: **Role of an atypical E2F transcription factor in the control of Arabidopsis cell growth and differentiation.** *Plant Cell* 2004, **16:**2350-2363.
46. Campanioni P, Nick P: **Auxin-dependent cell division and cell elongation. 1-Naphtaleneacetic acid and 2,4-Dichlorophenoxyacetic acid activate different pathways.** *Plant Physiol* 2005, **137:**939-948.
47. Morohashi K, Zhao M, Yang M, Read B, Lloyd A, Lamb R, Grotewold E: **Participation of the Arabidopsis bHLH factor GL3 in trichome initiation regulatory events.** *Plant Physiol* 2007, **145:**736-746.
48. von Dassow G, Odell GM: **Design and constraints of the Drosophila segment polarity module: robust spatial patterning emerges from intertwined cell state switches.** *J Exp Zool Mol Dev Evol* 2002, **294:**179-185.
49. Kwon YK, Cho KH: **Coherent Coupling of Feedback Loops: A Design Principle of Cell Signaling Networks.** *Bioinformatics* 2008, **24(17):**1926-1932.
50. Savage NS, Walker T, Wieckowski Y, Schiefelbein J, Dolan L, Monk N: **A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the Arabidopsis root epidermis.** *PLoS Biol* 2008, **6:**e235.

Apéndice C

Dynamic-module redundancy confers robustness to the gene regulatory network involved in hair patterning of Arabidopsis epidermis

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Dynamic-module redundancy confers robustness to the gene regulatory network involved in hair patterning of *Arabidopsis* epidermis.

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Abstract

Redundancy among dynamic modules is emerging as a potentially generic trait in gene regulatory networks. Moreover, module redundancy could play an important role in network robustness to perturbations. We explore the effect of dynamic-module redundancy in the networks associated to hair patterning in *Arabidopsis* root and leaf epidermis. Recent studies have put forward several dynamic modules belonging to these networks. Here, we define these modules in a discrete dynamical framework that was previously reported. Then, we addressed whether these modules are sufficient or necessary for recovering epidermal cell types and patterning. We achieved this by defining two quantitative estimates of robustness. We also compared the robustness of each module with that of a network coupling all the hair or root modules. We found that, considering certain assumptions, all the dynamic modules proposed so far are sufficient on their own for pattern formation, but reinforce each other during epidermal development. Furthermore, we found that networks of coupled modules are more robust to perturbations than single modules. These results suggest that dynamic-module redundancy might be an important trait in gene regulatory networks and points at central questions regarding network evolution, module coupling, pattern robustness and the evolution of development.

Keywords

Arabidopsis epidermis, dynamic modularity, gene regulatory network, redundancy

Introduction

Joint experimental and modeling approaches have contributed to understanding gene regulatory networks (GRN) involved in cell-fate determination and patterning (e.g. von Dassow et al., 2002; Espinosa-Soto et al. 2004; Jönsson et al, 2006) and are helping uncover generic GRN traits.

One of these GRN traits is dynamic-module redundancy (Kwon and Cho, 2008; Benítez et al., 2008), which implies the coherent coupling of regulatory modules that exhibit similar or equivalent behaviors. The partial or complete redundancy of interlinked modules or sub-networks has proven to result in highly robust global dynamics of signaling networks (Kwon and Cho, 2008). This characteristic could also be and important feature of development-related GRNs as semi-autonoumous dynamic modules may constitute building blocks in the evolution of developmental mechanisms. However, in contrast to gene redundancy, the origin and effect of dynamic-module redundancy in GRNs remains to be explored.

Cell-fate determination and patterning in root and leaf epidermis of the model plant *Arabidopsis thaliana* constitutes a very useful system to address key questions in the study of GRNs underlying development. In both types of epidermis, there are hair and non-hair cells arranged in non-trivial patterns: spaced-out in the leaf and striped in the root. The work of several laboratories has yielded a thorough characterization of the molecular aspects of this system (see reviews in Ishida et al., 2008; Schellmann et al., 2007; Schiefelbein et al., 2009) and some dynamical models for leaf and root epidermal patterning have been proposed (Mendoza and Alvarez-Buylla, 2000; Benítez et al., 2007, 2008; Bouyer et al., 2008; Savage et al., 2008; Digiuni et al., 2008). Such modeling efforts are now providing a rich view of the overall system's dynamics and suggest that this particular system exhibits dynamic-module redundancy (Benítez et al., 2008; Schiefelbein et al., 2009). Indeed, six non-exclusive and mutually reinforcing dynamic modules that may suffice for cell-type patterning have been described for the leaf and root epidermis (Benítez et al., 2008; Bouyer et al., 2008 and Digiuni et al., 2008 for the leaf; and Savage et al., 2008; Benítez et al., 2008; Schiefelbein and Kwak, 2008 for the root. See Table 1).

In order to study the role of module redundancy in this developmental process, we simulated each of the proposed mechanisms by means of a previously presented modeling framework (Benítez et al., 2008). We then put forward a method to assess whether the modules are necessary or sufficient for patterning and to compare the robustness of each single with that of the GRN with all modules coupled.

We found that the dynamic modules are indeed redundant as they all suffice for pattern formation. Moreover, our results show that dynamic-module redundancy contributes to the robustness of cell-fate determination and patterning in the face of mutations and stochastic perturbations.

Methods

We modeled all of the systems summarized in Table 1 with the use of a previously reported framework that enables exploring the spatio-temporal dynamics of discrete GRNs (Benítez et al., 2008). In this model, each GRN node's changes according to:

$g(t+1) = F_n(g_{n1}(t), g_{n2}(t), ..., g_{n3}(t))$,

where g_{ni} are the regulators of gene g_n, and F_n is a discrete function or logical rule. The model considers a discrete lattice of $n \times n$ elements in which each element (i, j) represents a cell with its own GRN. It has been experimentally shown that some proteins codified by elements of the GRNs under study are able to move among neighboring cells (e.g. Wada et al., 2002). Thus, in the spatial model certain elements are allowed to move among neighboring cells following the equation:

$g(t)_{T[i][j]} = H(g(t)_{[i][j]} + D(g(t)_{[i+1][j]} + g(t)_{[i-1][j]} + g(t)_{[i][j+1]} + g(t)_{[i][j-1]} - 4(g(t)_{[i][j]}))$,

where $g(t)_{T[i][j]}$ is the total amount of protein g in cell (i, j). D is a continuous variable that determines the proportion of g that can move from any cell to neighboring. H is a step function that converts the continuous values corresponding to the amount of g diffused into a discrete

variable that may attain values of 0, 1 or 2. Finally, $g(t)_{T\ [i]\ [j]}$ is considered in order to estimate the $g(t)_{[i]\ [j]}$ value according to the equation:

$g'(t)_{T\ [i]\ [j]} = g(t)_{\ [i]\ [j]} + g(t)_{T\ [i]\ [j]} - k$,

where k stands as a degradation constant and $g'(t)$ is taken as an input to evaluate the logical rules and again calculate $g(t)_{\ [i]\ [j]}$ until a steady state is reached for the whole lattice. In the steady state, each cell in the lattice exhibits a node activation profile that corresponds to a gene activation profile characterizing hair or non-hair cells. The GRN logical rules were all grounded on available experimental data (see Benítez et al., 2008 for detailed review).

In order to study the sufficiency and necessity, as well as the robustness of the different patterning modules put forward up to now, all the GRN and patterning mechanisms presented in Table 1 were modeled as described above (Benítez et al. 2008). According to empirical data, nodes modeled as mobile elements were GL3, EGL3, CPC and TRY in the root system, and TTG, CPC and TRY in the leaf one. Since there is no experimental information suggesting a particular regulatory sequence and for simplicity, all simulations were performed under synchronic updating schemes. Diffusion constants were always $D_{bHLH}=0.03$, $D_{CPC}=0.05$, $D_{bTRY}=0.05$. Initial conditions were set randomly. GRN graphs were elaborated with Graphviz. Programs were elaborated in C and are available upon request.

Results

Single dynamic modules are sufficient to recover leaf and root epidermal cell determination and patterns that resemble those observed in real plants.

We used the modeling framework described in Methods (see Benítez et al., 2008 for further details) in order to simulate the different patterning mechanisms that have been put forward for leaf and root epidermal hairs (Benítez et al., 2008; Bouyer et al., 2008; Digiuni et al., 2008; Savage et al., 2008; Schiefelbein and Kwak, 2008; see table 1 for graphic description and main assumptions of each proposed module). By comparing simulated and observed patterns, we first

evaluated whether each of the modules summarized in Table 1 was able to render GRN activation profiles that typify hair and non-hair cell types and whether these showed dotted and banded patterns typical of leaf and root epidermis. All the isolated modules that we simulated were able to do this (Table 1). It is important to note that the TTG-trapping/depletion mechanism (Bouyer et al., 2008) makes a strong assumption by not including *GL1*, a component of the leaf epidermis GRN. However, if the TTG-trapping/depletion assumptions are considered in the simulations, this mechanism alone also generates a dotted hair pattern. This mechanism may be the only one that is not strictly sufficient, but it is certainly important for patterning reinforcement as it enhances robustness of the GRN that couples this and other module (details below).

Given that more than one patterning modules are sufficient for epidermal cell-type determination and arrangement, none of these sub-modules is, in principle, absolutely necessary for pattern formation. All of them might be, however, contribute to the overall robustness of this developmental process.

Redundancy of dynamic modules confers robustness to GRNs underlying epidermal cell-fate determination and patterning.

In order to evaluate the contribution of dynamic modularity and redundancy to the robustness of the GRNs under study, we defined two measures of robustness. The first one estimates the robustness to single loss-of-function mutations and is obtained by dividing the neutral mutations (*m*) by all the possible single loss-of-function mutations in the module (*M*). Neutral mutations were defined in two ways. In the first one, *m* considers mutations that affect neither cell-fate determination nor pattern formation (FD&P column in Table 2). In the second more flexible definition of *m*, neutral mutations are taken as those that may or may not affect the pattern, but do not affect cell type determination (i.e. hair or non-hair cell types are formed). An average of these two estimations of *m*/*M* was also calculated (<> column in Table 2).

The second measure of module robustness was established in terms of the tolerance to random perturbations in the logical rules governing the GRNs dynamics. In order to obtain this

measure, all the outputs of the logical rules were randomly modified from their correct value with a probability *p*. All outputs were modified with the same probability in each run, but each output was perturbed independently of the others. We systematically tested values of *p* ranging from *0.00001* to *0.1* and took the value for which noise was high enough to alter the wild-type patterns.

Benítez and collaborators (2008) used the so-called *R* value (Larkin et al., 1996) and the hair clustering probability (*C*) to analyze if the simulated pattern was similar to that observed on in leaves, which has $R>1$ and $C\approx 0$. In the case of the root epidermis, the number of misplaced cells in the lattice, which would correspond to ectopic hairs, was used as an estimator of "pattern accuracy". These same measures were used to analyze if single mutations or noisy perturbations in the dynamic modules affected the typical leaf and root spatial patterns.

We found that for both the leaf and root GRN the systems containing all the dynamic modules were more robust to mutations and noise than single modules (Table 2). As it could be expected, it is clear from robustness analyses that simple modules that render the pattern with a *minimum* mechanism are more sensible to single loss-of-function mutations than modules integrating partially redundant genes. Then, robustness of the GRNs that couple all the modules could, in principle, have its origin on gene redundancy alone and not necessarily on redundancy of dynamic modules. However, the root GRN that couples all the modules does not have more redundant or partially redundant nodes than one of the single modules (GL3/EGL3 and CPC/TRY, Table 1), but still exhibits higher values of robustness. Therefore, overall robustness does not only depend on the presence of gene redundancy, but is also a consequence of dynamic-module redundancy.

Discussion

Our results show that the GRN underlying cell patterning in leaf and root epidermis of *Arabidopsis thaliana* incorporates several redundant dynamic modules that are each sufficient for cell-fate determination and spatial patterning. Furthermore, our simulations demonstrate for a

specific biological system that redundancy of such dynamic modules enhances the robustness of a developmental process in the face of different types of perturbations. Then, the behavior of the genetic system involved in epidermal cell-fate determination and pattering actually seems to reflect the coherent integration of several dynamic redundant modules, rather than the dynamics of a single patterning module. This will be an important consideration for future experiments and modeling efforts, as well as for evolutionary analyses. In particular, it would be interesting to study the dynamics of single and coupled modules by performing experiments in which these are selectively uncoupled or blocked.

It is worth noting that for the analyses of robustness regarding stochastic perturbations, all the logical rules underlying the GRNs dynamics were perturbed with the same probability. However, not all the rules or the nodes are equally sensible to this kind of perturbation. Moreover, in some modules, such as that put forward by Savage and collaborators (2008), noise in some of the rules may actually be required for pattern stabilization. This fact could explain the high tolerance of this particular module to stochastic perturbations (Table 2). Further explorations of the effect of noise in particular genes and in complete GRNs will be needed for a better understanding of how and in what ranges stochasticity may contribute to developmental processes.

In the leaf GRN it appears that the two measures of robustness, the one related to mutations and the one related to noise, are correlated. In the root GRN, however, this tendency is not conserved. Further analysis in other documented GRNs will be needed to establish the relation between these measures and the origin of the two types of robustness.
Importantly, in a recent contribution Kang and collaborators (2009) uncovered another positive feedback loop in the GRN underlying cell-fate determination in the root epidermis. As the authors mention, this loop, which involves the *MYB23* gene, could be part of another set of interactions that further enhance the overall GRN robustness.

The fact that all simulated modules were able to render the expected patterns when modeled with a common mathematical framework, different to that in which they were originally described, suggests that these modules are indeed dynamically relevant and relatively robust on their own, not depending on precise implementations or modeling details.

Our results open novel questions regarding module and network evolution. First, given that module redundancy reinforces the patterning process under study, and assuming that hair patterning or the presence of hair and non-hair cells is somehow adaptive, it could constitute a selected trait throughout GRN evolution. It is also possible that dynamic-module redundancy emerges as a consequence of how GRNs evolve and that gene duplication and divergence contribute to generating modularity (structural or dynamic).

The role of selection and of structural or historical restrictions in the origin and evolution of dynamic-module redundancy remains to be studied. It would also be relevant in terms of module evolution and coupling, to explore if these dynamic modules are represented in other plant species and if they are, whether the combinations of different modules are correlated with contrasting epidermal cell patterning phenotypes. Finally, different environmental cues of stressful conditions could perturbate the GRNs or the modules in different ways. Then, it would be helpful to study how redundancy of dynamic modules contributes to the response to these particular cues and conditions. In this work, we put forward a relatively simple method to investigate dynamic modularity that could help address some these issues.

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References

von Dassow, G., Meir, E., Munro, E., Odell, G., 2000. The segment polarity network is a robust developmental module. Nature 406, 188-193.

Espinosa-Soto, C., Padilla-Longoria, P., Alvarez-Buylla, E., 2004. A Gene Regulatory Network Model for Cell-Fate Determination during Arabidopsis thaliana Flower Development that is Robust and Recovers Experimental Gene Expression Profiles. Plant Cell 16, 2923-2939.

Jönsson, H., Heisler, M.G., Shapiro, B.E., Meyerowitz, E.M., Mjolsness, E., 2008. An auxin-driven polarized transport model for phyllotaxis. Proc Natl Acad Sci USA 103,1633-1638.

Kwon, Y.K., Cho, K.H., 2008. Coherent Coupling of Feedback Loops: A Design Principle of Cell Signaling Networks. Bioinformatics 24, 1926-1932.

Benítez, M., Espinosa-Soto, C., Padilla-Longoria, P., Alvarez-Buylla, E.R., 2008. Interlinked nonlinear subnetworks underlie the formation of robust cellular patterns in Arabidopsis epidermis: a dynamic spatial model. BMC Syst Biol 2, 98.

Ishida, T., Kurata, T., Okada, K., Wada, T., 2008. A Genetic Regulatory Network in the Development of Trichomes and Root Hairs. Annu Rev Plant Biol 59,365-86. 37.

Schellmann, S., Hülskamp, M., Uhrig, J., 2007. Epidermal pattern formation in the root and shoot of Arabidopsis. Biochem Soc Trans 35, 146-8.

Schiefelbein, J., Kwak, S.H., Wieckowski, Y., Barron, C., Bruex, A., 2009. The gene regulatory network for root epidermal cell-type pattern formation in Arabidopsis. J Exp Bot 60, 1515-21.

Mendoza, L., Alvarez-Buylla, E., 2000. Genetic regulation of root hair development in Arabidopsis thaliana: a network model. J Theor Biol 4, 311-326.

Benítez, M., Espinosa-Soto, C., Padilla-Longoria, P., Díaz, J., Alvarez-Buylla, E.R., 2007. Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in Arabidopsis root and leaf epidermis: a dynamic model. Int J Dev Biol 51, 139-155.

Bouyer, D., Geier, F., Kragler, F., Schnittger, A., Pesch, M., Wester, K., Balkunde, R., Timmer, J., Fleck, C., Hülskamp, M. 2008. Two-dimensional patterning by a trapping/depletion mechanism: the role of TTG1 and GL3 in Arabidopsis trichome formation. PLoS Biol 6:e141.

Savage, N.S., Walker, T., Wieckowski, Y., Schiefelbein, J., Dolan, L., Monk, N., 2008. A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the Arabidopsis root epidermis. PLoS Biol 6:e235.

Digiuni, S., Schellmann, S., Geier, F., Greese, B., Pesch, M., Wester, K., Dartan, B., Mach, V., Purushottam, B., Timmer, J., Fleck, C., Hulskamp, M., 2008. A competitive complex formation mechanism underlies trichome patterning on Arabidopsis leaves. Mol Syst Biol 4:217.

Kwak, S., Schiefelbein, J., 2008. A Feedback Mechanism Controlling SCRAMBLED Receptor Accumulation and Cell-Type Pattern in Arabidopsis. Curr Biol 18, 1949–1954.

Wada, T., Kurata, T., Tominaga, R., Koshino-Kimura, Y., Tachibana, T., Goto, K., Marks, M., Shimura, Y., Okada, K., 2002. Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation. Development 129, 5409-5419.

Larkin, J., Young, N., Prigge, M., Marks, M., 1996. The control of trichome spacing and number in Arabidopsis. Development 122, 997-1005.

Kang, Y.H., Kirik, V., Hulskamp, M., Hee Nam, K., Hagely, K., Min Lee, M., Schiefelbein, J., 2009. The MYB23 Gene Provides a Positive Feedback Loop for Cell Fate Specification in the Arabidopsis Root Epidermis. Plant Cell (online advanced publication).

Table1. Summary of the graphs and conditions for sufficiency and necessity for each sub-module put forward up to now. *See, however, Morohashi et al., 2007 and further details in Results.

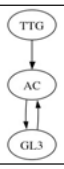
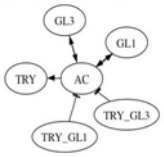
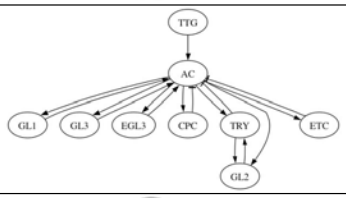
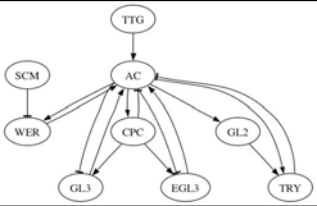
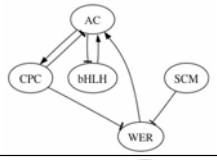
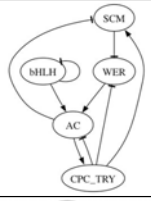
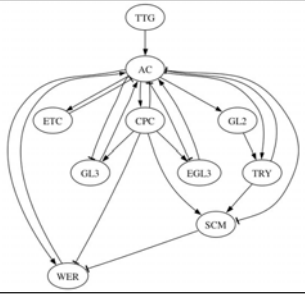
	Module description	Graph	Sufficient for spaced out pattern	Necessary
Leaf epidermis (TTG, TRY and CPC are able to move among cells)	TTG1-trapping/depletion mechanism (Bouyer et al., 2008)		Yes* (Assumes that AC activates *GL3* and *GL2* in a *GL1*-independent manner)	No (*GL3* overexpresion recovers *ttg* loss of function)
	Double competitive inhibition (Digiuni et al., 2008)		Yes (assumes that TTG is present in all cells)	No (Activator-inhibitor-like system also renders the dotted pattern)
	Meta-GRN (Benítez et al., 2008); also the coupled system of submodules.		Yes (Assumes that *GL1* is directly or indirectly self-activated)	No (Double competitive inhibition also generates the dotted pattern)
Root epidermis (bHLH, TRY and CPC proteins diffuse; in all cases SCM inhibits *WER* in the H position)	WER-self-activation model (Benítez et al., 2008)		Yes (Assumes WER's direct or indirect self-activation)	No (Other proposed mechanisms render the root pattern)
	Mutual support mechanism (Savage et al., 2008)		Yes (Assumes that *WER* and *GL3* are constantly expressed with a basal rate)	No (Other proposed mechanisms render the root pattern)
	SCM-accumulation system (Schiefelbein and Kwak 2008)		Yes (Assumes that WER and bHLH have a constant basal expression)	No (The two other proposed mechanisms render the root pattern)
	Coupled system of sub-modules		Yes (Incorporates assumptions of single sub-modules)	No (Single sub-modules are sufficient)

Table 2. Results for robustness assessment of each dynamic module. From left to right, columns indicate the type of epidermal system under study, the particular dynamic module, measure of robustness in the face of single loss-of-function mutations and measure of robustness to stochastic perturbations. In all the columns, values range goes from 0 to 1 (0 represents low robustness and 1 high robustness). For the FD&P column, neutral mutations, *m,* are taken as those that do not affect neither cell-fate determination or pattern formation. In the FD column, neutral mutations are taken as those that may or may not affect the pattern, but do not affect cell type determination (i.e. one of the two cell types is no longer formed). An average of these two estimates of *m/M* is presented in the <> column. *For this system, noise within certain range, particularly in the expression of *WER,* actually contributes to pattern formation. For this analysis, noise was considered for all elements and in addition to noise originally considered.

	Regulatory GRN tested	**Robustness to loss of function mutations: m/M**			**Robustness to stochastic perturbations**
		FD&P	**FD**	**<>**	
Leaf epidermis	TTG-trapping/depletion module (Bouyer et al., 2008)	0	0	0	0.001818
	TTG-independent mechanism (Digiuni, et al., 2008)	0	0.333	0.166	0.001428
	Meta-GRN (Benítez et al., 2008) considering TTG trapping and *TRY* double competitive inhibition.	0.625	0.75	0.687	0.003333
Root epidermis	Mutual support mechanism (Savage et al., 2008)	0	0.5	0.25	0.01
	WER-self-activation model (Benítez et al., 2008)	0.125	0.875	0.5	0.0005
	SCM-accumulation model (Schiefelbein&Kwak, 2008)	0	0.75	0.352	0.001
	All modules coupled	0.625	0.875	0.75	0.033

Apéndice D

Epidermal patterning in Arabidopsis: models make a difference

En preparación para la revista Development.

Epidermal patterning in *Arabidopsis*: models make a difference

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Abstract
The leaf and root epidermis in *Arabidopsis* provide ideal systems in which to explore the mechanisms that underlie the patterned assignment of cell fates during development. Extensive experimental studies have uncovered a complex interlocked feedback network operates within the epidermis that coordinates the choice between hair and non-hair fates. A number of recent studies using mathematical models have begun to unpick this network, highlighting new mechanisms that have subsequently been confirmed in model-directed experiments. These studies illustrate the potential of integrated modelling and experimentation to shed new light on developmental processes.

Introduction
The patterned assignment of alternative cell fates lies at the heart of many developmental processes. Understanding how neighbouring cells in a developing tissue adopt distinct fates therefore presents a key challenge in development. In principle, a range of mechanisms can contribute to patterned fate assignment, including cell lineage, imposition of spatial information from neighbouring tissues (induction), and pattern-generating interactions within the tissue being patterned.

Plant development provides many instances of patterned cell fate assignment. These provide attractive systems in which to explore the mechanisms underlying the emergence and maintenance of patterning, since patterning takes place throughout the entire life of the plant (often reiteratively) and does not involve cell rearrangement due to migration. Among the best studied examples are the leaf and root epidermis of *Arabidopsis* thaliana (Ishida et al., 2008; Schiefelbein et al., 2009). During early stages
of development of the epidermis, cells undergo a binary fate decision, with a subset adopting a fate leading to later differentiation as a hair-bearing cell. In the leaf, these trichome-bearing cells arise in a two-dimensional spacing pattern (Fig. 1a); in the root, trichoblasts—which have the potential to form root hair cells—arise in the meristem and form continuous files aligned along the long axis of the root, separated by files of atrichoblasts (Fig. 1b).

Advances in methodologies for probing the molecular state of cells have helped to provide increasingly detailed models of a range of dynamic cellular processes. However, these methodologies largely depend on the availability of uniform populations of cells or on single cell culture systems. Patterned fate assignment presents a more challenging problem: since the central feature under study is the emergence of differences between neighbouring cells, and changes in cell state are integrated with spatial patterning, it remains difficult to obtain detailed data on the dynamics of pattern formation. In spite of these challenges, a number of models have been developed recently that illustrate the
value of a range of mathematical and computational approaches in exploring the dynamics of regulatory networks in multicellular tissues (see reviews in Reeves et al., 2006; Alvarez-Buylla et al,. 2007; Jaeger et al., 2008; Grieneisen and Scheres, 2009; Oates et al., 2009; Jönsson and Krupinski, 2010). Indeed, the difficulty in obtaining detailed information on individual cell states in a multicellular context provides excellent opportunities to use iterative cycles of modelling and experimentation to refine understanding of the mechanisms underlying the patterning of these systems.

In this review we summarise a number of recent models of root and leaf epidermal patterning in *Arabidopsis*. After outlining the key features of the experimentally determined epidermal patterning network, we illustrate how each model allows distinct aspects of this network to be explored in detail, generating a range of specific predictions that have been confirmed by directed experiments. Finally, we discuss some general conclusions that can be drawn from the models about the robustness, plasticity and evolution of spatial pattering in *Arabidopsis* and other organisms.

Similar regulatory networks underlie fate choice in the root and leaf

Although patterning takes place in quite different contexts in the root and leaf epidermis, strikingly similar genetic regulatory networks (GRNs) operate in both (Lee and Schiefelbein, 2001; Ishida et al., 2008; Schiefelbein et al., 2009). The networks are centred on two classes of trimeric protein complexes comprising the WD40 repeat protein TRANSPARENT TESTA GLABRA 1 (TTG1), a basic-helix-loop-helix (bHLH) protein and a MYB protein. In both the root and the leaf, the bHLH proteins GLABRA3 (GL3) and ENHANCER OF GLABRA 3 (EGL3) play functionally equivalent roles in the complexes. The two classes of complexes are distinguished by the nature of the MYB protein. One—which we refer to as the Activator Complex (AC)—involves a R2R3 MYB protein (GLABRA1 (GL1) and WEREWOLF (WER) in the leaf and root, respectively).

The other complex—which we refer to as the Inhibitor Complex (IC)—involves a single R3 MYB protein. This role is filled primarily (albeit redundantly) by TRIPTYCHON (TRY) in the leaf and by CAPRICE (CPC) in the root (Schellmann et al., 2007). However, the three proteins ENHANCER OF TRIPTYCHON AND CAPRICE 1, 2 and 3 (ETC1, ETC2 and ETC3) can also contribute to the IC (Esch et al., 2004; Kirik et al., 2004a; Kirik et al., 2004b; Wester et al., 2009). Mutations in the genes coding for the single R3 MYB proteins show a range of severity of patterning defects in the leaf and root.

The AC is localised in the cell nucleus and up-regulates the transcription of the homeodomain transcription factor GLABRA2 (GL2) in both tissues (Larkin et al., 1997; Lee and Schiefelbein, 1999; Payne et al., 2000; Zhao et al., 2008). GL2 regulates a series of transcriptional events leading to the determination of the hair (trichome) fate in the leaf and the non-hair (atrichoblast) fate in the root (Di Cristina et al., 1996; Hung et al., 1998; Szymanski et al., 1998; Lee and Schiefelbein, 1999; Rerie et al., 1994; Szymanski et al., 1998; Payne et al., 2000). The IC, in contrast, does not have the capacity to regulate transcription. Accumulation of AC in atrichoblasts (root) and in nascent trichomes (leaf) is thus a key step in the patterning of cell fates. Establishment of these patterns depends on a combination of regulatory feedback and movement of a subset of proteins between epidermal cells.

Because the single R3 MYB proteins can compete for binding to GL3/EGL3 with the R2R3 MYB proteins, the two complexes are mutually competitive. A central feedback in the epidermal GRN results from the direct up-regulation of the single R3 MYB genes by the AC (Schnittger et al., 1999; Lee and Schiefelbein, 2002; Kirik et al., 2004; Srinivas, 2004; Koshino-Kimura et al., 2005; Ryu et al., 2005; Morohashi et al., 2007; Simon et al., 2007). Thus, the AC enhances production of its own competitor, resulting in negative feedback. In contrast, the AC represses transcription of GL3 (Lee and Schiefelbein, 2002; Morohashi et al., 2007).

Coordination of levels of AC between epidermal cells depends on the intercellular movement of single R3 MYB proteins. Genetic data show that TRY acts non-cell autonomously in leaves and experiments with fluorescent fusion proteins show directly that CPC protein moves between cells in both leaf and root epidermis (Wada et al., 2002; Kurata et al., 2005; Zhao et al., 2008). In contrast, the R2R3 MYB proteins do not move between cells (Zhao et al., 2008). The mobility of GL3/EGL3 and TTG1 is currently unclear. While fluorescent GL3 fusion proteins do not move between leaf epidermal cells (Zhao et al., 2008), their distribution in the root epidermis is suggestive of cell-to-cell movement (Bernhardt et al., 2005). Experiments using fluorescent TTG1 fusion proteins are currently inconclusive (Bouyer et al., 2008; Zhao et al., 2008), and there are no data pertaining to TTG1 movement in the root.

In the leaf, all evidence suggests that the epidermal GRN operates to generate the trichome spacing pattern independent of cell lineage and without any imposed bias. In contrast, in the root, the pattern of trichoblasts is strongly biased by positional information from the underlying cortical cells (Dolan et al., 1994; Berger et al., 1998).

The putative membrane receptor SCRAMBLED (SCM) appears to be a crucial component required in the epidermis to receive this bias (Kwak et al., 2005; Kwak and Schiefelbein, 2007). Although SCM is expressed uniformly in all epidermal cells during the initial establishment of cell fate, it acts to down-regulate WER transcription in epidermal cells overlying clefts between cortical cells (in the ‘H position’), thus biasing the state of the epidermal GRN (Kwak et al., 2005; Kwak and Schiefelbein, 2007).

The regulatory interactions and protein movements outlined above have been integrated into spatially-distributed GRNs that are summarized in Fig. 2. Although a “parts list” of the epidermal GRNs has been mapped out, this is not sufficient to furnish a full explanation of the mechanisms underlying the emergence and robustness of the spatial patterns of cell fate.

Efforts towards such an explanation have recently involved a number of studies that have integrated mathematical modelling with directed experiments in an attempt to identify and answer a number of key outstanding mechanistic questions.
These studies, described below, have provided both unexpected *insights* into the regulatory logic of epidermal patterning, and a valuable framework in which to explore the origin and evolution of epidermal patterning in *Arabidopsis* and other plants.

Models of epidermal cell fate patterning

The *Arabidopsis* epidermis provides an attractive system in which to develop approaches that integrate modelling and directed experimentation. Extensive experimental studies have provided a detailed picture of the components of the epidermal GRN, together with many of their regulatory interactions. Furthermore, the nature of the emergent patterns is simple, and can be characterized in terms of the expression of a single gene (GL2). This allows specific mechanistic questions to be posed with a clarity that is difficult in many other developmental processes. However, most of the available data provide only qualitative information on the GRN, while mechanistic mathematical models require specific quantitative representations and associated kinetic parameters (Reeves et al., 2006; Oates et al., 2009).
In the absence of appropriate quantitative data, phenomenological models that aim to capture the core regulatory logic of the epidermal GRNs provide a valuable starting point. These have the advantage of requiring only a few parameters to be specified, while allowing general mechanistic questions to be addressed. However, such models are unsuitable for probing mechanistic details. An iterative approach has thus been adopted, in which initial phenomenological modelling has been used to direct the
development of reduced mechanistic models. The results of the phenomenological modelling gives initial insight into the patterning potential of these models, allowing appropriate parameters to be determined by scanning.

Phenomenological models
The most basic question that can be addressed using modelling is whether the known reactions and movements of components of the epidermal GRNs are sufficient to generate spatial patterns. The epidermal GRNs exhibit some of the features of an activator-inhibitor system—a specific class of reaction-diffusion network that can generate spatial patterns (Turing, 1952; Gierer and Meinhardt, 1972). This led to the proposal that an activator-inhibitor mechanism may underlie the spacing patterns of trichomes and atrichoblasts, with GL1/WER and TRY/CPC playing the roles of the ‘activator’ and ‘inhibitor’, respectively. (Pesch and Hulskamp, 2004).

To explore this proposal, Benítez et al. (2007) set out to determine whether appropriate tissue-specific conditions in a single activator-inhibitor system could account for pattern formation in both the root and leaf epidermis. After demonstrating that discrete models of the root and leaf epidermal GRNs were equivalent in terms of key dynamical properties, they proposed a consensus GRN for both tissues, which was represented by a continuous activator-inhibitor model (see Box 1). They then confirmed
that the consensus model could account for root- and leaf-like patterns, given appropriate positional cues or cellular geometries, suggesting that cell geometry may play a role in the stabilisation of spatial cellular patterns.

While this phenomenological model supports the proposed activator-inhibitor mechanism, it has three important limitations. First, it assumes self-activation of the activator (GL1/WER), for which there is currently no experimental support. Second, the reduction of the epidermal GRNs to a two-variable model does not allow the expression patterns of all GRN components to be assessed. Third, model reduction results in the loss of important dynamic traits—such as robustness—of the original patterning processes.

Genetic Regulatory Network models for root hair patterning
Many of the limitations of phenomenological models can be overcome by using mechanistic models (Reeves et al., 2006). These are based on specific mathematical representations of basic regulatory and provide a valuable tool for exploring dynamic processes in terms of their steady states, dynamic transitions, robustness to perturbations, etc. (Alon, 2007). The first GRN model of *Arabidopsis* root hair patterning was developed by Mendoza and Alvarez-Buylla (2000), who developed a discrete model (in which each network component can take either two or three values—see Box 1) integrating the root epidermis GRN known at the time with the ethylene signalling pathway (a key plant hormone that acts after the fate assignment network). This integration allowed quantitative predictions of hair density and arrangement in the context of different pharmacological treatments to be made.

Since this model, many additional components of the root epidermal GRN have been identified. This additional information was used by Savage et al. (2008) to construct a more detailed model of fate assignment in young root epidermal cells. The

model took advantage of the highly stereotypical cross-sectional geometry of the early root epidermis in *Arabidopsis* (Dolan et al., 1994) to reduce the relevant geometry of the epidermis to a one-dimensional ring of cells (Savage et al., 2008). Given the lack of quantitative data on expression levels and the detailed nature of regulatory interactions, the GRN was initially represented using a discrete model in which each mRNA and protein variable can take one of two values ("on" or "off") and regulatory interactions are represented by logical rules (see Box 1). The majority of these rules encode cell-autonomous regulation; movement of CPC and GL3 between cells was incorporated by allowing the mRNA levels in one cell to influence the corresponding protein levels in the neighbouring cells.

While the previous phenomenological model (Benítez et al., 2007) assumed self-activation of WER expression, no evidence exists to support this. Genetic data, however, indicate that WER expression is down-regulated by CPC and by SCM activity in H position cells (Kwak et al., 2005; Kwak and Schiefelbein, 2007). A key question addressed by this the model was the nature the regulation of WER transcription. Two possibilities were considered: self-activation of WER, with CPC repression operating via competitive complex formation, or constitutive transcription of WER and direct repression by CPC. To avoid having to arbitrarily assign dominance to one of the two regulators of WER, the state of WER mRNA was represented by a random variable, that adopts the "on" and "off" states with probabilities that depend on the states of both of its regulators. This allows the relative strengths of the two regulators to be varied continuously (Savage et al., 2008).

Savage et al. demonstrated that both mechanisms of WER regulation could robustly generate appropriate expression patterns in wild type and mutant roots, showing that WER self-activation was not necessary for the root epidermal GRN model to drive appropriate cell fate patterning. Furthermore, some mutant phenotypes could be reproduced by the model only when WER self-activation was not incorporated, suggesting that a mutual support model—in which WER does not self-activate— provides a better representation of early root epidermal fate assignment.

An alternative discrete GRN model for epidermal patterning was developed by Benítez et al. (2008). This model was formed on a two-dimensional array of cells, allowing exploration of the potential interactions between cell geometry and signalling pathways to be explored. As in the model of Savage et al., it was found that WER self-activation is not necessary for wild-type expression patterns to arise; however, in the two-dimensional model, it was required to recover all mutant-like patterns (Benítez et al., 2008). Finally, the model results suggested that cell geometry may play an important role in pattern stabilization—as proposed in the earlier phenomenological model (Benítez et al., 2007). The model of Benítez et al. (2008) suggests that the mutual support mechanism proposed by Savage et al. may act redundantly with an activator-inhibitor mechanism incorporating WER self-activation to contribute to the formation and maintenance of robust root-hair patterns.

To explore directly the nature of WER regulation, Savage et al. carried out a careful examination of the expression pattern of a marker driven by the WER promoter in the meristem of wild type and mutant roots (Savage et al., 2008). Marker expression in the mutants was found to be indistinguishable from wild type, demonstrating that in the early stages of epidermal fate assignment, WER transcription does not depend on the presence of either functional WER protein or a functional AC. Furthermore, WER is initially expressed uniformly in the youngest epidermal cells, supporting the idea that WER transcription is uniformly up-regulated in all epidermal cells at the start of the patterning process. These results provide strong evidence that WER self-activation does not play a significant role in early fate assignment, lending support to the mutual support model (Savage et al., 2008).

An outstanding question is whether the results obtained in the one-dimensional model of Savage et al. (2008) can be reproduced in a growing two-dimensional geometry that would more accurately reflect the root epidermis. In particular, the model of Savage et al. encodes an implicit assumption that communication along the apical-basal axis of the epithelium can be neglected. The results of Benítez et al. (2008) suggest that some form of local self-activation may be necessary to stabilize patterning in a two-dimensional geometry. Interestingly, two separate forms of self-activation have recently been shown to operate later in root epidermal patterning, following the initial fate assignment process modelled by Savage et al. First, both SCM mRNA and SCM protein accumulate in H position epidermal cells as they age, providing positive feedback on WER expression that is necessary for normal patterning of hair cells (Kwak and Schiefelbein, 2008). Second, expression of the MYB23 gene—which codes for a R2R3 MYB protein that can functionally substitute for WER—is regulated by the components of the AC and IC, such that MYB23 accumulates in the nuclei of developing non-hair cells. MYB23 positively regulates its own expression, and thus generates a WER-induced local self-activation that operates downstream of the initial fate assignment GRN (Kang et al., 2009). Both SCM- and MYB23-dependent self-activation appear to act to reinforce the early-emerging complementary expression patterns of WER and CPC.

The root epidermal GRN models of Savage et al. (2008) and Benítez et al. (2008), taken together with expression data,

suggest that a mutual support mechanism acts early in the fate assignment process, and that the resulting expression patterns are then reinforced by at least two distinct local self-activation mechanisms. This redundancy may play an important role in allowing epidermal patterning to combine robustness and flexibility (Benítez et al., 2008; Schiefelbein et al., 2009).

Genetic Regulatory Network models for leaf hair patterning

As outlined above, the GRNs underlying epidermal patterning in leaves and roots are strikingly similar. A number of models investigating different aspects of leaf trichome patterning have been developed recently, each based on an explicitly two-dimensional cellular representation of the leaf epidermis, with diffusive movement of a subset of proteins between neighbouring cells (Benítez et al., 2008; Bouyer et al., 2008; Digiuni et al., 2008).

Digiuni et al. (2008) used a combination of modelling and experiments to address the open question of the nature of the competition between the AC and IC. Using a continuous model based on just four variables, representing GL1, GL3, AC (GL1-GL3 complex), and TRY, three possible forms of TRY-mediated inhibition of AC formation were explored. Simulations of the model demonstrated that while all three mechanisms were compatible with the wild type spacing pattern, not all were able to account for the results of over-expression of GL3 and TRY. Focused experiments, together with extensive simulations using a wide range of parameter values, allowed the authors to show that the binding of TRY to free GL3 was the most likely competitive inhibition mechanism, even though TRY can also bind to GL1 (Digiuni et al., 2008). Furthermore, while the model allows both TRY and GL3 to move between cells, simulations in which GL3 was cell autonomous more closely matched the observed degree of order in the trichome pattern. Using microparticle bombardment of cells with fluorescent fusion proteins, the authors were able to confirm that while TRY (and CPC) can move between leaf epidermal cells, GL3 is cell autonomous. This model demonstrated that competitive complex formation could provide a specific realisation of the proposed phenomenological activator-inhibitor models (Pesch and Hülskamp, 2004; Benítez et al., 2007).

In a separate combined modelling and experimental study, Bouyer et al. (2008) focused on the potential role of TTG1 movement between epidermal cells. The model of Digiuni et al. (2008) assumed implicitly that TTG1 plays only a permissive role in complex formation (Schnittger et al., 1998). In contrast, Bouyer et al. explored an alterative mechanism of pattern formation based on substrate depletion—whereby the AC effectively self-activates by depleting a mobile component (TTG1) of the complex from neighbouring cells (Gierer and Meinhardt, 1972; Schnakenberg, 1979). Exploration of the role of TTG1 was motivated by seemingly contradictory ttg1 mutant phenotypes; while strong ttg1 alleles result in the loss of trichomes, clusters of trichomes form in weak alleles (Schnittger et al., 1999; Larkin et al., 1999).

Using a continuous model of a highly reduced GRN incorporating only binding of GL3 and TTG1 to form AC and diffusion of TTG1 between neighbouring cells, Digiuni et al. demonstrated that local self-activation of AC (via positive regulation of GL3 14 expression), together with TTG1 diffusion could alone account for patterning similar to that observed in the leaf epidermis (Bouyer et al., 2008). Importantly, the model was able to reproduce the observed phenotypes of different ttg1 alleles. Bouyer et al. went on to show using microparticle bombardment that fluorescent TTG1 fusion proteins accumulate in developing trichomes, while being depleted in their immediate neighbours.

Taken together, the model and experiments suggest that a TTG1 depletion mechanism may act in parallel to an activator-inhibitor mechanism based on TRY-mediated inhibition (Bouyer et al., 2008). These two mechanisms may well be redundant, since bHLH over-expression can recover a wild-type phenotype in ttg1 loss of function mutants (Schnittger et al., 1998). Furthermore, similar microparticle bombardment experiments reported by Zhao et al. (2008) found no evidence of TTG1 movement between leaf epidermal cells. The extent to which TTG1 movement contributes to trichome patterning therefore remains an open question.

A third model of the leaf epidermal GRN was proposed by Benítez et al. (2008), using the same discrete formalism as in their root epidermis model (see above). In common with the models of Digiuni et al. and Bouyer et al., this model incorporated GL1 self-activation and diffusive protein movement. In addition to accounting for patterning in a detailed GRN model, this study identified the GL3/EGL3 and CPC genes as the most likely direct targets of plant hormone pathways (such as gibberelic acid and jasmonic acid) that can regulate the density of trichomes (Traw and Bergelson, 2003). This prediction is consistent with recent data showing that GL3 is directly regulated by hormonal pathways (Yoshida et al., 2009), and opens up new avenues for exploring the potential role of the fate assignment GRN as a signal integrator and an important regulator of plastic responses.

Generic features of epidermal patterning models

The recent crop of quantitative models and associated experimental studies suggest that epidermal patterning in *Arabidopsis* relies on redundant dynamic modules or sub-networks, each being sufficient (in principle) for cell-type determination and

pattern formation (Benítez et al., 2008; Bouyer et al., 2008; Digiuni et al., 2008; Savage et al., 2008). The full epidermal patterning networks comprise multiple interlocked feedback loops, making experimental assessment of their relative contributions to patterning difficult. In such circumstances, models provide invaluable exploratory tools. Each model uses a reduced representation of the full GRN to focus on different sub-networks centered on specific types of feedback. In addition to showing the sufficiency of each sub-network, each model highlights specific outstanding mechanistic questions, many of which have been answered by associated focused experimental studies.

Importantly, while all the proposed models are consistent with current molecular and genetic data, the validity of some of their assumptions remains unclear. All models apart from that developed by Savage et al. (2008) rely on some form of cell autonomous positive feedback that allows cells to "lock in" to a state of high AC activity and inhibit their neighbours from doing the same. In the model of Savage et al. the two alternative cell states mutually support each other, allowing model cells to change state in response to a change in position relative to the imposed spatial bias from the cortex. Such plasticity has been observed in response to both cell division and cell ablation in the root epidermis (Berger et al., 1998; Kidner et al., 2000). The recently reported local positive feedback loops involving SCM and MYB23 (Kwak and Schiefelbein, 2008; Kang et al, 2009) act at a later developmental stage than the initial fate assignment network modeled by Savage et al., suggesting that root epidermal cells first attain a labile state which is then reinforced, providing a balance between plasticity and robustness.

An additional feature that is highlighted by all the models is the importance of the intercellular mobility of a subset of proteins. It has been demonstrated convincingly, in both leaf and root, that single R3 MYB proteins can move between cells, while R2R3 MYB proteins cannot, and these features are incorporated in all the models. In contrast, the existence and potential importance of GL3/EGL3 and TTG1 movement remains unclear, and provides an important issue to be explored in future models. Another important outstanding question is how the actions of multiple single R3 MYB proteins—which show only partial functional redundancy—are integrated in the leaf epidermis. A recent combined modelling and experimental study has suggested that the mobility of single R3 MYB proteins is modulated by their binding affinity to GL3, providing an intriguing potential link between protein complex formation and protein mobility (Wester et al., 2009). Future detailed modelling studies should help to clarify whether these differences play a significant role in trichome patterning.

Outstanding issues

A simplification that is common to all the models discussed above is the assumption that the cellular array on which patterning occurs is static. In reality, however, epidermal patterning occurs in tissues comprising cells undergoing division and shape changes. The models of Benítez et al. (2007, 2008) show that cell geometry and other related traits— such as cell tension or cytoskeletal arrangement—could be important for the formation and stability of patterns. These features have been incorporated in a general modelling framework developed by Dupuy et al. (2007), who show that a reduced leaf epidermal GRN (with feedback and protein movement, but without explicit complex formation) can reproduce typical trichome spacing patterns on a growing leaf.

Although the initial pattern of fate assignment in the root apical meristem is robust, the later stages of epidermal development show significant plasticity. Not all root trichoblasts go on to differentiate as hair cells, the likelihood of differentiation being dependent on the details of the growth conditions such as nutrient availability (López-Bucio et al., 2003; Muller and Schmidt, 2004). Savage and Schmidt (2008) proposed that an activator-inhibitor mechanism acting downstream of fate assignment might account for plasticity. In one of the few modelling studies to consider interactions between a GRN and environmental factors, it is shown how a phenomenological model can recapitulate the observed dependence of the pattern of differentiated root hairs on levels of nutrients such as phosphate (Savage and Schmidt, 2008). The model provides an organising framework for a wealth of data and acts as a guide for the design of additional experiments. For example, different hypotheses concerning the interaction of nutrients and patterned cell fate with the activator-inhibitor mechanism predict different spatial distributions of root hairs (Savage and Schmidt, personal communication).

Conclusions

The studies of epidermal patterning outlined above provide specific illustrations of the potential of mathematical models to enhance our understanding of complex developmental processes. By focusing on reduced networks, the models allow interlocked regulatory feedbacks to be uncoupled, providing a conceptual decomposition of the full network into a set of dynamic patterning modules, each in principle capable of generating pattern. The theoretical insights gained provide a valuable complement to empirical data, since such a decomposition is difficult (if not impossible) to achieve experimentally. Importantly, by focusing squarely on specific features of the patterning network, the models yield specific predictions that

can (and have) been used to validate and refine the models.

The theoretical redundancy of the dynamic modules studied in the reduced network models raises the question of the potential role of module redundancy in development and evolution (Schlosser, 2004). A recent analysis has shown that while all the proposed dynamic epidermal patterning modules are indeed sufficient for pattern formation, the GRN model integrating all the modules is more robust to single mutations and stochastic perturbations than any of the single dynamic modules (Benítez and
Alvarez-Buylla, 2010).

These results suggest that redundancy of dynamic modules could have important consequences for the robustness of epidermal patterning. Modular redundancy could also contribute to flexibility, allowing the epidermal patterning networks to generate diverse patterns when subject to different cellular contexts (Meir et al., 2002; Benítez et al., 2007; Munteanu and Solé, 2008). In the context of *Arabidopsis* epidermal patterning, conserved redundant modules can generate contrasting cell types and spatial patterns in the leaf, root, and probably the hypocotyl (Dolan and Scheres, 1998). This suggests that patterning outcomes can depend on key contextual qualities (cell and domain geometry, positional bias, mechanical restrictions, etc.) and supports the idea that evolutionary changes in key contextual traits could underlie the origin of novel and diverse patterns, even if gene regulatory networks of cell fate determination remain unchanged. Experimental approaches that allow modules to be selectively uncoupled or disabled would help to provide further insight into the importance of modularity for
robust and flexible pattern formation.

A future challenge remaining for modelling studies of epidermal patterning (and other developmental processes) is their integration into a wider framework that considers not only genetic elements and cellular dynamics, but also factors such as geometry, environmental elements, stochasticity and mechanical forces (see, for example, Dupuy et al, 2007; Grieneisen and Scheres, 2009; Jönsson and Krupinski, 2010).

Box 1: Modelling glossary

Activator-inhibitor system. A class of a reaction-diffusion system that requires a self-activating substance (activator) that either does not diffuse or does so at short distances, and a long-range antagonist (inhibitor). The activator up-regulates the inhibitor, creating a mechanism of local activation and lateral inhibition that can spontaneously generate spatial patterns of activator concentration (Gierer and Meinhardt, 1972)

Continuous model. A type of model in which variables take continuous values. Typically based on differential equations, continuous models allow detailed quantitative analyses of physical, chemical and biological systems.

Deterministic model. A class of model in which the value of every variable is determined for all time, given an initial state.

Discrete model. A class of model in which one or more variables take only a discrete set of values (e.g. “on” or “off”). Model evolution is typically performed in discrete time steps.

Gene regulatory network (GRN) model. A class of network models used to study the collective dynamics of mutually-regulating genes. The nodes of the network represent genes, mRNAs, proteins or molecular complexes, while the edges represent regulatory interactions among the nodes.

Reaction-diffusion system. A continuous model describing the concentration in time and space of substances (morphogens) that diffuse and react at different rates. Under certain conditions, these systems can spontaneously generate patterns of morphogen concentration, such as spots, stripes, labyrinths, and spirals. First proposed as a potential mechanism for generating patterns in biology by Turing (1952), they have been widely used to model pattern formation during development.

Figure legends

Figure 1. Root and leaf hair patterns in *Arabidopsis*. (A) Leaf trichomes are arranged in two-dimensional spacing pattern. (B) Root epidermal cells are organized in alternating files of hair and non-hair cells.

Figure 2. Schematic representations of the epidermal patterning gene regulatory networks. The GRN underlying epidermal patterning in the leaf (A) and in the root (B). Ovals represent genes and red nodes represent genes that encode proteins that may move between neighboring epidermal cells. In the root, N and H position cells touch one and two underlying cortical cells, respectively.

References

Alon, U. (2007). An Introduction to Systems Biology: Design Principles of Biological Circuits. Chapman and Hall/CRC.

Alvarez-Buylla, E. R., Benítez, M., Balleza Dávila, E., Chaos, A., Espinosa-Soto, C. and Padilla-Longoria, P. (2007). Gene regulatory network models for plant development. Curr. Opin. Plant Biol. 10, 83–91.

Benít ez M. and Alvarez-Buylla, E. R. (2010). Dynamic-module redundancy confers robustness to the gene regulatory network involved in hair patterning of *Arabidopsis* epidermis. BioSystems (in press).

Benítez, M., Espinosa-Soto, C., Padilla-Longoria, P., Díaz, J. and Alvarez-Buylla, E. R. (2007). Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in *Arabidopsis* root and leaf epidermis: a dynamic model. Int. J. Dev. Biol. 51, 139–155.

Benítez, M., Espinosa-Soto, C., Padilla-Longoria, P. and Alvarez-Buylla, E. R. (2008). Interlinked nonlinear subnetworks underlie the formation of robust cellular patterns in *Arabidopsis* epidermis: a dynamic spatial model. BMC Syst. Biol. 2, 98.

Berger, F., Haseloff, J. Schiefelbein, J. and Dolan, L. (1998). Positional information in root epidermis is defined during embryogenesis and acts in domains with strict boundaries. Curr. Biol. 8, 421–430.

Bernhardt, C., Zhao, M., Gonzalez, A., Lloyd, A. and Schiefelbein, J. (2005). The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the *Arabidopsis* root epidermis. Development 132, 291–298.

Bouyer, D., Geier, F., Kragler, F., Schnittger, A., Pesch, M., Wester, K., Balkunde, R., Timmer, J., Fleck, C. and Hülskamp, M. (2008). Two-dimensional patterning by a trapping/depletion mechanism: the role of TTG1 and GL3 in *Arabidopsis* trichome formation. PLoS Biol. 6, e141.

Cui, H., Levesque, M. P., Vernoux, T., Jung, J. W., Paquette, A. J., Gallagher, K.L., Wang, J. Y., Blilou, I., Scheres, B. and Benfey, P. N. (2007). An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. Science 316, 421–425.

Digiuni, S., Schellmann, S., Geier, F., Greese, B., Pesch, M., Wester, K., Dartan, B., Mach, V., Srinivas, B. P., Timmer, J., et al. (2008). A competitive complex formation mechanism underlies trichome patterning on *Arabidopsis* leaves. Mol. Syst. Biol. 4, 217.

Dolan, L., Duckett, C. M., Grierson, C., Linstead, P., Schneider, K., Lawson, E., Dean, C., Poethig, S. and Roberts, K. (1994). Clonal relationships and cell patterning in the root epidermis of *Arabidopsis*. Development 120, 2465–2474.

Dolan, L. and Scheres, B. (1998). Root pattern: Shooting in the dark? Sem. Cell Dev. Biol. 9, 201–206.

Dupuy, L., Mackenzie, J., Rudge, T. and Haseloff, J. (2008). A system for modelling cell-cell interactions during plant morphogenesis. Ann. Bot. 101, 1255–1265

Esch, J. J., Chen, M., Sanders, M., Hillestad, M., Ndkium, S., Idelkope, B., Neizer, J. and Marks, M. D. (2003). A contradictory GLABRA3 allele helps define gene interactions controlling trichome development in *Arabidopsis*. Development 130, 5885– 5894.

Esch, J. J., Chen, M. A., Hillestad, M. and Marks, M. D. (2004). Comparison of TRY and the closely related At1g01380 gene in controlling *Arabidopsis* trichome patterning.Plant J. 40, 860–869.

Gallagher, K. L., Paquette, A. J., Nakajima, K. and Benfey, P. N. (2004). Mechanisms regulating SHORT-ROOT intercellular movement. Curr. Biol. 20, 1847–1851.

Gallagher, K. L. and Benfey, P. N. (2009). Both the conserved GRAS domain and nuclear localization are required for SHORT-ROOT movement. Plant J. 57, 785–797.

Gierer, A. and Meinhardt, H. (1972). A theory of biological pattern formation.Kybernetik 12, 30–39.

Grieneisen, V. and Scheres, B. (2009). Back to the future: evolution of computational models in plant morphogenesis. Curr. Opin. Plant Biol. 12, 606–614.

Hülskamp, M., Misera, S., and Jürgens, G. (1994). Genetic dissection of trichome cell development in *Arabidopsis*. Cell 76, 555–566.

Ishida, T., Kurata, T., Okada, K. and Wada, T. (2008). A genetic regulatory network in the development of trichomes and root hairs. Annu. Rev. Plant Biol. 59, 365–386.

Jaeger, J., Irons, D. and Monk, N. (2008). Regulative feedback in pattern formation: towards a general relativistic theory of positional information. Development 135, 3175–3183.

Jönsson, H. and Krupinski, P. (2010). Modeling plant growth and pattern formation. Curr. Opin. Plant Biol. 13, 5–11.

Kang, Y. H., and Kirik, V., Hulskamp, M., Nam, K. H., Hagely, K., Lee, M. M. and Schiefelbein, J. (2009). The MYB23 gene provides a positive feedback loop for cell fate specification in the *Arabidopsis* root epidermis. Plant Cell 21, 1080–1094.

Kidner, C., Sundaresan, V., Roberts, K. and Dolan, L. (2000). Clonal analysis of the *Arabidopsis* root confirms that position, not lineage, determines cell fate. Planta 211,191–199.

Kirik, V., Simon, M., Hulskamp, M. and Schiefelbein, J. (2004a). The ENHANCER of TRY and CPC1 (ETC1) gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in *Arabidopsis*. Dev. Biol. 268, 506–513.

Kirik, V., Simon, M., Wester, K., Schiefelbein, J. and Hulskamp, M. (2004b). ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of *Arabidopsis*. Plant Mol. Biol. 55, 389–398.

Koshino-Kimura, Y., Wada, T., Tachibana, T., Tsugeki, R., Ishiguro, W. and Okada, K. (2005). Regulation of CAPRICE transcription by MYB proteins for root epidermis differentiation in *Arabidopsis*. Plant Cell Physiol. 46, 817–826.

Kurata, T., Ishida, T., Kawabata-Awai, C., Noguchi, M., Hattori, S., Sano, R., Nagasaka, R., Tominaga, R., Koshino-Kimura, Y., Kato, T., et al. (2005). Cell-to-cell movement of the CAPRICE protein in *Arabidopsis* root epidermal cell differentiation.

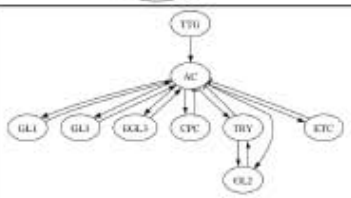
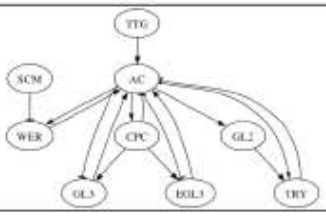
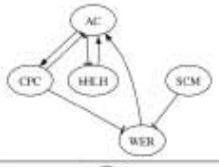
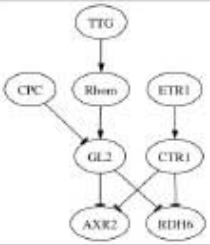
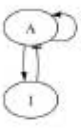
Developme nt 132, 5387–5398. Kwak, S., Shen, R. and Schiefelbein, J. (2005). Positional signaling mediated by a receptor-like kinase in *Arabidopsis*. Science 307, 1111–1113.

Kwak, S. and Schiefelbein, J. (2006). The role of the SCRAMBLED receptor-like kinase in patterning the *Arabidopsis* root epidermis. Dev. Biol. 302, 118–131.

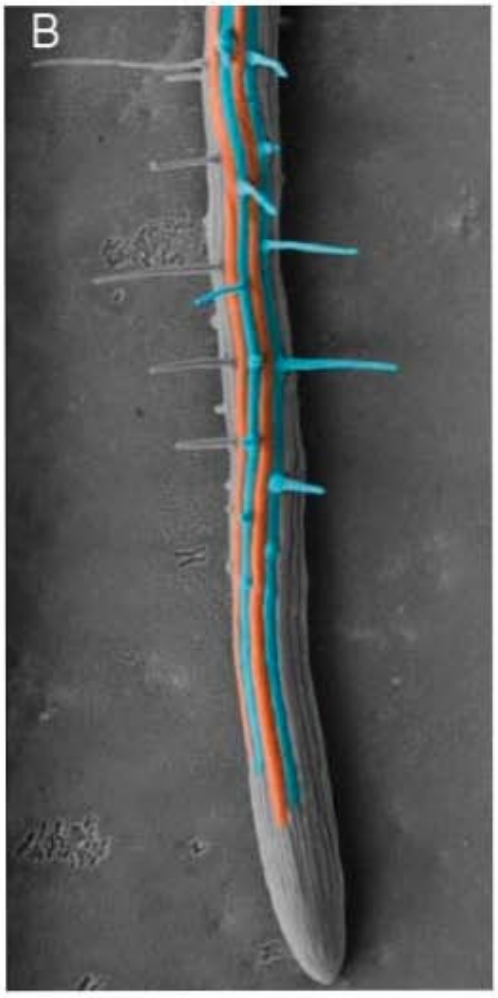
Kwak, S.-H. and Schiefelbein, J. (2008). A feedback mechanism controlling SCRAMBLED receptor accumulation and cell-type pattern in *Arabidopsis*. Curr. Biol. 18, 1949–1954.

Larkin, J. C., Walker, J. D., Bolognesi-Winfield, A. C., Gray, J. C. and Walker, A. R. (1999). Allele-specific interactions between ttg and gl1 during trichome development in *Arabidopsis* thaliana. Genetics 151, 1591–1604.
Lee, M. M. and Schiefelbein, J. (2001). Developmentally distinct MYB genes encode functionally equivalent proteins in *Arabidopsis*. Development 128, 1539–1546.
Lee, M. M. and Schiefelbein, J. (2002). Cell pattern in the *Arabidopsis* root epidermis determined by lateral inhibition with feedback. Plant Cell 14, 611–618.
López-Bucio, J., Cruz-Ramirez, A. and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. Curr. Opin. Plant Biol. 6, 280–287.
Meir, E., von Dassow, G., Munro, E. and Odell, G. M. (2002). Robustness, flexibility, and the role of lateral inhibition in the neurogenic network. Curr. Biol. 12, 778–786.
Mendoza, L. and Alvarez-Buylla, E. (2000). Genetic regulation of root hair development in *Arabidopsis* thaliana: a network model. J. Theor. Biol. 4, 311–326.
Morohashi, K., Zhao, M., Yang, M., Read, B., Lloyd, A., Lamb, R. and Grotewold, E. (2007). Participation of the *Arabidopsis* bHLH factor GL3 in trichome initiation regulatory events. Plant Physiol. 145, 736–746.
Muller, M. and Schmidt, W. (2004). Environmentally induced plasticity of root hair development in *Arabidopsis*. Plant Physiol. 134, 409–419.
Munteanu, A. and Solé, R. V. (2008). Neutrality and robustness in evo-devo: emergence of lateral inhibition. PLoS Comput. Biol. 4, e1000226.
Oates, A. C., Gorfinkiel, N., González-Gaitán, M. and Heisenberg, C.-P. (2009).
Quantitative approaches in developmental biology. Nat. Rev. Genet. 10, 517–530.
Pesch, M. and Hülskamp, M. (2004). Creating a two-dimensional pattern de novo during *Arabidopsis* trichome and root hair initiation. Curr. Opin. Genet. Dev. 14, 422–427.
Reeves, G., Muratov, C., Schüpbach, T. and Shvartsman, S. (2006). Quantitative models of developmental pattern formation. Dev. Cell 11, 289–300.
Ryu, K. H., Kang, Y. H., Park, Y., Hwang, I., Schiefelbein, J. and Lee, M. M. (2005).
The WEREWOLF MYB protein directly regulates CAPRICE transcription during cell fate specification in *Arabidopsis* root epidermis. Development 132, 4765–4775.
Savage, N. S. and Schmidt, W. (2008). From priming to plasticity: the changing fate of rhizodermic cells. BioEssays 30, 75–81.
Savage, N. S., Walker, T., Wieckowski, Y., Schiefelbein, J., Dolan, L. and Monk, N. A. M. (2008). A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the *Arabidopsis* root epidermis. PLoS Biol. 6, e235.
Schellmann, S., Hülskamp, M. and Uhrig, J. (2007). Epidermal pattern formation in the root and shoot of *Arabidopsis*. Biochem. Soc. Trans. 35, 146–148.
Schiefelbein, J., Kwak, S. H., Wieckowski, Y., Barron, C. and Bruex, A. (2009). The gene regulatory network for root epidermal cell-type pattern formation in *Arabidopsis*. J. Exp. Bot. 60, 1515–1521.
Schlosser, G. (2004). The roles of modules in development and evolution. In Modularity in Development and Evolution (ed. G. Schlosser and G. P. Wagner), pp. 519–582.Chicago: University of Chicago Press, Chicago.
Schnackenberg, J. (1979). Simple chemical reaction systems with limit cycle behavior J. Theor. Biol. 81, 389–400.
Schnittger, A., Jürgens, G. and Hulskamp, M. (1998). Tissue layer and organ specificity of trichome formation are regulated by GLABRA1 and TRIPTYCHON in *Arabidopsis*. Development 125, 2283–2289.
Schnittger, A., Folkers, U., Schwab, B., Jürgens, G. and Hulskamp, M. (1999). Generation of a spacing pattern: The role of TRIPTYCHON in trichome patterning in *Arabidopsis*. Plant Cell 11, 1105–1116.
Traw, M. B. and Bergelson, J. (2003). Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. Plant Physiol. 133, 1367–1375.
Turing, A. M. (1952). The chemical basis of morphogenesis. Philos. Trans. R. Soc. London Ser. B 237, 37–72.
Wester, K., Digiuni, S., Geier, F., Timmer, J., Fleck, C. and Hülskamp, M. (2009). Functional diversity of R3 single-repeat genes in trichome development. Development 136, 1487–1496.
Zhao, M., Morohashi, K., Hatlestad, G., Grotewold, E. and Lloyd, A. (2008). The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. Development 135, 1991–1999.
Yoshida, Y., Sano, R., Wada, T., Takabayashi, J. and Okada, K. (2009). Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in *Arabidopsis*. Development 136, 1039–1048.

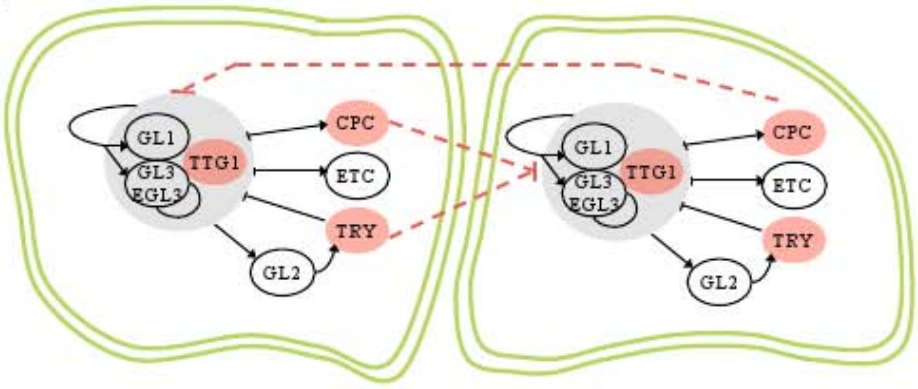
Table 1. Summary of models of leaf and root epidermal patterning

	Type of model	Graphical description	Results
Leaf epidermis	TTG1-trapping/depletion mechanism (Bouyer et al., 2008) Continuous model on a 2D cellular lattice.		Recovers typical trichome patterns. Accounts for previously unexplained data *ttg* mutants.
	Competitive inhibition (Digiuni et al., 2008) Continuous model on a 2D cellular lattice.		Recovers trichome patterns. Predicts that *TRY* binds *GL3* and/or *GL1* to inhibit the AC. Also predicts that *TRY* and *CPC* move among neighboring cells, while *GL1* and *GL3* do not.
	Meta-GRN (Benítez et al., 2008) Discrete model on a 2D cellular lattice.		Recovers wild type and mutant trichome patterns. Predicts the way a hormone signaling pathway could affect the GRN and that *GL1* self-activates.
Root epidermis	WER-self-activation model (Benítez et al., 2008) Discrete model on a 2D cellular lattice.		Recovers wild type and mutant trichoblast pattern. Predicts a stabilizing effect of cell elongation in pattern stability and that *WER* self-activates.
	Mutual support mechanism (Savage et al., 2008) Discrete and continuous models and on a 1D cellular lattice.		Recovers wild type and mutant trichoblast pattern. Predicts that *WER* self-activation is not necessary for pattern formation.
	Cell-level GRN model (Mendoza and Alvarez-Buylla, 2000) Cell-autonomous discrete model.		Recovers the gene activation profiles that specify trichoblasts and atrcichoblasts. Predicts phenotypic effects of pharmacological treatments and new potential GRN interactions.
Both types of epidermis	Phenomenological Activator-Inhibitor system (Benítez et al., 2007, proposed by Pesch and Hülskamp, 2004) Continuous model on a continuous spatial domain		Generates spaced (trichome) and banded (trichoblast) patterns when subject to different contexts. The leaf and root patterning systems are dynamically equivalent activator-inhibitor systems. Cell elongation stabilizes the banded pattern


A


B

A


GL1
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TTG1
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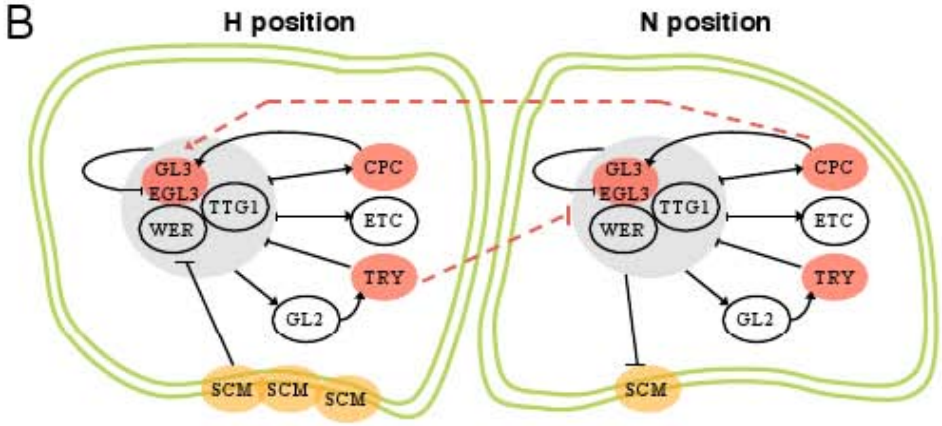
B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Apéndice E

Gene regulatory network models for plant development

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Gene regulatory network models for plant development

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Accumulated genetic data are stimulating the use of mathematical and computational tools for studying the concerted action of genes during cell differentiation and morphogenetic processes. At the same time, network theory has flourished, enabling analyses of complex systems that have multiple elements and interactions. Reverse engineering methods that use genomic data or detailed experiments on gene interactions have been used to propose gene network architectures. Experiments on gene interactions incorporate enough detail for relatively small developmental modules and thus allow dynamical analyses that have direct functional interpretations. Generalities are beginning to emerge. For example, biological genetic networks are robust to environmental and genetic perturbations. Such dynamical studies also enable novel predictions that can lead to further experimental tests, which might then feedback to the theoretical analyses. This interplay is proving productive for understanding plant development. Finally, both experiments on gene interactions and theoretical analyses allow the identification of frequent or fixed evolutionary solutions to developmental problems, and thus are contributing to an understanding of the genetic basis of the evolution of development and body plan.

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Introduction

The way in which the concerted action of multiple genes, along with environmental factors, regulates cell differentiation and development is still an open question in biology. Given the overwhelming number of genes and complexity of interactions that are involved in these processes, schematic and intuitive models are not sufficient to describe them. Hence, quantitative and integrative tools, such as mathematical representations and computational simulations, are becoming paramount. These tools enable structural and dynamic studies of complex assemblages of interconnected genes, proteins and other molecules, which we refer to as gene regulatory networks (GRN).

In Box 1 (see also [1]), we summarize some mathematical and simulation approaches that have been used recently to integrate experimental data on GRN. Such formal theory and methods provide improved understanding of biological systems. During the 20th century, several mathematical models for development were proposed (see timeline in Figure 1). These constitute a solid theoretical framework that might help to pose hypotheses about the conditions that are necessary and sufficient for cell differentiation and pattern formation, but they incorporated unrealistic assumptions about genetic mechanisms or, until very recently, could not be validated because of the scarcity of data. For example, the first GRN models [2], which aimed to represent dynamic and structural aspects of collections of interacting genes, assumed randomly connected networks with the same average number of regulatory interactions per node. By contrast, recent data suggest that actual GRN exhibit skewed distributions and preferred local connectivity patterns [3,4].

The accumulation of data from classical molecular genetic studies of development and functional genomics enables more realistic dynamic GRN models of cell differentiation and morphogenesis. In GRN models, genes, mRNA or proteins correspond to the network nodes and the links among nodes stand for regulatory interactions. These models are being developed with two main approaches. One uses functional genomics to reverse engineer the identity of the network nodes and the regulatory interactions among them (e.g. [5••]). The second approach uses detailed molecular genetic experiments to propose models of GRN architectures for relatively small gene networks (e.g. [6••]). Such networks can be studied thoroughly in relative isolation from the whole, allowing direct functional interpretations. They have enabled analyses of the temporal change of concerted gene activities (i.e. network dynamics) and of the way in which genes are connected to each other (i.e. network structure or architecture).

Box 1 From genes and molecules to gene regulatory networks: mathematical models.

There is always tension between generality and level of detail (and thus tractability) in a model. Depending on the scale involved and the nature of the available information, a suitable mathematical framework can be selected. We present basic terminology and concepts in GRN models and, in this context, introduce different types of models (see review in [48]). As development involves a wide range of scales and mechanisms, a combination of the models presented below, and others, will surely become necessary to understand fundamental aspects of morphogenesis.

Gene regulatory network models

In GRN models, the nodes correspond to genes, messengers or proteins and the edges represent regulatory interactions (activations or inhibitions) among the network components. In these models, gene regulatory interactions are translated into a set of updating rules that determines the nodes' states at every moment (Box Figure 1). These rules make it possible to follow the trajectory from one gene activity configuration to another, starting in each one of the possible configurations of gene activities (initial conditions). Configurations can be tracked until they reach a state that, given the network rules, remains unchanged. This state is called a fix point attractor. Configurations can also follow a trajectory that leads to a so-called periodic attractor, which corresponds to a collection of states among which the configuration cycles indefinitely once it is reached. The set of all initial configurations that lead to a specific attractor, be it fix point or periodic, conform the attractor's basin of attraction. A frequently used analogy is that of a landscape with valleys, in which the bottom of the valleys correspond to attractors and the valley's basin to the basins of attraction. Continuing with the analogy, every point on the landscape matches a gene activity configuration and if a bead were located on one of these points, it would follow a path on the rugged landscape, a trajectory, until it reached a valley. Finally, the topography of the valley, i.e. the number and identity of attractors, is determined by the set of logical rules and network architecture. Cell states that can be characterized by a certain fixed or cyclic gene activity configuration might indeed correspond to the attractors of a complex dynamic system [2,49•].

Discrete *versus* continuous GRN models

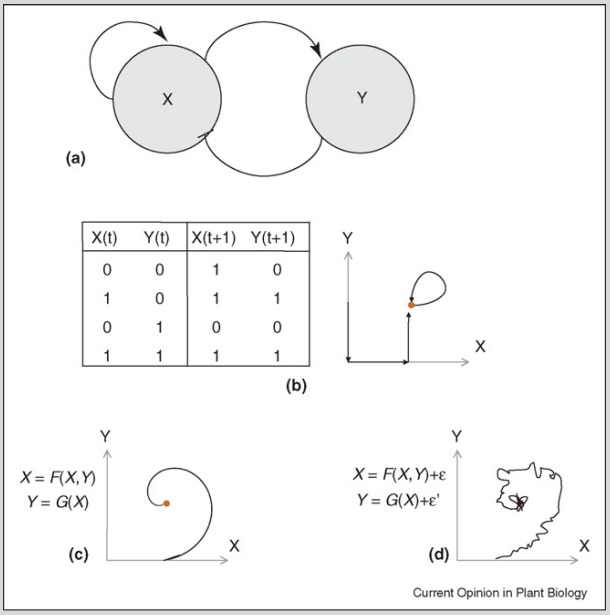
GRN models might incorporate continuous functions (differential equations; e.g. [22]) or discrete functions (difference equations; e.g. [6••]) to describe the rules that govern gene activation kinetics. Continuous implementations can include more detail and yield quantitative predictions, but experimental data that provide parameter estimates for such models are scarce. Despite this, continuous models have proven to be very useful for investigating signal transduction pathways and the circadian clock, both relevant processes in plastic plant development ([34••,35•,50,51•]; see [52] for review).

Different analyses of topologically equivalent continuous and discrete models have shown that both yield equivalent dynamic results ([24,53], although see [54]). In networks that have many non-linearities, the behaviour of the system seems to depend mostly on the GRN topology rather than on specific parameter values. In addition, if gene expression functions and pattern formation time scales are considerably distinct, qualitative discrete systems might be useful. Finally, recent experimental evidence suggests that gene expression is digital and stochastic at the individual cell level, although in cell aggregates gene expression might appear to be continuous [55,56,57•]. Given this, qualitative GRN models that have discrete kinetics of gene activation (e.g. [6••]) (0 ['OFF'] or 1 ['ON'] in the simplest Boolean case [32]) might be the most appropriate representation of complex gene regulatory logics.

Deterministic versus stochastic GRN dynamics

If logical rules or equations that govern the updating of the network states allow us to determine the fates of all states at every moment, the system is deterministic (e.g. [22]). By contrast, stochastic models [58] consider the noise inherent to natural systems that is caused by small numbers of molecules or other sources of uncertainties. In these models, the updating depends partially on a stochastic variable, which introduces a certain amount of uncertainty into the system dynamics.

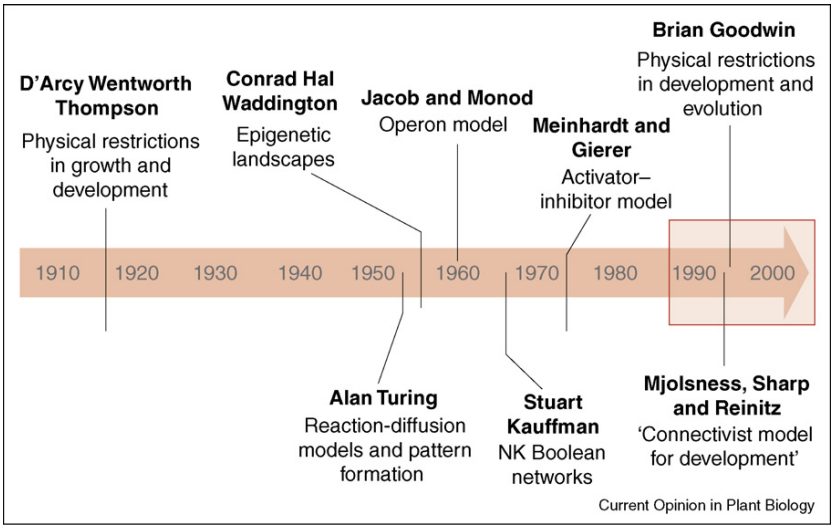
Box Figure 1



Example of a simple two-gene GRN dynamic model. **(a)** Two (X and Y) element GRN with positive (arrowhead edges) and negative (flat end edges) regulatory interactions. **(b)** Logical rules and graphical representation of a two-dimensional discrete trajectory that results from applying the set of rules (explicit only for the 0,1, Boolean case). Note that the 1,1 corresponds to a fix point attractor and the rest of the conditions that lead to this state to its basin of attraction. **(c)** Continuous deterministic case, given by differential equations, and graphical representation of a dynamic scenario qualitatively equivalent to that presented for the Boolean case. **(d)** Stochastic case of a similar dynamical scenario (ε and ε' are sufficiently small stochastic variables).

Here, we review studies of these two approaches for plant systems, also touching upon relevant animal examples. We then highlight the general findings that are emerging from these studies, the efforts to model morphogenesis from coupled GRN in explicit spatiotemporal domains, and the utility of formal dynamical analyses for evolutionary studies. We conclude that the two approaches are complementary for understanding the interplay between the structure and dynamics of GRN, and for uncovering general rules in the logic of the regulation of developmental processes and its links with signalling pathways and other cellular processes.

Figure 1



Some of the twentieth century theoreticians of development and their contributions. The boom of functional genomic technologies and system biology approaches is framed in the red rectangle.

From functional genomic data to gene regulatory networks

Recent powerful experimental technologies and novel statistical methods are being developed to infer GRN architectures from genomic data obtained in microarray experiments (reviewed in [7,8,9•]; for plants see [10]; and see Figure 2). Efficient reverse engineering of GRN architectures depends on collecting data that guarantee a wide exploration of perturbation conditions [11] or phenotypic variations of a cell type [12••], so that correlations among expression levels of different genes can be thoroughly investigated.

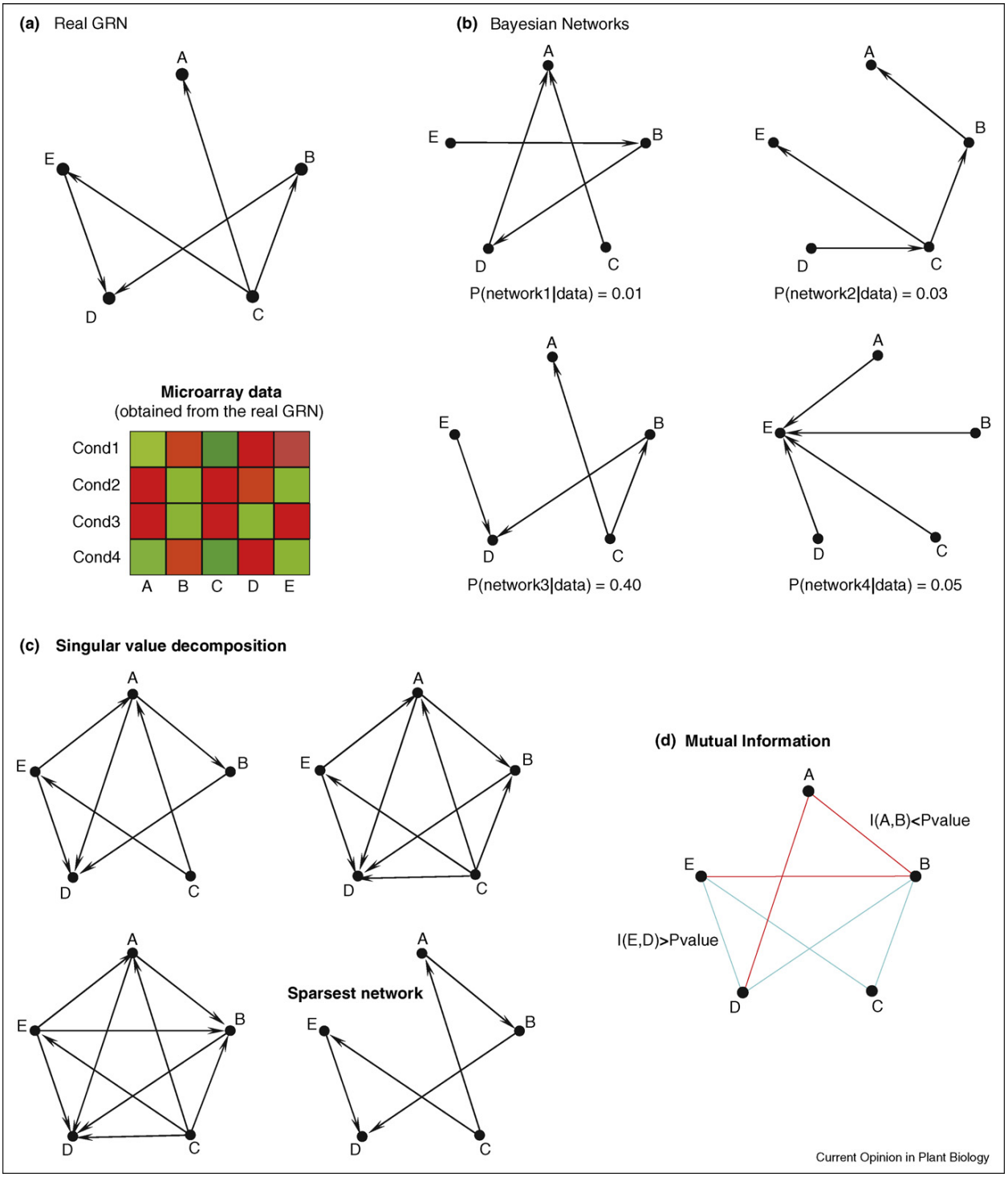
Two GRN architecture inference methods are widely accepted and have a particularly sound theoretical basis (Figure 2). More importantly, networks obtained by these two methods have been extensively validated with experimental data; although to our knowledge, they have not been applied to data from plant systems. The first method is based on Bayesian inference theory ([13]; Figure 2). When applied to gene regulation, the goal of this theory is to find the most probable GRN given the observed expression patterns of the genes to be considered in the network. Thus, the regulatory interactions among genes and their directions are derived from expression data. Different network structures are proposed and then scored on the basis of how well they explain the data. This method has been applied, for example, to infer regulatory modules in *Saccharomyces cerevisiae* [14].

The second method uses 'mutual information' as a measure of correlation between gene expression patterns ([15,16]; Figure 2). A regulatory interaction between two genes is established if the mutual information on their expression patterns is significantly larger than a P-threshold value calculated from the mutual information between random shufflings of the same patterns. In contrast to the Bayesian theory, which tries out whole networks and selects the one that best explains the observed data, the mutual information method constructs a network by selecting or rejecting regulatory interactions between pairs of genes or GRN nodes. This method does not provide the direction of regulatory interactions and has been tested for GRN that underlie the differentiation of human cell types [12••].

Recently, a third method for inferring GRN has been put forward (Figure 2). It assumes that GRN operate near a steady state and approximates its dynamics by a system of linear differential equations. The matrix of the linear system gives the type and strength of regulatory interactions. The system is solved to yield a matrix of gene interactions that matches the gene expression data. This method has been improved to take into account sparcity of connections and to incorporate different sets of microarray data [17]. To our knowledge, this is the first method that has been used to model the structure of an *Arabidopsis thaliana* GRN [5••].

Other recent methods have been tested using detailed data for relatively small model networks (for plant examples see [18,19], and for *Drosophila* [20•]) and might be useful as complementary techniques. Reverse engineering methods, in general, should be part of a recursive

Figure 2



The organism whose GRN is to be inferred is exposed to different conditions and microarray data is obtained accordingly. **(a)** A known or 'real' GRN consisting of A, B, C, D and E genes. Note that the expression patterns of the genes vary under different environmental conditions (cond1, . . ., cond4); the 'richness' of the patterns, which depends in part on the conditions tested, is important to discover gene interactions. **(b)** In Bayesian Network inference, different architectures are discriminated by calculating the probability of a network architecture given the observed data, P(network/data). The network that has the highest probability is selected. In this case, we see that the network that best explains

process in which previous or additional functional data are also considered to propose and validate former and novel regulatory interactions.

Dynamic models for small GRN of developmental modules

Development consists of processes that can be logically isolated, probably because of an underlying modularity in the global GRN [21]. This encourages the analysis of gene sub-networks for modules that are structurally and functionally isolated from the rest and that have been thoroughly studied in terms of molecular genetics, therefore allowing the introduction of dynamic models.

Pioneering work on this approach, developed in Odell's laboratory [22,23], shows that the gene network that determines *Drosophila* segment polarity is robust for different initial conditions or parameter values that affect, among other things, the strength of interactions and the exact kinetic functions of the genes (or proteins) [22]. Robustness to initial conditions supports evidence suggesting that the studied GRN performs its function semi-autonomously, irrespective of its interactions with other genes outside the network [21]. The robustness of the GRN studied by von Dassow and collaborators is further supported by the fact that a Boolean model of the segment polarity GRN recovers the same patterns for the *Drosophila* segment polarity genes as those recovered by the continuous model [22,24]. Similarly, the neurogenic and proneural GRN in *Drosophila*, also studied in Odell's laboratory [25,26], is a robust module. This latter study further suggested that structural alterations, and not only parameter changes, are tolerated by the GRN.

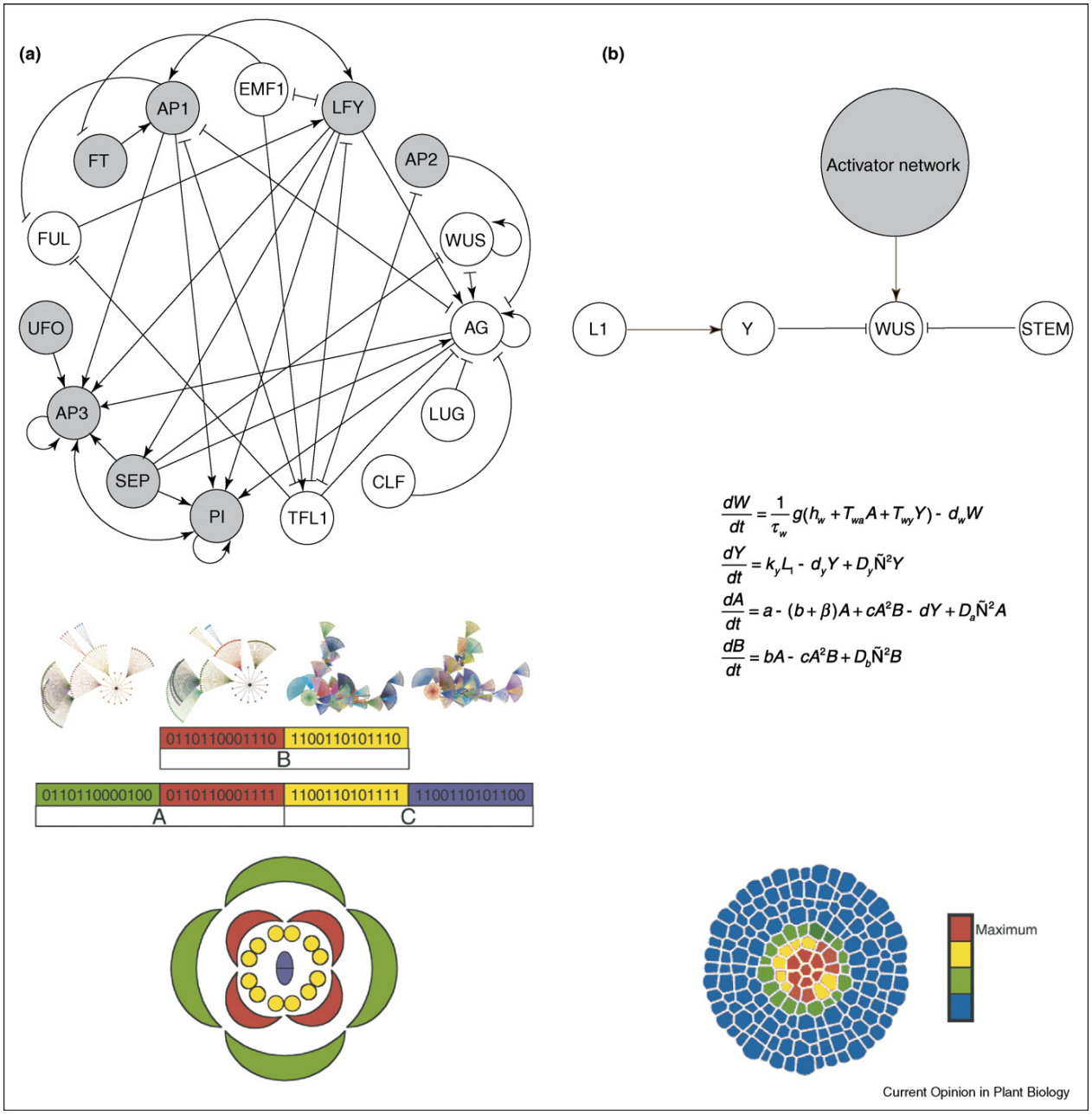
Few similar studies have been put forward for plant systems. The first dynamic models of plant GRN were proposed for the specification of floral organ and root epidermis cell types in *A. thaliana* [27,28]. These studies used Boolean models, grounded on available experimental data, to describe the gene activity profile that characterizes the state of single cells. Thomas [29] proposed a logical approach to analyze the dynamics of Boolean GRN (Box 1) that was based on identifying functional positive and negative feedback loops. Such analyses might also be used to identify functional sub-modules within GRN and were applied to *A. thaliana* floral identity GRN [30]. The results mostly coincided with predictions made by the now classical ABC model of floral organ specification [31] or with the steady states predicted by a different approach [28].

The ABC model postulates that the combination of three classes of genes (A, B and C) underlies the specification of primordial floral organ cells in plants; with A genes alone specifying sepal primordia, A+B petals, B+C stamens and C alone carpel. Furthermore, ABC functions seem to be conserved over a wide array of flowering plant species. The ABC combinatorial model does not, however, explain the conservation of this model, or the logic and dynamics of gene regulation involving ABC and non-ABC genes that underlie the gene activation profiles observed in floral organ specification. An updated GRN floral model [6••] showed that, given the interaction rules extracted from experimental information, all possible initial gene activity configurations converge to few fixed gene activity states, also called attractors (Box 1). Such attractors match the gene expression profiles of the cells of inflorescence meristems and of sepal, petal, stamen and carpel primordia. In addition, genetic perturbations of this GRN reproduce patterns observed in reported mutants ([6••]; Figure 3a). By recovering all of the gene activation profiles that correspond to either inflorescence meristem cells or cells of each one of the four floral organs, and no more, this GRN model [6••] provides a dynamic explanation for the ABC model. It seems to incorporate the key elements of a developmental module that underlies the ABC model of floral organ specification. This study also showed that the steady states are robust to changes in the interaction rules [6••,32], consistent with the fact that the overall floral plan is widely conserved among flowering plants. Furthermore, this study suggests that, even though qualitative models do not consider the detailed kinetic functions of gene activation (Box 1), such models might provide an adequate representation of the logic of regulation, and are useful integrative tools for detecting holes in experimental data and for generating novel predictions. For example, the floral organ identity GRN ([6••]; see [28,30] for other examples) predicted that the gene *AGAMOUS* should self-activate. This was confirmed by independent parallel experiments [33].

Another recent example of a relatively small plant network that has been grounded on detailed experimental data has identified the essential components of the abscisic acid signal transduction pathway, which controls stomatal opening and closure depending on the water balance of the plant [34••]. Although this GRN controls a physiological rather than a developmental process, it constitutes another example of the power of qualitative representations to integrate data into a dynamic analysis. The model outputs are consistent with experimental

(Figure 2 Legend continued) the data is the third one (P[network/data] = 0.40). **(c)** Using Singular Value Decomposition, the family of feasible solution architectures, constricted by the data, are obtained. The final network is selected on the assumption that the most (biologically) relevant network is the sparsest one. The example shows the family of solution networks that are consistent with the data. At the end, the sparsest one is selected. **(d)** The Mutual Information (I) of different pairs of genes is measured. If I is lower than a P-threshold value, then the interaction is rejected (red links in the example network). If I is larger than the P-threshold value, the interaction is accepted (blue links). In this way, the GRN is constructed interaction by interaction.

Figure 3



Gene regulatory network models for plants. **(a)** Single cell GRN dynamic model for cell specification in *Arabidopsis* floral organs [6••]. The topology of the 15 gene GRN is shown according to [6••], with activations as arrowheads and repressions as flat heads. Below the GRN, the basins of attraction that lead to each of the four floral primordial cell types (sepals, green; petals, red; stamens, yellow; and carpels, purple) are shown. Each attractor is defined by the steady-state activations of the fifteen genes (OFF - '0' and ON - '1'), and they match those observed experimentally and predicted by the ABC model. Shaded genes in the GRN correspond to those active ('1') in the petal attractor. **(b)** Continuous spatiotemporal model for the mRNA expression pattern of the *WUSCHEL* (*WUS*) gene in the *Arabidopsis* SAM [22]. The simple GRN proposed is shown with nodes and edges as in (a). The equations of the model proposed are shown, with concentration of *WUS* (W) and A, Y and B as variables of a reaction–diffusion Brussellator system. Below the equations, WUS protein concentration is shown in a cellular lattice simulation of the SAM (modified from [45••]).

data on stomata dynamics in wildtype and in mutant or pharmacologically treated plants. The model also allowed a number of clear and novel predictions, some of which have been tested experimentally. This work also suggests that network modules of signal transduction pathways are robust in the face of diverse perturbations (see also [35•]).

From GRN to morphogenetic patterns and evolution

Experimentally supported GRN models have made it possible to propose some generic aspects of development. For example, dynamic GRN models of the functional gene modules studied to date suggest that cell-type determination depends mainly on global aspects of GRN architecture and dynamics, rather than on the precise values of parameters for kinetic functions such as gene activation or protein degradation. Additionally, GRN characterization in diverse systems tends to support the claim of some theoreticians (Figure 1) that a limited number of mechanisms are capable of generating and maintaining heterogeneities during morphogenesis. For example, the so-called activator–inhibitor system has been frequently found among documented GRN [36]. This system is a type of reaction–diffusion system, which has been widely used to address how spatial heterogeneities or patterns might arise in living organisms. In activator–inhibitor systems, two elements interact (the activator element positively regulates itself and the inhibitor element, whereas the latter negatively regulates the activator) and diffuse, giving rise to different spatial patterns.

As described above, GRN that are grounded on experimental data generally also seem to be functionally robust under perturbations. Apparently, particular structural traits (e.g. feedback loops) could underlie such generic robustness [29,37]. Further documentations of GRN should provide a more complete analysis of the interplay between structure and function. This might be more feasible in small and well-characterized sub-networks or modules in which functional and evolutionary interpretations are more direct than in global networks that have been inferred from genomic data. Nonetheless, the modular and reverse engineering genomic approaches should feedback from each other, and from more theoretical approaches that aim at uncovering general principles for network assemblage and dynamics [38,39•].

Comparative approaches are promising in searching for generalities, and, in their broadest sense, these approaches should consider both plants and animals [40]. Correlations of structural and dynamic aspects of GRN with variation in the morphological traits that are regulated by such networks [41••,42] are already being explored. For example, von Dassow and collaborators [21,22] suggested that the robustness and alterations of the segment polarity gene network in insects could underlie the overall conservation of body plan and the origin of long and short germ-band insects, respectively. Also, the GRN for floral organ specification ([6••], Figure 3a) seems to be robust even in the face of gene duplications, and thus could underlie the conserved basic floral plan of Eudicot flowering plants. At the same time, it could also account for the observed divergent phenotypes of mutants of flowering species that have duplicated floral genes [6••].

Conclusions

Mathematical models have proven to be very useful in developmental biology, but we still lack a formulation based on experimental facts that can account for biological phenomena at different scales and, most importantly, for the emergence of robust yet evolvable spatiotemporal patterns. There has been a recent burst of mathematical models invoking non-linear dynamic mechanisms to address spatiotemporal patterns of morphogens in plants (see review in this issue, e.g. [43•]) and animals (e.g. [44•]). However, these do not incorporate explicit complex GRN. The first efforts in this direction for plant systems are being made by Jonsson and collaborators [45••]. These authors used quantitative gene expression data from *in vivo* live confocal microscopy to create dynamic and spatially explicit computational templates. They have explicitly incorporated a small GRN that regulates shoot apical meristem (SAM) size and maintenance and have modeled the expression pattern of the gene *WUSCHEL* in a simulated SAM domain (Figure 3b). For this end, they applied the so-called 'connectivist model' [46,47], which unfortunately has the limitation of allowing only for paired gene–gene interactions.

Although simplified versions of GRN are relatively tractable, they might not be helpful in recovering the robust patterns observed in organisms [25,26,37]. This encourages the consideration of experimentally grounded complex GRN coupled in realistic cellular contexts. To this end, multidisciplinary work aimed at building hybrid models (see Box 1) and at incorporating available empirical information will definitely help to address the major task of building models that accurately capture the essential aspects of multi-scale processes during development.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:• of special interest•• of outstanding interest

1. Bower JM, Bolouri H (Eds): *Computational Modeling of Genetic and Biochemical Networks*. London: MIT Press; 2001.

2. Kauffman S: **Metabolic stability and epigenesis in randomly constructed genetic nets**. *J Theor Biol* 1969, **22**:437-467.

3. Barabási A-L, Oltvai ZN: **Network biology: understanding the cell's functional organization**. *Nat Rev Genet* 2004, **5**:101-113.

4. Milo R, Itzkovitz S, Kashtan N, Levitt R, Shen-Orr S, Ayzenshtat I, Sheffer M, Alon U: **Superfamilies of evolved and designed networks**. *Science* 2004, **303**:1538-1542.

5. •• Wang Y, Joshi T, Zhang X-S, Xu D, Chen L: **Inferring gene regulatory networks form multiple microarray datasets**. *Bioinformatics* 2006, **22**:2413-2420.

This paper puts forward a method for inferring gene regulatory networks from multiple data sets, which was tested for data relating to the yeast cell cycle and to stress responses in *Arabidopsis*.

6. •• Espinosa-Soto C, Padilla-Longoria P, Alvarez Buylla E: **A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles**. *Plant Cell* 2004, **16**:2923-2939.

The authors put forward a dynamic GRN model for genes that are involved in cell-type specification during floral organ development. Their model recovers few steady gene activity configurations that are robust, all of which correspond to observed patterns of gene activation in primordial cells of sepals, petals, stamens and carpels of *Arabidopsis* flowers. Thus, this work offers a dynamic explanation for the ABC model and seems to include the key elements of a robust developmental module that underlies this classical model of plant development.

7. Quackenbush J: **Computational analysis of microarray data**. *Nat Rev Genet* 2001, **2**:418-427.

8. Affymetrix: *Gene Chip Expression Analysis Technical Manual, 2004*. http://www.affymetrix.com.

9. • Ball CA, Awad IAB, Demeter J, Gollub J, Hebert JMM, Hernandez-Boussard T, Jin H, Matese JC, Nitzberg M, Wymore F, Zachariah ZK, Brown PO, Sherlock G: **The Stanford microarray database accommodates additional microarray platforms and data formats**. *Nucleic Acids Res* 2005, **33**:D580-D582.

The authors describe one of the best public microarray databases. They also feature a tutorial on two-colour spotted microarray data normalization.

10. Wellmer F, Riechmann JL: **Gene network analysis in plant development by genomic technologies**. *Int J Dev Biol* 2005, **49**:745-759.

11. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO: **Genomic expression programs in the response of yeast cells to environmental changes**. *Mol Biol Cell* 2000, **11**:4241-4257.

12. •• Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A: **Reverse engineering of regulatory networks in human B cells**. *Nat Genet* 2005, **37**:382-390.

This paper is a milestone in work on the mutual information method. The regulatory network of B cells is inferred. The proto-oncogene MYC turns out to be a hub of the network; many of the MYC predicted interactions are biochemically validated.

13. Heckerman D: *A Tutorial on Learning with Bayesian Networks*. Microsoft Research, Advanced Technology Division. 1996. Technical Report MSR-TR-95-06.

14. Segal E, Shapira M, Regev A, Pe'er D, Botstein D, Koller D, Friedman N: **Module networks: identifying regulatory modules and their condition-specific regulators form gene expression data**. *Nat Genet* 2003, **34**:166-176.

15. Liang S, Fuhrman S, Somogyi R: **REVEAL, a general reverse engineering algorithm for inference of genetic network architectures**. *Pacific Symposium on Biocomputing* 1998, **3**:18-29.

16. Steuer R, Kurths J, Daub CO, Weise J, Sebig J: **The mutual information: detecting and evaluating dependencies between variables**. *Bioinformatics* 2002, **18**:S231-S240.

17. Yeung MKS, Tegnér J, Collins JJ: **Reverse engineering gene networks using singular value decomposition and robust regression**. *Proc Natl Acad Sci USA* 2002, **99**:6163-6168.

18. Aracena J, Demongeot J: **Mathematical methods for inferring regulatory networks interactions: application to genetic regulation**. *Acta Biotheor* 2004, **52**:391-400.

19. Perkins TJ, Hallett M, Glass L: **Inferring models of gene expression dynamics**. *J Theor Biol* 2004, **230**:289-299.

20. • Perkins TJ, Jaeger J, Reinitz J, Glass L: **Reverse engineering the gap gene network of *Drosophila melanogaster***. *PLoS Comput Biol* 2006, **2**:e51.

The authors provide a novel method to infer gene network structure from functional genomic data and explore the architecture of the *Drosophila* gap gene network. They propose a network, validate it with experimental results and, among other things, use their model to find inconsistencies in experimental data.

21. von Dassow G, Munro E: **Modularity in animal development and evolution: elements of a conceptual framework for EvoDevo**. *J Exp Zool Mol Dev Evol* 1999, **285**:307-325.

22. von Dassow G, Meir E, Munro EM, Odell GM: **The segment polarity network is a robust developmental module**. *Nature* 2000, **406**:188-193.

23. von Dassow G, Odell GM: **Design and constraints of the *Drosophila* segment polarity module: robust spatial patterning emerges from intertwined cell state switches**. *J Exp Zool Mol Dev Evol* 2002, **294**:179-215.

24. Albert R, Othmer HG: **The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in *Drosophila melanogaster***. *J Theor Biol* 2003, **223**:1-18.

25. Meir E, Munro EM, Odell GM, von Dassow G: **INGENEUE: a versatile tool for reconstituting genetic networks, with examples from the segment polarity network**. *J Exp Zool Mol Dev Evol* 2002, **294**:216-251.

26. Meir E, von Dassow G, Munro E, Odell GM: **Robustness, flexibility, and the role of lateral inhibition in the neurogenic network**. *Curr Biol* 2002, **12**:778-786.

27. Mendoza L, Alvarez-Buylla ER: **Genetic regulation of root hair development in *Arabidopsis thaliana*: a network model**. *J Theor Biol* 2000, **204**:311-326.

28. Mendoza L, Alvarez-Buylla ER: **Dynamics of the genetic regulatory network for *Arabidopsis thaliana* flower morphogenesis**. *J Theor Biol* 1998, **193**:307-319.

29. Thomas R: **Regulatory networks seen as asynchronous automata: a logical description**. *J Theor Biol* 1991, **153**:1-23.

30. Mendoza L, Thieffry D, Alvarez-Buylla ER: **Genetic control of flower morphogenesis in *Arabidopsis thaliana*: a logical analysis**. *Bioinformatics* 1999, **15**:593-606.

31. Coen ES, Meyerowitz EM: **The war of the whorls: genetic interactions controlling flower development**. *Nature* 1991, **353**:31-37.

32. Chaos A, Aldana M, Espinosa-Soto C, García Ponce de León B, Garay A, Alvarez-Buylla RE: **From genes to flower patterns and evolution: dynamic models of gene regulatory networks.** *J Plant Growth Regul* 2006, in press.

33. Gómez-Mena C, de Folter S, Costa MMR, Angenent GC, Sablowski R: **Transcriptional program controlled by the floral homeotic gene *AGAMOUS* during early organogenesis**. *Development* 2005, **132**:429-438.

34. •• Li S, Assmann SM, Albert R: **Predicting essential components of signal transduction networks: a dynamic model of guard cell abscisic acid signaling**. *PLoS Biol* 2006 doi: 10.1371/journal.pbio.0040312.

An elegant application of the Boolean formalism to study the dynamic behaviour of a complex signal transduction pathway. The model, which is grounded in experimental evidence, integrates the concerted action of

more than 40 components and identifies essential components of the abscisic acid response pathway, which regulates stomata closure and water loss in plants. One of the first successful applications of a Boolean formalism to describe a complex signal transduction pathway.

35. Diaz J, Alvarez-Buylla ER: **A model of the ethylene signaling**
• **pathway and its gene response in *Arabidopsis thaliana*: pathway cross-talk and noise-filtering properties**. *Chaos* 2006, **16**:023112.
This study integrates experimental data on the ethylene signal transduction pathway into a dynamic model. This model makes it possible to explore how the effects of different temporal patterns of environmental signals on the studied pathway impact gene expression dynamics. It also shows that the pathway is robust to parameter perturbations.

36. Meinhardt H: **From observations to paradigms; the importance of theories and models. An interview with Hans Meinhardt by Richard Gordon and Lev Beloussov**. *Int J Dev Biol* 2006, **50**:103-111.

37. Furusawa C, Kaneko K: **Robust development as a consequence of generated positional information**. *J Theor Biol* 2003, **224**:413-435.

38. Salazar-Ciudad I, Newman S, Solé R: **Phenotypic and dynamical transitions in model genetic networks. I. Emergence of patterns and genotype–phenotype relationships**. *Evol Dev* 2001, **3**:84-94.

39. Arenas A, Díaz-Guilera A, Pérez-Vicente CJ: **Synchronization**
• **reveals topological scales in complex networks**. *Phys Rev Lett* 2006, **96**:114102.
This letter proposes a method for studying how huge networks have been constructed or assembled. This is accomplished by studying the emergence of synchronization patterns in these systems, which has been shown to be closely related to the underlying topology of interactions. This approach is useful in revealing coupled behaviour of closely related elements.

40. Meyerowitz EM: **Plants compared to animals: the broadest comparative study of development**. *Science* 2002, **295**:1482-1485.

41. Davidson EH, Erwin DH: **Gene regulatory networks and the**
•• **evolution of animal body plans**. *Science* 2006, **311**:796-800.
An outstanding comparative analysis that, by showing conserved GRN structure among distant animal phyla, allows the authors to postulate the existence of 'kernels'. Kernels are sub-networks that are fixed irrespective of other components and are thought to be responsible for major body plan traits.

42. Abouheif E, Wray GA: **Evolution of the gene network underlying wing polyphenism in ants**. *Science* 2002, **297**:249-252.

43. Jonsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E:
• **An auxin-driven polarized transport model for phyllotaxis**. *Proc Natl Acad Sci USA* 2006, **103**:1633-1638.
The authors put forward a model for plant phyllotactic patterns that is based on recent experiments related to auxin transport and its effects on bud outgrowth. Their model considers an explicit cellular domain, includes cell growth and proliferation, and recovers the widespread leaf arrangement patterns. Importantly, the authors propose a pattern-generating mechanism that might be important during morphogenesis.

44. Umulis DM, Serpe M, O'Connor MB, Othmer HG: **Robust,**
• **bistable patterning of the dorsal surface of the *Drosophila* embryo**. *Proc Natl Acad Sci USA* 2006, **103**:11613-11618.
This authors present a computational model for dorsal surface patterning in *Drosophila* and show how transient morphogens can transduce into a switch-like system.

45. Jonsson H, Heisler M, Reddy GV, Agrawal V, Gor V, Shapiro BE,
•• Mjolsness E, Meyerowitz EM: **Modeling the organization of the *WUSCHEL* expression domain in the shoot apical meristem**. *Bioinformatics* 2005, **21**:i232-i240.
One of the first attempts to integrate GRN in a spatial and cellular context in a dynamic model. The authors propose a simplified GRN model for genes that are involved in *Arabidopsis* SAM regionalization and size maintenance, with special focus on the spatiotemporal pattern of the gene *WUSCHEL*.

46. Mjolsness E, Sharp DH, Reinitz J: **A connectionist model of development**. *J Theor Biol* 1991, **152**:429-453.

47. Shapiro BE, Levchenko A, Meyerowitz EM, Wold BJ, Mjolsness ED: **Cellerator: extending a computer algebra system to include biochemical arrows for signal transduction simulations**. *Bioinformatics* 2003, **19**:677-678.

48. Eungdamrong NJ, Iyengar R: **Computational approaches for modeling regulatory cellular networks**. *Trends Cell Biol* 2004, **14**:661-669.

49. Huang S, Eichler G, Bar-Yam Y, Ingber DE: **Cell fates as high-**
• **dimensional attractor states of a complex gene regulatory network**. *Phys Rev Lett* 2005, **94**:128701.
This is one of the recent papers that explore the idea of cell types corresponding to gene configurations attractors.

50. Locke JCW, Millar AJ, Turner MS: **Modeling genetic networks with noisy and varied experimental data: the circadian clock in *Arabidopsis thaliana***. *J Theor Biol* 2005, **234**:383-393.

51. Rand DA, Shulgin BV, Salazar D, Millar AJ: **Design principles**
• **underlying circadian clocks**. *J R Soc Interface* 2004, **1**:119-130.
One of the first dynamic mathematical models of signal transduction in plants.

52. Bhalla US: **Models of cell signaling pathways**. *Curr Opin Genet Dev* 2004, **14**:375-381.

53. Thomas R, Thieffry D, Kaufman M: **Dynamical behaviour of biological regulatory networks — I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state**. *Bull Math Biol* 1995, **57**:247-276.

54. Mochizuki A: **An analytical study of the number of steady states in gene regulatory networks**. *J Theor Biol* 2005, **236**:291-310.

55. Elowitz MB, Levine AJ, Siggia ED, Swain PS: **Stochastic gene expression in a single cell**. *Science* 2002, **297**:1183-1186.

56. Paulsson J: **Summing up the noise in gene networks**. *Nature* 2004, **427**:415-418.

57. Gurol MS, Garcia-Ojalvo J, Liberman LM, Elowitz MB: **An**
• **excitable gene regulatory circuit induces transient cellular differentiation**. *Nature* 2006, **440**:545-550.
This is one of the several papers that show the important role of noise in cell state switching and in cell differentiation and patterning.

58. Shmulevich I, Dougherty ER, Kim S, Zhang W: **Probabilistic Boolean networks: a rule-based uncertainty model for gene regulatory networks**. *Bioinformatics* 2002, **18**:261-274.

Apéndice F

Phenotypic evolution is restrained by complex developmental processes

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HFSP Journal

COMMENTARY

Phenotypic evolution is restrained by complex developmental processes

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The paper, "Evolution and development of inflorescence architectures" by Przemyslaw Prusinkiewicz, Yvette Erasmus, Brendan Lane, Lawrence D. Harder and Enrico Coen [*Science*, 316, 1452–1456 (2007)], sets to accomplish a longstanding goal: to explain, for the first time, how and to what extent developmental constraints restrict phenotypic evolution. Prusinkiewicz and collaborators provide a relatively simple model that accounts for the variety of patterns of inflorescence architecture found among angiosperms, in which only a few of all possible types are observed. [DOI: 10.2976/1.2749445]

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Why are certain phenotypic types, the occurrence of which is theoretically possible, actually not observed? In general, the absence of certain phenotypical types that have equivalent fitness effects than those observed among present living organisms, may be due to constraints of developmental processes. Development involves interactions among parts (genes, cells, tissues, etc.) which affect phenotypic traits in nonindependent manners, thus generating correlated changes among morphogenetic relevant variables. Such developmental constraints could explain the absence of certain phenotypic types.

Until the last decades, developmental processes could not be understood in terms of the interactions of molecular and structural factors at different levels of organization. Such levels go from gene regulatory networks, to collections of cells bearing such networks coupled in spatio-temporal dynamics by intercellular communication processes. At the same time, structural characteristics of the system's components and emerging organs limit the patterns arising during morphogenesis. Mathematical and computational models are required to integrate the concerted action of the many interacting components with correlated and nonlinear behaviors that underlie the complex nature of development. Some recent models of this kind are showing that the whole structure and collective dynamics of gene regulatory networks are important to obtain robust morphogenetic patterns (Hogeweg, 2000). This implies that models which oversimplify development may predict phenotypic patterns that are superficially similar to those observed in nature, but may not underlie the formation of robust patterns in real organisms.

Prusinkiewicz and co-workers acknowledge that studying the variational properties of developmental processes is important in order to understand phenotypic evolution. Such an endeavor entails postulating explicit molecular genetic models of development that explain why and how the configuration of a morphospace is restricted during the course of evolution. Prusinkiewicz and collaborators propose an imaginative model that yields inflorescence two-dimensional branching patterns that coincide with those observed among angiosperms: panicles, cymes and racimes (Fig. 1). They then provide molecular data for two genes that have been shown to be key in controlling inflorescence branching patterns: *LEAFY (LFY)* and *TERMINAL FLOWER1 (TFL1)* to justify their proposed model for morphogenetic mechanisms. Finally, they map the inflorescence types in a plane with two orthogonal variables and construct a fitness landscape which provides predictions on expected inflorescence types under contrasting environments and enables speculations on evolutionary changes of types.

http://hfspj.aip.org/doi/10.2976/1.2749445

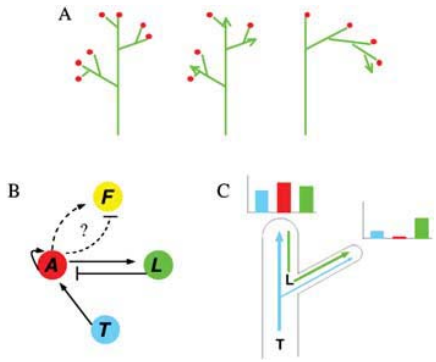

Figure 1. Alternative simple model that yields the three main types of inflorescence architectures. Types of inflorescences: panicles, racemes and cymes from left to right (A). Network underlying the proposed 'toy model' (B). Dynamics of the toy model, which also renders the inflorescence architectural types observed among real plants (C).

The paper of Prusinkiewicz and collaborators is certainly an innovative and important effort in the direction of integrating development and phenotypic evolutionary models. However, we argue that their model might be one of several ad-hoc models yielding inflorescence structures similar to those observed in nature. Thus, it might not be relevant for studying the generation of inflorescence architecture in plants, unless the proposed mechanism could be validated with a broader set of experimental evidence.

The most relevant trait that distinguishes racemose and cymose inflorescences is the relationship between location and age of floral meristems. In racemose inflorescences the youngest flowers are located in the distal part of the inflorescence, just beneath the indeterminate inflorescence main (apical) meristem. In contrast, the main meristem in cymose inflorescences is the first one to become determinate and turn into a flower, while the lateral meristems keep on branching reiterating the same rules: the main meristem terminates in a flower and the derived meristems branch. This results in the youngest flowers occurring farthest from the tip of the main stalk. Hence, any mechanism in which there is a structure that grows by branching will render racemes if, after a bifurcation, the younger tip turns into a floral (determinate) meristem, and the older one does not. In contrast, if the older tips turn into floral (determinate) meristems before the younger ones, cymes will be formed.

The "transient mechanism" proposed by Prusinkiewicz and collaborators is an algorithmical rewrite of the definition of cymes, racemes and panicles. The authors consider two kinds of meristems: young (B) and old meristems (A), and each kind requires different lengths of time to achieve flowering (T_A and T_B). When $T_A < T_B$, the older meristem within an inflorescence transits to flowering before derived lateral meristems, hence cymes are formed. When $T_A > T_B$, the younger lateral meristems turn into flowers before the apical meristem that maintains its indeterminacy, and so racemes are formed. Panicles are branched racemose inflorescences where the flower stalk divides into two or more parts, and therefore flowers are arranged in groups of two or more. When $T_A = T_B$, either the two resulting meristems of a branching event develop as floral meristems or they do not, giving rise to panicles.

Prusinkiewicz and collaborators attempt to use the transient mechanism to explain some experimental data for two floral meristem identity genes in *Arabidopsis, LFY* and *TFL1*. In order to do that, it is necessary to make some assumptions about the interactions between these two genes and the rest of the system's components. The authors recover activity patterns of *LFY* and *TFL1* that resemble those reported experimentally: *LFY* is expressed in young meristems, while *TFL1* is not, but in older meristems *LFY* is not expressed, while *TFL1* is. The authors conclude from this data that their model provides an explanation for the expression patterns observed in *Arabidopsis*. However, this is not the case, since they had assumed, in order to construct this version of the model, that *TFL1* activity is repressed and *LFY* expression is enhanced in young, but not in older, meristems. The model is fed with the observed expression patterns as assumptions, and therefore, does not explain them. In conclusion, what the authors call the transient mechanism is a description of the pattern and not an explanatory mechanism.

It is important to explore and explicitly model developmental processes because only then can we begin to understand how morphological patterns arise as a consequence of the variational properties of such processes, thus enabling the study of phenotypic evolution. However, a valid and useful hypothetical mechanism would have to generate observed patterns from interactions at a lower scale, otherwise, the model would be descriptive but it would not be explanatory. For instance, consider a chessboard pattern. We could propose that the "mechanism" that leads to it consists of alternately placing white and black squares. Such simple recipe would certainly yield the pattern, but would not provide any information regarding underlying proximal causes of the pattern because it only describes or "draws" it. Moreover, this hypothesis cannot be tested because the only way to achieve this would imply showing that such a pattern does not exist. However, this is impossible because this would be done once such patterns are documented and thus makes such a hypothesis scientifically futile. Alternatively, we could propose that the edges of white squares have a high affinity for the edges of black squares and *vice versa*. In such a system, a chessboard is the only two-dimensional pattern that is steady and stable if there are as many black as white squares. In this alternative mechanism we are explaining the pattern as a consequence of the properties of the system's components. This hypothetical mechanism could be tested experimentally, and hence it is scientifically useful. For example, we could test it by designing white squares with black edges and mix

them with "wild type" white squares and analyze the patterns achieved.

The lack of explanatory power of the morphogenetic mechanism for inflorescence types proposed in the paper by Prusinkiewicz and co-workers is not the main limitation of the paper. Indeed, the transient mechanism restrains developmental possibilities to a set that excludes many variants not found in nature. However, it seems likely that there are a number of different mechanisms that could recover a similar set of inflorescence two-dimensional architectural types. The necessary rules of all such alternative mechanisms would imply producing, after a branching event, exclusively one of three types of structures: (1) those in which older meristems develop into flowers and younger ones do not, (2) those in which younger meristems develop into flowers and older ones do not, and (3) those in which old and young meristems flower at the same time. These cases correspond to cymes, racemes and panicles, respectively.

Here we provide an example of a set of rules that, just as the transient mechanism, produces the observed types of inflorescences. Consider a hypothetical protein "*T*" that is synthesized in leaves and travels through the phloem towards shoot meristems. Some real molecules, such as the messenger RNA of the *FLOWERING LOCUS T (FT)* gene, show this kind of behavior (Wigge *et al.*, 2005). Let us also assume two proteins, "A" and "L," that are synthesized and are enabled to exert their function only in meristematic cells. *A* promotes its own synthesis, and that of *L*. *L* is a small protein that inhibits the synthesis of *A*, and diffuses to nearby meristems, where it can also repress the synthesis of *A*.

It is reasonable to consider that main (apical) meristems receive an amount of *T* that is higher than that received by derived (lateral) meristems, maybe because of differences in the width of vascular bundles in the main and derived shoots. Besides, we consider that T promotes *A* activity. Hence, in the apical meristems there will be a high level of *A* and *L* expression, and in nearby lateral meristems there will only be *L* activity, because of the diffusive and regulatory properties of *A* and *L*. This mechanism suffices to distinguish between apical and derived meristems. Now, let us also assume that the activity of *A* affects somehow the decision of turning into a floral meristem. If *A* represses flowering, then the apical meristems will remain indeterminate and lateral meristems, where there is *L* but not *A* activity, will turn into floral meristems; hence racemes will arise. In contrast, if *A* promotes flowering, then the apical meristems will flower and lateral meristems will keep on branching; therefore cymes will result. Different kinds of racemes and cymes could be obtained by tuning parameter values such as branching, decay and diffusion rates. We will refer to this hypothetical process of inflorescence development as our "toy model" (Fig. 1).

We are not claiming that the transient mechanism, by Prusinkiewicz and collaborators, is wrong and our toy mechanism corresponds to a more accurate description of inflorescence development. The point we are trying to make is that there might be several alternative mechanisms, such as the transient and toy ones, that could reproduce similar sets of patterns. Hence, the replication of patterns observed in nature does not guarantee that the mechanism that underlies it in the real world has been discovered or explained. More importantly, two different mechanisms that produce the same structures might generate different adaptive landscapes if the relative positions of the structures inside the landscape depend on the developmental model being postulated (see Fig. 2). Hence, development does not restrain evolution just by limiting the set of available forms, but also by making some structures more probable than others given an ancestral phenotype, therefore biasing the production of variation (Lewontin, 1974; Maynard-Smith *et al.*, 1985). It follows that not any mechanism that generates the set of observed structures will be useful to predict the proximity of phenotypes within the morphospace, and hence the likelihood of going from one to another one during the course of evolution.

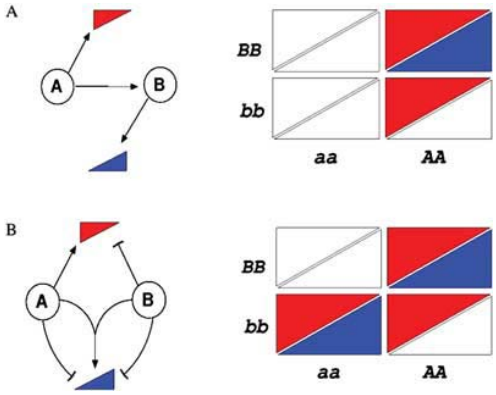

Figure 2. Different developmental models give rise to different morphospaces which may include the same types of "phenotypes" ("blue but no red" is not observed in either case), but these have different distributions in the morphospace and different transition probabilities among them. Blue and red triangles stand for the presence of a certain phenotypic trait. Whereas in the top panel a single genetic change suffices to transit from "red but no blue" phenotype to the "neither red nor blue" phenotype, two changes are required for the same change in the bottom panel. 'B' denotes a functional allele, and 'b' denotes a null nonfunctional allele of the B locus, whereas 'A' denotes a functional allele, and 'a' denotes a null nonfunctional allele of the A locus.

There are two nonexclusive and mutually supporting ways to discern among alternative developmental mechanisms. The first one is to ground the model on experimental evidence. This implies incorporating all interactions that have been supported experimentally, and not only taking *a priori* components which behaviors seem to be sufficient to generate similar patterns to those of structures found in nature. Then, such models can be validated by altering them *in silico* and addressing if such alterations recover observed

patterns in mutants. Recently, gene network models grounded on experimental data aiming at uncovering the logic of gene regulation, that underlies observed patterns of gene expression during development in plants and animals, have been proposed (e.g. von Dassow *et al.*, 2000; Albert and Othmer, 2003; Espinosa-Soto *et al.*, 2004; Jonsson *et al.*, 2005; Li *et al.*, 2006; Benítez *et al.*, 2007). However, these gene regulatory network models have not yet been explicitly incorporated in evolutionary models of the studied structures.

The second way to validate if a proposed mechanism is congruent with experimental data is to test if predicted behaviors derived from the models assumptions are indeed found in nature or can be induced in some way. For example, the model by Prusinkiewicz and collaborators creates patterns because each meristem is able to sense its own age and follow some rules accordingly. Hence, it is assumed that the fate of a meristem depends only on its intrinsic dynamics and not on interactions with other meristems or on communication mechanisms between this and the other parts of the plant. If the transient mechanism indeed operates in plants during inflorescence development, one would predict that it would be possible to find somatic mutations that could change the behavior of some meristems resulting in chimeric inflorescences. However, this might not be the case because communication mechanisms, such as diffusion of morphogens (Crick, 1970; Kim and Zambryski, 2005), active transport such as that of the phytohormone auxin; (Vieten *et al.*, 2007), signaling among cell layers (as in meristem patterning by WUS and CLV proteins; Doerner *et al.*, 2000) among many others, might constitute mechanisms important for coordinated responses in organisms. However, our skepticism is not sufficient to discard the transient mechanism, and experiments would be needed to this end.

In an attempt to validate their transient mechanism, the authors postulate a developmental model that incorporates the action of only two genes (*LFY* and *TFL*) on a continuous hypothetical variable (*veg*) which levels determine if shoots are vegetative or reproductive and may therefore represent a flower identity factor. *LFY* and *TFL1* are components of a larger and complex regulatory network of which many more components have been characterized already. To reduce all the known elements and regulatory interactions relevant for this system to a pair of interacting genes implies averaging, at least, the genetic context in which these genes are embedded. But biological systems are typically nonadditive and, therefore, the loss of information due to averaging is hard to justify, especially in a case where a large amount of experimental information is available. To elaborate on this issue, to us critical, we mention below just some of the elements and interactions that were mistakenly obviated in the developmental account presented by Prusinkiewicz and collaborators.

It has been shown that *LFY* activates different targets depending on its interactions with other factors (e.g., Parcy *et al.*, 2002) whose spatio-temporal expression patterns are far from trivial. Furthermore, *LFY* and *TFL1* themselves are regulated in a complex manner by other genes that are not included in the author's account, such as *APETALA1*. Importantly, the genes that regulate *TFL1* and *LFY* do not seem to be all acting in the same direction as plant age in their model and, more importantly, some of them are also regulated by *TFL1* or *LFY*, so they cannot be collapsed in one input with no feedback. *LFY*, *TFL1* and other floral and shoot meristem identity genes have been shown, instead, to hold complex interactions (Baurle and Dean, 2006; and references in Prusinkiewicz *et al.*, 2007).

In the transient mechanism the levels of a hypothetical substance, veg, define whether a meristem is reproductive or vegetative. However, we argue that the available experimental evidence suggests very different properties for a meristem identity crucial factor. For example, the MADS-box gene, *APETALA1*, is a floral identity marker, however veg may not represent *AP1* because the effect of *LFY* and *TFL1* over *AP1* is opposite to that proposed for veg and *AP1* does not change gradually as veg and *LFY* do. The existence of veg could be compatible with the existence of *AP1* if veg mediated the interactions between *LFY* and *AP1*, but this is not the case, since *LFY* binds directly to the *AP1* promoter (Wagner *et al.*, 1999). Furthermore, the activity of *AP1* does not depend only on *LFY* and *TFL1*, but also on other independent factors, such as *FT* and *FD* (Wigge *et al.*, 2005). Therefore, there is no reason to reduce the network by excluding *AP1* among other characterized genes that affect inflorescence architecture. Moreover, including *AP1* could avoid making up a new variable, such as *veg*, which behavior may not be monitored in real plants. Furthermore, other genes, such as *AGL24* seem to have an important role on the decision of vegetative *vs.* floral meristems in *Arabidopsis* inflorescences and has a feedback regulation with *LFY* (Yu *et al.*, 2004), being only one more of the factors that should be included in a model that addresses inflorescence patterning.

The unjustified reduction of a complex network constituted by several genetic and nongenetic elements, to a pair of interacting genes, leads to conclusions that might not be accurate. The evolution of development and phenotypical traits is a longstanding question in evolutionary biology. However, to address this issue by studying the variational properties of a developmental mechanism, this has to be independently validated. Otherwise, even if the evolutionary assumptions and formalisms are correct, and if the fitness value associated to each genotype is accurate, we can end up studying the evolutionary consequences of a mechanism that has never existed. Therefore, the inferred dynamics and transitions might be misleading. In conclusion, the transient mechanism, by Prusinkiewicz *et al.*, seems too simplistic and *ad hoc* to yield the observed types of inflorescence architectures.

In conclusion, we think that the paper has the merit of addressing how developmental processes constrict phenotypic morphospaces and how fitness values can be associated with every point in these morphospaces for a specific case. In doing so it attempts to integrate an architectural model, some molecular genetic data for the trait under analyses and a fitness landscape for evolutionary inferences under contrasting environments. Such an integrative approach could in turn be used to make predictions about which transitions among phenotypes are possible and more probable during evolution. The latter should be approached by evolutionary developmental biology studies in which transitions among types assuming alternative developmental models are mapped onto phylogenetic trees. This is certainly important, innovative and necessary in order to understand phenotypic evolution. However, a developmental approach to this issue should capture the complexity of the processes underlying morphogenetic restrictions, and be validated with data other than the expected results. The challenge remains ahead.

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REFERENCES

Albert, R, and Othmer, HG (2003). "The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in Drosophila melanogaster." *J. Theor. Biol.* **223**, 1–18.

Baurle, I, and Dean, C (2006). "The timing of developmental transitions in plants." *Cell* **125**(4), 655–64.

Benítez, M, Espinosa-Soto, C, Padilla-Longoria, P, Díaz, J, and Álvarez-Buylla, ER (2007). "Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in Arabidopsis root and leaf epidermis: a dynamic model." *Int. J. Dev. Biol.* **51**, 139–155.

Crick, FH (1970). "Diffusion in embryogenesis." *Nature (London)* **225**, 420–422.

Doerner, P (2000). "Plant stem cells: the only constant thing is change." *Curr. Biol.* **10**, R826–R829.

Espinosa-Soto, C, Padilla-Longoria, P, Alvarez-Buylla, E (2004). "A gene regulatory network model for cell-fate determination during Arabidopsis thaliana flower development that is robust and recovers experimental gene expression profiles." *Plant Cell* **16**, 2923–2939.

Hogeweg, P (2000). "Evolving mechanisms of morphogenesis: on the interplay between differential adhesion and cell differentiation." *J. Theor. Biol.* **203**, 317–333.

Jonsson, H, Heisler, M, Reddy, GV, Agrawal, V, Gor, V, Shapiro, BE, Mjolsness, E, Meyerowitz, EM (2005). "Modeling the organization of the WUSCHEL expression domain in the shoot apical meristem." *Bioinformatics* **211**, Suppl. 1, i232–240.

Kim, I, and Zambryski, PC (2005). "Cell-to-cell communication via plasmodesmata during Arabidopsis embryogenesis." *Curr. Opin. Plant Biol.* **8**, 1–7.

Lewontin, R (1974). The Genetic Basis of Evolutionary Change, Columbia University Press, NY.

Li, S, Assmann, SM, and Albert, R (2006). "Predicting essential components of signal transduction networks: a dynamic model of guard cell abscisic acid signaling." *PLoS Biol.* **4**(10), e312.

Maynard-Smith, J, Burian, R, Kauffman, S, Alberch, P, Campbell, J, Goodwin, B, Lande, R, Raup, D, and Wolpert, L (1985). "Developmental constraints and evolution: a perspective from the Mountain Lake Conference on development and evolution." *Q. Rev. Biol.* **60**, 265–287.

Parcy, F, Bomblies, K, and Weigel, D (2002). "Interaction of LEAFY, AGAMOUS and TERMINAL FLOWER1 in maintaining floral meristem identity in Arabidopsis." *Development* **129**, 2519–27.

Prusinkiewicz, P, Erasmus, Y, Lane, B Harder, LD, and Coen, E (2007). "Evolution and development of inflorescence architectures." *Science* (in press).

Vieten, A, Sauer, M, Brewer, PB, and Friml, J (2007). "Molecular and cellular aspects of auxin-transport-mediated development." *Trends Plant Sci.* **12**(4), 160–168.

von Dassow, G, Meir, E, Munro, EM, and Odell, GM (2000). "The segment polarity network is a robust developmental module." *Nature (London)* **406**, 188–193.

Wagner, D, Sablowski, RW, and Meyerowitz, EM (1999). "Transcriptional activation of APETALA1 by LEAFY." *Science* **285**, 582–584.

Wigge, PA, Kim, MC, Jaeger, KE, Busch, W, Schmid, M, Lohmann, JU, and Weigel, D (2005). "Integration of spatial and temporal information during floral induction in Arabidopsis." *Science* **309**, 1056–1059. Erratum in *Science* **312**, 1600.

Yu, H, Ito, T, Wellmet, F, and Meyerowitz, EM (2004). "Repression of *AGAMOUS-LIKE 24* is a crucial step in promoting flower development." *Nat. Genet.* **36**, 157–161.

Apéndice G

Floral morphogenesis: stochastic explorations of a gene network epigenetic landscape

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Floral Morphogenesis: Stochastic Explorations of a Gene Network Epigenetic Landscape

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Abstract

In contrast to the classical view of development as a preprogrammed and deterministic process, recent studies have demonstrated that stochastic perturbations of highly non-linear systems may underlie the emergence and stability of biological patterns. Herein, we address the question of whether noise contributes to the generation of the stereotypical temporal pattern in gene expression during flower development. We modeled the regulatory network of organ identity genes in the *Arabidopsis thaliana* flower as a stochastic system. This network has previously been shown to converge to ten fixed-point attractors, each with gene expression arrays that characterize inflorescence cells and primordial cells of sepals, petals, stamens, and carpels. The network used is binary, and the logical rules that govern its dynamics are grounded in experimental evidence. We introduced different levels of uncertainty in the updating rules of the network. Interestingly, for a level of noise of around 0.5–10%, the system exhibited a sequence of transitions among attractors that mimics the sequence of gene activation configurations observed in real flowers. We also implemented the gene regulatory network as a continuous system using the Glass model of differential equations, that can be considered as a first approximation of kinetic-reaction equations, but which are not necessarily equivalent to the Boolean model. Interestingly, the Glass dynamics recover a temporal sequence of attractors, that is qualitatively similar, although not identical, to that obtained using the Boolean model. Thus, time ordering in the emergence of cell-fate patterns is not an artifact of synchronous updating in the Boolean model. Therefore, our model provides a novel explanation for the emergence and robustness of the ubiquitous temporal pattern of floral organ specification. It also constitutes a new approach to understanding morphogenesis, providing predictions on the population dynamics of cells with different genetic configurations during development.

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Introduction

"*All [the] epistemological value of the theory of probability is based on this: That large scale random phenomena in their collective action create strict, non random regularity*". (From: B.V. Gnedenko and A.N. Kolmogorov, Limit Distributions for Sums of Independent Random Variables, Reading, Ma: Addison-Wesley, 1954).

The development of multicellular organisms consists of cell differentiation and spatiotemporal patterning. Since these processes arise from complex interactions among genetic and non-genetic elements, mathematical and computational models are useful to study the concerted action of these elements. Gene regulatory network (GRN) models, which are grounded in experimental data, have been able to recover fixed profiles of gene activation, that mimic those characterizing different cell types in both plants and animals (e.g., [1–3]). Such profiles correspond to the attractors of these networks, and have been interpreted as cell fates [4–7].

Some studies have explored cell-fate decisions by modeling transitions among attractors with stochastic gene regulatory networks (e.g. [8,9]); however, models grounded in experimental data that are able to recover patterns of cell-fate attainment for a particular living system are only now starting to appear. Herein, we attempted to construct an integrative model driven by noise that explores the patterns of temporal cell-fate attainment in the experimental plant, *Arabidopsis thaliana* (L.) Heynh.

In plants, morphogenesis takes place during the entire life cycle from groups of undifferentiated cells called meristems. Within meristems, cell fate is mostly determined by position rather than by cell lineage [10]. Flower meristems are formed from the flanks of

the inflorescence meristem, which is found at the apex of an *Arabidopsis thaliana* plant once it has reached a reproductive stage (Figures 1A and B). Early in flower development, a floral meristem is sequentially partitioned into four regions, from which the floral organ primordia are formed and eventually give rise to sepals in the outermost whorl, then to petals in the second whorl, stamens in the third, and carpels in the fourth whorl in the central part of the flower (Figures 1B and C). This spatio-temporal sequence is widely conserved among the quarter of a million flowering plant species [11]; however, the dynamic mechanisms underlying this robust pattern are not yet understood.

In this study, we used a previously characterized Boolean GRN, which converges to ten attractors (Figure 1), to explore the dynamics of cell-fate decisions during the early stages of flower development. The ten attractors correspond to the main cell types observed during early flower development, namely, meristematic

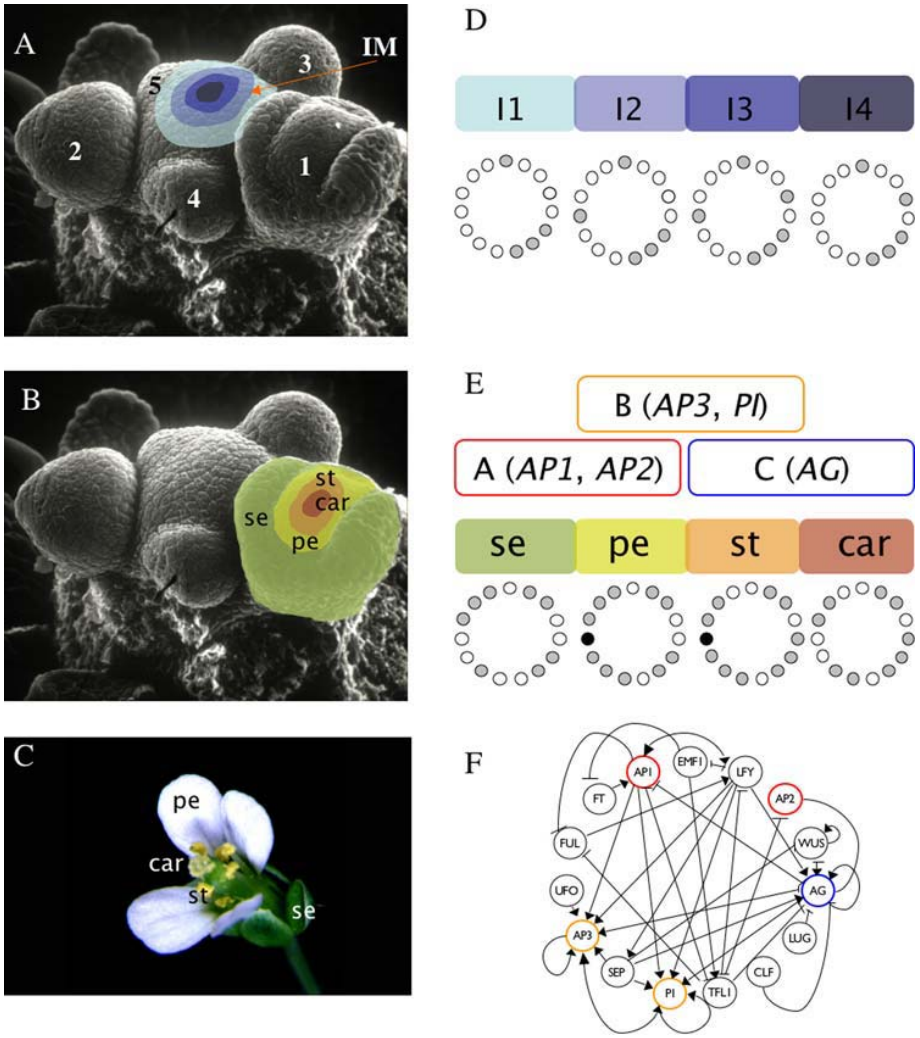

Figure 1. Flower development and gene network underlying primordial floral organ cell-fate determination in *Arabidopsis thaliana*. (A) The inflorescence meristem (IM in the Scanning Electron Micrography) is found at the apex of a reproductively mature plant. Within the IM, four regions can be distinguished. Interestingly, the experimentally observed gene activation configurations of each one of these regions are mimicked by the I1, I2, I3, and I4 attractors of the 15-gene GRN. Flower meristems arise in a helicoidal pattern from the flanks of the IM. The order in which floral meristems appear is indicated with numbers (1, oldest; 5, youngest). (B) Young flower meristems can be subdivided into four regions, each one containing the primordial cells that will eventually develop into the flower organs. In each floral meristem, the outermost region, which is first determined, will give rise to the sepal (se) primordium, the next to petals (pe) and finally, the primordial corresponding to stamens (st) and carpels (car) are determined in the center third and fourth whorls of the flower bud, respectively. (C) The mature flower of *Arabidopsis thaliana*. (D) I1, I2, I3, and I4 regions of the IM correspond to four of the attractors of the 15-gene GRN model. The expressed genes for each attractor are represented as gray circles, while the non-expressed genes correspond to white circles. (E) The other six attractors of the GRN model match gene expression profiles characteristic of sepal, petal (p1 and p2), stamen (st1 and st2), and carpel primordial cells. Black circles represent a gene (*UFO*) that can be either expressed or not expressed in the petal and stamen attractors, thus yielding two attractors for petal and stamen primordial cell-type. The gene activation profiles of the attractors recovered for the 15-gene GRN are congruent with the combinatorial activities of A, B, and C-type genes predicted by the ABC model of floral organ determination. See the Results section and [3,12] for details. (F) Gene regulatory network model underlying cell fate determination in the IM and the flower meristem. A-genes (red), B-genes (yellow), and C-genes (blue) from the ABC model are indicated in the network.

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cells of the inflorescence, which is itself partitioned into four regions (I1, I2, I3, and I4; Figures 1A and D), and sepal, petal (P1 and P2), stamen (S1 and S2), and carpel primordial cells within flower meristems (Figures 1B and E) [3,12]. This network was grounded in experimental data for 15 genes, wherein their interactions were formalized as logical functions. Among the 15 genes, five are grouped into three classes (A-type, B-type, and C-type), whose combinations are necessary for floral organ cell specification [13]. A-type genes (*AP1* and *AP2*) characterize sepal identity, A-type together with B-type (*AP3* and *PI*) petal identity, B-type and C-type (*AGAMOUS*) stamen identity, and the C-type gene (*AG*) alone for carpel primordia cell identity. The so-called ABC model describes such combinatorial activities during floral organ determination (Figures 1E and F) [13].

Different sets of initial conditions (basins of attraction) of the 15-gene regulatory network converge to the ABC-gene combinations necessary for floral organ determination [3,12] (Figures 1E and F); however, this deterministic GRN does not enable studies of the transitions among the attractors. In this study, we investigated the temporal sequence with which attractors are visited in this GRN when noise or random perturbations to the output of the updating rules drive the system from one attractor to any other.

The obtained results demonstrate that noise alone is able to drive transitions among attractors with temporal patterns that mimic the sequence with which ABC-genes are activated (first A genes, then B genes, and finally the C gene) during early flower development [13]. These results are in line with the finding that the GRN in question is a robust developmental module that is widely conserved among flowering plant species [3]. Furthermore, the temporal cell-fate pattern during early stages of flower development seems to emerge from such a robust network in the presence of noisy perturbations. The results presented herein support the idea that random fluctuations in a system may be important for physiological adaptation, plasticity, and cell differentiation (examples in: [14–24]).

Results

A stochastic Boolean model of the GRN enables the study of transitions among network attractors

We first present the results obtained from the Boolean model of the GRN, and in the next section, we present the equivalent results obtained from a continuous model. The Boolean approach focuses on the state of genes' expression rather than on the concentration of their products. Thus, each gene in the network is represented by a Boolean variable x that takes the value $x = 1$, if the corresponding gene is expressed, and the value $x = 0$, if it is not expressed. The state of expression of the genes in the entire network (herein, configurations of the GRN, which correspond to "dynamic state of the network" used by some authors), is then represented by a vector with the set of Boolean variables $\{x_1,x_2,\ldots,x_N\}$, where x_n is the state of expression of the n^{th} gene and N is the total number of genes in the network. The state of expression of each gene changes in time according to the dynamic equation:

$$x_n(t+\tau) = F_n(x_{n_1}(t),x_{n_2}(t),\ldots,x_{n_k}(t)). \quad (1)$$

In the above equation, $\{x_{n_1}(t),x_{n_2}(t),\ldots,x_{n_k}(t)\}$ are the regulators of the gene x_n, and F_n is a Boolean function, also called a logical rule, which is constructed according to the combinatorial action of the regulators of x_n. The additional parameter τ is a measure of the *relaxation time*, namely, of the time that it takes for a gene to change its state of expression under a change in the expression of its regulators. In the Boolean model, it is common to take $\tau = 1$. Each gene in the network has its own associated Boolean function. This particular GRN includes 15 genes (Figure 1) whose logical functions are grounded in experimental biological data, as explained in [3]. The updated truth tables used here are available in [12].

Note that the dynamics given by Eq. (1) is deterministic: For a given set of Boolean functions, the configuration of the network at time t completely determines the configuration of the network at the next time step $t+\tau$. Also note that since the number of dynamic states or configurations of the network is finite ($\Omega = 2^N$), under the dynamics given in Eq. (1), the network will eventually come back to a previously visited configuration, after which the network enters into a periodic pattern of expression. Such a periodic pattern is called an *attractor*, and all the initial configurations that eventually fall into that attractor constitute its *basin of attraction*. The deterministic version of the Boolean GRN modeled here recovered 10 fixed point attractors, each with a period equal to one, implying that the GRN remains in one of the 10 fixed 15-gene configurations after it reaches one of them.

Therefore, in the deterministic model defined in Eq. (1), once the system reaches an attractor, it remains there for all subsequent iterations; however, if noise is introduced into the logical rules, there is a finite probability for the system to "jump" from one basin of attraction to another. Our central aim herein was to address whether noisy perturbations of the logical rules in *A. thaliana* GRN are sufficient to recover the observed sequences of transitions among attractors (i.e., gene activity configurations characteristic of the primordial cell types within the floral meristem) during the development of this particular biological system.

The ten attractors of the 15-node GRN used here are as follows (Figure 1): Four corresponding to the four regions of the inflorescence meristem (I1, I2, I3, and I4), and six to the four floral organ primordial cells within the flower meristem (S, P1, P2, S1, S2, and C). The two attractors corresponding to petals (P1 and P2) are identical except for the state of activation of the *UFO* gene, and the same holds for the two stamen attractors (S1 and S2).

In the simulations of the stochastic versions of the GRN presented in this work, we did not consider the inflorescence attractors (I1–I4) because they are substantially separated from the floral primordia attractors. The distance between the two sets of attractors (inflorescence and floral) is clearly depicted by the way they are grouped in a phenogram (Figure 2). This is a branching diagram that groups entities according to their similarity (see Methods). The inflorescence meristem and floral organ primordia attractors cluster into two clearly distinct groups (Figure 2). Indeed, in simulations that considered all of the attractors, we found that, for a wide range of noise levels, the system never leaped out of the inflorescence attractors. On the other hand, when large noise magnitudes were considered, the system went from the inflorescence attractors to the carpel or stamen attractors, without visiting the sepal and petal attractors. Dismissing the I1–I4 attractors in the simulations allows for a better exploration of the temporal pattern in which the attractors corresponding to each of the four floral organ primordial cells are attained.

We used the GRN depicted in Figure 1 to examine which of the attractors (S, P1, P2, S1, S2, and C) the system is most likely to reach when it is initialized at a particular attractor and then is driven by noise to a different one. In order to obtain the transition probabilities among the different attractors (i.e., the entries of the so-called Markov matrix, see the detailed description below), the possible initial configurations of the system were exhaustively explored. Given any possible configuration (defined by an array of 15 entries with zeros and ones representing the activation states of

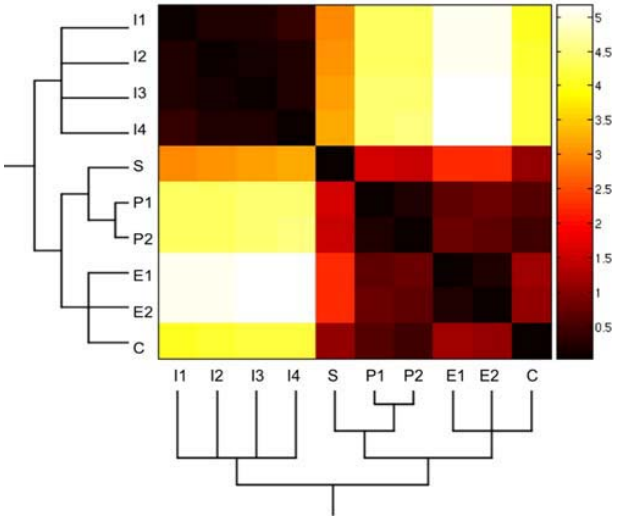

Figure 2. Heat map of the similarity matrix among the ten attractors of the GRN. A strict consensus phenogram was obtained for the GRN attractors (vectors of zeros and ones) by using the Manhattan distance similarity index (see Methods). This phenogram is shown below the attractors that are ordered along the X and Y axes of the heat map. Attractors that group together had the highest similarity indexes between them (i.e. the lowest Manhattan distance). Color scale: darker colors indicate more similar, while lighter ones indicate more different attractors in the pairs compared.
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the genes), the system was updated every iteration step according to the deterministic logical rules [12] with an error probability η. In other words, at each time step, each gene "disobeys" its Boolean function with a probability η, such that the dynamic rule in the presence of noise can be given by:

$$x_n(t+\tau)=\begin{cases} F_n(t) & \text{with prob.} \quad 1-\eta \\ 1-F_n(t) & \text{with prob.} \quad \eta \end{cases} \qquad (2)$$

Note that the above equation reduces to Eq. (1) for $\eta=0$. [In order to simplify the notation, we have written just $F_n(t)$ instead of $F_n(x_{n_1}(t),x_{n_2}(t),\ldots,x_{n_k}(t))$.] These perturbations are applied independently and individually to each gene at each iteration.

If, after applying noise in one time step, the system remains in the same attractor or the same basin of attraction that it was before the noise was applied, one count is added to the main diagonal in the entry of the Markov matrix corresponding to that basin of attraction. If the configuration ended up in a different basin, a count is added to the row corresponding to the recipient basin in the Markov matrix (Table 1). This was repeated 10000 times for each of the $\Omega=2^N$ possible initial conditions. The number of realizations was fixed to a considerably larger number than that at which the matrix entries become stable (data not shown). The transition probabilities $P(n|m)$ of the Markov matrix (Table 1) give the probability that a network in attractor m jumps to attractor n in the presence of noise, and are calculated by dividing the number of counts in each matrix entry by the total number of configurations that started in the corresponding matrix row.

Since we wanted to find the most probable sequence of transitions among the attractors representing the various cell types, we followed the changes in the probability of reaching a certain attractor throughout time given that the system was initialized in a particular attractor at time $t=0$ (see Figure 3). In order to achieve this, note that the Markov matrix (herein denoted as **M**) in Table 1 contains the conditional probabilities $P(n|m)$ of reaching attractor n at time $t+\tau$, given that the system is at attractor m at time t. In order to obtain the temporal sequence in which attractors are most likely reached, it is necessary to repeatedly multiply the Markov matrix **M** by the vector $\vec{v}(t)$, whose entries contain the fraction of cells at each attractor in a given population at time t. In other words, $\vec{v}(t)=(v_1(t),v_2(t),\ldots,v_m(t))$, where $v_1(t)$ is the fraction of cells in the population whose configurations at time t are in the basin of attraction of the first attractor, $v_2(t)$ is the fraction of cells at time t in the basin of attraction of the second attractor, and so on. Starting out from a population with a given distribution $\vec{v}(0)$ of cells among the attractors, the distribution of cells at time t is given by: $\vec{v}(t)=\vec{v}(0)[\mathbf{M}]^t$.

Table 1. Markov matrix.

	sep	pe1	pe2	st1	st2	car
sep	0.939395	0.001943	0.009571	0.000083	0.00049	0.048517
pe1	0.036925	0.904162	0.00925	0.0339	0.000488	0.015275
pe2	0.009067	0.000464	0.941609	0.000024	0.048374	0.000461
st1	0.000084	0.001893	0.00002	0.936514	0.00996	0.05153
st2	0.00002	0.000001	0.002074	0.000356	0.987953	0.009597
car	0.002045	0.000034	0.00002	0.001951	0.01002	0.98593

Matrix of transition probabilities among all possible pairs of attractors. The entries of each column in this matrix correspond to the probabilities *P(n|m)* of reaching attractor n, given that the system is at attractor m at time *t = 0* (see Results and Methods, noise magnitude used for this case is 1%).
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Since we did not consider the four inflorescence attractors, only six attractors are involved in the dynamics. Therefore, **M** is a 6×6 matrix and $\vec{v}$ is a 6-dimensional vector. We also assumed that the total number of cells in the population always remains constant; hence, the sum of the six components of $\vec{v}$ must sum to 100 (there are no "probability leaks" because transitions to the inflorescence attractors are extremely rare for the error levels used).

It is worth noting that the different attractors have basins of vastly different sizes. For instance, the basins of attraction of sepals and petals are very small in comparison to those of stamens and carpels. Therefore, the absolute probabilities for the attractors of sepals and petals are inevitably smaller than those of stamens and carpels; hence, in order to clearly observe the time at which each attractor attains its maximum probability, we divided each absolute probability value by the maximum of each attractor's curve, and plotted the relative probabilities for each attractor probability distribution. Note that since each curve was normalized in relation to its own maxima, the probabilities in these graphs no longer add up to 1 at every moment.

It is important to notice that although the Markov matrix **M** provides information about the probability of going from any attractor m at time t to any attractor n at time $t+\tau$, this matrix alone is not sufficient to derive the most probable sequence of transitions among attractors. The latter is only evident when the matrix **M** is recursively multiplied by the vector $\vec{v}$ containing the fraction of cells per attractor, ideally until the system reaches a steady probability distribution.

Since sepal cells are the first to attain their fate in flower development, we used an initial vector $\vec{v}(0)$ with $v_1(0)=100$ and $v_m(0)=0$ in all of the other entries (the first entry corresponds to the sepal configuration). Thus, initially, all of the population of cells

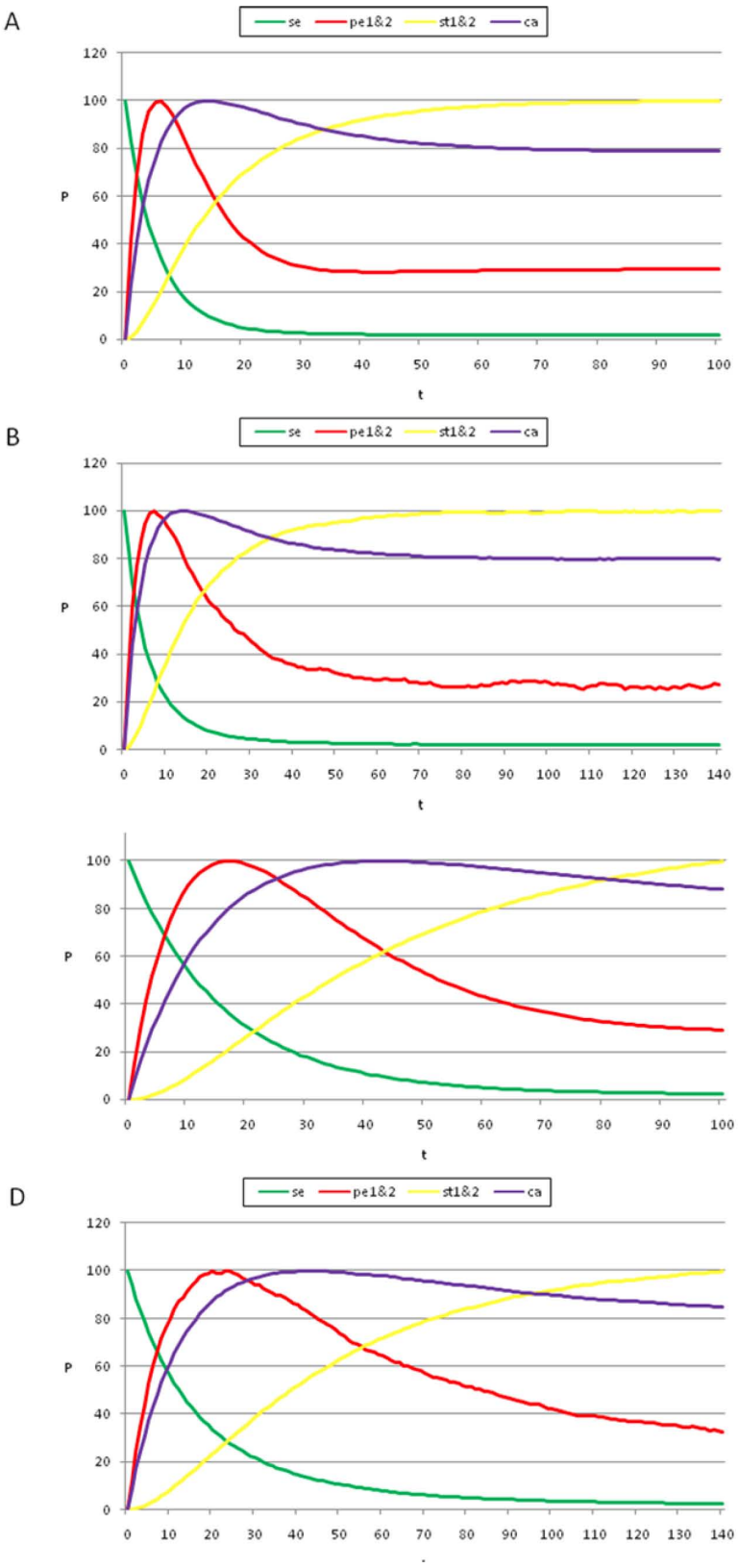

Figure 3. Temporal sequence of cell-fate attainment patterns under the Boolean dynamics with noise. Maximum relative probability ("Y" axis) of attaining each attractor, as a function of iteration number or time ("X" axis). (A) Probability of attaining each attractor (i.e., cell type) obtained by multiplying the Markov matrix **M** by a population vector $\vec{v}$ initialized at the sepal attractor. The error probability in computing this graph

was $\eta = 0.03$. The most probable sequence of cell attainment is: Sepals, petals, carpels, and stamens. (B) Probability of attaining each attractor (i.e., cell type) at each iteration when 80000 randomly chosen ''sepal'' configurations were selected and followed for 140 steps. Noise was introduced in the updating of each gene independently, with a $\eta = 0.03$ probability at each iteration. The probabilities for the petal (p) and stamen (st) attractors correspond to the sum of p1+p2 and st1+st2, respectively. All maxima correspond to 100 because each absolute probability value was divided by the maximum of each attractor's curve (see Results and Methods). Equivalent graphs to those in (A) and (B) for $\eta = 0.01$ are shown in (C) and (D), respectively.
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within a floral primordia is in the sepal attractor. We then followed the changes in the probability of reaching each one of the other attractors over time, given that the entire system started in the sepal configuration (see Figure 3A). Every attractor has a maximum or peak in the probability of being reached at particular times. This maximum corresponds to the moment at which the corresponding primordial cell fate is most likely.

The use of the probability peaks to determine the time at which each cell multigenic configuration is most probable follows the standard reasoning in deriving maximum likelihood estimators in statistics [25]. The time at which the probability peak appears corresponds to the maximum of the associated transition probability for that particular attractor. The order of appearance of the peaks shown in Figure 3 matches the order of formation of the maxima of the transition probabilities. Recall that when using the maximum likelihood methodology [25], the main assumption is that the set of real data is precisely observed because they are more likely to happen than other possible data sets. In other words, they maximize the probability of being observed among all possible samples of the same size. Conversely, if we want to know when a specific event is more likely to happen, the most natural assumption is that it will be at a maximum of the corresponding probability distribution. This is precisely what we claim based on the graphs of the frequencies of visits to each attractor. Also notice that the locations of the maxima are not affected by normalization.

This interpretation hence implies that, given that a particular attractor will be reached (i.e. that a specific event will occur), it is natural to assume that the most likely time for it to occur is when the probability of reaching that particular attractor is maximal. Therefore, we propose that the temporal sequence in which attractors are attained will correspond to the sequence in which their maximum probabilities are reached.

A related important issue has to do with the interpretation of the transition probabilities. There are at least two possibilities that are consistent with the traditional approaches in statistical studies of collective behavior [26]. First, it is possible to consider that each agent (in this case, a single cell) will spend some time at each equilibrium configuration and then will jump to another with a certain probability. This would imply that each cell transits through different configurations. In our case, for example, a particular cell might attain a sepal primordia identity, then transit to a petal primordial cell, then to a stamen primordial cell, and finally to a carpel primordial cell. An alternative interpretation is that, from a given initial population of cells, the number of individual cells at a certain attractor at any given time, is proportional to the transition probability of reaching that particular attractor.

These two interpretations are equivalent or are assumed to be so (ergodic hypothesis) in many applications of statistical physics. This is often summarized by saying that averaging quantities in time is the same as averaging them in space [26]; however, in the case we have considered here, the second interpretation seems more appropriate. Future experimental studies that actually follow gene configurations over time at the individual cellular level will directly test these two alternative interpretations. For now, if we accept the overall population of undifferentiated cells in the floral meristem as our system, it is consistent to assume that the proportion of them reaching a particular configuration will be in accordance with the transition probabilities.

Therefore, we present a stochastic GRN that can be interpreted as a model of cell population dynamics. This model describes the dynamics of cells within the flower meristem, in which different fractions of cells sequentially attain distinct configurations. Therefore, it does not imply that individual cells transit through different identities or configurations, but rather that once in a floral meristem, one set of cells attains a certain identity first (sepal primordia) and then, from the remaining cells, another fraction attains a second cell fate (petal primordia), and so on, until all the cells in the floral primordium have reached an identity corresponding to each of the four floral organ primordia. Later in development, primordia will grow and differentiate to form the four floral mature organs: Sepals, petals, stamens, and carpels. The latter events are regulated by other GRNs. We explored whether the observed dynamics of cell-fate attainment can be recovered by the stochastic Boolean GRN model presented here.

Simulated temporal transitions among attractors (cell types) mimicked the sequence in which A, B, and C genes are expressed in real flower meristems

By following the procedure presented above, we found that, by starting from the gene configuration associated with sepal primordial cells ($t = 0$ in Figures 3 A and C), the next maximum probability was observed in the petal curves, P1 plus P2 ($t = 18$ in Figure 3A). Afterwards, the peaks for the probability of attaining first the carpel and then the stamen (S1 plus S2) identity appeared ($t \approx 45$, $t \approx 100$ in Figures 3A and C). Interestingly, the same sequence was observed when applying a range of noise magnitudes from 0.5 to 10%; however, the peaks corresponding to the stamen and carpel cell fates became closer, almost simultaneous, as the noise magnitudes increased (compare Figures 3A–C). Nonetheless, it is noteworthy that the probability peak of the carpel configuration appeared before the peak of the stamen configuration.

The sequence resulting from the aforementioned model mimics the observed temporal pattern for A, B, and C gene expression: A-genes are expressed first, followed by B-genes, and finally by the C-gene [27,28]. Furthermore, our model predicts that the gene configuration characteristic of carpels most probably appears before that corresponding to stamens during early flower development. This would, in fact, be the case if the C gene was first expressed in the flower center and then its expression expanded to the peripheral whorls. This should be tested experimentally by gathering data on the population dynamics of cells with different genetic configurations during early stages of flower development.

It is noteworthy that, among all of the tested noise levels, the only non-trivial temporal sequence of A, B, and C gene combinations recovered was: A, then AB, then C and finally BC. Although the latter two appeared almost simultaneously as error magnitudes used increased. This sequence is congruent with the ABC temporal pattern in *Arabidopsis thaliana* (Figures 3A and C) in which the A genes are turned on first, then the B and finally the C genes; hence BC and C combinations are defined at the same time. The trivial behaviors are: i) remaining in the initial configuration forever, and ii) transitions depending only on the size of the basins of attraction

(i.e., the system behaves according to only noise). If the magnitude of the noise is increased, for example to 50%, the system goes from sepal to stamen1 or carpel configurations directly. This is because the basins of attraction corresponding to petals are very small in comparison to those of stamens and carpels.

In addition to the Markov matrix approach, we also performed simulations by directly following trajectories starting in randomly chosen configurations from the basin of attraction corresponding to the "sepal" configuration. We followed each of 80000 such configurations for 140 iterations in order to compute the probabilities of directly attaining each attractor at each iteration (see Methods). This latter simulation is directly comparable to that performed for the Glass system discussed in the following section. It is noteworthy that the sequence of probability peaks we found for each attractor over time is the same as the one that we had obtained using the Markov Matrix approach: Sepal, petal, carpel, and stamen (Figures 3B and D).

Continuous GRN model with noise

In order to develop a continuous model based on the differential equations of the flower development GRN considered here, one would need to know all of the kinetic reaction constants, promoter affinities, degradation rates, and many other parameters involved in the dynamics. To the best of our knowledge, these have not yet been identified; however, a first step towards a continuous description of this GRN is to implement the Glass dynamics in the network [29]. This can be accomplished by considering the parameter τ in Eq. (1) as a small quantity, and expanding the left-hand side of that equation to the first order in powers of τ, which gives:

$$\frac{dx_n(t)}{dt} = \alpha[F_n(x_{n_1}(t), x_{n_2}(t), \ldots, x_{n_k}(t)) - x_n(t)], \qquad (3)$$

where $\alpha = 1/\tau$ is a measure of the "relaxation" time in the gene expression profile. Although the above equation is formally correct, it has the problem that the Boolean function F_n on the right-hand side has to be evaluated using discrete variables, whereas the derivative on the left-hand side treats the x_n's as continuous variables. Therefore, each continuous variable x_n has to be transformed into a discrete variable in order to evaluate the Boolean function. This is accomplished by introducing the discrete variables $\hat{x}_n$ defined as:

$$\hat{x}_n = H(x_n - \theta_n), \qquad (4)$$

where θ_n is a threshold, and $H(x)$ is the Heaviside function. ($H(x) = 1$ if $x \geq 0$ and $H(x) = 0$ if $x < 0$). Thus, each continuous variable x_n, representing the *level of expression* of a given gene, has an associated discrete variable $\hat{x}_n$ that represents the *state of expression* of that gene: "ON" if x_n is above the threshold θ_n, and "OFF" if x_n is below θ_n. In principle, each gene x_n could have its own threshold θ_n. Our simulations show that the results are qualitatively the same if we randomly assign the thresholds in the interval $\theta_n \in [0.35, 0.65]$. Thus, in what follows, we fixed $\theta_n = 0.5$ for all of the genes.

The continuous piece-wise linear Glass dynamics of the network can thus be given by:

$$\frac{dx_n(t)}{dt} = \alpha[F_n(\hat{x}_{n_1}(t), \hat{x}_{n_2}(t), \ldots, \hat{x}_{n_k}(t)) - x_n(t)] \qquad (5)$$

We will refer to the set of continuous values $\{x_1(t), x_2(t), \ldots, x_N(t)\}$ as the *microscopic configuration* of the network, and to the set of corresponding discrete values $\{\hat{x}_1(t), \hat{x}_2(t), \ldots, \hat{x}_N(t)\}$ as the *Boolean* configuration of the network. Note that there are infinitely many microscopic configurations compatible with the same Boolean configuration. Finally, we will refer to the dynamics generated by Eq. (5) as *Glass dynamics*.

It has been pointed out that the discrete model given in Eq. (1) and the corresponding continuous piece-wise linear model defined in Eq. (5) are not necessarily equivalent, since the attractors of the two models can be different, even when the Boolean functions F_n are the same in both cases. Nonetheless, our numerical simulations show that for the *A. thaliana* network, the Glass dynamics generate exactly the same ten point attractors obtained in the Boolean model, and only those ten attractors. Therefore, from now on, we will make no distinction between the attractors of the Boolean model and the attractors of the continuous model, referring to them simply as the attractors of the floral GRN.

Even when the Boolean dynamics and the Glass dynamics produce the same ten attractors, their basins of attraction do change from one model to the other. This is so because two different initial microscopic configurations that correspond to the same Boolean configuration may end up in two different attractors under the Glass dynamics. In order to show that this is indeed the case, for each of the $\Omega = 2^N$ Boolean configurations of the network, we probed 10,000 compatible microscopic configurations. We evolved these 10,000 microscopic configurations in time until an attractor was reached, and determined the configuration in which the network fell. Figure 4 depicts in a color map the probability $P_G(n|m)$ that the network ends up in attractor n under the Glass dynamics, given that it started in a microscopic configuration whose corresponding Boolean configuration was in the basin of attraction of attractor m. As can be seen, the highest probabilities lie along the diagonal; however, the non-vanishing off-diagonal elements indicate that two different microscopic configurationss corresponding to the same Boolean configuration may end up in two different attractors.

On the other hand, Table 2 shows the fractional sizes of the basins of attraction in both the Boolean and the continuous

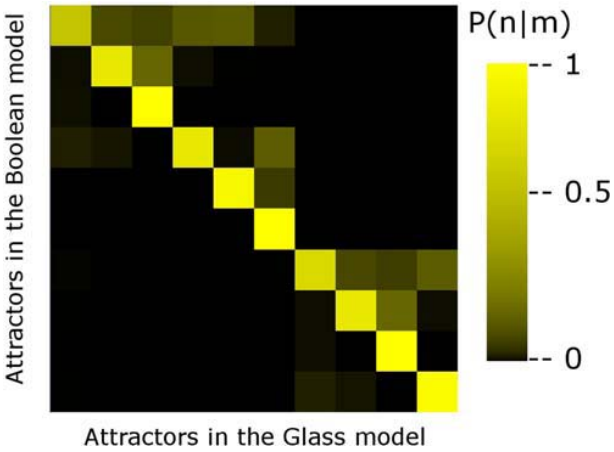

Figure 4. Changes in the basins of attraction of the continuous model with respect to the Boolean model. Color map of the probability $P(n|m)$ that a microscopic configuration whose associated Boolean configuration belongs to the basin of attraction of attractor m, ends up in attractor n using Glass dynamics. Note that the main transitions occur along the diagonal where attractors are reached by both dynamics (Boolean and Glass); however, the non-diagonal elements indicate that two microscopic configurations that correspond to the same Boolean configuration may end up in different attractors.
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Table 2. Basins of attraction.

Attractor	Boolean Dynamics	Glass Dynamics
Inflorescence 1	0.0156	0.0500
Inflorescence 2	0.0156	0.0500
Inflorescence 3	0.0078	0.0380
Inflorescence 4	0.0078	0.0381
Carpel	0.4404	0.2622
Sepal	0.0185	0.0670
Stamen 1	0.4570	0.3331
Stamen 2	0.0166	0.0710
Petal 1	0.0195	0.0786
Petal 2	0.000976	0.0116

This table shows the fractional sizes of the basins of attraction in the Boolean and Glass models. The data for the Glass dynamics were obtained by sampling 10,000 microscopic configurations for each of the $\Omega = 2^N$ Boolean configurations, and by counting the frequency with which these microscopic configurations end up in each of the ten attractors.
doi:10.1371/journal.pone.0003626.t002

models. It is apparent from this table that, when passing from the Boolean to the continuous description, the largest basins of attraction (carpel and stamen1) lose about 30 to 40 percent of their configurations, which are redistributed among the smaller basins of attraction. Thus, even when the predicted cell types (attractors) are the same in the two models, the basins of attraction are not.

The stochastic continuous model of the GRN yields a cell-fate attainment sequence similar to the Boolean stochastic model

In order to implement noise in the continuous model, we followed a procedure similar to the one indicated in Eq. (2); namely, with a probability η, each gene will disobey its Boolean function F_n, replacing it by $1-F_n$; however, since the system in this case is governed by differential equations, this "perturbation" will occur during a *finite time interval* Δt_p, rather than being instantaneous. In other words, if at time t one particular gene x_n is perturbed and chosen to disobey its Boolean function, then from time t to time $t+\Delta t_p$ its state will not be determined by Eq. (5), but rather by the equation:

$$\frac{dx_n(t)}{dt} = \alpha[1 - F_n(\hat{x}_{n_1}(t), \hat{x}_{n_2}(t), \ldots, \hat{x}_{n_k}(t)) - x_n(t)] \qquad (6)$$

After the time interval Δt_p, the state of x_n will be determined again by Eq. (5), and a new set of "disobeying genes" will be chosen. We will call these disobeying genes the *perturbed genes*.

We have to choose the value of Δt_p in such a way that the gene has enough time to relax to its new state after the perturbation has been produced. In other words, Δt_p has to be larger (or at least of the same order of magnitude) than the relaxation time $\tau = \alpha^{-1}$ appearing in Eq. (5). Figure 5 shows two typical noisy realizations of the temporal evolution of a particular $x_n(t)$ as a function of time, for two different choices of τ and Δt_p: One for $\Delta t_p = 2.5$ and $\tau = 1$ (black curve), and the other for $\Delta t_p = 2.5$ and $\tau = 1/20$ (red curve). The two realizations started out from the same initial conditions, and underwent the same set of perturbations. The only difference was the value of τ. As can be seen from this figure, the trajectories are qualitatively the same as long as $\Delta t_p > \tau$. In what follows, we selected $\Delta t_p = 2.5$ or 1 (Figures 6A and B, respectively), and $\tau = 1$ to simulate Glass dynamics with noise (see methods for further details).

In order to determine the cell-fate attainment patterns in the *A. thaliana* network under Glass dynamics with noise, we analyzed the transitions between attractors over time in a population of 80 000 cells subject to the perturbations described above. At time $t = 0$, all of the cells were initialized in different random microscopic configurations corresponding to the sepal basin of attraction. In every cell, each gene was independently chosen to be perturbed with a probability $\eta = 0.03$. The non-perturbed genes then evolved in time according to Eq. (5), whereas the perturbed genes evolved following Eq. (6). After a time interval $\Delta t_p = 2.5$ or 1 (for Figures 6A and B, respectively), a new set of perturbed genes in the entire population was chosen again, and so on.

At each unit of time, we looked at the microscopic configuration of each cell and determined to which attractor this microscopic configuration would have evolved in the absence of perturbations. This allowed us to associate a given attractor at each unit of time to each cell configuration. The results of this simulation are

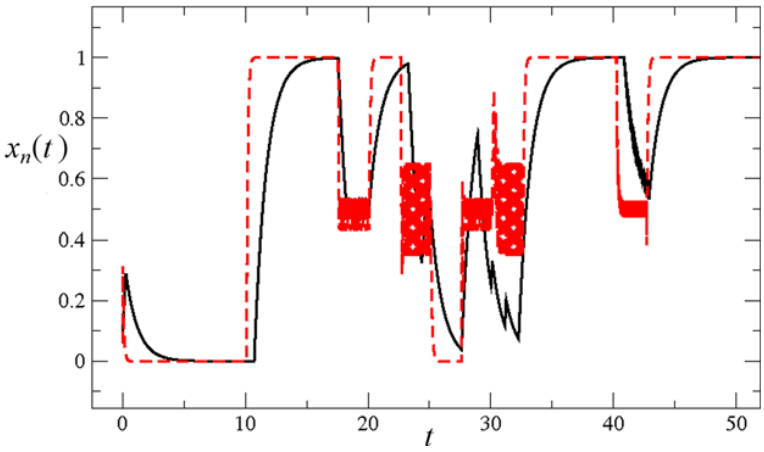

Figure 5. Effects of the choice of the relaxation time on Glass dynamics with noise. Two typical realizations of Glass dynamics for a given gene x_n showing that the choices of the relaxation time τ and the perturbation time Δt_p do not affect the qualitative dynamics, so long as $\Delta t_p > \tau$. Both trajectories started from the same initial conditions, and were followed through the same set of perturbations. The black trajectory corresponds to $\Delta t_p = 2.5$ and $\tau = 1$, whereas the red trajectory corresponds to $\Delta t_p = 2.5$ and $\tau = 1/20$.
doi:10.1371/journal.pone.0003626.g005

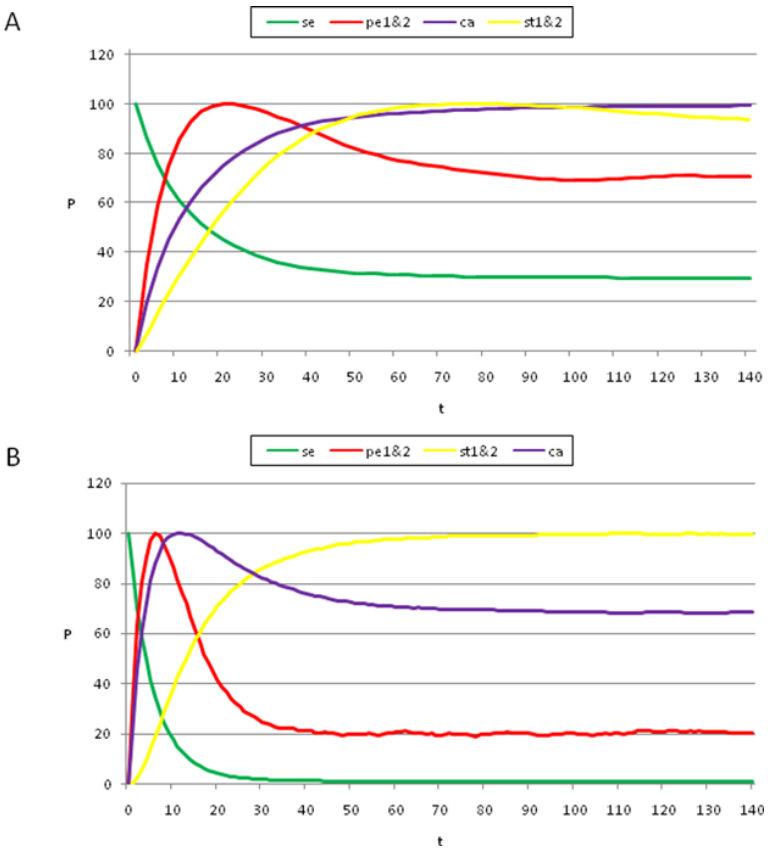

Figure 6. Temporal sequence of cell-fate attainment patterns under the Glass dynamics with noise. Maximum relative probability ("Y" axis) of attaining each attractor as a function of iteration number or time ("X" axis). (A) The maxima of the cell-fate curves are attained in a particular sequence in time, which in this case is sepal, petal, stamen, and carpel. Parameters used: dt = 0.01, $\tau = 1$, and $\Delta t_p = 2.5$. (B) When the simulations mimic the Boolean case (dt = 1, $\tau = 1$ and $\Delta t_p = 1$; see Results and Methods), a temporal pattern identical to that of the Boolean dynamics was obtained, with a sequence of sepal, petal, carpel and stamen. The noise used in both cases was $\eta = 0.03$. Although the Boolean and Glass dynamics need not coincide in general, for the case of the *A. thaliana* GRN, both models provide similar predictions. Simulations show that the order of emergence of the stamen and carpel maxima, as compared to the Boolean model, may depend on the precise values of the kinetic constants.
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reported in Figure 6, which shows the evolution in time of the population of cells, and shows how the cells redistribute among the sepal, petal, stamen, and carpel attractors. Similar patterns were recovered for other values of noise (data not shown), ranging between $\eta = 0.005$ and $\eta = 0.1$, as well as for other values of Δt_p.

As can be observed in Figure 6, the results obtained using Glass dynamics are analogous to those obtained for the Boolean model, in that the addition of noise to the dynamics produces the emergence of cell-fate attainment patterns in a population of cells in a specific temporal order. Thus, the use of the Glass model, based on piece-wise linear differential equations, reveals that the time ordering in the emergence of the cell-fate patterns is not an artifact of the synchronous updating in the Boolean model; however, the stamen and carpel peaks are reversed in time between the Boolean and Glass models (Figure 3 vs. Figure 6A).

In real flowers, A genes are first "ON", followed by the B genes that turn "ON," thus defining the A (sepal) to AB (petal) transition. This is recovered by both models (Figures 3 and 6), and is observed in real flowers. The C genes then turn "ON," and hence, the BC (stamens) and C (carpels) configurations are defined at the same time. While the Boolean dynamics predict that the carpel primordia cell fate (C alone) will be attained before that of the stamen (BC), in the Glass model, these two are reversed (Figure 3 vs. Figure 6A). Interestingly, when this model is simulated to mimic the Boolean model (Figure 6B), both systems recover the same sequence: "Sepal-petal-carpel-stamen" (Figure 3 vs. Figure 6B) and in both cases the time at which stamen and carpel configurations are determined converge as noise levels are increased. Detailed experimental data on the precise spatio-temporal dynamics of the gene activation profiles of cells in the developing flower meristem are needed to test which of the two peaks is observed first in real floral buds. Such data will also be useful to determine which of the two models predicts the most realistic frequency distributions of cell types over time. The latter will be related to the relative sizes of the basins of attraction.

Glass system simulations indicate that the order of appearance of the two peaks (stamen or carpel) may depend on the precise values of the reaction-kinetic constants and degradation times, as well as some other epigenetic processes not taken into consideration in the simple analysis presented here. The important conclusion of both models is that noise in the gene-expression dynamics is necessary and sufficient to qualitatively recover the temporal transitions among the ABC-gene configurations observed during early flower development.

Discussion

Robust morphogenetic patterns that are recreated over the life cycles of individuals from the same species, or even from distantly related species, have led to the prevailing view of development as a deterministic process; however, we have shown here that the stereotypical temporal pattern with which floral organs are determined may result from a stochastic dynamic system associated with a highly non-linear GRN.

This study supports recent work that has concluded that random fluctuations in a system may be important for cell behavior and pattern formation ([14–21]), and contrasts with deterministic and preprogrammed views of development. Intrinsic noise (noise arising from the system itself) has its origin in molecular fluctuations due, for example, to slight modifications in temperature, and in random events due to sampling, given that the number of molecules is not infinite during transcription and translation [16,19,14].

Stochastic implementations of a GRN model as pursued in this study were proposed by C. H. Waddington many years ago ([22]; see review in [23]). He understood development as a complex dynamic system, with genes, proteins, metabolites, and environmental factors constituting complex dynamic networks. The attractors of such networks represent a specific configuration of the system (e.g. cell types). The number, depth, width, and relative position of these attractors are represented by the hills and valleys of his "Epigenetic Landscape" metaphor [22,7]. The study presented here actually explored such an Epigenetic Landscape for the flower organ determination GRN (Figures 1 and 7). Other recent studies have also explored this idea for GRNs [30].

In the case presented here, a GRN generates the overall temporal morphogenetic pattern (Figures 3 and 6) observed during flower development of *Arabidopsis thaliana* [31,32]: A genes are expressed first, followed by B genes, and finally C genes, in a rather broad range of noise magnitudes, and in two different modeling approaches. Therefore, our results provide a possible explanation for the conservation, among many flowering plant species [27,28,31–34], of the temporal transitions of A, B, and C-gene expression, and to some extent, of the observed cell fate attainment patterns.

Our results support the hypothesis that biological systems may not only cope with random perturbations, but that the noise may have been incorporated during evolution in the generation of biological patterns (*e.g.* [30,35–37]). Central to the constructive role of noise is the existence of non-linear dynamic systems [38] that converge to robust attractors for a range of noise magnitudes. Stochastic implementations of GRNs, such as the one presented here, may guide predictions of actual noise magnitudes experienced in biological systems.

Nevertheless, deterministic signals or inducers of flower development cannot be dismissed. Indeed, our results hold when focusing on the attractors corresponding to the four types of floral organ primordia. However, if all of the attractors (including I1–I4) are considered, and the system is initialized in one of the inflorescence basins, the system hardly ever transits into the floral basins when small noise levels are used, or else it directly jumps to one of the largest basins (stamens1 or carpels) when larger magnitudes of noise are simulated. These results enable us to speculate on the role of reported non-random inducing signals in the transition from cell fates in the inflorescence meristem to those in the flower meristem. Genes such as *FLOWERING LOCUS T*,

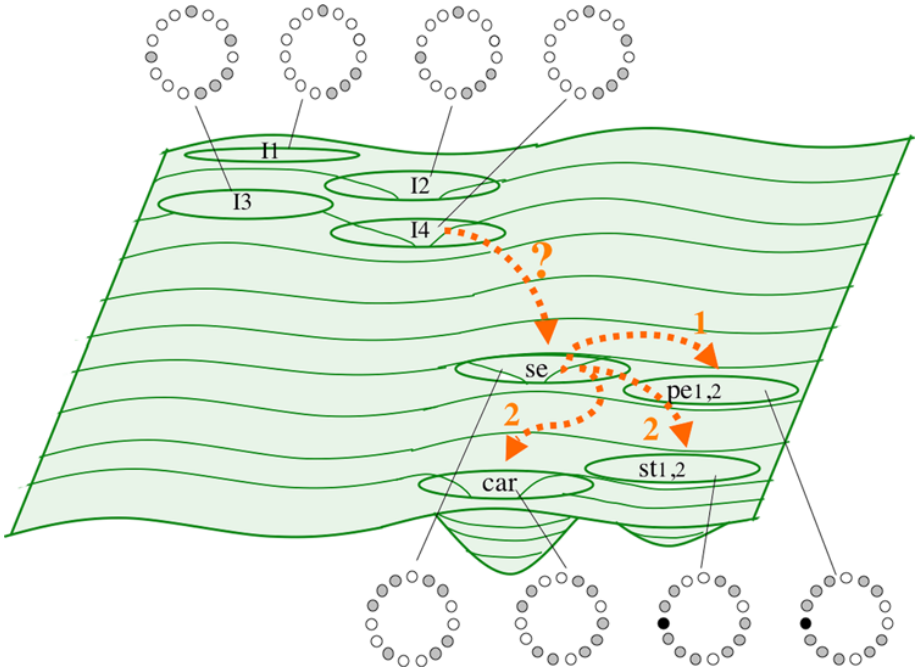

Figure 7. Schematic representation of the epigenetic landscape generated by a stochastic exploration of the GRN for flower development. This schematic landscape is equivalent to the Epigenetic Landscape proposed by C.H. Waddington (1957). Basins comprise the cell genetic configurations that lead to attractors (in this case, gene arrays characteristic of floral organ primordial cell-types: Sepals, petals, stamens, and carpels. See Figure 1 and Discussion). Each cell fate is associated to the GRN configuration corresponding to each of the attractors. The arrows represent transitions among attractors. The transition from inflorescence to sepal attractor might be biased or determined by an inducer. The numbers associated to the arrows represent the sequence of transitions among attractors: From sepals to petals, and then to carpels and stamens.
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SUPPRESSOR OF OVEREXPRESSION OF CO 1, or *CONSTANS* (see [39] for a review) could constitute or mediate such signals.

The type of model put forward here will enable the predictions of the real magnitudes of stochastic fluctuations once such deterministic biasing signals are considered. They will also be useful to test what mutations may cause alterations in the epigenetic landscape and alter the temporal order with which attractors are visited. Such models will guide the search of genetic alterations underlying atypical morphogenetic patterns during the evolution of flowering plant species [40].

One possible interpretation of our model is to assume that, once most cells have attained a certain attractor within a primordium, these are canalized to develop into a particular organ type. One possible explanation for this is that noise does not drive the cells out of each configuration once a certain proportion of them attain an attractor, or that the noise is "frozen" at some point, maybe because irreversible differentiation or synchronization events take place. We may speculate that, in the developmental system we have studied, non-autonomous cell function of key transcription factors [41–44] could play a relevant role in this process, as it could effectively freeze the stochastic fluctuations or synchronize the configuration of the cells within a primordium, and thus, contribute to the formation of the observed spatio-temporal patterns. We could further speculate that the activity of pre-patterning genes (e.g., *WUSCHEL* or *UNUSUAL FLORAL ORGANS*; [43,45,46]) may play important roles during spatio-temporal pattern formation.

Models such as those presented here enable novel predictions about the genetic regulation of cell differentiation and morphogenetic patterns. For example, the stochastic GRN dynamic system eventually attains a stationary distribution of attractor probabilities. The distribution reflects the probability of the cells being in each attractor, and may be interpreted as the proportion of primordial cells fixed to each GRN configuration. In the floral organ specification network, such proportions would correspond to the regions within the floral meristem with A, A+B, B+C, and C function configurations; however, this distribution may only be observed at the very early stages of the partitioning of the floral bud into four concentric rings. This event occurs before cells committed to a certain cell-type start further differentiation and acquire distinct division and elongation rates; hence, the final amount of cells in a certain organ or organ primordium would not necessarily coincide with that predicted by the models presented herein.

Another prediction derived from this model states that the carpel attractor appears either before (Figure 3) or after (Figure 6A) that of stamens. This prediction does not contradict the fact that, in most plants, carpels are the last organs to be fully formed because, again, cells have different division and elongation rates after cell-type differentiation, and therefore, the order in which organogenesis takes place may not match the sequence in which organ primordia cells are determined during early flower development, before the primordia actually emerge.

The discussion above suggests that models that incorporate GRN associated to cellular growth and proliferation, as well as spatial aspects of the system presented here, will eventually be needed to understand the dynamics by which cells attain their fate and proliferate in the floral spatio-temporal domain. In this paper, we have restricted ourselves to exploring the temporal patterns of cell-fate establishment early in flower development, assuming that cells differentiate independently of one another; however, in real organisms, cell-cell communication, cellular dynamics, domain geometry, and growth or mechanical interactions, are all likely to alter the proportion of cells across space and time that are set aside for each type in early flower development [10].

Kauffman's Boolean model for cell differentiation has been criticized because it is said to oversimplify the gene regulatory interactions and the way activation states of all genes are updated (synchronically in Kauffman's proposal); however, Boolean GRN models grounded in experimental data have been able to recover observed multi-gene expression arrays characteristic of certain cell types in several biological systems [2,3,7,36]. These results suggest that the logic of regulation considered in Boolean networks suffices to qualitatively reproduce the dynamics of biological GRNs. Furthermore, theoretical studies have suggested that the details of the kinetic functions are not relevant in determining the system's attractors. In particular, updating schemes do not seem to affect the number and identity of fixed-point attractors [47], as is the case of the attractors recovered in the network used here.

Given that the identity of the attractors and the temporal sequence in which these were attained are the same (Figures 3A–C vs Figure 6B) or very similar (Figures 3A–C vs Figure 6A) using Boolean and Glass dynamics, this study reveals that the time ordering in the emergence of cell-fate patterns is not an artifact of synchronous updating in the Boolean model; however, the sizes of the basins of attraction differ between the two models. In Glass dynamics, the basins corresponding to stamen and carpel primordia cells are smaller, and those of sepals and petals are larger (Table 2); hence, the proportion of cells at each fate along time predicted by the Glass and Boolean dynamics differ, which suggests that the updating schemes might be relevant to recovering the actual temporal cell population dynamics in biological systems. Experimental data on the temporal fluctuations of primordial cells with different multi-gene expression arrays will test which of the two systems and updating hypotheses better reproduces the real system.

Eventual formalizations of stochastic multicellular GRN dynamics in explicit spatial domains may require "hybrid" approximations that enable large computational explorations, and allow, for instance, the explicit incorporation of developmental processes into models of network or phenotypic evolution [48], or the study of the epigenetic landscapes that emerge from GRN related to complex diseases, such as cancer [9].

In conclusion, we put forward a stochastic approach to model the Boolean and continuous dynamics of an experimentally-based GRN, and thus, take Waddington's Epigenetic Landscapes into a specific biological framework: Flower organ specification in *Arabidopsis thaliana*. The theoretical framework of this proposal could also be useful for studying the behavior of other networks, including, for instance, ecological, epidemiological, immunological, engineering, or social networks. Finally, our results emphasize that complex networks and stochastic processes are central to understanding the biological development and emergence, as well as the stability, of morphogenetic patterns.

Methods

Construction of phenogram of attractors

We obtained six phenograms by estimating the Manhattan distance index to infer the relationships among the 10 attractors for the 15-gene system. This index was obtained by comparing the vectors of zeros and ones of each attractor. We then used the clustering method by the unweighted pair-group method with arithmetic average (UPGMA) to group the attractors. We obtained six different phenograms, with which we constructed a strict consensus that kept the branches that were recovered in all of the six phenograms. In Figure 2, the consensus phenogram is shown below the attractors ordered along the X and Y-axes of the heat map, corresponding to the Similarity Matrix.

Implementation of noise in the GRN model

Boolean case. The GRN has 15 elements; two of them (LUG and CLF) are constitutively expressed in the flower meristem, and thus, their activation states were fixed to 1. The transition probabilities among attractors in the Boolean GRN implementation were obtained by introducing noise to the updating logical rules in 10, 000 realizations for each possible configuration of the system. The analyses of the Boolean model were performed with the "Atalia" software, which is publically available (http://www.ecologia.unam.mx/achaos/Atalia/atalia.htm).

Another equivalent method to obtain the Markov matrix entries would be to follow the system's trajectory for every possible initial configuration. For certain levels of noise, the system never remains at a particular basin, and it is hard to determine when to stop the computation for the corresponding initial condition. Nonetheless, we performed a similar type of simulation in order to mimic that of the Glass system. We selected a random configuration from those in the "sepal" basin. Each gene was updated according to its true table, except that with a certain probability (0.01 and 0.03), the rule was violated, and if the true table predicted that a state should be "1," it was set to "0," and vice versa. The new basin was registered, and this procedure was continued for 140 iterations. 80,000 such realizations were obtained (i.e., 80,000 randomly chosen configurations from the "sepal" basin were chosen).

Glass system. The model is explained in the Results section. We numerically integrated the set of differential equations (5) and (6) using the Euler method with an integration step $dt = 0.01$. The results do not change by choosing smaller values of dt; however, if we take $dt = \tau = \Delta t_p = 1$, then the continuous model given in equations (5) and (6) becomes completely equivalent to the Boolean model given in Eq. (2). The results for this latter case are shown in Figure 6B. In order to recover the temporal sequence, in which attractors (cell-fate) were attained in the *A. thaliana* network using Glass dynamics with noise, we followed transitions for 140 time-steps, starting with a population of 80 000 cells (configurations from the "sepal" basin of attraction), in which each gene was independently chosen not to be updated according to its logical functions (set to "1" if the predicted value was "0," and vice versa), with a probability $\eta = 0.03$; hence, the non-perturbed genes evolved in time according to Eq. (5), while the perturbed genes evolved following Eq. (6). After a time interval $\Delta t_p = 2.5$ for Figures 6A, and 1 for Figure 6B, a new set of perturbed genes in the entire population was chosen again, and so on until 140 iterations were completed. Qualitatively similar results were obtained for a noise of 0.01. The code for the Glass system simulations was developed in JAVA, and is available upon request.

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Author Contributions

Conceived and designed the experiments: ERAB AC MA PPL. Analyzed the data: ERAB AC MA MB YCP CES DHF RBL DM GJES PPL. Wrote the paper: ERAB MA MB RBL PPL. Overall project design, coordination and integration: ERAB. Implemented and planned simulations of Boolean Dynamics: ERAB. Ran simulations of Boolean Dynamics: ERAB AC. Designed simulations for Boolean Dynamics: AC. Conceptual contributions: AC MB CES DM GJES PPL. Figures: AC MB. Ran and designed simulations for the Glass Dynamics: MA. Singular value decomposition calculations: YCP. Programming and ran simulations for the Glass Dynamics: DHF. Programming for Boolean Dynamics: DM. Simulations: GJES. Revised paper: GJES PPL.

References

1. Von Dassow G, Meir E, Munro EM, Odell GM (2000) The segment polarity network is a robust developmental module. Nature 406: 188–193.
2. Albert R, Othmer HG (2003) The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in Drosophila melanogaster. J Theor Biol 223: 1–18.
3. Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla ER (2004) A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles. Plant Cell 16: 2923–39.
4. Kauffman SA (1993) The Origins of Order: Self-organization and Selection in Evolution. Oxford: Oxford University Press.
5. Davidson E, Rast JP, Oliveri P, Ransick A, Calestani C, et al. (2002) A genomic regulatory network for development. Science 295: 1669–1678.
6. Acar M, Becskei A, van Oudenaarden A (2005) Enhancement of cellular memory by reducing stochastic transitions. Nature 435: 228–32.
7. Huang S, Eichler G, Bar-Yam Y, Ingber DE (2005) Cell fates as high-dimensional attractor states of a complex gene regulatory network. Phys Rev Lett 94: 128701.
8. Lipniacki T, Paszek P, Brasier AR, Luxon BA, Kimmel M (2006) Stochastic regulation in early immune response. Biophys J 90: 725–42.
9. Huang S, Ingber DE (2007) A non-genetic basis for cancer progression and metastasis: self-organizing attractors in cell regulatory networks. Breast Dis 26: 27–54.
10. Scheres B (2001) Plant cell identity. The role of position and lineage. Plant Physiol 125: 112–114.
11. Rudall P (1987) Anatomy of flowering plants: An introduction to structure and development. Edward Arnold, USA.
12. Chaos A, Aldana M, Espinosa-Soto C, García-Ponce B, Garay-Arroyo A, Alvarez-Buylla ER (2006) From genes to Flower patterns and evolution: dynamic models of gene regulatory networks. Plant Growth Regul 25: 278–289.
13. Coen E, Meyerowitz E (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353: 31–37.
14. Hume DA (2000) Probability in transcriptional regulation and its implications for leukocyte differentiation and inducible gene expression. Blood 96: 2323–2328.
15. Fedoroff N, Fontana W (2002) Genetic networks. Small numbers of big molecules. Science 297: 1129–31.
16. Paulsson J (2004) Summing up the noise in gene networks. Nature 427: 415–8.
17. Martínez-Arias A, Hayward P (2006) Filtering transcriptional noise during development: concepts and mechanisms. Nature Reviews (Genetics) 7: 34–44.
18. Wang J, Huang B, Xia X, Sun Z (2006) Funneled landscapes leads to robustness of cell networks: yeast cell cycle. PloS Computational Biology 2(11): e147.
19. Wang J, Huang B, Xia X, Sun Z (2006) Funneled landscapes leads to robustness of cellular networks: MAPK signal transduction. Biophysical Journal: Biophysical Letters doi: 10.1529/biophysj.106.086777: L54–L56.
20. Han B, Wang J (2007) Quantifying robustness and dissipation cost of yeast cell cycle network: the funneled energy landscape perspectives. Biophysical Journal 92: 3755–3763.
21. Kaneko K (2007) Evolution of robustness to noise and mutation in gene expression dynamics. PloS ONE 2: e434.
22. Waddington CH (1957) The Strategy of the Genes. London: Geo Allen & Unwin.
23. Slack JM (2002) Conrad Hal Waddington: the last Renaissance biologist? Nat Rev Genet 3: 889–95.
24. Sasai M, Wolynes PG (2003) Stochastic gene expression as a many-body problem. Proc Natl Acad Sci U S A 100: 2374–9.
25. Aldrich J (1997) R.A. Fisher and the making of maximum likelihood 1912–1922. Statist Sci 12: 162–176.
26. Huang K (1990) Statistical Mechanics. Wiley, John & Sons.
27. Yu H, Ito T, Zhao Y, Peng J, Kumar P, Meyerowitz EM (2004) Floral homeotic genes are targets of gibberelin signaling in flower development. Proc Natl Acad Sci USA 101: 7827–7832.
28. Bomblies K, Dagenais N, Weigel D (1999) Redundant enhancers mediate transcriptional repression of AGAMOUS by APETALA2. Dev Biol 216: 260–264.
29. Glass L (1975) Classification of biological networks by their qualitative dynamics. J Theor Biol 54: 85–107.
30. Wang Z, Hou Z, Xin H, Zhang Z (2006) Engineered internal noise stochastic resonator in a gene network: A model study. Biophys Chem 125: 281–285.
31. Bowman J, ed. (1994) *Arabidopsis*. An Atlas of Morphology and Development. U.S.A.: Springer-Verlag.

32. Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, et al. (2003) Dissection of floral induction pathways using global expression analysis. Development 130: 6001–12.
33. Whipple CJ, Ciceri P, Padilla CM, Ambrose BA, Bandong SL, Schmidt RJ (2004) Conservation of B-class floral homeotic gene function between maize and Arabidopsis. Development 131: 6083–91.
34. Adam H, Jouannic S, Morcillo F, Verdeil JL, Duval Y, Tregear JW (2007) Determination of flower structure in *Elaeis guineensis*: do palms use the same homeotic genes as other species? Ann Bot 100: 1–12.
35. Li F, Long T, Lu Y, Ouyang Q, Tang C (2004) The yeast cell-cycle network is robustly designed. Proc Natl Acad Sci U S A 101: 4781–4786.
36. Escudero C (2006) Stochastic resonance due to internal noise in reaction kinetics. Phys Rev E Stat Nonlin Soft Matter Phys 74: 010103.
37. Sigal A, Milo R, Cohen A, Geva-Zatorsky N, Klein Y, et al. (2006) Variability and memory of protein levels in human cells. Nature 444: 643–6.
38. Gammaitoni L, Hanggi P, Jung P, Marchesoni F (1998) Stochastic Resonance. Rev Mod Phys 70: 233.
39. Baurle I, Dean C (2006) The timing of developmental transitions in plants. Cell 125: 655–64.
40. Ambrose BA, Espinosa-Matías S, Vázquez-Santana S, Vergara-Silva F, Martínez E, Márquez-Guzmán J, Álvarez-Buylla ER (2006) Comparative developmental series of the Mexican Triurids support a euanthial interpretation for the unusual reproductive axes of Lacandonia schismatica (Triuridaceae). Am J Bot 93: 15–35.
41. Lucas WJ (1995) Plasmodesmata: intercellular channels for macromolecular transport in plants. Curr Opin Cell Biol 7: 673–80.
42. Sessions A, Yanofsky MF, Weigel D (2000) Cell-cell signaling and movement by the floral transcription factors *LEAFY* and *APETALA1*. Science 289: 779–781.
43. Parcy F, Nilsson O, Busch MA, Lee I, Weigel D (1998) A genetic framework for floral patterning. Nature 395: 561–566.
44. Wu X, Dinneny J, Crawford K, Rhee Y, Citovsky V, Zambryski P, Weigel D (2003) Modes of intercellular transcription factor movement in the *Arabidopsis* apex. Development 130: 3735–3745.
45. Lohmann J, Hong RL, Hobe M, Busch MA, Parcy F, et al. (1995) Selective trafficking of *KNOTTED1* homeodomain protein and its mRNA through plasmodesmata. Science 270: 1980–1983.
46. Lenhard M, Bohnert A, Jürgens G, Laux T (2001) Termination of stem cell maintenance in Arabidopsis floral meristems by interactions between WUSCHEL and AGAMOUS. Cell 105: 805–14.
47. Gershenson C (2004) Updating Schemes in Random Boolean Networks: Do They Really Matter? In: Pollack J, Bedau M, Husbands P, Ikegami T, Watson RA, eds. Artificial Life IX, Proceedings of the Ninth International Conference on the Simulation and Synthesis of Living Systems. MIT Press. pp 238–243.
48. Alvarez-Buylla ER, Benítez M, Dávila EB, Chaos A, Espinosa-Soto C, Padilla-Longoria P (2007) Gene regulatory network models for plant development. Curr Opin Plant Biol 10: 83–91.

Apéndice H

From ABC genes to regulatory networks, epigenetic landscapes and flower morphogenesis: Making biological sense of theoretical approaches

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Review

From ABC genes to regulatory networks, epigenetic landscapes and flower morphogenesis: Making biological sense of theoretical approaches

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ABSTRACT

The ABC model postulates that expression combinations of three classes of genes (A, B and C) specify the four floral organs at early stages of flower development. This classic model provides a solid framework to study flower development and has been the foundation for multiple studies in different plant species, as well as for new evolutionary hypotheses. Nevertheless, it has been shown that in spite of being necessary, these three gene classes are not sufficient for flower organ specification. Rather, flower organ specification depends on complex interactions of several genes, and probably other non-genetic factors. Being useful to study systems of complex interactions, mathematical and computational models have enlightened the origin of the A, B and C stereotyped and robust expression patterns and the process of early flower morphogenesis. Here, we present a brief introduction to basic modeling concepts and techniques and review the results that these models have rendered for the particular case of the *Arabidopsis thaliana* flower organ specification. One of the main results is the uncovering of a robust functional module that is sufficient to recover the gene configurations characterizing flower organ primordia. Another key result is that the temporal sequence with which such gene configurations are attained may be recovered only by modeling the aforementioned functional module as a noisy or stochastic system. Finally, modeling approaches enable testable predictions regarding the role of non-genetic factors (noise, mechano-elastic forces, etc.) in development. These predictions, along with some perspectives for future work, are also reviewed and discussed.

Contents

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1. ABCs of flower development: from schemes to dynamic models

Although 20 years have passed since the publication of the ABC model of flower development [1–3], we still do not know why it works. We now have a thorough description of the spatiotemporal patterns of ABC gene expression patterns and mutant phenotypes (e.g., [4,5]) yet we do not know how the interactions among these and other genes dynamically render such patterns. Completely unraveling the dynamic mechanisms that underlie the ABC gene expression patterns and the stable ABC gene combinations necessary for floral organ specification and arrangement is still a challenge. These are, indeed, major issues since gene expression patterns, which are required for development to take place, are not a fixed or predetermined background field. Neither are they specified by single or simple combinations of genes, but are originated and dynamically maintained by several genetic and non-genetic factors that interact among themselves in non-linear manners [6,7]. Thus, in order to fully understand development, evolution and phenotypic transformation, it is essential to understand how gene expression patterns arise, and how such expression patterns coordinate with non-genetic factors during development.

We know that the ABC model works in *Arabidopsis thaliana, Antirrhinum majus* [3], as well as in other flowering species [8]. This suggests a robust underlying mechanism that resists environmental variations and that has been conserved throughout evolution. We have also learned from molecular genetics experiments that the genes involved in this model are necessary for the specification of the primordial cells that will eventually form sepals, petals, stamens and carpels from the floral primordium periphery to the center as flower development progresses [9]. However, we also know that these genes alone are not sufficient for this process [10,11]. Mathematical and computational models that integrate information systematically and help studying the dynamical aspects of flower development are useful for understanding how steady gene expression patterns, like those characterizing the ABC model, are generated. Actually, models that are able to follow the concerted and dynamic action of the ABCs with several other interactors have been used to postulate a regulatory module that is sufficient to recover ABC combinations in conjunction with other genes and proteins that have been shown to co-express with them (see review [12]). In contrast to schematic representations that depict gene regulatory interactions, dynamic models may consider the non-linear aspects of regulation and explore the way gene expression changes in time, both in wild type and perturbed simulated systems. Certainly, these models have helped to provide a dynamic account of the ABC model, as well as novel predictions and input for experimental studies. Nevertheless, important challenges, such as a complete understanding of the mechanisms and processes that lead to the observed morphogenetic patterns characteristic of flower development, are still open.

1.1. The gene regulatory module underlying cell-fate determination and morphogenesis during early flower development: the ABC genes are necessary, but not sufficient for flower organ specification

The ABC model integrates three classes of genes (A, B and C genes) and postulates that the combined expression of subsets of these classes specifies the different flower organs at early stages of flower development: A genes are necessary for sepal specification; A and B for petals; B and C for stamens; and C for carpels (reviewed in: [1,3–5], and elsewhere in this issue). A rather complete set of data concerning the interactors of the ABC genes has been gathered [5,12–15] for the model plant *A. thaliana* and, therefore, most modeling efforts concerning the ABCs have been done for this species.

Most plant species, including *A. thaliana* (Fig. 2), and especially eudicots, share an overall conserved flower body plan consisting of concentric whorls of organs: the two outermost rings are non-reproductive organs (sepals and petal) and the innermost are the reproductive organs (stamens and carpels) [9]. The temporal order with which ABC genes are expressed and the corresponding floral organ types specified are also quite conserved among higher eudicots. The A genes are turned on and the sepals determined first, then the B genes, and hence the AB combination and petals, and almost at the same time, the stamen and carpel cells are specified once the C genes are turned on and both BC and C genes alone are expressed ([16]; see Fig. 3).

Developmental processes, such as flower development, are often organized in a modular way, so different semi-autonomous processes or functions may be defined [17]. In this review, we focus on the regulatory module responsible for primordial cell-fate determination during early stages of flower development, in which the floral meristem is subdivided into the four concentric rings of cells that will form the floral organs [12,18,19]. We define a regulatory module as a set of molecules, signals or other kinds of interacting entities that constitute a functional unit and are sufficient for a process to occur. As units, modules can function fairly robustly and independently from other regulatory modules or entities (see, for e.g., [20,21]). Importantly, the gene regulatory module associated with cell-fate determination during the early stages of flower development seems to integrate environmental and internal signals (e.g., plant hormones) that also affect the flower meristem behavior, as well as to connect with other genetic modules involved in later stages of flower development (see review in [12]), for instance, in cell-type sub-differentiation and organogenesis processes.

Since the description of the ABC model, many other genes involved in flower organ determination have been described (reviewed in [14,15]). Several experiments have now demonstrated that flower organ determination depends on the expression and inter-regulation of the ABC genes (Fig. 2), but also of several non-ABC genes like *FLOWERING LOCUS T* (*FT*), *LEAFY* (*LFY*), *TERMINAL FLOWER1* (*TFL1*), *SEPALLATA genes* (*SEP1*, *SEP2*, *SEP3)* and *WUSCHEL* (*WUS*). The study of all of these genes has been instrumental for understanding flower development (e.g., [22–26]; and other reviews in this special issue) and has pointed at the genes and gene interactions that are fundamental to flower organ determination. Nevertheless, the characterization of isolated genes or of paired interactions is not enough to fully describe and understand the regulatory module that is necessary and sufficient for the spatial and temporal processes associated with flower organ determination. As we will review, studies of the collective action of many interacting genes are required and such studies have used mathematical models.

We now turn to the basic concepts and definitions of the models proposed by us and other groups in order to integrate molecular genetic experimental data on development. We then review the

achievements of such models, especially for understanding the ABC model.

2. GRN models for understanding development

Developmental processes involve complex interactions among multiple genetic and non-genetic elements, interactions that occur in a wide range of spatiotemporal scales. Consequently, different kinds of mathematical formalisms may be chosen depending on the scales relevant to the system under study and on the nature of the available data. Integrative approaches aiming at further understanding development may, however, encompass more than one type of model. Here, we summarize some of the basic mathematical methods and concepts related to gene regulatory network (GRN) modeling (see reviews by: [27,28]).

GRN models have been widely used to study gene collective dynamics as these and their regulatory interactions may be intuitively represented as dynamic networks. Moreover, these relatively simple models capture the non-linear character of the interactions associated with the logic of gene regulation and seem to be valid abstractions of such complex regulatory interactions, as they recover important features of their dynamics and observed stable multigenic configurations of cells at different stages of development. In these models, the nodes of the network correspond to genes, RNA, proteins or complexes that take part in gene regulation, while the edges stand for the positive or negative regulatory interactions among the network elements.

2.1. Discrete and continuous GRN models

There are two main approaches to modeling GRNs, namely, the *discrete* and *continuous* methods. These two approaches differ in scope; yet, they often yield equivalent qualitative results when applied to concrete biological systems (e.g., [21]). This equivalence suggests that the logic and overall dynamics of gene regulation depend mostly on the network architecture and signs of the interactions, rather than on the details of the mathematical specification of the GRN.

2.1.1. Discrete models

The simplest case of discrete GRNs is that of the so-called Boolean networks, first put forward by Kauffman ([29]; see Fig. 1). In such models, nodes can be in one of two activation states in time t, 0 (off) or 1 (on), depending on the state at time $t-1$ of the elements that regulate each of them. The regulatory interactions determining the changes in gene activation states are defined as logical rules in which logical connectors such as OR, AND or NOT are used. Equivalently, the logical rules might be represented as tables providing a corresponding output for a given input gene profile.

For GRNs based on empirical evidence, the information needed to define logical rules is obtained from diverse experimental results (gene expression patterns, loss and gain of function phenotypes, protein interaction assays, etc.). Then, the activation state of every gene is given by:

$$g_n(t+1) = F_n(g_{n_1}(t), g_{n_2}(t), \ldots, g_{n_{k_n}}(t)), \quad (1)$$

where $\{g_{n_1}, g_{n_2}, \ldots, g_{n_{k_n}}\}$ are the regulators of the gene g_n, and F_n is the discrete function or logical rule.

Given the set of logical rules defining the GRN dynamics, it is possible to update the gene states. In many cases of interest, they all eventually attain a steady or equilibrium state. Those GRN steady states at which all of the nodes (initial conditions) end up are usually referred to as *attractors*. Kauffman [29] suggested that GRN attractors corresponded to sustained gene activation profiles characteristic of particular cell types and this has been substantiated experimentally and validated in a handful of modeled systems (e.g., [21,30,31]; see Fig. 2). Additional experimental and theoretical studies suggest that regulation at the transcriptional level might be better represented by discrete, rather than continuous models (e.g. [32]).

2.1.2. Continuous models

Continuous GRNs are defined by coupled non-linear differential equations describing changes in the concentration (rather than the state of expression) of the molecules involved in the gene regulation processes. In continuous GRNs, nodes can take an infinite number of state values and the equilibrium points may be found by analytic means. Just like the attractors of discrete GRNs, these equilibrium points are thought to match gene activation configurations typical of particular cell types. This description stands on largely developed analytical tools and is especially useful when the system under consideration has few nodes, since large networks become intractable with this approach.

The continuous approach can incorporate effects such as active transport, diffusion, and elastic or mechanical phenomena, among others. Moreover, this kind of implementation can include more detail on the kinetics of gene regulation and yield quantitative predictions that have provided insights into signal transduction and oscillatory systems. The detailed experimental data to obtain parameter values for the differential equations representing such models are, however, extremely scarce.

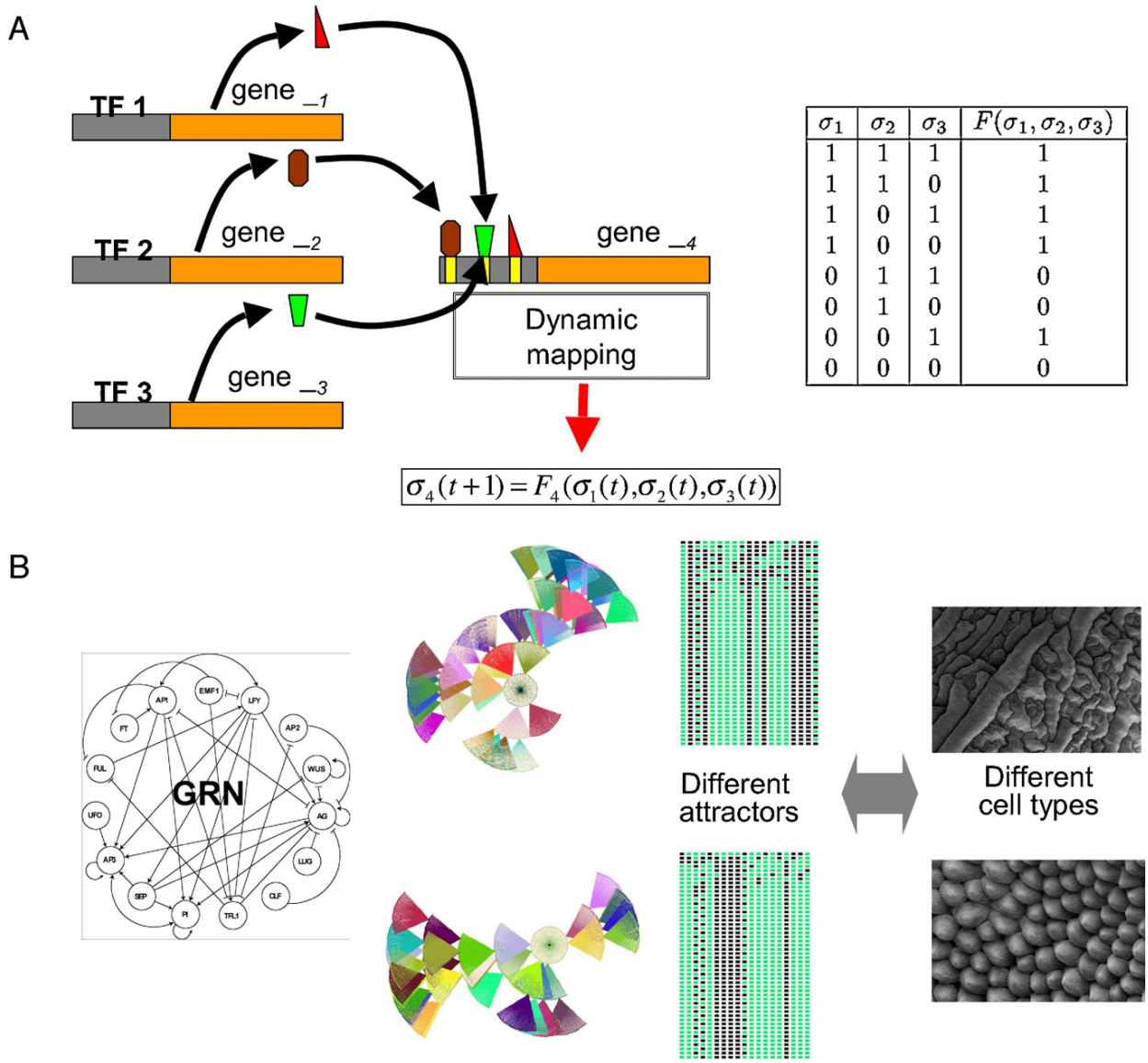
As mentioned above, both discrete and continuous GRN models enable a characterization of the collective gene regulatory dynamics in terms of, among other features, the number and nature of attractors and equilibrium points.

2.2. Deterministic and stochastic GRN dynamics

Deterministic GRNs are those for which it is possible to know the state of every node at every moment, given an initial configuration. In contrast, stochastic GRNs consider the noise originated, for instance, by small numbers of molecules (example of a source of intrinsic stochastic fluctuation due to the small sample sizes), environmental fluctuations (example of a source of external stochastic fluctuations) or other sources of uncertainties. In stochastic models, the updating rules, discrete or continuous, depend partially on a stochastic variable, which, instead of attaining a fixed value, has a distribution of possible values or states. This is, in fact, a more realistic representation of a biological system (review in [33]). Introducing stochasticity into GRN models has provided interesting results suggesting, for example, that noise may play a constructive role in biological systems (details below and in [34]).

3. Morphogenetic models encompassing GRNs

Even though GRN models are indeed useful to study development and other biological processes, these generally constitute abstractions of the one-cell level gene regulatory processes and do not consider cell-to-cell communication, spatial components of development or cellular dynamics, among other important aspects of morphogenesis. In general, to understand cell-fate determination, one has to explain how identical cells become sufficiently different as to appear as a particular type of cell (Fig. 1). It is an undeniable fact that the expression of genes, through their organization in GRNs, is a central issue in the whole process of differentiation: undifferentiated cells share the same gene expressions and differentiated ones certainly express their genes differently. However, it is evident that the genes by themselves have no means of canalizing which attractors of their GRN to "choose", and it is mostly seen that this "decision" is driven by the situation (e.g., depending on its



σ_1	σ_2	σ_3	$F(\sigma_1, \sigma_2, \sigma_3)$
1	1	1	1
1	1	0	1
1	0	1	1
1	0	0	1
0	1	1	0
0	1	0	0
0	0	1	1
0	0	0	0

Fig. 1. The dynamic modeling of gene regulatory networks (GRNs). (A) GRNs are established by the non-linear cross-regulation of genes. Here, three transcription factors that regulate a target gene are exemplified. Each gene or node in a GRN is regulated by *k* transcription factors. If GRNs are modeled with discrete dynamic models, each gene has a truth table as the one shown here, in which the combinations of gene activation states (gene configurations of *k* entries, in this example, $k = 3$) leading to an "ON" or "OFF" activation state of the target gene are tabulated. Hence, the dynamics of the expression state of each gene is given by a Boolean logical function (Fn), which, in turn, depends on the expression states in previous time steps, of the genes that regulate it. Each gene configuration (conformed in the Boolean case of 0's and 1's or initial configuration of a GRN, will lead to a stable configuration, which is called an attractor state. Attractors can be of the fixed-point type (a single configuration), or cyclic (several configurations). Kauffman proposed that GRNs underlying cell differentiation may attain fixed-point attractors, in which configurations correspond to multigenic profiles characterizing each cell type. (B) In this figure, we show a diagram for the set of initial configurations that lead to the sepal and petal stable configurations or attractors. All of the configurations that lead to a given attractor are called basins of attraction. Hence the basins of attraction of sepal and petal primordial cells are shown in the form of colored fan diagrams with the attractors' configurations in the point in the central part or to which all other converge via connecting lines in these fan diagrams and all the possible configurations in the points around such central one. The colors, lengths or angles of the lines in the fan diagrams do not have any meaning and are drawn as such just for design reasons. However the number of points and lines do indicate the number of configurations that lead to each attractor (point to which all others converge). In tabular form, to the right of the basins of attraction, an example of how different initial configurations ("ON" in green and "OFF" states in black) lead to different attractors is shown. On the right, scanning electron micrographs of sepal (top) and petal (bottom) cell types are shown.

lineage) or position of the cell in the organism. Therefore, it is essential that each cell extracts temporal and positional information from its environment, and for this we need to postulate processes that generate this positional information at all times, and produce changes in the operation of the identical GRN accordingly (Fig. 4). That is, short-range (cell-to-cell) and long-range communication are needed to allow a complete relative positional information and the regulation of sizes and dimensions of tissues, as well as the relative position of organs.

This is why one of the challenges ahead consists of rendering spatio-temporal models that consider GRNs in cellularized domains and that encompass cellular dynamics, chemical gradients, cell-to-cell communication, etc. (Fig. 4). There have now been some efforts in this direction (e.g., [35,36]). For instance, GRN models have recently contributed to specify the metaphor of *epigenetic landscapes* [37,38] for particular systems. In such a metaphor, developmental processes are viewed as a ball rolling through pathways in a landscape, and the topology of the landscape is defined by the

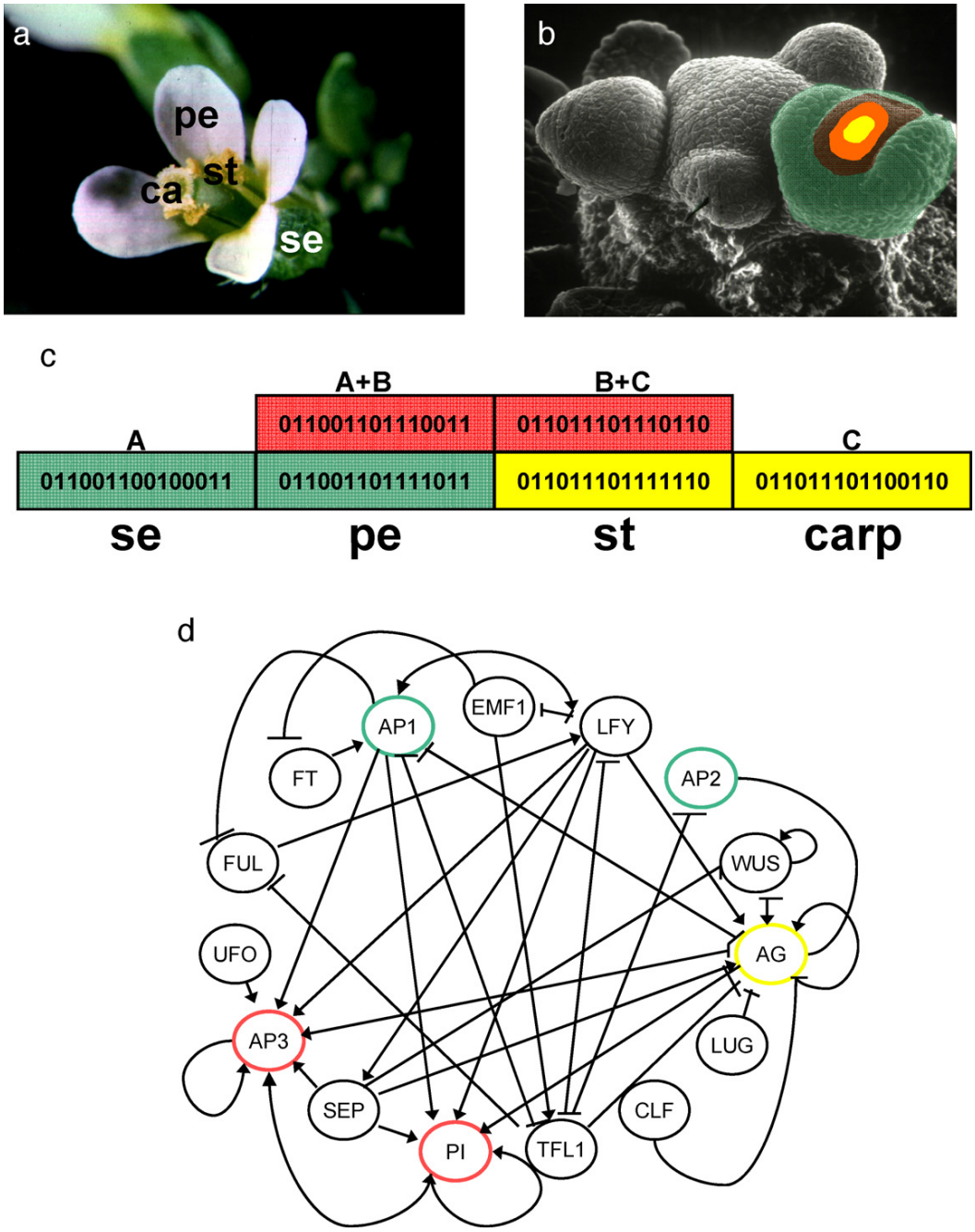


Fig. 2. The *Arabidopsis thaliana* flower has the stereotypical floral arrangement of eudicots, with sepals, petals, stamens and carpels from the periphery to the flower center. (a) Flower meristems form in the axils of rudimentary bracts that appear in the flanks of the inflorescence meristem of which a scanning electron micrograph is shown in (b). Here a flower meristem in which the sepal primordia are already visible is colored with the primordial cells of sepals in green (A function alone), those of petals in brown grey (green and red from A + B functions), the stamens in orange (yellow and red from B + C functions) and the carpels in yellow (C function alone). In (c), the ABC model and the floral organ determination GRN (FOS-GRN) stable configurations that correspond to A, A + B, B + C and C gene combinations necessary for sepal, petal, stamen and carpel development, respectively. The activation states correspond to each of the GRN nodes starting on the left with "EMF1" and consecutively progressing clockwise the rest of the genes in the GRN shown in (d). (d) The FOS-GRN that underlies the dynamic attainment of the ABC combinations, with the A, B and C genes colored as in (c). In the FOS-GRN, arrows correspond to activations and blunt ended edges to repressions.

interactions among genes and other elements (Fig. 3). Continuing with the metaphor, the bottoms of valleys correspond to steady states or final stages of development (e.g., the attractive states of GRNs as defined above). Yet further work needs to be done in order to generate integrative morphogenetic models. Some general ideas for advancing these kinds of models for the case of flower development will be discussed below.

4. The floral organ specification GRN (FOS-GRN): recovering the ABC gene configurations characteristic of primordial cell types during early flower development

We have modeled the experimentally grounded GRN model for flower organ specification (FOS-GRN) that integrates molecular genetic data for the ABC genes and their main interactors

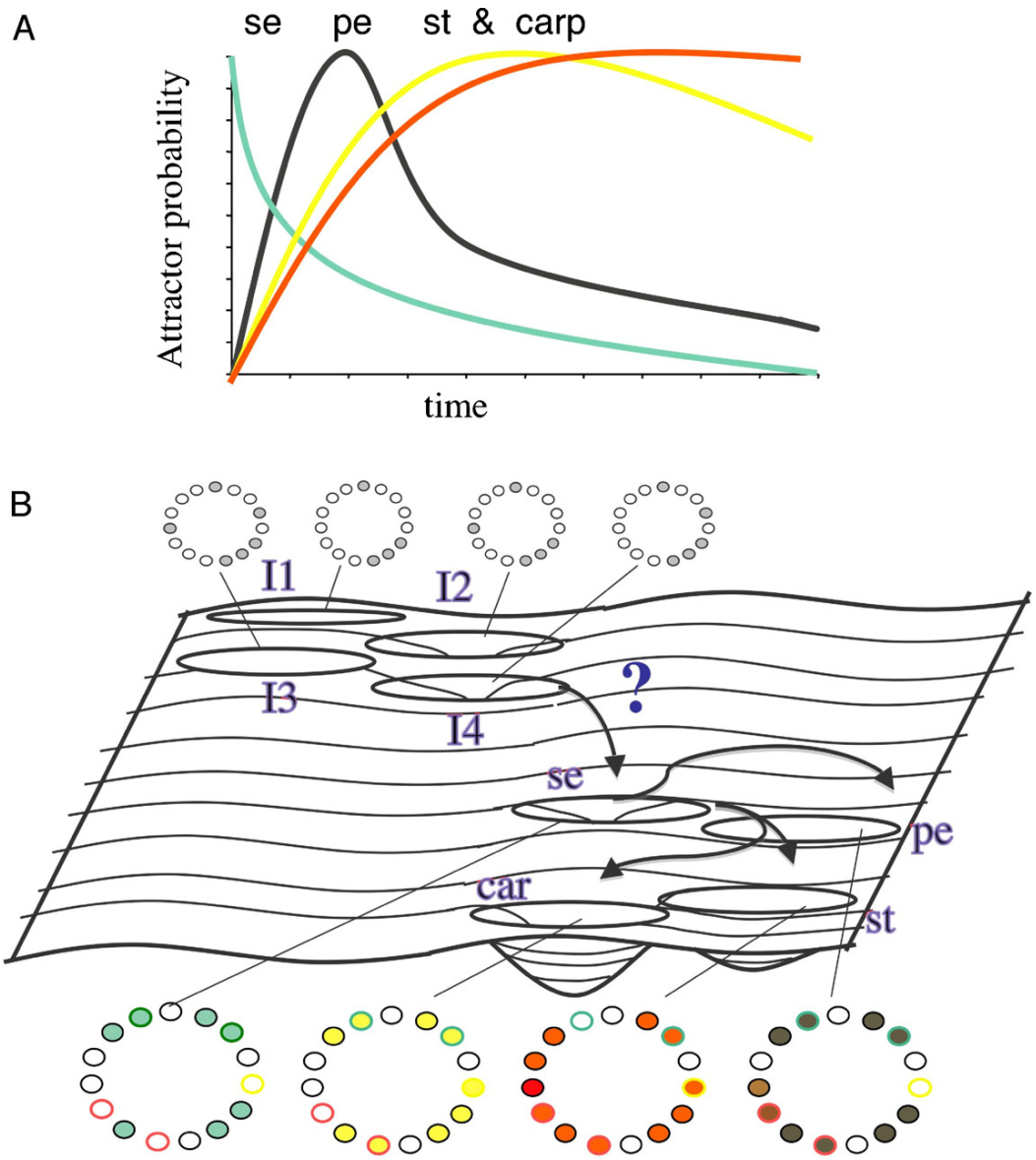


Fig. 3. The four floral organs appear in a well-defined temporal sequence that is recovered by a stochastic GRN model. Sepal primordial cells are the first to be specified (green), then petals (brown) and finally, almost at the same time, stamens (orange) and carpels (yellow). (A) Alvarez-Buylla et al. [34] demonstrated that stochastic perturbations in the FOS-GRN in Fig. 2 are sufficient to recover the observed temporal sequence of ABC gene expression and floral organ specification. (B) A schematic representation of the epigenetic landscape generated by a stochastic exploration of the FOS-GRN for flower development. This schematic landscape is equivalent to the epigenetic landscape proposed by Waddington [37]. Basins comprise the cell genetic configurations that lead to attractors (in this case, gene configurations characteristic of floral organ primordial cell-types: sepals, petals, stamens, and carpels. Se: Sepals (green), pe: petals (brown), st: stamens (orange), car: carpels (yellow).

([30,34,39,40]; Figs. 1 and 2). This GRN includes key regulators underlying the transition from shoot apical meristem once it produces the apical inflorescence (we call it the inflorescence meristem, IM) to flower meristem (FM) (*FT, TFL, EMF, LFY, AP1*, *FUL*), the ABCs and some of their interacting genes (*AP1*, *AP3*, *PI*, *AP2*, *AG*, *SEP*), as well as some genes that link floral organ specification to other modules regulating primordia formation and homeostasis (*AG*, *CLF* and *WUS)*, and some regulators of organ boundaries (*UFO* and *LUG)*.

Interestingly, the postulated discrete regulatory module has over 130,000 different 15-gene activation configurations, but it only converges to ten attractors: four corresponding to gene combinations characteristic of four sub-regions in the IM, and the rest to gene activation states configurations observed in sepal (one attractor), petal (two attractors; with and without *UFO* "ON"), stamen (two attractors; with and without *UFO* "ON") and carpel (one attractor) primordia (Figs. 1 and 2; see [30]). This first result is outstanding, as it is extremely rare that randomly generated complex networks of the size of this one attain so many fixed-point attractors (ten in this case). This result also suggests that the postulated regulatory module is robust to certain alterations (i.e., those stemming from its connections to other components not considered here and which modify the initial configurations of the FOS-GRN) and that this GRN is sufficient to specify gene configurations characteristic of primordial cells during the first stages of flower organ development, thus constituting a functional module.

Various robustness analyses were performed on the FOS-GRN and showed that the recovered attractors are also robust in the

face of permanent alterations of the logical functions of gene interactions and gene duplications [30,40,41]. These results suggest that the multigenic configurations that are sufficient for flower organ specification, dynamically and robustly emerge from complex networks of molecular components, rather than from a series of linear or hierarchical gene interactions, or from the action of particular genes or simple gene combinations. Moreover, the robustness analyses indeed support that such a GRN could account for the overall widespread conservation of the ABC patterns and floral bauplan (particularly that of eudicots; see [9,42,43]).

Since the first publications of the FOS-GRN [39,44], this GRN has been continuously updated. However, the basic structural characteristics of it have been stable and the additions, while providing important new insights, have not substantially altered the main conclusions originally put forward. Recent updates have been implemented with the new software, ATALIA (available at: http://www.ecologia.unam.mx/~achaos/Atalia/atalia.htm), developed in the Alvarez-Buylla laboratory by Alvaro Chaos. This software can run a GRN dynamics and obtain its attractors, basins of attraction, among other significant dynamic signatures (see examples in [12]).

In conclusion, the basic FOS-GRN proposed has provided a sufficient explanation for the observed ABC patterns and the stable gene expression configurations observed in IM and FM during early flower development in *A. thaliana*. Such dynamic account of the robustness and overall dynamic effects of particular genetic alterations would have been impossible to achieve without integrative approaches considering mathematical models and computer simulations.

5. Temporal and spatial patterns of cell-fate attainment during early flower development

The FOS-GRN reviewed above only considers a deterministic, single-cell GRN that is able to recover different gene configurations, which can be compared to observed gene profiles in different primordial cell types at early stages of flower development. However, in real developmental processes, groups or populations of cells attain distinct fates with certain spatial and temporal patterns. Thus, as mentioned above, we need morphogenetic models that are able to recover and enlighten such temporal and spatial morphogenetic patterns.

We have addressed the challenge of studying the temporal pattern of flower organ determination by exploring the sequence with which attractors for primordial flower organs are attained. We achieved this goal by introducing stochasticity into the GRN model described in the previous section. Since biological systems have evolved in inherently noisy environments, it is postulated that GRNs have been assembled in such a way that observed patterns are recoverable under noisy conditions. Indeed, recent studies show that stochasticity at the molecular scale may actually contribute to the formation of spatio-temporal patterns at higher levels of organization during development in other systems (see reviews in [33,45]).

Considering that noise could trigger transitions among attractors, we expected that in a stochastic model for a population of cells initialized in the sepal state (A genes expressed), the next most probable state would be AB (petals), then BC (stamens) and C (carpels) [9,46,47]. By introducing a certain degree of error in the updating of the dynamical rules of the FOS-GRN we actually recovered such a temporal sequence of cell-fate and ABC gene activation combinations ([34]; Fig. 3). In the latter study, this result was repeated with two GRN versions: the Boolean one described above and a continuous implementation that mimics the discrete case.

6. Recovering the epigenetic landscape of the FOS-GRN

Having shown that noisy fluctuations alone are able to drive transitions among attractors and that those transitions follow temporal patterns that reproduce the sequence with which ABC-genes are activated, it is reasonable to use the stochastic GRN model to postulate an epigenetic landscape (EL; [37,38]) associated with flower organ determination. Such an EL would be generated by the stochastic GRN dynamics and the GRN attractors would constitute the landscape's valleys (Fig. 3). It is interesting to note that, far from what could be intuitively expected, noise should not always be considered as a perturbation favoring disorder or instability. On the contrary, in view of these results, noise should be considered as a necessary factor in order for some developmental features to emerge in a robust way; in this case, the typical temporal sequence of activation of some genes, and probably also the spatial arrangement of gene configurations to some extent.

Studies with the stochastic version of the FOS-GRN also concluded that the relative position of the landscape's basins (GRN attractors) is important in determining the most probable temporal sequence of cell-fate attainment referred above [34]. This fascinating result certainly suggests that the stereotypical temporal pattern of cell-fate specification within the floral meristem at early stages of flower development may be an emergent and robust consequence of the complex GRN underlying cell-fate determination. In principle, such temporal sequence of cell differentiation could take place in the absence of inductive signals, emerging only as a result of the stochastic fluctuations that occur during transcriptional regulation [12].

Conceptually, this analysis was performed in a population of cells (or GRNs), updating each genetic configuration independently of each other. The next modeling step will be the integration of a collection of these networks, corresponding to the meristem, in a spatio-temporal framework (Fig. 4). This will allow a better understanding of the morphogenetic implications of the structure of the EL and is the object of current research. Moreover, this approach can also be used to test specific hypotheses. Questions of special interest are: (i) what is the mechanism by which the spatial disposition of the floral whorls is established, and (ii) what is the minimum number of necessary changes in the FOS-GRN and in the corresponding EL required for the whorls corresponding to stamens and carpels to be exchanged as in the atypical plant *Lacandonia schismatica* ([48,49]).

In another theoretical study, Lenser et al. [50] formulated a computational model that enabled the testing of the role of joint self-regulation of DEF-like and GLO-like floral homeotic genes in the robustness of petal and stamen development when stochastic noise is considered. In accordance with the results reviewed above, the authors found that the heterodimerization of these B floral homeotic proteins enhanced the robustness of cell-fate organ determination in the presence of stochasticity. Furthermore, their analysis suggests that mechanisms such as protein heterodimerization may play a central role in the canalization of flower development and evolution. This kind of prediction can hardly be formulated or tested without the aid of dynamical models.

7. Not in the genes: the role of geometrical and mechanical forces during development

A complete understanding of flower morphogenesis will continue to require multidisciplinary approaches and modeling tools that help at underpinning the coupling of such single-cell GRNs in explicit spatial and cellularized domains (e.g., [35,36,51]), with aspects like morphogen (e.g., auxin) metabolism, signaling pathways, gradients, cell growth and proliferation, mechanical forces,

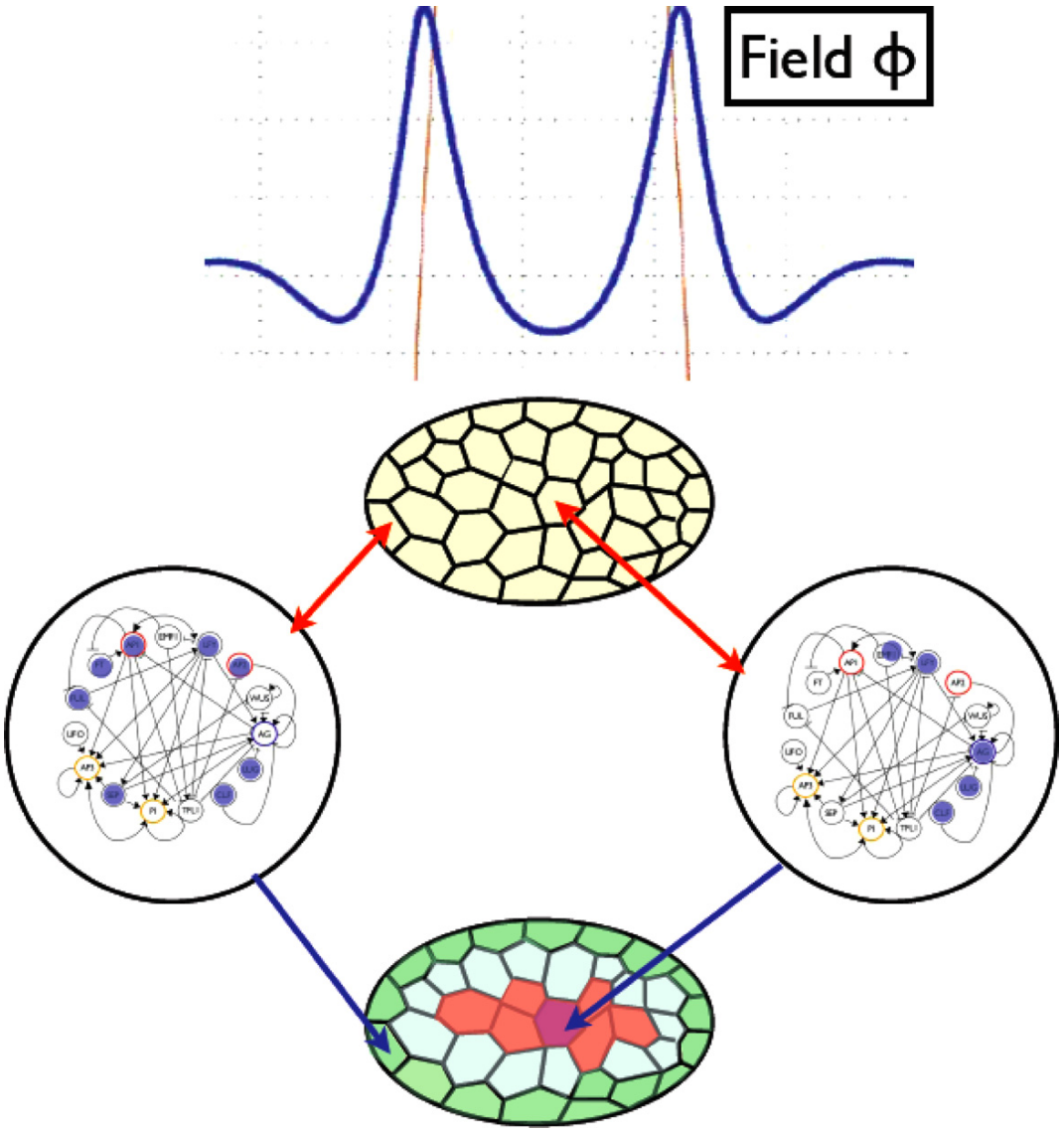


Fig. 4. Feedback between the FOS-GRN and physical fields could lead to the establishment of different regions within the meristem, each reaching the same stable multigene expression configurations or attractors. An idealized diagram illustrating the coevolution process of cell differentiation in a meristem is shown. A group of undifferentiated or stem cells (in yellow) secrete substances that produce a physical field of concentrations (ϕ) that forms a spatial pattern. According to the value of this field, the GRN of each cell (encircled) attains a different attractor, with a particular gene expression configuration that locally modifies the field, as well (double red arrows). Eventually, such a feedback process will allow cells to attain a different fate and become, for example, committed to form sepals or petals (cells with different colors at the bottom), depending on their relative position in the meristem.

and cell–cell communication mechanisms. All such aspects are likely to interact in non-linear ways both to and from the intracellular GRNs regulating cell differentiation and proliferation.

A central question in developmental biology is whether and how mechanical forces serve as cues for cellular behavior and thereby regulate morphogenesis [52]. Perhaps the most challenging aspect in understanding how mechanics, geometry and growth contribute to the formation of functional and robust structures is that these aspects not only influence each other, but are also coupled with at least two other fundamental interactions, genetic regulation and the cell cycle, at different time and spatial scales.

Several processes of cell communication may be relevant to understanding development, either by direct contact through their membranes, by the release of chemicals to the intercellular space, by electrical signals [53] or by the detection of pressure gradients and changes of curvature in tissue or organ surfaces [54]. Let us concentrate on the chemical signals, because meristematic cells are continuously exchanging and releasing all sorts of compounds (such as mRNAs, proteins, amino acids, hormones, etc.) to the extracellular matrix. These compounds must arrange themselves in space to form a macroscopic pattern of molecules, which, in turn, should be detected by GRNs in each cell altering their gene expression configurations accordingly. In other words, in order to accomplish this extraordinary choreography (without a choreographer!), the behavior of the chemicals and communication mechanisms should be coupled to the dynamics of the GRN in such a way that the chemical composition dictates the attractor of the GNR, and at the same time, the modified gene activity configuration of the GRN regulates the spatial pattern of chemical concentrations.

As mentioned before, the famous ABC model is able not only to predict the specification of the four whorl types in the flower, but also the different mutations found when one or several of the

ABC gene functions is lost. The problem is that this model does not explain why the spatial disposition of organs is ordered in concentric regions with the correct geometry or spatial arrangement and in the correct temporal sequence, that is, it does not address the central problem of self-organization in space–time during cell differentiation. In our opinion, this central issue remains unsolved.

The problem could be stated as follows: the GRN in each cell in the meristem is in a state of undifferentiated complacency, yet producing certain chemicals that act as transcription factors. These substances move in the intercellular space and respond to some physical field that dictates their concentrations in different regions of space, forming a geometrical pattern in space, which, in turn, provides each genetic network with a chemical environment that depends on this geometry (Fig. 4). The pertinent GRN for flower development in each cell senses this composition of chemicals and changes its configuration, expressing its genes in a different way, reaching a particular attractor (stable configuration of gene expression states), and consequently differentiating.

One could think of various physical fields that provide the size and form of the macroscopic domain where the microscopic processes (pertaining to the intracellular GRN dynamics) take place. A Turing mechanism is the most immediate and simple way of obtaining spatial stationary patterns through the diffusion of chemicals [51,55], but other mechanisms could be involved. In an ongoing work, we have proposed that a phase field with spontaneous curvature could be one of the mechanisms on which the accommodation of the ABC genes in space relies.

In other attempts to integrate physical fields and forces into models of development, meristem growth has been modeled using the principles of continuum mechanics (see review in [56–58]). Also, some quantitative mesoscopic models for flower development and growth in *A. thaliana* and other angiosperms have been put forward (e.g., [59–62]). More recently, it was shown that cells in the *A. thaliana* shoot apical meristem orient their microtubules along mechanical stress patterns generated during tissue formation, and this then affects the mechanical properties of the cell, thus establishing a feedback loop [52].

Even though, during the last years, genetics based approaches have been favored, it is recently being accepted that the richness and robustness of biological forms are not encoded only in the genes. Recent research is consistently showing that there are non-trivial interactions at all the levels mentioned above and, therefore, in order to achieve a global understanding of development, an integrated view has to be adopted [63,64].

If this program is to be carried out, the implementation of mathematical models becomes necessary. From the methodological perspective, modeling should be done in a multi-scale framework [65]. For the mechanical effects, the standard tool is continuum mechanics. There are several possibilities for GRN modeling, and assessing the performance of different models is still the subject of intensive research. However, we can safely say that no matter what formal mathematical description is used (a Boolean network, a system of ordinary differential equations, etc.), a non-linear dynamical system will account for the behavior of the genetic interactions.

However, this is only part of the required mathematical models, since, as it was pointed out before, all these three complexity levels interact non-trivially. We already mentioned that a central issue is how such levels of complexity are coupled. For example, what might have been considered as a parameter at some level becomes a variable dependent on variables at the other scales.

8. Conclusions

Understanding the emergence of spatiotemporal patterns that underlie organ formation during development remains a major challenge in biology. Moreover, understanding the origin and evolution of such patterns and developmental processes remains central to comprehending phenotypic transformations and is thus key to evolutionary biology in general [66–69]. Mathematical and computational modeling are playing a key role in the study of these central aspects of evolutionary developmental biology, as they (i) provide ways to uncover functional modules that are necessary and sufficient for developmental processes to occur, and (ii) can then be used to test how genetic or other kinds of variation in these modules that render new phenotypes. Pursuing this type of study could indeed shed light on the problem of the origin of morphological *themes* in plant and animals, this is, on the origin of structures that are observed in several lineages and that remain unchanged under a wide range of environmental conditions. Moreover, such research could help account for the origin of variations of these themes and morphogenetic novelties.

Modeling has proven particularly useful in the study of plant morphogenetic themes, specifically that of flower organ specification and arrangement. As reviewed above, theoretical efforts based on a vast set of experimental data resulted in the formulation of a functional module that includes the ABC genes and underlies cell-fate determination during early flower development. Furthermore, a morphogenetic model that could account for the spatiotemporal patterns that characterize flower organ arrangement is now being developed. We are starting to understand how the collective action of ABC genes, other genes and non-genetic factors give rise to the robust flower development theme. It is now possible to perform joint theoretical and experimental work aimed at exploring the variations that could underlie diversification and the generation of novelties throughout flower evolution.

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References

[1] Bowman JL, Smyth DR, Meyerowitz EM. Genes directing flower development in Arabidopsis. Plant Cell 1989;1(1):37–52.
[2] Bowman JL, Smyth DR, Meyerowitz EM. Genetic interactions among floral homeotic genes of Arabidopsis. Development 1991;112:1–20.
[3] Coen HS, Meyerowitz EM. The war of the whorls: genetic interactions controlling flower development. Nature 1991;353:31–7.
[4] Schwarz-Sommer Z, Davies B, Hudson A. An everlasting pioneer: the story of Antirrhinum research. Nat Rev Genet 2003;4(8):657–66.
[5] Jack T. Molecular and genetic mechanisms of floral control. Plant Cell 2004;16(Suppl.):S1–17.
[6] Jaeger J, Reinitz J. On the dynamic nature of positional information. Bioessays 2006;28:1102–11.
[7] Newman SA, Müller GB. Epigenetic mechanisms of character origination. J Exp Zool 2000;288(4):304–17.
[8] Ferrario S, Immink RG, Angenent GC. Conservation and diversity in flower land. Curr Opin Plant Biol 2004;7(1):84–91.
[9] Rudall P. Anatomy of flowering plants. 3rd ed. Cambridge University Press; 2007.
[10] Honma T, Goto K. Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 2001;409:525–9.
[11] Pelaz S, Tapia-López R, Alvarez-Buylla ER, Yanofsky MF. Conversion of leaves into petals in Arabidopsis. Curr Biol 2001;11:182–4.
[12] Alvarez-Buylla ER, Benítez M, Corvera-Poiré A, Chaos CA, de Folter S, Gamboa A, et al. Flower development. The Arabidopsis book. Rockville, MD: American Society of Plant Biologists; 2010, in press [http://www.aspb.org/publications/arabidopsis/].

[13] de Folter S, Busscher J, Colombo L, Losa A, Angenent GC. Transcript profiling of transcription factor genes during silique development in Arabidopsis. Plant Mol Biol 2004;56(3):351–66.
[14] Sablowski R. Flowering and determinacy in Arabidopsis. J Exp Bot 2007;58(5): 899–907.
[15] Sablowski R. Genes and functions controlled by floral organ identity genes. Semin Cell Dev Biol 2009;(September) [Epub ahead of print].
[16] Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, et al. A gene expression map of Arabidopsis development. Nat Genet 2005;37:501–6.
[17] Wagner GP, Mezey J, Calabretta R. In: Callebaut W, Rasskin-Gutman D, editors. Modularity: understanding the development and evolution of complex systems. Cambridge, MA: MIT Press; 2004.
[18] Smyth DR, Bowman JL, Meyerowitz EM. Early flower development in Arabidopsis. Plant Cell 1990;2:755–67.
[19] Urbanus SL, de Folter S, Shchennikova AV, Kaufmann K, Immink RG, Angenent GC. In planta localization patterns of MADS domain proteins during floral development in *Arabidopsis thaliana*. BMC Plant Biol 2009;12(9):5.
[20] von Dassow G, Meir E, Munro EM, Odell GM. The segment polarity network is a robust developmental module. Nature 2000;406:188–93.
[21] Albert R, Othmer HG. The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in *Drosophila melanogaster*. J Theor Biol 2003;223:1–18.
[22] Ruiz-García L, Madueño F, Wilkinson M, Haughn G, Salinas J, Martínez-Zapater JM. Different roles of flowering-time genes in the activation of floral initiation genes in Arabidopsis. Plant Cell 1997;9(11):1921–34.
[23] Ratcliffe OJ, Bradley DJ, Coen ES. Separation of shoot and floral identity in Arabidopsis. Development 1999;126(6):1109–20.
[24] Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF. B and C floral organ identity functions require SEPALLATA MADS-box genes. Nature 2000;405(6783):200–3.
[25] Komeda Y. Genetic regulation of time to flower in *Arabidopsis thaliana*. Annu Rev Plant Biol 2004;55:521–35.
[26] Kieffer M, Stern Y, Cook H, Clerici E, Maulbetsch C, Laux T, et al. Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance. Plant Cell 2006;18(3):560–73.
[27] Bower JM, Bolouri H. Computational modeling of genetic and biochemical networks. MIT Press; 2001.
[28] de Jong H. Modeling and simulation of genetic regulatory systems: a literature review. J Comput Biol 2002;9(1):67–103.
[29] Kauffman S. Metabolic stability and epigenesis in randomly constructed genetic nets. J Theor Biol 1969;22:437–67.
[30] Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla ER. Gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles. Plant Cell 2004;6:2923–39.
[31] Huang S, Ingber DE. A non-genetic basis for cancer progression and metastasis: self-organizing attractors in cell regulatory networks. Breast Dis 2006;26:27–54.
[32] Paulsson J. Summing up the noise in gene networks. Nature 2004;427:415–8.
[33] Macarthur BD, Ma'ayan A, Lemischka IR. Systems biology of stem cell fate and cellular reprogramming. Nat Rev Mol Cell Biol 2009;10:672–81.
[34] Alvarez-Buylla ER, Chaos A, Aldana M, Benítez M, Cortes-Poza Y, Espinosa-Soto C, et al. Floral morphogenesis: stochastic explorations of a gene network epigenetic landscape. PLoS ONE 2008;3(11):e3626, doi:10.1371/journal.pone.0003626.
[35] Savage NS, Walker T, Wieckowski Y, Schiefelbein J, Dolan L, Monk NA. A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the Arabidopsis root epidermis. PLoS Biol 2008;6(9):e235.
[36] Benítez M, Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla ER. Interlinked nonlinear subnetworks underlie the formation of robust cellular patterns in Arabidopsis epidermis: a dynamic spatial model. BMC Syst Biol 2008;17(2):98.
[37] Waddington CH. The strategy of the genes. A discussion of some aspects of theoretical biology. New York, NY: MacMillan; 1957.
[38] Slack JM. Conrad Hal Waddington: the last Renaissance biologist? Nat Rev Genet 2002;3:889–95.
[39] Mendoza L, Alvarez-Buylla ER. Dynamics of the genetic regulatory network for *Arabidopsis thaliana* flower morphogenesis. J Theor Biol 1998;193:307–19.
[40] Chaos A, Aldana M, Espinosa-Soto C, García Ponce de León B, Garay-Arroyo A, Alvarez-Buylla ER. From genes to flower patterns and evolution: dynamic models of gene regulatory networks. J Plant Growth Regul 2006;25: 278–89.
[41] Vandenbussche M, Zethof J, Royaert S, Weterings K, Gerats T. The duplicated B-class heterodimer model: Whorlspecific effects and complex genetic interactions in Petunia hybrida flower development. Plant Cell 2004;16: 741–54.
[42] Whipple CJ, Ciceri P, Padilla CM, Ambrose BA, Bandong SL, Schmidt RJ. Conservation of B-class floral homeotic gene function between maize and Arabidopsis. Development 2004;131:6083–91.
[43] Adam H, Jouannic S, Morcillo F, Verdeil JL, Duval Y, Tregear JW. Determination of flower structure in Elaeis guineensis: do palms use the same homeotic genes as other species? Ann Bot 2007;100:1–12.
[44] Mendoza L, Thieffry D, Alvarez-Buylla ER. Genetic control of flower morphogenesis in *Arabidopsis thaliana* a logical analysis. Bioinformatics 1999;15:593–606.
[45] Raj A, van Oudenaarden A. Nature, nurture, or chance: stochastic gene expression and its consequences. Cell 2008;135:216–26.
[46] Yu H, Ito T, Zhao Y, Peng J, Kumar P, Meyerowitz EM. Floral homeotic genes are targets of gibberelin signaling in flower development. Proc Natl Acad Sci USA 2004;101:7827–32.
[47] Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, et al. Dissection of floral induction pathways using global expression analysis. Development 2003;130:6001–12.
[48] Vergara-Silva F, Espinosa S, Ambrose A, Vázquez-Santana S, Martínez-Mena A, Márquez-Guzmán J, et al. Inside-out flowers characteristic of *Lacandonia schismatica* evolved at least before its divergence from a closely related taxon, *Triuris brevistylis*. Int J Plant Sci 2003;164:345–57.
[49] Ambrose BA, Espinosa-Matías S, Vázquez-Santana S, Vergara-Silva F, Martínez E, Márquez-Guzmán J, et al. Comparative developmental series of the Mexican triurids support a euanthial interpretation for the unusual reproductive axes of *Lacandonia schismatica* (Triuridaceae). Am J Bot 2006;93:15–35.
[50] Lenser T, Theissen G, Dittrich P. Developmental robustness by obligate interaction of class B floral homeotic genes and proteins. PLoS Comput Biol 2009;5(1):e1000264.
[51] Jönsson H, Heisler M, Reddy GV, Agrawal V, Gor V, Shapiro BE, et al. Modeling the organization of the WUSCHEL expression domain in the shoot apical meristem. Bioinformatics 2005;21:i232–40.
[52] Hamant O, Heisler MG, Jönsson H, Krupinski P, Uyttewaal M, Bokov P, et al. Developmental patterning by mechanical signals in Arabidopsis. Science 2008;322:1650–5.
[53] Barlow PW. Reflections on 'plant neurobiology'. Biosystems 2008;92(2): 132–47.
[54] Kwiatkowska D. Flower primordium formation at the Arabidopsis shoot apex: quantitative analysis of surface geometry and growth. J Exp Bot 2006;57:571–80.
[55] Turing A. The chemical basis of morphogenesis. Philos Trans R Soc Lond B Biol Sci 1952;237:37–72.
[56] Kwiatkowska D. Flowering and apical meristem growth dynamics. J Exp Bot 2008;59(2):187–201.
[57] Baskin TJ. Anisotropic expansion of the plant cell wall. Annu Rev Cell Dev Biol 2005;21:203–22.
[58] Goodall CR, Green PB. Quantitative analysis of surface growth. Bot Gaz 1986;147:1–15.
[59] Rolland-Lagan J, Bangham A, Coen E. Growth dynamics underlying petal shape and asymmetry. Nature 2003;422:161–3.
[60] Lee HC, Chioub DW, Chenc WH, Markhartd AH, Chene YH, Lin TY. Dynamics of cell growth and endoreduplication during orchid flower development. Plant Sci 2004;166:659–67.
[61] Skryabin KG, Alekseev DV, Ezhova TA, Kozlov VN, Kudryavtsev VB, Nosov MV, et al. A mathematical model of genetic control of determination of floral organ identity in *Arabidopsis thaliana*. Biol Bull 2004;31:346–53.
[62] Mündermann L, Erasmus Y, Lane B, Coen E, Prusinkiewicz P. Quantitative modeling of Arabidopsis development. Plant Physiol 2005;139:960–8.
[63] Geitmann A, Ortega JK. Mechanics and modeling of plant cell growth. Trends Plant Sci 2009;14(9):467–78.
[64] Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM. Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. Proc Natl Acad Sci USA 2009;106(38): 16529–34.
[65] Alarcón T, Byrne HM, Maini PK. Towards whole-organ modelling of tumour growth. Prog Biophys Mol Biol 2004;85(2–3):451–72.
[66] Hall BK. Evo-Devo: evolutionary developmental mechanisms. Int J Dev Biol 2003;47:491–5.
[67] Carroll SB. EvoDevo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 2008;134:25–36.
[68] Sommer RJ. The future of evo-devo: model systems and evolutionary theory. Nat Rev Genet 2009;10(6):416–22.
[69] Müller GB. Evo-devo: extending the evolutionary synthesis. Nat Rev Genet 2007;8:943–9.

Apéndice I

Gene Regulatory Models for Plant Development and Evolution

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Chapter 1
Gene Regulatory Models for Plant Development and Evolution

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1.1 Introduction: the Need for Mathematical Models to Understand Plant Development

During development, complex interactions amongst genetic and non-genetic elements give rise to robust spatiotemporal patterns. Moreover, an important feature of biological systems is the nontrivial flow of information at several scales. When we consider the scale determined by the cell, we observe that it integrates information coming from gene regulatory networks (GRNs), biochemical pathways, and other microscopic processes. If we consider larger scales, then intercellular communication, mechanical and geometric effects (such as growth, shape, and size), and environmental influences have to be taken into account. This is why understanding how patterns arise during development requires the use of formal dynamical models able to follow the concerted action of so many elements at different spatiotemporal scales.

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The fact that biological entities and scales often interact nonlinearly makes mathematical modeling of biological systems, and in particular of gene regulatory networks, a nontrivial problem. From the mathematical point of view, the incorporation of all these interactions can be taken into account only by implementing hybrid models, that is, by incorporating both discrete and continuous elements, as well as deterministic and stochastic frameworks. In fact, depending on the specific space-time scale at which a process is being observed, it might appear discrete or continuous, deterministic or random. For instance, the levels of gene expression might be taken as discrete (the gene is "on" or "off") when seen at rough space-time scales, but when observed with a finer gauge, these levels appear as continuously varying.

Mathematical models of GRNs provide an integrative tool, a systematic way of putting together and interpreting experimental information about the concerted action of gene activity. They also offer new insights on the mechanisms underlying biological processes, in particular developmental ones, as well as a means to make informed predictions on the behavior of such complex systems.

1.2 Dynamic GRN Models

Today, one of the most important challenges in systems biology is to relate the gene expression patterns of an organism with its observed phenotypic traits. Since these patterns result from the mutual activation and inhibition of all the genes in the genome in a coordinated way, the above problem is equivalent to relating the dynamical properties of the underlying genetic network with the organism's phenotype (Hasty et al. 2001; Levine and Tjian 2003). In order to achieve this goal, one must decide first how to model the dynamics of the genetic network.

Amongst the several theoretical approaches that have been proposed to model the genetic dynamics, two stand out, namely, the *continuous* and the *discrete* (Smolen et al. 2000; Bower and Bolouri 2001). The continuous approach is based on systems of coupled nonlinear differential equations that describe the temporal evolution of the concentration of the chemicals involved in the gene regulation processes (proteins, enzymes, transcription factors, metabolites). This description is particularly suitable when the systems under consideration consist of a small number of components (Arkin et al. 1998; Vilar et al. 2003). However, large-scale genome analysis has revealed that the coordinated expression of dozens, or even hundreds of genes is required for many cellular processes to occur, such as cell division or cell differentiation (Whitfield et al. 1992; Rustici et al. 2004). For such processes, the continuous approach becomes intractable due to the great number of components and equations involved.

The discrete approach to model the dynamics of genetic networks was first introduced by Kauffman to describe, in a qualitative way, the processes of gene regulation and cell differentiation (Kauffman 1969). This approach focuses on the state of expression of the genes, rather than on the concentration of their products.

Thus, the level of expression of a given gene is represented by a discrete variable g that usually takes the values g=0 if the gene is not expressed, and g=1 if the gene is fully expressed. The genome is considered then as a set of N discrete variables, $g_1, g_2, \ldots, g_N$, their values changing in time according to:

$$g_n(t+1) = F_n\left[g_{n_1}(t), g_{n_2}(t), \ldots, g_{n_{k_n}}(t)\right] \tag{1.1}$$

In this equation, $(g_{n_1}, g_{n_2}, \ldots, g_{n_{k_n}})$ are the k_n regulators of the gene g_n, and F_n is a discrete function (also known as a logical rule) constructed according to the nature of the regulators. The advantage of the discrete model is that it can incorporate a much larger number of components than the continuous models. Furthermore, recent work shows evidence that, in spite of the simplicity of the discrete approach, it is able to reproduce the gene expression patterns observed in several organisms (Huang and Ingber 2000; Mendoza and Alvarez-Buylla 2000; Albert and Othmer 2002; Espinosa-Soto et al. 2004; Davidich and Bornholdt 2008). This evidence has been obtained for relatively small genetic networks for which both the regulators and the logical rules are known for each gene.

Accumulated data on molecular genetics and current high-throughput technology (see next section) have made available a great amount of data regarding GRNs, yet information for all the regulators and logical rules in entire genomes is not available yet for any organism. Nonetheless, it is important to emphasize that, for the small genetic modules or sub-networks that have been thoroughly documented experimentally, the discrete approach gives accurate predictions.

Arguably, one of the most important results of the discrete model is the existence of *dynamical attractors*. Starting out from an initial state $[g_1(0), g_2(0), \ldots, g_N(0)]$ in which some genes are active and some others inactive, Eq. (1.1) generates dynamics in which each gene goes through a transient series of active/inactive states until the whole network enters into a periodic pattern of expression (Fig. 1.1). Some genes reach a constant value that does not change in time anymore, whereas some others keep "blinking" in a periodic way. This periodic state of expression of the entire network is the dynamical attractor. The set of all the possible initial states that after a transient time fall into the same attractor is called the *basin of attraction* of that attractor. Each attractor is uniquely identified by its set of active genes. In other words, particular sets of genes are expressed in different attractors, and this is precisely the characteristic that identifies the different functional states of the cell. For this reason, Kauffman formulated the hypothesis—confirmed experimentally—that the dynamical attractors of the genetic network correspond to the different cell types or cell fates observed in the organism.

Since the level of expression of each gene is discretized into a finite number of values, the total number, Ω, of dynamical states in which the network can be found is also finite, and is given by $\Omega = \prod_{n=1}^{N} m_n$, where m_n is the number of discrete values that g_n can acquire. Under Eq. (1.1), the dynamical space of the network (i.e., the possible Ω states) is partitioned into disjoint sets consisting of the attractors and their corresponding basins of attraction (Fig. 1.1). This structure of the dynamical

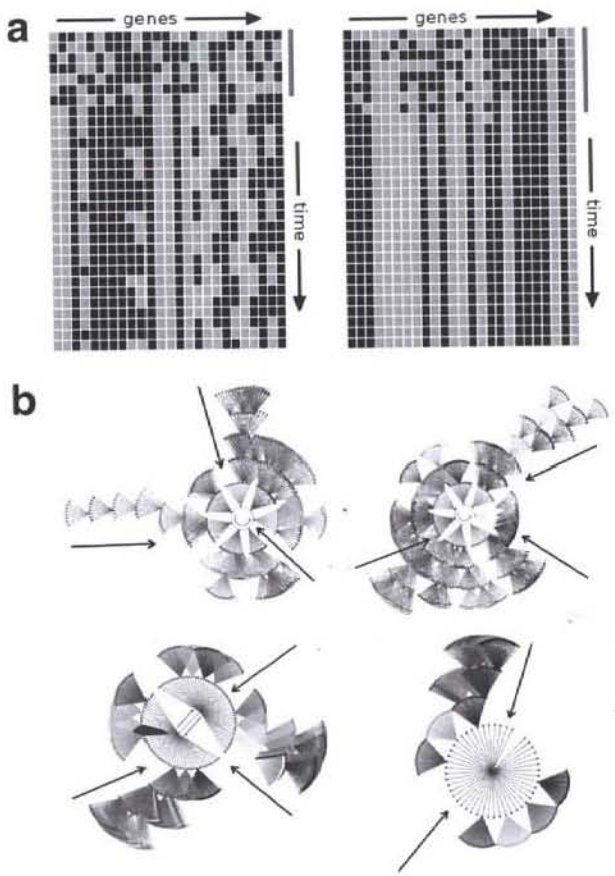

Fig. 1.1 Attractors and attractor basin in a GRN. **(a)** Visual representation of the dynamical attractors of a genetic network. Each square represents a gene, in *gray* if it is expressed, and in *black* if it is not. The genes are lined up horizontally so that each row represents the state of expression of the entire genome at a given time. Time flows downward. After a transient time (indicated with a *vertical line*), the whole network reaches a periodic pattern of expression, which is the dynamical attractor. As shown, two different initial states (the *uppermost rows*) can lead to different attractors. The attractor on the *left* has period six, whereas the attractor on the *right* consists of only one state. **(b)** Visual representation of the attractor landscape for a randomly constructed network with $N=12$ genes. Each *dot* represents a dynamical state of the network (i.e., one of the rows in **a**), and the *lines* represent discrete time steps. Two dots are connected if they are successive states under the dynamics given by Eq. (1.1). The fan-like structures reflect the fact that many states can have the same successor in time (the dynamics are dissipative). The *arrows* indicate the direction of the dynamical flow. In this particular example, the state-space of possible dynamical states organizes into four disjoint sets consisting of the attractors and their respective basins of attraction

space is called the *attractor landscape*, and constitutes a representation of the epigenetic landscape conceived by Waddington (1957) to qualitatively understand the different functional states of the cell. It has been shown recently for several cases that many important phenotypic traits of the organism, such as the cell type or the cell cycle, are encoded in the entire attractor landscape.

1.3 Inference of GRN Topology from Microarray Experiments

GRN architecture inference is the process by means of which information on the regulators is obtained from experimental data. In some cases, network structure has been inferred from thorough data of molecular genetics experiments, enabling novel insights and predictions for particular developmental systems (Mendoza and Alvarez-Buylla 2000; Albert and Othmer 2002; Espinosa-Soto et al. 2004). Nevertheless, the current available technology enables the generation of large sets of genomic information, commonly acquired from microarray experiments. This experimental technique allows observing the expression pattern of a set of genes at different sample points in time or under different experimental conditions, and has generated a vast data base.

Although powerful, microarray experiments and their data have two difficulties. First, an enormous number of experiments are necessary in order to confidently infer all the logical rules in a given genome. Second, the data obtained are very noisy, which is why uncovering structural or dynamic information is anything but trivial. We briefly introduce some of methods and approaches that have addressed the need of formal frameworks in this area.

Reverse engineering is the process of discovering the functional principles of a device, object, or system through analysis of its structure, function, or operation. In the context of GRNs, it constitutes the process of network structure inference from the analysis of experimental data on gene expression under diverse conditions, often derived from microarray experiments. Despite the particular method to be used to analyze microarray data, the overall goal of GRN reverse engineering is to find mathematical evidence supporting the proposition of an interaction between the nodes of the network.

Two main classes of methods have been proposed to infer GRN architectures via reverse engineering methods. The first class relies on probability theory, and its objective is to find the most probable network architecture given a genetic expression pattern, or to quantify the existent correlation between pairs of genes. Bayesian networks, both traditional and their dynamic variant, fall into the first approach, while mutual information methods fall into the second one. The second class of methods is based on continuous analysis. It involves ordinary differential equations (ODEs), and is supported by the theory and methods from stability analysis of dynamic systems.

1.3.1 Bayesian Networks

A Bayesian network is an acyclic graph of a joint probability distribution where the nodes are the random variables, and the directed edges are causal influences. Bayesian network models have proven to be useful to infer a GRN structure (Imoto et al. 2002). However, one of the major drawbacks of traditional Bayesian models is that, by definition, cycles cannot be found, and cycles or feedback loops constitute a very important feature of biological GRNs. However, dynamical Bayesian network models (Kim et al. 2003) allow both the inclusion of cycles and the representation of a different temporal behavior for each gene of the network, and offer a promising alternative for reverse engineering of GRNs.

1.3.2 Mutual Information

Mutual information is a technique that allows inferring GRN architecture with a more general criterion than that of the more common statistical methods, which focus mainly on linear correlations, as it enables consideration of any functional relationship (see review in Steuer et al. 2002). Despite the advantage of being rooted in a well-known probabilistic framework, these methods are computationally intensive, due to the high amount of nodes, and the estimation of the unknown temporal delays for each node, which has to be approximated, thus limiting the possibility of studying GRNs composed of a large number of nodes.

1.3.3 Continuous Analysis Models

These methods consider a network of n genes as a system of ODEs where the change in the level of expression of gene i is denoted as (x_i), and its dynamic is described as:

$$\frac{dx_i}{dt} = f_i(x_1, x_2, \ldots, x_n) \text{ for } i = (1, 2, \ldots, n) \tag{1.2}$$

Thus, the influence that node x_j inflicts on node x_i is obtained by computing the partial derivative of f_i with respect to x_j. Moreover, the sign of each of these partial derivatives determines whether the interaction between a couple of nodes corresponds to up- or downregulation. The set of all so-defined partial derivatives constitutes the Jacobian matrix of the system, and hence, the GRN architecture is obtained as a graphical representation of the signs of the elements of the Jacobian matrix (Aguda and Goryachev 2007). An alternative method to compute the sign of these derivatives consists of perturbing each f_i (see Kholodenko et al. 2002; Sontag et al. 2004; Andrec et al. 2005). In fact, near a steady state, both the perturbation and the Jacobian matrix methods are theoretically equivalent, and thus yield the same results.

A slightly different approach is suggested by Cho et al. (2006). In this method, each column of a matrix represents the expression profile of gene i at times $t_1, t_2, \ldots, t_k$. This may be regarded as a set of measurements of a random variable x_i. Each time series x_i is then plotted in a phase portrait against each and every x_j such that $j \neq i$. In this case, the direction of the interaction is given by a winding index WI, and the type of interaction by a slope index SI. For instance, considering a two-node network with components x_1 and x_2 whose time-series expression profiles are measured at k even sampling points, SI and WI of x_1 and x_2 are given by:

$$\mathrm{SI}(x_1, x_2) = \frac{1}{k-1} \sum_{i=1}^{k-1} sign\left[\frac{x_2(i+1) - x_2(i)}{x_1(i+1) - x_1(i)}\right] \tag{1.3}$$

$$\mathrm{WI}(x_1, x_2) = \frac{1}{k-2} \sum_{i=1}^{k-2} sign\ [\mathrm{d}(i)] \tag{1.4}$$

where

$$\mathrm{d}(i) = \det \begin{bmatrix} x_1(i) & x_1(i+1) & x_1(i+2) \\ x_2(i) & x_2(i+1) & x_2(i+2) \\ 1 & 1 & 1 \end{bmatrix} \tag{1.5}$$

There are still very few examples of successful applications of these methods of reverse engineering to plant data (cf. review in Alvarez-Buylla et al. 2007). In contrast, dynamic GRN models grounded on detailed molecular genetic plant data have been successful at reproducing observed patterns of gene expression. We, therefore, focus here on such an approach for small sub-networks of plant development.

1.4 GRN Models for Modules of Plant Development

Dynamic network models have been recently used to study plant development, since they are able to capture important aspects of biological complexity. Furthermore, these models integrate empirical evidence, and thus provide a useful tool for novel hypothesis testing by detecting missing or contradictory data, generating predictions, and delimiting future experiments. As mentioned above, most of such models have been based on relatively small and thoroughly described subnetworks associated to a particular developmental process. This has enabled a rather direct interpretation of the model results, and a more profound understanding of certain aspects of development.

1.4.1 *Single-Cell Gene Regulatory Network Models: the Case of Arabidopsis* Flower Organ Primordial Cell Specification

In plants, the flower is the most complex and well-studied structure from a developmental perspective. It characterizes angiosperms or flowering plant species, and exhibits a stereotypical conserved structure in the great majority of flowering plant species (Rudall 1987). Early during flower development, the bud or flower meristem is partitioned into four concentric regions, each one comprising the primordia that will eventually form mature floral organs. Floral organs appear from the outermost to the inner part of the plant in the sequence sepals, petals, stamens, and carpels.

There is a great amount of detailed and high-quality data for the molecular interactions that regulate flower development. In fact, on the base of these data, a now classical model of flower development has been proposed, namely, the "ABC" model. This model establishes that the combinatorial activities of genes grouped in three types or functions (*A* type, *B* type, and *C* type) are needed to specify floral organs. *A* genes alone are needed for sepal identity, *A*+*B* for petal, *B*+*C* for stamen, and *C* alone for carpel identity (Coen and Meyerowitz 1991).

A GRN Boolean model grounded on experimental data (Mendoza and Alvarez-Buylla 1998; Mendoza et al. 1999; Espinosa-Soto et al. 2004; Chaos et al. 2006) recovers the profiles of gene activation that characterize primordial sepal, petal, stamen, and carpel cells during early *Arabidopsis thaliana* (L.) Heynh. flower development (Fig. 1.2). This was the first published Boolean GRN model that was validated with experimental data, and generated testable predictions. Since then, other systems have been studied with the same approach.

The results of the floral GRN model coincide with the *ABC* model, but also provide a dynamic explanation for the robust attainment of the combinatorial gene activations involved in floral organ determination. In addition, this GRN model enabled hypotheses on the sufficiency and necessity of particular gene regulatory interactions among the ABC and other genes. Computer simulations of this flower GRN also show that its attractors are robust to random perturbations on the logical rules (Espinosa-Soto et al. 2004; Chaos et al. 2006), hinting on an explanation for the evolutionary conservation of flower structure. In conclusion, this model incorporates the key components of the GRN underlying the ABC model, and provides a dynamical explanation for cell type determination in flower buds.

1.4.2 *Spatiotemporal Models of Coupled GRN Dynamics*

The models presented above are useful to explore cell-fate attainment in isolated cells. However, in order to understand the emergence of spatiotemporal cell patterns during development, models that couple such single-cell GRN models in explicit spatial domains are needed.

Most models addressing the origin of cellular patterns consider "toy networks", or dismiss intracellular GRN topology altogether, and provide only mesoscopic

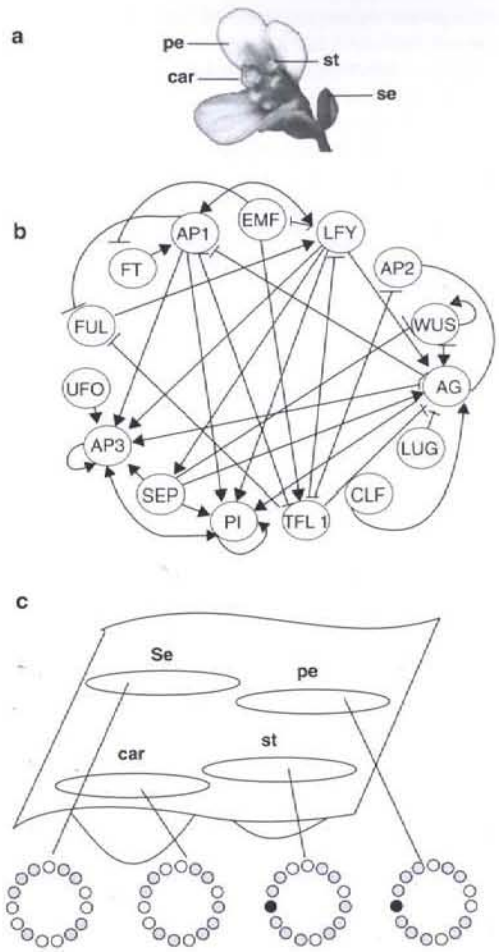

Fig. 1.2 Gene regulatory network underlying cell type determination during early flower development in *Arabidopsis*. **(a)** Mature flower showing the four floral organs: sepals, petals, stamens, and carpels. **(b)** The GRN depicted here underlies the attainment of the primordial cellular identities during flower development. Nodes represent genes, and edges denote regulatory interactions among them (*arrows* correspond to positive regulation, "*flat arrows*" to negative regulation). **(c)** The GRN represented in **b** attains steady states that match the gene activation profiles characteristic of the four primordial cell types. In a schematic landscape, each cell type corresponds to a local minimum, and is associated to a particular GRN configuration (nodes in *white* are "off", those in *gray* are "on", and those in *black* may be in either of the two states)

models of morphogenetic dynamics, while the majority of experimentally grounded GRN models ignore cellular-scale interactions. Therefore, one of the challenges remaining today is to achieve multi-scale models, most likely by the postulation of hybrid models that integrate GRNs in cellular contexts.

During plant development, cells commit to a certain fate according mainly to their position in a region of the plant, rather than in relation to their cellular lineage as is the case in most animal systems (Scheres 2001). Hence, understanding how positional information is generated and maintained comprises a paramount task for developmental biology. GRN dynamics, geometry of the domain, mechanical restrictions, and hormonal and environmental factors all play relevant roles in this process. Below we present two developmental models that partially incorporate some of these aspects.

The GRNs responsible for cell type determination in the leaf and root epidermis of *A. thaliana* have been thoroughly described, and provide an excellent system for addressing the origin of cellular patterning during development. It has been suggested that this network may behave qualitatively as an activator-inhibitor system (Pesch and Hülskamp 2004), which is able to generate stable complex patterns de novo. This has been further explored with the use of a dynamic spatial model (Benítez et al. 2007). In this approach, the authors first used a discrete GRN model, and found that its attractors match two epidermal cell types, corresponding to hair and non-hair cells. Then, the authors simulated a simplified version of the network in a spatial domain, and provided evidence supporting that leaf and root GRNs, although slightly different, are qualitatively equivalent in their dynamics. This study also showed that cell shape may have a relevant role during cell pattern formation in the root epidermis.

Another model that considers a GRN in a spatial domain is that proposed by Jönsson et al. (2005), in which the authors used in vivo gene expression data to simulate a cellularized template incorporating a relatively small GRN. This GRN, which includes the gene *WUSCHEL* (*WUS*), seems to regulate the meristem's size and maintenance, and was modeled with the use of the so-called connectionist model (Mjolsness et al. 1991). By doing this, the authors postulated a mechanism that could underlie the spatial gene expression pattern of *WUSCHEL* (*WUS*) in the A. thaliana shoot apical meristem, and provided a useful experimental and computational platform to improve developmental models. Recently, several platforms helpful for integrating GRNs in a cellularized domain and modeling plant development have been proposed (Holloway and Harrison 2007; Buck-Sorlin et al. 2008; Dupuy et al. 2008). These will be useful for further models of coupled GRNs.

1.4.3 Auxin Transport Is Sufficient to Generate Morphogenetic Shoot and Root Patterns

Morphogene gradients are the key for pattern formation. In plants, auxin is a hormone that provides important positional information during *A. thaliana*

development (Vieten et al. 2007). Recently, some mesoscopic models for auxin-driven pattern formation in the shoot and root have integrated the accumulated experimental evidence, and contributed to the understanding of these systems.

In the growing *A. thaliana* shoot, new leaves and flower primordia emerge at defined positions along the flanks of the shoot apical meristem. Primordia patterning, and therefore phyllotactic arrangements, seem to be determined by auxin peaks that determine site or primordia initiation and activation. On the base of empirical data, Jönsson et al. (2006) suggested a mechanism in which this plant hormone influences its own polarized flux within the shoot apical meristem by directing localization of its own transporters (PIN and AUX proteins). The mathematical model for auxin transport proposed by Jönsson et al. (2006) recovered peaks of auxin concentration at positions where actual new primordia emerge. Their cell-based model revealed that the auxin feedback loop, in which the hormone regulates its own transport, is sufficient to generate the regular spatial patterning of primordia.

The above model is able to generate the complex phyllotactic patterns observed in plants under different parameters. However, in contrast to what has been observed in a great majority of plants, the patterns generated by this model are not stable. We hypothesize that the stability of observed phyllotactic patterns may depend upon the complex GRNs that underlie PIN, AUX, and other protein regulation.

A recent paper (Grieneisen et al. 2007) proposed a computational model that addresses the generation of a robust and information-rich auxin pattern in *A. thaliana* roots. This model assumes certain internal distribution of the PIN auxin transport facilitators, and incorporates diffusion and permeability, as well as the *A. thaliana* root structure. Interestingly, the patterns recovered by this root model are robust to alterations on several parameters, as well as to cell division and expansion. Given the PIN layout in the root, the model is useful to explain the phenotypes of pin loss-of-function mutants, and also accounts for slow changes in root zonation (meristematic and elongation zones) when feedback from cell division and expansion are introduced. According to this work, the auxin pattern depends on a capacitor-like mechanism that may buffer the absence of auxin from the shoot, or auxin leakage and decay.

The study of Grieneisen et al. (2007) is a wonderful example of how a mathematical and computational model can be useful to provide explanations about developmental mechanisms and patterns, and to generate novel hypotheses that can be tested experimentally. Yet, this model stands on the assumption that the auxin transporters maintain a fixed polarized distribution within the cell. Since it has been shown that the transporters' localization is affected by the auxin flux itself, a more general model should incorporate a dynamic mechanism for the mutual regulation of transporter position and auxin flux.

Both models show that transport-dependent auxin gradients constitute a powerful mechanism to generate developmental information, and will certainly provide a solid base to incorporate the genetics of PIN distribution, as well as the role of other components of plant morphogenesis.

1.4.4 Signal Transduction Models

In living organisms, GRNs are often interacting with other sub-networks, or with signaling pathways that act as an input to GRNs. This is particularly clear in plants—being sessile, they respond to environmental challenges by plastic developmental responses. Signaling pathways frequently integrate environmental cues, and are the key for developmental plasticity. These pathways are usually hierarchical, and in a first approximation may be represented as cascade processes. However, these pathways often show complex dynamics, e.g., oscillations and chaos, and crosstalk among them seems to be the rule in plants, which is why dynamical models will certainly be useful for a better understanding of these processes. Some recent models aim at simulating the dynamics of pathways in plants, plastic processes of development, and the coupled dynamics of pathways and GRNs.

Díaz and Alvarez-Buylla (2006) proposed a continuous model that endeavors at studying the signaling pathway associated to the hormone ethylene in *A. thaliana*, as well as the effect of different ethylene concentrations on downstream transcription factors. This model predicts dose-dependent gene activity curves that are congruent with the dose-dependent observed phenotypes, and interestingly, it also leads to the prediction that signaling pathways may filter certain stochastic or rapid fluctuations of hormone concentration.

Also focusing on the dynamics of plant hormones is the model presented by Li et al. (2006). Their model consists of a Boolean network approach that integrates the great amount of experimental findings related to the abscisic acid pathway, and stomata opening and closure dynamics. Such a model is able to predict and test network alterations leading to qualitative changes in the behavior of stomata. Models like this contribute to a better understanding of plant physiology, as well as to the development of better techniques for crop management.

As mentioned above, cell type determination in the *A. thaliana* epidermis has been thoroughly studied, and it has been found that root hair arrangement is plastic with respect to nutrient availability. Savage and Schmidt (2008) present a hypothesis that is congruent with available molecular and physiological data, and that attempts to account for root hair arrangement in a context of developmental plasticity. The mechanism they postulate and simulate relies on a well-known Turing-like patterning mechanism, and remains to be tested experimentally. This is an example of how computational models of plant development may lead to, or eventually support, precise and novel non-intuitive hypotheses.

1.5 The Constructive Role of Stochasticity in GRN and Other Complex Biological Systems

All the above models are deterministic. Historically, noise has been considered as a nuisance, and efforts to control or minimize it have been undertaken. However, the pioneering works of Benzi et al. (1981) and Nicolis (1981, 1982) changed this

perspective, as they showed that noise may play an important role in the appearance of patterns in complex systems.

Benzi et al. (1981) introduced the concept of stochastic resonance (SR) for processes, in which the presence of random fluctuations (noise) amplifies the effects of a weak deterministic signal (Gammaitoni et al. 1998). More recently, the number of studies addressing the interaction of noise and deterministic signals in complex systems has increased (Gang et al. 1993; Pikovsky and Kurths 1997; Russell et al. 1999), and numerous new constructive roles of noise have been acknowledged in diverse natural processes.

Noise is ubiquitous in genetic processes (Rao et al. 2002; Blake et al. 2003; Paulsson 2004; Cai et al. 2006). It can arise from at least two different sources in cells. First, statistical fluctuations from a finite number of molecules make the transcriptional and translational processes intrinsically stochastic (Blake et al. 2003). Second, small variations in temperature and environmental perturbations provide the source for extracellular noise. It appears that GRNs are not only robust to stochastic fluctuations, but in some cases they incorporate noise in a constructive way (Wang et al. 2007). Most studies of this phenomenon have documented noise-enhanced heterogeneity (Rao et al. 2002), which has been proposed as a means for improving sensitivity of intracellular regulation to external signals (Paulsson et al. 2000). A related phenomenon is noise-induced selection of attractors (Kaneko 1998; Kraut et al. 1999), which enables dynamical switching to multistability in systems that are deterministically monostable.

In the context of developmental biology, it has been postulated that cell-fate differentiation can be driven by noise (Huang and Ingber 2007). Therefore, considering noise in dynamic models could be important for analyzing the spatiotemporal sequence with which cell fates are determined during development. For instance, GRNs that underlie cell determination could be viewed as a stochastic dynamical system (Davidson et al. 2002). This approach rescues the original proposal of an epigenetic landscape explored by random perturbations (Waddington 1957) as a metaphor for understanding the dynamical patterns of transitions among different functional states of the cell during development.

1.6 GRN Structure and Evolution

Besides the use of GRNs for understanding the development of extant organisms, such models are useful for exploring hypotheses on organismal evolution. A particularly interesting phenomenon recently reported is that, after the duplication and divergence (through mutations) of a single gene in a network, new attractors can appear (Aldana et al. 2007). Thus, not only can the duplicated and diverged gene acquire a new function (Li and Graur 1991), e.g., a new signaling molecule or structural protein, but also the entire genetic network can develop new phenotypes and functional states. Attractors of GRNs can be interpreted as

characters, cell types, or functions (Huang and Ingber 2000; Espinosa-Soto et al. 2004; Huang et al. 2005), and the number of these affect the possibilities to evolve and adapt. Thus, the emergence of new attractors allows for the possibility of evolving, constituting the raw material upon which natural selection could act.

A second possibility for GRN evolution is the integration of two networks in a way similar to that of an engineer working with capacitors, transistors, and other modular elements. These are combined in various ways to create new devices. This evolutionary process may occur by duplicating the whole network, or by linking two or more independent networks, each one with a particular set of functions. In this way, both networks can continue to yield their original functions, but the interaction between them can originate new functions.

In the different types of GRNs, and thus organismal evolution, particular restrictions operate. Under the second one (network coupling), the resulting network must maintain its original attractors, or at least most of them. If the original attractors were eliminated, it would be very difficult for the organism to survive, because its phenotype would be drastically affected. This mechanism could underlie key evolutionary events—for example, the appearance of eukaryotic cells from the combination of prokaryotic cells, or that of multicellularity from combining unicellular organisms (Margulis and Sagan 1986). Indeed, multicellular organisms are ensembles of complex networks that could have originally underlied single-celled organisms. Therefore, methods enabling the dissection of large networks into subnetworks or modules that have a shared history will be useful to understanding the evolution of large and complex GRNs.

Biological networks are modular and composed of some reiterating subgraphs, but little is known about the evolutionary origin of such components or GRN building blocks. Several contributions on modularity have attempted to understand the connectivity, topology, synchronization, and organization of modules (Ravasz et al. 2002; Kashtan and Alon 2005; Quayle and Bullock 2006; Arenas et al. 2006; Irons and Monk 2007; Wang and Zhang 2007; Siegal et al. 2007). For instance, initial approaches to understanding how networks are locally connected have identified certain types of sub-graphs, called motifs, with a particular connection pattern. The simplest motifs are of three nodes. If the graphs are directed, there are 13 different motifs or connective configurations of three nodes. The relative abundance of these motifs in real networks is not random; different types of networks have different motifs over- or underrepresented (Milo et al. 2004).

Such motif representation patterns may have been selected for, or maybe have resulted as a byproduct of the way networks are assembled—in other words, as a result of neutral processes (Solé and Valverde 2006). Evidence to support both cases exists, and therefore it is still unclear why some motifs are more, or less, common than others. Nevertheless, understanding how biological networks have assembled during the course of evolution is fundamental to comprehend how changes in GRNs map unto evolutionary alterations of developmental processes, and therefore, unto organismal phenotypes.

1.7 Conclusions

Mathematical models grounded on experimental data are now both possible and necessary in order to study the concerted action of the many entities that, at several spatiotemporal scales, intervene during development. Plants are becoming paradigmatic systems to meet the challenge of building these models.

We have reviewed two widely used types of models, discrete and continuous. Nevertheless, the central task of considering the various levels at which developmental processes occur in integrative and realistic models still remains ahead, and it is likely that hybrid models will be needed. So far, dynamical models, and more precisely, gene regulatory network models have provided a powerful means to integrate vast empirical information, test or postulate hypotheses and predictions, and reach novel insights on the nature and evolution of plant developmental processes. Such models will certainly continue to be useful tools as feedback to and from experimental approaches in plant developmental biology.

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References

Aguda BD, Goryachev AB (2007) From pathways databases to network models of switching behavior. PLoS Comput Biol 3:e152

Albert R, Othmer HG (2002) The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in *Drosophila melanogaster*. J Theor Biol 223:1–18

Aldana M, Balleza E, Kauffman S, Resendis O (2007) Robustness and evolvability in gene regulatory networks. J Theor Biol 245:433–448

Alvarez-Buylla RE, Benítez M, Chaos A, Espinosa-Soto C, Padilla-Longoria P, Balleza E (2007) Gene regulatory network models for plant development. Curr Opin Plant Biol 10:83–91

Andrec M, Kholodenko BN, Levy RM, Sontag E (2005) Inference of signaling and gene regulatory networks by steady-state perturbation experiments: structure and accuracy. J Theor Biol 232:427–441

Arenas A, Díaz-Guilera A, Pérez-Vicente C (2006) Synchronization reveals topological scales in complex networks. Phys Rev Lett 96:114102

Arkin A, Ross J, McAdams HH (1998) Stochastic kinetic analysis of developmental pathway bifurcation in phage-infected *Escherichia coli* cells. Genetics 149:1633–1648

Benítez M, Espinosa-Soto C, Padilla-Longoria P, Díaz J, Alvarez-Buylla ER (2007) Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in Arabidopsis root and leaf epidermis: a dynamic model. Int J Dev Biol 51:139–155

Benzi R, Sutera A, Vulpiani A (1981) The mechanism of stochastic resonance. J Phys A 14:L453–L457

Blake WJ, Kaern M, Cantor CR, Collins JJ (2003) Noise in eukaryotic gene expression. Nature 422:633–637

Apéndice J

Flower Development

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Nota: Por razones de espacio, se incluyen únicamente la carátula de este capítulo y la sección de modelos matemáticos del mismo.

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Flower Development

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Flowers are the most complex structures of plants. Studies of *Arabidopsis thaliana*, which has typical eudicot flowers, have been fundamental in advancing the structural and molecular understanding of flower development. The main processes and stages of Arabidopsis flower development are summarized to provide a framework in which to interpret the detailed molecular genetic studies of genes assigned functions during flower development and is extended to recent genomics studies uncovering the key regulatory modules involved. Computational models have been used to study the concerted action and dynamics of the gene regulatory module that underlies patterning of the Arabidopsis inflorescence meristem and specification of the primordial cell types during early stages of flower development. This includes the gene combinations that specify sepal, petal, stamen and carpel identity, and genes that interact with them. As a dynamic gene regulatory network this module has been shown to converge to stable multigenic profiles that depend upon the overall network topology and are thus robust, which can explain the canalization of flower organ determination and the overall conservation of the basic flower plan among eudicots. Comparative and evolutionary approaches derived from Arabidopsis studies pave the way to studying the molecular basis of diverse floral morphologies.

1. INTRODUCTION: WHEN DID THE FLOWER EVOLVE?

The flower is the most complex structure of plants. Flowers distinguish the most recently diverged plant lineage, the angiosperms or flowering plants, from the other land plants (Figure 1). Embryophytes originated approximately 450 million years before present (MYBP) and have as distinctive features a thick cuticle resistant to desiccation, sporopollenin, pores or true stomata that aid in gas exchange, a glycolate oxidase system that improves carbon fixation at high oxygen tensions, and importantly, distinctive multicellular diploid (sporophytic) and haploid (gametophytic) stages within their life cycles (Judd et al., 2002). The major extant land plant lineages are Bryophytes (Liverworts, Hornworts and Mosses), which do not have a vascular system, and Tracheophytes, vascular plants. Within the large latter group, Lycophytes, ferns, and seed bearing plants (Spermatophytes) can be distinguished. The Spermatophyte group has been further divided into Gymnosperms (originating 380-325 MYBP) and Angiosperms. According to the fossil record, flower-like structures originated 160-147 MYBP (Frohlich, 2006). A general trend within land plant evolution is the appearance of heterospory: the existence of a megagametophyte, including the female gametes, and a microgametophyte, including the male gametes, a progressive reduction in gametophyte size (sexual reproductive structures), and within the seed plants, the presence of a diploid embryo. While these characteristics are shared among both extant and extinct seed plant lineages, the defining features of the angiosperm flower are: (1) a closed carpel bearing the ovules, which are each generally comprised of two integuments and (2) a nucellus that contains the embryo sac within which, after double fertilization, a diploid embryo and a triploid endosperm (nutritional tissue for the embryo) will develop to form a seed (Judd et al., 2002). Another character-

3.4.7 Nectaries

Little is known about the molecular genetics of nectary development. It is clear that nectaries are ABC-independent because *ap2-2 pi-1 ag-1* triple mutant flowers develop nectaries, although in these mutants nectaries are clearly smaller. However, ABC genes may play a role in nectary patterning as *pi-1 ag-1* and *ap3-3 ag-3* double mutants lack them (Baum *et al*., 2001). Also, single mutant *lfy* and *ufo* plants show reduced nectary formation (Lee *et al*., 2005a).

Several genes have been found to be expressed in the nectaries (e.g., *SHP1*, *ALC, SPL, MS35* and *XAL1*), but no detectable defect is observed in their respective mutants (Figure 5G; Schiefthaler et al., 1999; Roeder and Yanofsky, 2006; Yang et al., 2007b; Tapia-López et al., 2008). The only gene that has been clearly related to nectary development is *CRC,* which is also important for gynoecial development (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). The regulation of *CRC* in the nectaries seems to be independent of its expression in the gynoecium. Expression of this gene is already detectable at stage 6 of flower development in the area where the nectaries will be formed. Thus, *CRC* may be important for the early specification of nectary cells (Bowman and Smyth, 1999). *CRC* may also be necessary for maturation or maintenance of the nectaries, because it is expressed at high levels when they develop (Bowman and Smyth, 1999) and *crc* mutants have defects in nectary development. But *CRC* is not sufficient for nectary development, because its ectopic expression does not yield ectopic nectaries (Lee et al., 2005b). Bioinformatic and functional molecular genetic approaches have been taken to discover components of the regulatory network in which *CRC* participates during nectary and carpel development. A combination of floral homeotic gene activities acting redundantly with each other, involving *AP3*, *PI* and, *AG* and in combination with SEP proteins, activate *CRC* in both organs (Lee et al., 2005a). Interestingly in another study, *CRC* was also found to be a direct target gene of AG (Gómez-Mena et al., 2005) and to be indirectly regulated by *LFY* and *UFO* (Lee et al., 2005a). A model has been proposed in which *LFY* and *UFO* activate downstream MADS-box genes which could be working in conjunction with region-specific factors to activate *CRC* during nectary and carpel development (Lee et al., 2005a).

Evolutionary studies have indicated that the *CRC* gene may have been recruited as a regulator of nectary development in the core eudicot plant lineage, but its ancestral function could have been related to carpel development (Lee et al., 2005b).

4. THEORETICAL MODELS: INTEGRATIVE AND DYNAMIC TOOLS FOR UNDERSTANDING FLOWER DEVELOPMENT

As shown throughout this chapter, morphogenetic patterns underlying flower development arise from complex, often non-additive, interactions among several molecular and other kinds of components (e.g., cells) and factors (e.g., morphogen gradients, physical and geometrical constraints) at different levels of organization. Dynamical models can be used to study the concerted action of many elements at different spatio-temporal scales and levels of organization; an approach which is becoming both necessary and possible for understanding how morphogenetic patterns emerge and are maintained during development in general, and in particular, in flower development (for reviews Alvarez-Buylla et al., 2007; Grieneisen and Scheres, 2009). At the level of GRN, mathematical and computational models provide useful tools for integrating and interpreting molecular genetic information, or for detecting gaps and contradictions in the evidence for particular functional regulatory modules. At other levels, two or three-dimensional morphogenetic models of coupled GRNs within cells or among cells are useful for understanding spatiotemporal cell patterning in individual organs and overall plant architecture; and this enables novel insights into the mechanisms underlying developmental processes to be made. Such morphogenetic models are also a way of posing informed non-trivial predictions, testing hypotheses, uncovering potentially generic mechanisms underlying conserved features, and performing *in silico* investigations that guide novel experiments in biological development.

As the amount of experimental evidence increases and novel theoretical approaches and techniques develop, there continue to arise experimentally-grounded models of development and theoretical tools useful in posing predictions amenable to further experimental testing. These advances contribute to discussions of central issues in developmental and evolutionary biology (e.g., Kauffman, 1969; Berg et al., 2004; Milo et al., 2004; Wagner, 2005; Alvarez-Buylla et al., 2007; Balleza et al., 2008). In Arabidopsis, early flower development has been studied using dynamic gene regulatory network (GRN) models. Such models have helped capture the logic of gene regulation, mostly at the transcriptional level, during cell-type specification in various systems (e.g. von Dassow et al., 2000; Espinosa-Soto et al., 2004; Huang and Ingber, 2006; Li et al., 2006; Benítez et al., 2008). In this section we focus on this modeling approach and present some of the main results derived from network modeling in flower development.

4.1 Gene Regulatory Network Models

In this section we review some central notions in GRN theory and the main assumptions that are made and present some of the main results derived from network modeling in flower development. GRN models are composed of nodes, which stand for genes or proteins, and edges or connections, which represent the interactions among nodes (arrows for upregulation and bars for downregulation; for an example see Figure 20). Genes in the GRN model may take different activation states, depending on the activation states of their inputs. Given the architecture of the network and the sign of the interactions, it is possible to define a set of rules or kinetic functions that govern the GRN dynamics, that is, the way activation states of the genes change over time. These rules or kinetic functions may be defined and studied in the context of different mathematical frameworks, some of which have been thoroughly reviewed elsewhere (Gibson and Mjolsness, 2004; Alvarez-Buylla et al., 2007). In experimentally-based GRNs, the dynamic rules may be obtained from reported molecular genetics data as well as from functional genomics datasets.

The kinetic functions of gene activation depend on the states of the input nodes and are multivariate. These may be modeled with discrete or continuous functions. In the former, Boolean functions that allow only “0” (OFF; not expressed) or “1” (ON; expressed) values for the nodes have been successfully used

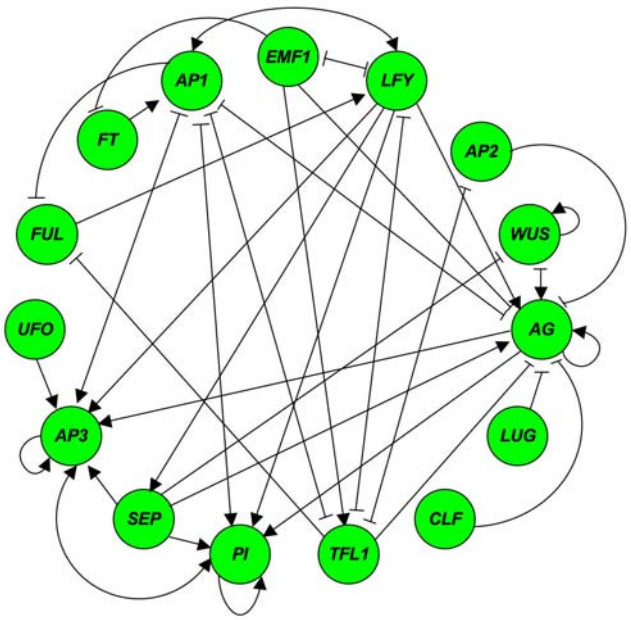

Figure 20. Floral organ specification gene regulatory network (FOS-GRN) model.

The diagram shows GRN topology where circles or nodes correspond to genes or proteins, and arrows and bars correspond to positive and negative regulatory interactions, respectively. The *SEP* node represents the *SEP1*, *2*, and *3* genes together. The interactions are updated with respect to previous publications (Espinosa-Soto et al., 2004; Chaos et al., 2006). The GRN attractors or steady states match the gene expression profiles that characterize inflorescence meristem regions and flower organ primordia. See text and Table 1 for details and experimental data supporting this model (and Table S2 for the dynamics truth tables).

to recover the key qualitative aspects of GRNs (e.g., Albert and Othmer, 2003; Espinosa-Soto et al., 2004). In Boolean networks, parameters of specific kinetic functions are not required. It is appropriate to assume that the GRN nodes are Boolean variables given that: (1) transcriptional regulation may be discrete and take place in the form of pulses, rather being continuous (Ross et al. 1994, Fiering et al., 2000, Ozbudak et al., 2002); (2) the experimental data at hand can be readily formalized as logical rules (see detailed discussions in Albert and Othmer, 2003; Espinosa-Soto et al., 2004; Chaos et al., 2006), while there are no or very few available data on parameters required to postulate continuous functions; and (3) in complex GRNs with many components interacting in non-linear manners, the overall topology of the GRN and the form of the logical rules of gene interaction, rather than the details of the kinetic functions, are what determine the qualitative network dynamics.

Independently of the mathematical formalism used, dynamical analyses of GRNs mostly focus on finding the steady gene activation profiles, that is, the configurations of the network that, once reached, remain in that configuration. These configurations are called *attractors*. The GRN model may be initialized on a particular gene-activation configuration known as an *initial condition* and then the elements of the GRN change their activation state according to the dynamic rules until they reach an attractor. Kauffman (1969) proposed that Boolean GRN attractors correspond to the activation profiles typical of different cell types and therefore that exploring the GRN architecture and dynamics is fundamental to understanding cell-type determination processes. This idea has now been verified experimentally and explored further (e.g. Albert and Othmer, 2003; Huang and Ingber, 2006; Alvarez-Buylla et al., 2007).

Another helpful notion in GRN dynamical studies is that of *basins of attraction*. Given the dynamic rules of the network, the set of initial conditions that lead to each of the attractors is known as its basin of attraction. As we discuss below, the concepts of GRN - attractor, initial condition and basin of attraction - may be useful in addressing some pertinent aspects of flower development.

4.1.2 Functional Modules in Flower Development

The functional data on genes involved in flower development reviewed in this Chapter suggest several regulatory modules exist that act at different stages and in different structures (Figures 9, 15-17 and 19). We define a regulatory module as a set of interacting genes that have more interactions among themselves than with other genes. These modules are semi-autonomous, meaning that their dynamic outcomes are fairly independent of other modules. In Figure 15 we have presented the best-studied modules associated with flower development. The approach described here for the functional module that includes the ABC genes could in principle be used for all of these modules as sufficient nodes have been identified and their regulatory interactions characterized. Eventually, models of coupled GRN that consider several such models together, both within and among cells, will be possible. For now, we have focused in just one such regulatory module.

In previous studies, we have proposed and analyzed the regulatory module, which includes the ABC genes as well as other components, that is sufficient to regulate the partitioning of the inflorescence and floral meristems into subregions of primordial cells. In the case of the flower meristem, each one of the four subregions is composed of the primordial cells that eventually give rise to each of the four types of floral organs: sepals, petals, stamens and carpels.

We use this functional module as a working example of the protocol that has been used in order to assemble an experimentally grounded gene regulatory network (GRN) model corresponding to a functional module. Then we demonstrate how once such a GRN model is postulated, it is possible to follow its dynamics, and explore how the concerted action of multiple interconnected molecular components eventually lead to stable gene expression profiles that may be compared to those characterizing different cell types. Then we delineate some theoretical approaches put forward to model coupled GRN dynamics that may underlie pattern formation and morphogenesis during the early stages of flower development, when the floral meristem is partitioned into four concentric rings of primordial cells. Finally, we review other modeling approaches that are useful to study signal transduction pathways.

4.2 Arabidopsis Flower Organ Specification GRN (FOS-GRN)

Soon after flowering is induced, the flower meristem is partioned into four concentric regions of primordial cells from which floral organs will later form. During the last decade, an experimentally-grounded GRN model for flower organ specification (FOS-GRN) has been built and investigated (Figure 20; Mendoza and Alvarez-Buylla 1998; Espinosa-Soto et al., 2004; Chaos et al., 2006; Alvarez-Buylla et al., 2008). This model incorporates the intricate regulatory interactions among ABC genes themselves and among ABC and non-ABC genes that are key to this process. This functional module includes: some key regulators underlying the transition from IM to FM (*FT, TFL, EMF*, *LFY, AP1*, *FUL*); the ABCs and some of their interacting genes (*AP1*, *AP3*, *PI*, *AP2*, *AG*, *SEP*); some genes that link floral organ specification to other modules regulating primordia formation and homeostasis (*AG*, *CLF* and *WUS)*; and some regulators of organ boundaries (*UFO* and *LUG*; Figures 9, 15 and 20).

The main result obtained from analyzing this GRN is that the postulated network converges to only ten attractors—even though it can be initialized in more than 130,000 different configurations of gene activation. Furthermore, the attractors—the stable configurations recovered—match gene activation profiles typical of the four inflorescence meristem regions (i.e., a region lacking *WUS* and *UFO*, two regions with either one of these two genes turned on, and a fourth region with both genes turned on; see Espinosa-Soto et al., 2004), as well as those of primordial sepal, petal, stamen and carpel cells (Figure 21). This shows that the FOS-GRN is suf-

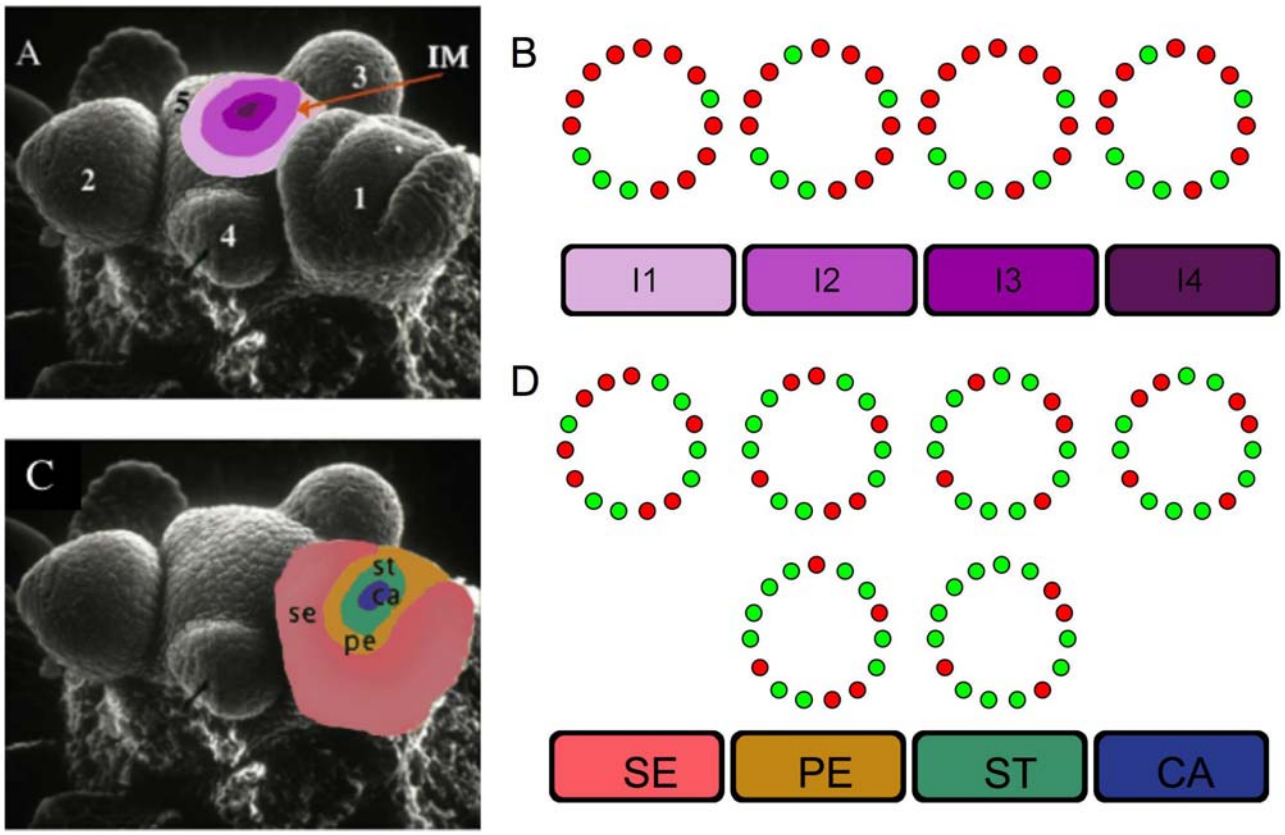

Figure 21. *Arabidopsis* inflorescence and flower development and FOS-GRN.

(A) SEM colored where four regions I1, I2, I3 and I4 are distinguished within the IM. FMs are also seen arising from the flanks of the IM,1 the oldest and 5 the youngest.

(B) I1, I2, I3 and I4 regions of the IM correspond to four of the FOS-GRN attractors. Expressed genes for each attractor are represented as green circles, while non-expressed genes correspond to red circles (nodes are in the same relative position as in Figure 20). Note that this model recovers the respective regions in the IM with both *WUS* and *UFO*, with either one of these two genes, or with neither expressed.

(C) SEM colored to distinguish four types of primordial cells in young flower meristems. Each will eventually develop into the different flower organs, from the flower periphery to the center, sepals (se), petals (pe), stamens (st) and carpels (car).

(D) The six attractors of the FOS-GRN model match gene expression profiles characteristic of sepal, petal (p1 and p2), stamen (st1 and st2) and carpel primordial cells. The gene activation profiles of the attractors are congruent with the combinatorial activities of A, B, and C genes described in the ABC model of floral organ determination (adapted from Alvarez-Buylla et al., 2008).

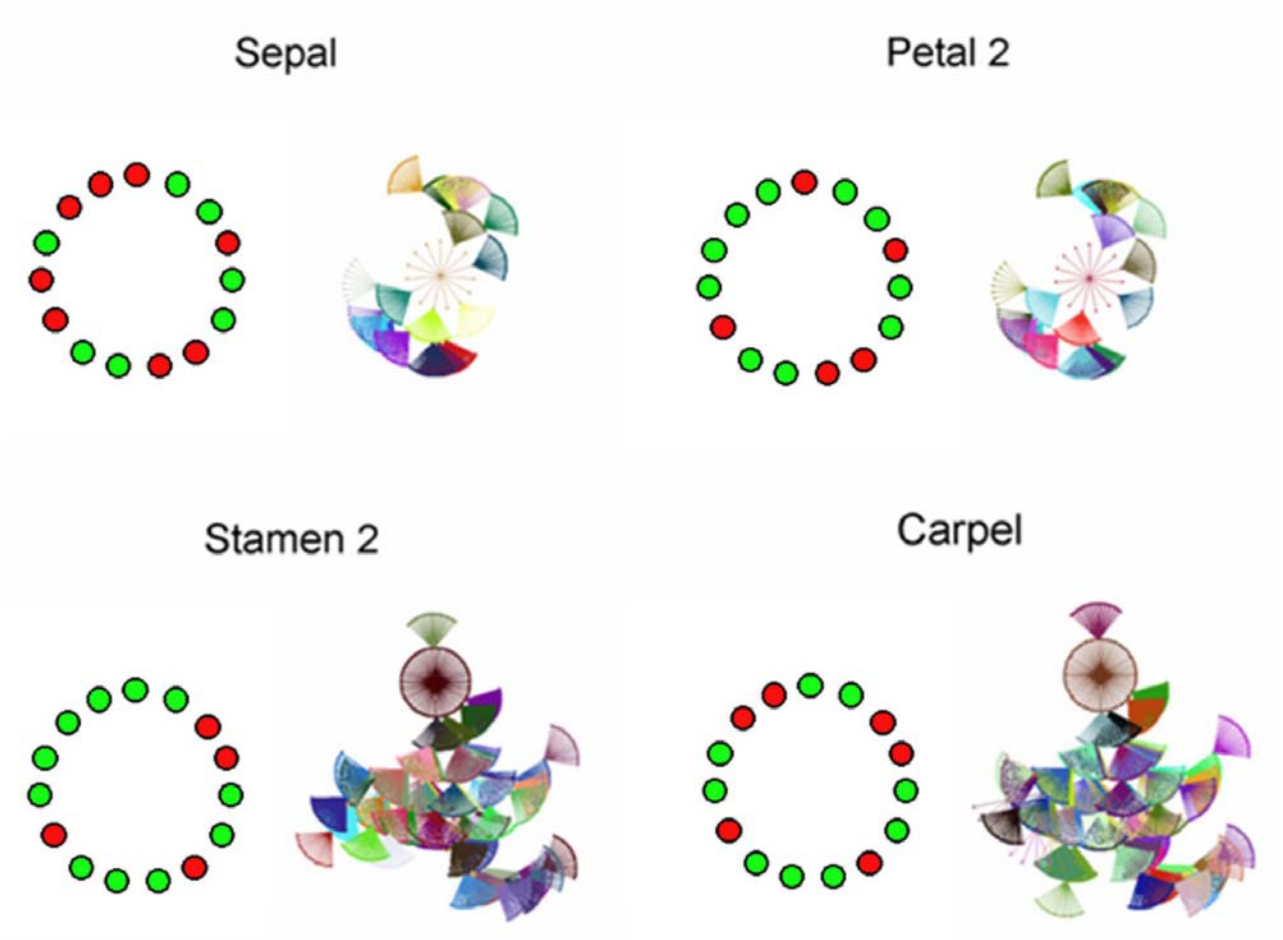

Figure 22. Basins of attraction for the four flower organ FOS-GRN attractors.

Attractors of FOS-GRN match the gene expression profiles of the four types of floral organ primordia of young floral buds (sepal, petal, stamen and carpel). The fan diagrams depict the GRN configurations (combinations of 0s and 1s corresponding to gene activation profiles) that lead to each of the attractors. Points in the outermost layers of these fan diagrams correspond to initial configurations of the network and they are linked to the transitory configurations. Petal2 and Stamen2 stand for one of the two possible attractors for each one of these organs. Relative position of nodes and their colors as in Figure 20.

ficient to recover the gene activation profiles required to specify primordial cells during the first stages of flower organ development. Therefore the GRN itself constitutes a functional module that robustly leads to the gene configurations that characterize different regions of inflorescence and flower meristems during early flower development; and this independently of the activation states of additional genes that are connected to this elucidated regulatory module. Furthermore, various robustness analyses have been performed showing that the recovered attractors are also robust in response to permanent alterations in the logical functions of gene interactions and the inclusion of gene duplications. Therefore, these results (Espinosa-Soto, et al., 2004; Chaos et al., 2006) suggest that FOS dynamically and robustly emerges from complex networks of molecular components, rather than from a series of linear or hierarchical gene interactions or from the action of particular genes. The FOS-GRN model not only recovers the ABC gene combinations that are necessary for FOS, but it also provides a dynamic explanation for the formation of such gene combinations, and postulates a set of gene interactions with the ABC genes, that are also sufficient for FOS. The functions and interactions of the genes included are reviewed earlier in this chapter.

The FOS-GRN was validated by using this model to simulate the effect of loss-of-function mutations or overexpression, and comparing the results recovered from the model with the gene activation profiles determined experimentally in mutant or overexpressor lines. The mutants were simulated by fixing the state of the gene to 0 for loss of function, and to 1 for gain of function or overexpression (Figure 20; Table 1 and Table S2). In all cases tested, the simulated and empirically-reported profiles matched (Espinosa-Soto et al., 2004).

In addition, this GRN model has enabled investigations to be made into the sufficiency and necessity of particular gene regulatory interactions, which have led to novel predictions. For example, these analyses predicted that *AG* upregulate itself (Espinosa-Soto el al., 2004), which seemed somewhat counterintuitive at the time, but which was then verified by independent experiments (Gómez-

Mena et al., 2005). Also, computer simulations of the FOS-GRN that show that its attractors are robust to different types of perturbation and to duplications (Espinosa-Soto et al., 2004; Chaos et al., 2006) can account for the overall conservation of the flower structure throughout angiosperm (particularly eudicot) evolution (Rudall, 2007; Whipple et al., 2004; Adam et al., 2007).

Since the FOS-GRN model was based on thorough molecular data and is one of the few well-characterized regulatory modules, it has been used as a "model GRN" for further methodological, theoretical and conceptual developments in GRN and systems biology research (Table 2). The main conclusions obtained from the first versions of this GRN have been confirmed. New data regarding FOS are continuously being generated (novel data are also summarized in Table 1) and the FOS-GRN constitutes a basic theoretical framework in which to integrate it alongside previous data. Here, we have updated the FOS-GRN taking these novel data into account and conclude that the basic module originally put forward (Espinosa-Soto et al., 2004; Chaos et al., 2006) is robust to the addition of these newly discovered interactions. We consider, for instance, that *EMF1* downregulates *AG* (Calonje et al., 2008), and *AP3*/*PI* downregulate *AP1* (Sundström et al., 2006), so the postulated module seems to be robust to the addition of intermediary components or previously missing interactions.

Simulations of the updated FOS-GRN have been performed with the new software, ATALIA (http://www.ecologia.unam.mx/~achaos/Atalia/atalia.htm) developed in the Alvarez-Buylla laboratory by A. Chaos-Cador. This tool can be used to readily update this and other GRN models and explore their dynamics. We illustrate the use of this software with a visualization of the attractors' basins (Figure 22) and a simulation of the updated wild-type and certain mutant FOS-GRN dynamics (Figure 23).

In the simulated FOS-GRN, genes can take only two activation states: 0 for no expression and 1 for expression. Hence, by using combinations of 0s and 1s, we can describe all the possible initial conditions of the GRN. Figure 22 presents the so-called *fan diagrams* that show all the GRN configurations leading to each of the attractors. Knowing the relative sizes of the basins of attraction of each steady state is the key to exploring the robustness of each attractor in the face of perturbations.

ATALIA can also calculate the attractor that every possible initial condition will eventually reach and show this information in a tapestry of destinies. In such tapestries, each possible configuration of the GRN is represented by a square in a lattice and is colored according to the attractor it reaches. Moreover, ATALIA can draw a tapestry that represents the difference between the original wild-type tapestry and a mutant one (Figure 23). For example, if we want to know whether an *ap2* mutation has a more or less drastic effect in terms of the GRN dynamics than a *pi* mutation, we can analyze the tapestries of *ap2* and *pi* shown in Figure 23 and conclude that *ap2* mutation has stronger dynamic effects than *pi* given the GRN postulated up to now. Given the complexity of the network involved, such predictions are impossible to make without a tool like ATALIA. As the regulatory interactions in other modules that participate in flower development are gradually uncovered, for each one the experimental data can be exhaustively mined and formalized in the form of a GRN topology and logical rules governing its components' interactions. ATALIA can then be used to explore their dynamics, validate the proposed GRN models by simulating experimental reports of mutants or overexpressing lines, and to postulate novel interactions. Eventually, two or more functional modules may be interconnected via common components to postulate GRN aggregates. Such an approach will be useful in beginning to uncover the types of microtopological trait that characterize the nodes connecting different functional modules, for example.

We have illustrated the potential of using dynamic GRN models to understand cell differentiation using a relatively small and well-characterized module. Approaches used for small regulatory modules that are well-characterized in terms of molecular genetics, should feedback from functional genomic efforts that span the dynamics of a larger number of genes or proteins under diverse conditions and developmental stages or tissues.

4.2. Temporal and Spatial Patterns of Cell-fate Attainment During Early Flower Development

In real biological systems, populations of meristematic cells differentiate into different cell types in stereotyped temporal sequences and spatial patterns. The first primordial cells to be determined in the flower meristem are those of sepals, then those of petals, stamens and carpels going from the periphery to the center of the floral meristem. This suggests that in the population of meristematic cells the most probable temporal order in which each attractor is visited follows the same sequence (Alvarez-Buylla et al., 2008). Recent results from another theoretical approach show that the sequence of floral organ determination can be recovered by introducing some level of stochasticity (random noise) in the GRN dynamics, namely, a degree of error in the updating dynamical rules of the GRN (Alvarez-Buylla et al., 2008). These results are consistent with a handful of other recent studies showing that stochasticity at the molecular scale may contribute to the formation of spatiotemporal patterns in development (see review in Raj and van Oudenaarden, 2008). Studies with the stochastic version of the FOS-GRN also concluded that the relative position of the basins is important in determining the most probable temporal sequence of cell-fate attainment referred to above (Alvarez-Buylla et al., 2008). This fascinating result certainly suggests that the stereotypical temporal pattern of cell fate specification at early stages of flower development may be an emergent and robust consequence of the complex GRN underlying cell-fate determination and that, in principle, it could take place in the absence of inducing signals, emerging only as a result of the stochastic fluctuations that occur during transcriptional regulation (Alvarez-Buylla et al., 2008). Ongoing modeling efforts are explicitly focusing on spatial domains, and exploring the need and sufficiency of different cell-cell communication mechanisms or physical fields (e.g., created by curvature or tension forces) that could provide positional information for spatio-temporal cell patterning during early stages of flower development.

It is important to mention that the FOS-GRN modeled up to now is an abstraction of the qualitative regulatory logic underlying the IM and FM subregionalization during early stages of flower development when the ABC patterns are established. However, other regulatory modules for meristem positioning, growth and polarity, among others, still need to be considered in order to fully understand spatiotemporal cell patterning and morphogenesis of IM and FM. Some genes interacting with FOS-GRN components

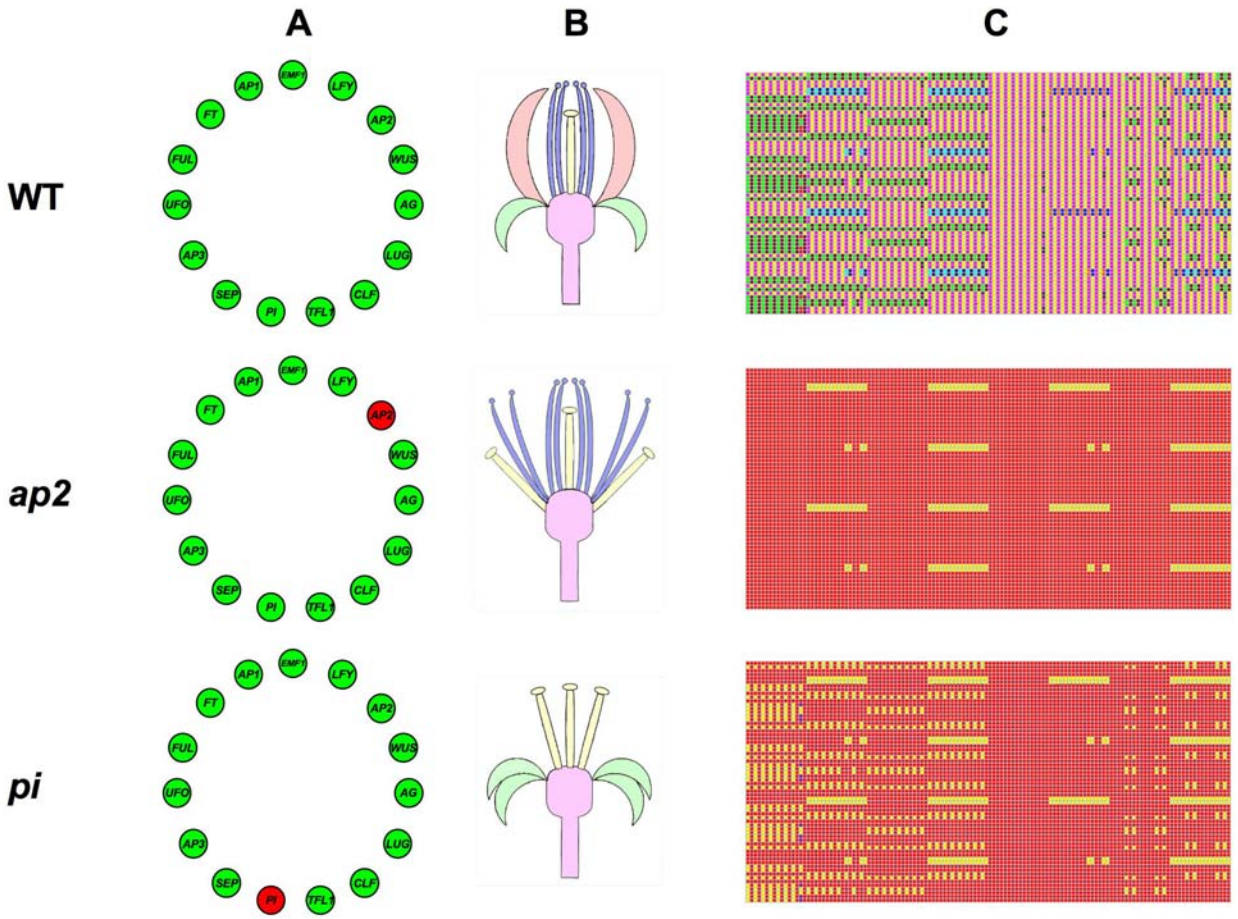

Figure 23. Simulation results for wild type (WT) and two mutants.

(A) Simplified representation of the FOS-GRN. The mutated genes are in red (nodes are in the same relative position as in Figure 20). Mutations were simulated by constitutively turning "off" (loss-of-function) mutated genes regardless of the dynamical rules.
(B) Floral diagrams showing floral organs of the simulated WT and mutant plants. These correspond to the steady-state gene expression arrays (attractors) attained in the simulation.
(C) Tapestries of gene configuration destinies corresponding to the simulated WT and mutant lines. In the WT simulation each square in the tapestry represents an initial condition and they are colored according to the attractor they eventually reach. In the mutant simulation for *ap2* and *pi*, the tapestries illustrate the difference between the WT tapestry of destinies and that obtained for the mutant simulations. Yellow squares, configuration attained is the same attractor as in the WT; red squares, configurations that reached a new attractor; purple squares, configurations that attained a pre-existing attractor but not the same one reached in the WT simulations. Images generated with ATALIA (http://www.ecologia.unam.mx/~achaos/Atalia/atalia.htm).

(e.g. *AGL24*, *BEL*, *RBE* and those described in the last section of Table 1) that do not seem to directly affect cell-type determination in the floral meristem, could link the FOS-GRN with: a) signaling pathways (e.g. Díaz and Alvarez-Buylla, 2006); b) genes involved in cell growth and proliferation both before and after the partitioning of the floral meristem into the four concentric regions; and c) other types of downstream genes or modules that are important during cell sub-differentiation and organogenesis at later stages of flower development.

A complete understanding of flower morphogenesis will continue to require multidisciplinary approaches and modeling tools that help unravel how such single-cell GRNs are coupled in explicit cellularized spatial domains and physicochemical fields (e.g. Jönsson et al., 2005, Savage et al., 2008; Benítez et al., 2008), including metabolism, signaling, and emergent gradients of morphogens (e.g., auxin), cell growth and proliferation, mechanical forces and cell-cell communication mechanisms. All of these are likely to feedback in non-linear ways from and to the GRNs underlying cell differentiation or proliferation (for example see Hamant et al., 2008).

It is important to keep in mind, for example, that plant cell growth in meristems is symplastic. This implies that the contacts

Table 1. Summary of evidence for the FOS-GRN gene interactions shown in Figures 20-23 (ChIP, chromosome immunoprecipitation; EMSA, electrophoretic mobility shift assays; arrows indicate gene induction and bars repression; Espinosa-Soto et al., 2004; Chaos et al., 2006).

INTERACTIONS	EXPERIMENTAL EVIDENCE	REFERENCE
AG (AT4G18960) → *AG*	ChIP shows that AG interacts *in vivo* with predicted regulatory sequences of *AG* .	Gómez-Mena et al., 2005.
AP1 (AT1G69120) --| *AG*	Sepals are replaced by carpels, and petals by stamens in *ap1* mutants. *AG* mRNA found in all flower primordia of *ap1-1* plants. First whorl organs are sometimes carpelloid, and second whorl organs are staminoid in *ap1* mutants.	Bowman et al., 1993; Weigel and Meyerowitz, 1993; Liu and Meyerowitz, 1995.
CLF (AT2G23380) --| *AG*	In *clf* mutants, first whorl sepals are frequently carpelloid, second whorl organs are staminoid petals and *AG* mRNA is detected in sepals. It is likely that CLF is part of a complex with EMF2, MSI1, and FIE that epigenetically regulate *AG*.	Goodrich et al., 1997; Calonje et al., 2008.
LFY (AT5G61850) → *AG*	Expression of *AG* is reduced in *lfy-6* flowers. The expression of *LFY* fused to a strong activation domain produces increased and ectopic AG expression. LFY binds to the first intron of *AG*, and cooperates with the WUS homeodomain to activate it.	Weigel and Meyerowitz, 1993; Parcy et al., 1998; Busch et al., 1999; Lohmann et al., 2001.
LUG (AT4G32551)--| *AG*	*AG* is ectopically expressed in *lug-1* mutants. LUG functions as a repressor of *AG* via its the second regulatory intron.	Liu and Meyerowitz, 1995; Sieburth and Meyerowitz, 1997; Deyholos and Sieburth, 2000; Gregis et al., 2006.
SEP3 (AT1G24260) → *AG*	There is *AG* expression in rosette leaves of *35S:SEP3* plants. In addition, *35S:AG 35S:SEP3* plants have more pronounced carpelloid features.	Castillejo et al., 2005.
TFL1 (AT5G03840) --| *AG*	Stigmas and styles of terminal flowers in *lfy ap1* double mutants are normal if the *tfl1* mutation is added.	Shannon and Meeks-Wagner, 1993.
WUS (AT2G17950) → *AG*	*wus* mutants lack carpels and most stamens. In *AP3:WUS* transgenic plants, second whorl organs are carpelloid stamens instead of petals, whereas in *AP3:WUS* ag plants, second and third whorl organs do not differentiate into carpelloid stamens.	Laux et al., 1996; Lenhard et al., 2001; Lohmann et al., 2001.
AG --| *AP1*	*AP1* mRNA accumulates uniformly in *ag-1* mutant flowers.	Gustafson-Brown et al., 1994.
FT (AT1G65480) → *AP1*	In *ft lfy* double mutants, there is no *AP1 mRNA* unlike in the respective single mutants, suggesting that at least one of these two genes needs to be active for *AP1* activation	Ruiz-García et al., 1997.
LFY → *AP1*	*AP1* expression is delayed in *lfy-6* null mutants, ectopic in *35S:LFY* plants and increased when *LFY-VP16* is induced. LFY directly binds the *AP1* promoter and activates this gene.	Parcy et al., 1998; Liljegren et al., 1999. Weigel and Nilsson, 1995; Wagner et al., 1999.
TFL1 --| *AP1*	In *tfl1* mutants, *AP1* is ectopically expressed in the basal lateral meristems and in terminal flowers. *AP1* expression is also retarded in *35S:TFL1*	Gustafson-Brown et al., 1994; Ratcliffe et al., 1998.
TFL1 --| *AP2* (AT4G36920)	The absence of petals in *tfl1 ap2* flowers and the presence of petals in *tfl1* single mutants suggest there is ectopic *AP2* activity in the terminal flowers of *tfl1* single mutants.	Shannon and Meeks-Wagner, 1993.
AG → *AP3* (AT3G54340)	There is weaker *GUS* expression in the third whorl of *ag-1 AP3:GUS* flowers than in the transgenic control. *AG* may maintain *AP3* expression because cauline leaves of *35S:PI 35S:AP3 35S:SEP3 35S:AG* are converted into stamen-like organs. ChIP shows that AG interacts *in vivo* with predicted regulatory sequences of *AP3*. Also, *AP3 RNA* is absent from the center of the *ag-1* meristem.	Hill et al., 1998; Honma and Goto, 2001; Gómez-Mena et al., 2005; Zhao et al., 2007.
AP1 → *AP3*	*AP3* expression is quite normal in *ap1* mutants but is almost undetectable in *lfy ap1* double mutants, indicating that *AP1* can act with *LFY* to regulate *AP3* expression. Futhermore, AP1 seems to bind *AP3 cis*-regulatory elements.	Weigel and Meyerowitz, 1993; Hill et al., 1998; Ng and Yanofsky, 2001; Lamb et al., 2002.
AP3 → *AP3*	Endogenous *AP3* is upregulated in *35S:AP3-GR* plants induced with dexamethasone, supporting the notion that *AP3* self-activates.	Hill et al., 1998; Honma and Goto, 2000.

(Continued)

Table 1. (continued)

INTERACTIONS	EXPERIMENTAL EVIDENCE	REFERENCE
LFY+*UFO* (AT1G30950) → *AP3*	Both the amount and the domain of *AP3* expression are reduced in *lfy-6* mutants. *ufo-2* plants have less *AP3* protein and less AP3 mRNA. Both *LFY* and *UFO* have to be overexpressed to induce ectopic expression of *AP3*. EMSA show that LFY binds directly to sequences in the *AP3* promoter. ChIP shows that UFO associates with the *AP3* promoter. This association was abolished when ChIP was performed using extracts from *lfy-26* plants harboring the *35S:UFO-Myc* transgene.	Weigel and Meyerowitz, 1993; Meyerowitz, 1995; Parcy et al., 1998; Lamb et al., 2002; Levin and Chae, 2008.
SEP (AT5G15800, AT3G02310, AT1G24260, AT2G03710) → *AP3*	In *AP3:GUS 35S:PI 35S:AP3 35S:AP1* mutants, AP3-GUS is expressed throughout the plant supporting the idea that full activation of the B-function genes requires tetramer formation to include SEP. The ectopic expression of *SEP3* resulted in the induction of ectopic *AP3* expression. Stronger *35S:SEP3* lines are also capable of activating *AP3:GUS* ectopically	Honma and Goto, 2001; Castillejo et al., 2005.
LFY --| *EMF1* (AT5G11530)	Ectopic *LFY* expression in *emfl-1* mutants increases the severity of the *emf* phenotype.	Chen et al., 1999.
EMF1 --| *FT*	*FT RNA* levels are higher in the *emf1-1* mutant and are detected earlier than in the wild type.	Moon et al., 2003.
AP1 --| *FUL* (AT5G60910)	*FRUITFULL* is ectopically expressed in *ap1* mutants.	Mandel and Yanofski, 1995b; Ferrándiz et al., 2000a.
TFL1 --| *FUL*	TFL1 has been postulated to be an inhibitor but it also is possible that other factors have this posttranscriptional inhibitory role. This interaction is necessary as when the negative posttranscriptional regulation of FUL by TFL1 is not considered, the nonfloral gene steady states disappear. No experimental evidence.	Espinosa-Soto et al., 2004.
AP1 → *LFY*	In *ap1* and *ap1 cal* double mutants, *LFY* expression is reduced. Additionally, *LFY* is activated earlier in *35S:AP1* plants than in the wild type.	Bowman et al., 1993; Kempin et al., 1995; Weigel and Nilson, 1995; Piñeiro and Coupland, 1998; Liljegren et al., 1999.
EMF1 --| *LFY*	Double mutants of the weak *emf1-1* allele and *lfy-1* bear *lfy*-like flowers suggesting that, for this trait, *lfy* is epistatic. These genes have antagonistic activities.	Yang et al., 1995.
FUL → *LFY*	Even though *LFY* expression is the similar in wild type and *LFY:GUS ful-2* plants, there is less expression in *ful ap1 cal* triple mutants than in *ap1 cal*, suggesting that the role of *FUL* in *LFY* upregulation is only important when *AP1* is inactive.	Ferrándiz et al., 2000a.
TFL1 --| *LFY*	In *tfl1* mutant plants *LFY* is ectopically expressed in the shoot apex.	Weigel et al., 1992; Ratcliffe et al., 1999.
LFY→ *PI* (AT5G20240)	Amount and domain of *PI* expression are reduced in *lfy-6* mutants. There is no *GUS* expression in early *lfy PI:GUS* flowers.	Weigel and Meyerowitz, 1993; Honma and Goto, 2000.
PI → *PI*	AP3 and PI co-immunoprecipitate. *AP3* and *PI* mRNA levels are not maintained in *ap3-3 pi-1* double mutants. In *AP3:GUS 35S:PI 35S:AP3 35S:AP1* mutants, *AP3:GUS* is expressed throughout the plant supporting the idea that full activation of the B-function genes requires PI	Jack et al., 1992; Goto and Meyerowitz, 1994; Honma and Goto, 2001.
LFY → *SEP*	Microarray experiments show that the group of *LFY* dependent genes includes the homeotic cofactors *SEP1-3*.	Schmid et al., 2003.
AP1 --| *TFL1*	In *35S:AP1, TFL1* expression is greatly diminished. *TFL1* is ectopically expressed in *ap1 cal* double mutants.	Liljegren et al., 1999.
AP2 --| *TFL1*	The *tfl1-1* mutation partially suppresses the *ap2-1 ap1-1* inflorescence phenotype.	Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993.
EMF1 → *TFL1*	In *emf1-2 tfl1* double mutants, the *emf1-2* mutation is epistatic with respect to flower initiation. These genes do not have antagonistic activities. This suggests that *EMF1* upregulates *TFL1*.	Chen et al., 1997.
LFY --| *TFL1*	The *35S:LFY* plants resemble the *tfl1* mutant and have no *TFL1* expression. *LFY* can inhibit *TFL1* at the transcriptional level. *TFL1* is also ectopically expressed in *lfy* mutants.	Weigel and Nilsson, 1995; Liljegren et al., 1999; Ratcliffe et al., 1999.
AG --| *WUS*	There is strong *WUS* expression in the center of ag floral meristem.	Lenhard et al., 2001; Lohmann et al., 2001.

Table 1. (continued)

INTERACTIONS	EXPERIMENTAL EVIDENCE	REFERENCE
SEP --| *WUS*	*SEP* activity is required for *WUS* downregulation by *AG* because *sep1 sep2 sep3* triple mutant plants bear indeterminate flowers.	Pelaz et al., 2000.
WUS --| *WUS*	No experimental evidence. Assumption of model.	Espinosa-Soto et al., 2004.
UPDATES (Chaos et al., 2006 and this chapter)		
EMF1 --| *AG*	In ChIP experiments, *EMF1* is associated with sites in the promoter and second intron of AG. *EMF1* interferes with transcription by RNA polymerase II and T7 RNA polymerase *in vitro*.	Calonje et al., 2008.
AP3 --| *AP1*	*AP1* transcript levels are significantly higher in *ap3-3* mutant plants than in both *WT* and *35S:AP3*.	Sundström et al., 2006.
PI --| *AP1*	ChIP shows that PI binds to target sequences in the *AP1* promoter	Sundström et al., 2006
MiR172 (AT2G28056, AT5G04275, AT3G11435) + *HEN1* (AT4G20910) --| *AP2*	Elevated *miR172* accumulation results in floral organ identity defects similar to those in loss-of-function *ap2* mutants. On the other hand, the *miR172* abundance depends on the activity of DICER-like protein HUA ENHANCER 1 (HEN1), which is expressed through the plant. This observation suggests that a cofactor expressed in the inner floral whorls is required to give specificity to the HEN1-dependent repression of *AP2*. The need for *AG* inactivity for *AP2* function is added to the *AP2* logical rules	Chen et al., 2002; Park et al., 2002; Chen et al., 2004; Zhao et al., 2007.
LFY → *SEP1-3*	Microarray experiments show that the group of *LFY* dependent genes includes the homeotic cofactors *SEP1-3*.	Schmid et al., 2003.
INTERACTIONS NOT INCLUDED IN THE MODEL		
AGL24 (AT4G24540) + *SVP* (AT2G22540) --| *AG*	In the *agl24 svp* double mutant, *AG* mRNAs are detected in the inflorescence and floral meristems as early as stage 1, indicative of early *AG* expression. In later stages, *AG* is still expressed in all floral organs. Probably, this interaction is part of a different GRN that ocurs before the cell fate determination	Gregis et al., 2006.
BLR (AT5G02030) --| *AG*	*AG* is expressed ectopically in *blr* mutants. *BLR* directly binds to *AG cis* elements (identified by EMSA). This interaction is probably important in organogenesis.	Bao et al., 2004.
RBE (AT5G06070) --| *AG*	In *rbe* mutants, there is ectopic expression of *AG* in second-whorl cells. This interaction may be important in organogenesis.	Krizek et al., 2006.
SEU (AT1G43850) --| *AG*	The direct *in vivo* association of SEUSS (SEU) with the *AG cis*-regulatory element was shown by ChIP. SEU interacts with LUG in a repressor complex to regulate *AG*, and LUG is already considered in the GRN model.	Sridhar et al., 2006.
AGL24+SVP --| *AP3*	An *in situ* analysis shows that in the *agl24 svp* double mutant, *AP3* is expressed in all parts of the floral meristem and later in all floral organs. Probably, this interaction is part of a different GRN occurring before the cell fate determination.	Gregis et al., 2006.
LFY → *CAL*(AT1G26310)	Using posttranslational activation of LFY-GR, it is demonstrated that CAL is a direct LFY target. cis-regulatory elements in the putative CAL promoter are bound by LFY. AP1 forms heterodimers with CAL and AP1 is already included.	William et al., 2004.
AP3 --| *FUL*	The domain of *FUL* expression is expanded to the third whorl in stage-3 *ap3* mutants, but no direct interaction is detected by ChIP analysis.	Mandel and Yanofsky, 1995b; Sundström et al., 2006.
FT --| *FUL*	*FUL* is expressed at higher levels in 35S:FT-VP16. It is not considered because this interaction could be mediated by TFL1 and LFY.	Teper-Bamnolker and Samach, 2005.
PNY (AT5G02030) → *LFY* *PNF* (AT2G27990) → *LFY*	The transcripts of *LFY* are substantially reduced in shoot apices of *pny pnf* double mutants after floral induction. *pny pnf* double mutants do not produce flowers but, *35S:LFY pny pnf* plants do produce flowers. This interaction is part of a different GRN.	Anrar et al., 2008.
AP2 → *PI*	*In situ* hybridization shows there is less *PI RNA* occupying a smaller area in *ap2-2* flowers than in wild type. Probably an indirect effect.	Zhao et al., 2007.
AG --- *SEP3*	ChIP shows that AG interacts *in vivo* with predicted regulatory sequences of *SEP3*. Insufficient experimental data.	Gómez-Mena et al., 2005.
FT → *SEP3*	Overexpression of *FT* causes ectopic expression of *SEP3* in leaves. No further experimental evidence.	Teper-Bamnolker and Samach, 2005.

Table 2. Some of the contributions that have used the flower organ specification GRN model in order to test, advance or discuss novel conceptual or methodological approaches.

Contribution	Reference
Logical analysis of the flower organ specification (FOS) GRN.	Mendoza et al., 1999
Introduction of the transsys formalism to represent GRN and implementation of FOS-GRN in this framework.	Kim, 2001
Method for gene network inference based on nonlinear differential equations and logical approaches. Predictions were tested using FOS-GRN.	Perkins et al., 2004
New method for automatically inferring gene regulation functions modeled as logical functions. The method is applied to FOS-GRN.	Bozek et al., 2006
Automatic Petri-net-based method, applied to FOS-GRN, for finding stationary states.	Gambin et al., 2006
Analysis of the dynamic role of feedback loops in networks including FOS-GRN.	Kwon and Cho, 2007
Application of the GenYsis software to model the discrete and multiple valued FOS-GRN.	Garg et al., 2007
Analysis of the effect of feedback loops on the robustness of Boolean networks, such as that of flower organ specification.	Kwon and Cho, 2008
Dynamic study of FOS-GRN and other GRNs with the finding that these exhibit a property known as criticality.	Balleza et al., 2008
Formal analysis of the main sources of perturbation and their effects in biological regulatory networks, with the FOS-GRN as example.	Demongeot et al., 2008

between cells are preserved because there is no displacement or sliding at middle lamellas that join neighboring cells (Priestley, 1930 and Erickson, 1986; cited in Kwiatkowska, 2008). Therefore, overall plant growth could be modeled using the principles of solid body mechanics (see review in Kwiatkowska, 2008). However plant cells also grow anisotropically which implies a variation in the directional growth rates at a given point (Baskin, 2005). Hence, meristem growth has rather been modeled using the principles of continuum mechanics, computing variables that characterize plastic strain (Goodall and Green, 1986; for review see Green, 1999).

Some quantitative mesoscopic models for flower development and growth in Arabidopsis and other angiosperms have been put forward (e.g., Rolland-Lagan et al., 2003; Lee et al., 2004; Skryabin et al., 2004; Mündermann et al., 2005). A finite element model of the SAM has also shown, for example, that lateral bulging of the meristem surface leading to the formation of a primordium results in a gradient of shear stresses with high shear stress at the point where the future primordium emerges (Selker et al., 1992; reviewed in Kwiatkowska 2008). More recently, it was shown that cells in the Arabidopsis SAM orient their cortical microtubules along lines of mechanical stress generated during tissue formation, and this then affects the mechanical properties of the cell, thus establishing a feedback loop (Hamant et al., 2008). This seems to be particularly relevant during the formation of the groove between the apical meristem and the primordium of lateral organs, but less so during growth and differentiation, because the lateral organ primordia are not affected when the microtubular network is disintegrated by a drug (Hamant et al., 2008). This implies that the mechanical feedback loop described is likely to act in parallel with the previously described auxin-mediated patterning mechanism (Laufs et al., 2009). Similar morphogenetic mechanisms are likely to be at work in flower meristem and floral organ development, and both morphogenetic mechanisms connected to the functional regulatory modules, including FOS-GRN and others that have been partly elucidated and reviewed in this Chapter.

5. CONCLUSIONS AND PERSPECTIVES

Arabidopsis has been indispensable in unraveling the molecular genetic bases of the stereotypical and most conserved aspects of flower development. It has also been used to resolve some basic mechanisms of floral meristem determination, as well as floral organ cell differentiation and morphogenesis. The challenge ahead will be to understand how modules regulating each aspect of flower development are interconnected among themselves and with signal transduction pathways that respond to environmental and internal cues to yield coupled GRN spatiotemporal dynamics during flower development. Such dynamics likely involve feedback from physical or mechanical forces, structural and geometric characteristics of domains of activity and from cell dynamics (cell growth and division) in complex ways still requiring multiple theoretical multilevel models and coordinated experimental research. Different functional modules are now being characterized (Figure 24 and Table S1) and shown to regulate some of the main processes involved in flower development. Some of these modules or their components may participate in one or more flower developmental process and data on the functions and interactions of genes are becoming available to enable new dynamic computational models of GRN and signaling pathways during flower development (Figure 24 and Table S1).

Computational models for the gene regulatory module that underlies patterning of the inflorescence meristem and determination of the primordial cell types during early stages of flower organ specification, have demonstrated the potential and need of theoretical dynamic approaches in understanding complex GRN underlying flower development. But information on each regulatory module and the interconnections between modules and with signal transduction pathways is still scanty.

It would be fascinating to unravel which molecular components, circuits, or sub-networks underlie the development and evolution of the diversity of flower forms and the variations

Bibliografía

Albert, R., & Othmer, H. G. 2003. The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in Drosophila melanogaster. *J Theor Biol*, **223**, 1–18.

Ancel, L. W. 1999. A quantitative model of the Simpson-Baldwin Effect. *J Theor Biol*, **196**(2), 197–209.

Baldwin, J. M. 1896. A new factor in evolution. *Am. Nat.*, **30**, 441–451; 536–553.

Benítez, M., Espinosa-Soto, C., Padilla-Longoria, P., Díaz, J., & Alvarez-Buylla, E. R. 2007. Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in Arabidopsis root and leaf epidermis: a dynamic model. *Int. J. Dev. Biol.*, **51**, 139–155.

Benítez, Mariana, Espinosa-Soto, Carlos, Padilla-Longoria, Pablo, & Alvarez-Buylla, Elena R. 2008. Interlinked nonlinear subnetworks underlie the formation of robust cellular patterns in Arabidopsis epidermis: a dynamic spatial model. *BMC Syst Biol*, **2**, 98.

Bernhardt, C., Zhao, M., Gonzalez, A., Lloyd, A., & Schiefelbein, J. 2005. The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis. *Development*, **132**, 291–298.

Bouyer, Daniel, Geier, Florian, Kragler, Friedrich, Schnittger, Arp, Pesch, Martina, Wester, Katja, Balkunde, Rachappa, Timmer, Jens, Fleck, Christian, & Hülskamp, Martin. 2008. Two-dimensional patterning by a trapping/depletion mechanism: the role of TTG1 and GL3 in Arabidopsis trichome formation. *PLoS Biol*, **6**(6), e141.

Bowman, J. (ed). 1994. *Arabidopsis. An Atlas of Morphology and Development.* U.S.A.: Springer-Verlag.

Digiuni, Simona, Schellmann, Swen, Geier, Florian, Greese, Bettina, Pesch, Martina, Wester, Katja, Dartan, Burcu, Mach, Valerie, Srinivas, Bhylahalli Purushottam, Timmer, Jens, Fleck, Christian, & Hulskamp, Martin. 2008. A competitive complex formation mechanism underlies trichome patterning on Arabidopsis leaves. *Mol Syst Biol*, **4**, 217.

Dolan, L. 1996. Pattern in the root epidermis: an interplay of diffusible signals and cellular geometry. *Ann. Bot.*, **77**, 547–553.

Draghi, J., & Wagner, G. P. 2009. The evolutionary dynamics of evolvability in a gene network model. *J Evol Biol*, **22**(3), 599–611.

Espinosa-Soto, C., Padilla-Longoria, P., & Alvarez-Buylla, E. R. 2004. A gene regulatory network model for cell-fate determination during Arabidopsis thaliana flower development that is robust and recovers experimental gene expression profiles. *Plant Cell*, **16**, 2923–2939.

Garland, Theodore, & Kelly, Scott A. 2006. Phenotypic plasticity and experimental evolution. *J Exp Biol*, **209**(Pt 12), 2344–2361.

Gilbert, S. F. 2006. *Developmental biology.* 8 edn. Sunderland, MA: Sinauer Associates.

Goodwin, B. 2001. *How the leopard changed its spots. The evolution of complexity.* 2 edn. Princeton, NJ: Princeton Science Library.

Hintze, A., & Adami, C. 2008. Evolution of complex modular biological networks. *PLoS Comput Biol*, **4**, e23.

Huang, Sui, Eichler, Gabriel, Bar-Yam, Yaneer, & Ingber, Donald E. 2005. Cell fates as high-dimensional attractor states of a complex gene regulatory network. *Phys Rev Lett*, **94**(12), 128701.

Jaeger, Johannes, & Reinitz, John. 2006. On the dynamic nature of positional information. *Bioessays*, **28**(11), 1102–11.

Jönsson, H., Heisler, M.G., Shapiro, B.E., Meyerowitz, E.M., & Mjolsness, E. 2006. An auxin-driven polarized transport model for phyllotaxis. *Proc. Natl. Acad. Sci. (USA)*, **103**, 1633–1638.

Kaplan, Jonathan Michael. 2008. The end of the adaptive landscape metaphor? *Biol Philos*, **23**, 625–638.

Kashtan, N., & Alon, U. 2005. Spontaneous evolution of modularity and network motifs. *Proc Natl Acad Sci USA*, **102**, 13773–13778.

Kauffman, S. A. 1969. Metabolic stability and epigenesis in randomly constructed genetic nets. *J. Theor. Biol.*, **22**, 437–467.

Kitano, H. 2004. Biological robustness. *Nat. Rev. Genet.*, **5**, 826–837.

Kwak, S.-H., & Schiefelbein, J. 2007. The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis. *Dev. Biol.*, **302**, 118–131.

Kwak, S.-H., Shen, R., & Schiefelbein, J. 2005. Positional signaling mediated by a receptor-like kinase in Arabidopsis. *Science*, **307**, 1111–1113.

Kwak, Su-Hwan, & Schiefelbein, John. 2008. A feedback mechanism controlling SCRAMBLED receptor accumulation and cell-type pattern in Arabidopsis. *Curr Biol*, **18**(24), 1949–54.

Kwak, Su-Hwan, & Schiefelbein, John. 2009. Regulated accumulation of the SCRAMBLED receptor and position-dependent cell type patterning in Arabidopsis. *Plant Signal Behav*, **4**(4), 332–5.

Kwon, Y.-K., & Cho, K.-H. 2008. Quantitative analysis of robustness and fragility in biological networks based on feedback dynamics. *Bioinformatics*, **24**, 987–994.

Lande, Russell. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J Evol Biol*, **22**(7), 1435–1446.

Larkin, J. C., Oppenheimer, D. G., Lloyd, A., Paparozzi, E. T., & Marks, M. D. 1994. The roles of GLABROUS1 and TRANSPARENT TESTA GLABRA genes in Arabidopsis trichome development. *Plant Cell*, **6**, 1065–1076.

Lewontin, R. C. 2000. *Preface to The Ontogeny of Information, by Oyama, S.* Duke University Press. Chap. 1.

Maes, Lies, Inzé, Dirk, & Goossens, Alain. 2008. Functional specialization of the TRANSPARENT TESTA GLABRA1 network allows differential hormonal control of laminal and marginal trichome initiation in Arabidopsis rosette leaves. *Plant Physiol*, **148**(3), 1453–64.

Meinhardt, H. 1982. *Models of biological pattern formation.* London: Academic Press.

Meinhardt, H., & Gierer, A. 2000. Pattern formation by local self-activation and lateral inhibition. *BioEssays*, **22**, 753–760.

Mendoza, L., & Alvarez-Buylla, E. R. 2000. Genetic regulation of root hair development in Arabidopsis thaliana: a network model. *J Theor Biol*, **204**, 311–326.

Meyerowitz, E. M. 2002. Plants compared to animals: The broadest comparative study of development. *Science*, **295**, 1482–1485.

Müller, G. B. 2007. Evo-devo: extending the evolutionary synthesis. *Nat. Rev. Genet.*, **8**, 943–949.

Newman, Stuart A, & Bhat, Ramray. 2007. Activator-inhibitor dynamics of vertebrate limb pattern formation. *Birth Defects Res C Embryo Today*, **81**(4), 305–19.

Newman, Stuart A, & Bhat, Ramray. 2009. Dynamical patterning modules: a "pattern language" for development and evolution of multicellular form. *Int J Dev Biol*, **53**(5-6), 693–705.

Newman, Stuart A, Forgacs, Gabor, & Muller, Gerd B. 2006. Before programs: the physical origination of multicellular forms. *Int J Dev Biol*, **50**(2-3), 289–99.

Olson, Mark E, & Arroyo-Santos, Alfonso. 2009. Thinking in continua: beyond the .ªdaptive radiation"metaphor. *Bioessays*, **31**(12), 1337–46.

Oyama, S. 2000. *The Ontogeny of Information: Developmental systems and Evolution.* 2nd edn. Duke University Press.

Perazza, Vachon, & Herzog. 1998. Gibberellins promote trichome formation by Up-regulating GLABROUS1 in arabidopsis. *Plant Physiol*, **117**(2), 375–83.

Pesch, M., & Hülskamp, M. 2004. Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation. *Curr. Opin. Genet. Devel.*, **14**, 422–427.

Salazar-Ciudad, I., Garcia-Fernández, J., & Solé, R.V. 2000. Gene networks capable of pattern formation: from induction to reaction-diffusion. *J. Theor. Biol.*, **205**, 587–603.

Savage, Natasha Saint, Walker, Tom, Wieckowski, Yana, Schiefelbein, John, Dolan, Liam, & Monk, Nicholas A M. 2008. A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the Arabidopsis root epidermis. *PLoS Biol*, **6**(9), e235.

Scheres, B. 2001. Plant cell identity. The role of position and lineage. *Plant Physiol.*, **125**, 112–114.

Schiefelbein, John, Kwak, Su-Hwan, Wieckowski, Yana, Barron, Christa, & Bruex, Angela. 2009. The gene regulatory network for root epidermal cell-type pattern formation in Arabidopsis. *J Exp Bot*, **60**(5), 1515–21.

Schnittger, A., Folkers, U., Schwab, B., Jürgens, G., & Hulskamp, M. 1999. Generation of a spacing pattern: the role of TRIPTYCHON in trichome patterning in Arabidopsis. *Plant Cell*, **11**, 1105–1116.

Serna, L. 2005. Epidermal cell patterning and differentiation throughout the apical-basal axis of the seedling. *J. Exp. Bot.*, **56**, 1983–1989.

Slack, J. M. W. 2002. Conrad Hal Waddington: the last Renaissance biologist? *Nat. Rev. Genet.*, **3**, 889–895.

Sommer, Ralf J. 2009. The future of evo-devo: model systems and evolutionary theory. *Nat Rev Genet*, **10**(6), 416–22.

Telfer, A., Bollman, K.M., & Poethig, R.S. 1997. Phase change and the regulation of trichome distribution in Arabidopsis thaliana. *Development*, **124**, 645–654.

Tononi, G, Sporns, O, & Edelman, G M. 1999. Measures of degeneracy and redundancy in biological networks. *Proc Natl Acad Sci U S A*, **96**(6), 3257–62.

Tsukaya, Hirokazu, & Horiguchi, Gorou. 2005. [Leaf morphogenesis]. *Tanpakushitsu Kakusan Koso*, **50**(6 Suppl), 627–32.

Turing, A. M. 1952. The chemical basis of morphogenesis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **237**, 37–72.

von Dassow, G., & Munro, E. M. 1999. Modularity in animal development and evolution: elements of a conceptual framework for EvoDevo. *J. Exp. Zool. (Mol. Dev. Evol.)*, **285**, 307–325.

von Dassow, G., & Odell, G. M. 2002. Design and constraints of the Drosophila segment polarity module: robust spatial patterning emerges from intertwined cell state switches. *J Exp Zool (Mol Dev Evol)*, **294**, 179–215.

Wada, T., Kurata, T., Tominaga, R., Koshino-Kimura, Y., Tachibana, T., Goto, K., Marks, M. D., Shimura, Y., & Okada, K. 2002. Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation. *Development*, **129**, 5409–5419.

Waddington, C. H. 1953. Genetic assimilation of an acquired character. *Evolution Int. J. Org. Evolution*, **7**, 118–126.

Waddington, C. H. 1957. *The strategy of the genes. A discussion of some aspects of theoretical biology.* New York, NY: MacMillan.

Wagner, A. 2005. *Robustness and evolvability in living systems.* Princeton Studies in Complexity. Princeton, NJ: Princeton University Press.

Wagner, A. 2008. Neutralism and selectionism: a network-based reconciliation. *Nat. Rev. Genet.*, **9**, 965–974.

Wagner, G. P. 2009. Bio. *Evol Dev*, **11**(2), 139–141.

Wagner, Günter P, Pavlicev, Mihaela, & Cheverud, James M. 2007. The road to modularity. *Nat Rev Genet*, **8**(12), 921–31.

Walbot, V. 2000. Arabidopsis thaliana genome. A green chapter in the book of life. *Nature*, **408**(6814), 794–5.

West-Eberhard, M. J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press.

West-Eberhard, M. J. 2005. Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci. USA*, **102**, 6543–6549.

Wolpert, L. 1996. One hundred years of positional information. *Trends Genet.*, **12**, 359–364.

Yoshida, Yuki, Sano, Ryosuke, Wada, Takuji, Takabayashi, Junji, & Okada, Kiyotaka. 2009. Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in Arabidopsis. *Development*, **136**(6), 1039–48.