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Programa de Maestría y Doctorado en Ciencias Bioquímicas

DESARROLLO DE MODELOS *IN SILICO* PARA EL ESTUDIO DE LA DIFERENCIACIÓN DE LOS LINFOCITOS B

TESIS
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P r e s e n t e

Los miembros del Subcomité Académico en reunión ordinaria del día 13 de marzo del presente año, conocieron su solicitud de asignación de **JURADO DE EXAMEN** para optar por el grado de **DOCTORADO EN CIENCIAS**, con la réplica de la tesis **"Desarrollo de modelos in silico para el estudio de la diferenciación de los linfocitos B"**, dirigida por el/la Dr(a) **Mendoza Sierra Luis Antonio**.

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"Por mi raza hablará el espíritu"
Cd. Universitaria, Cd. Mx., a 13 de marzo de 2017.
C O O R D I N A D O R

Dr. ROBERTO CORIA ORTEGA

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HSC:	Células troncales hematopoyéticas
MPP:	Progenitores multipotentes
LMPP:	Progenitor multipotente tendente al linaje linfoide
ELP:	Progenitor linfoide temprano
CLP:	Progenitor linfoide común
EP:	Progenitor eritroide
MegP:	Progenitor de megacariocitos
GP:	Progenitor de granulocitos
DC:	Células dendríticas
NK:	Natural killer
LI:	Linfocitos innatos
SQUAD:	Método de Sistemas Dinámicos Cualitativos Estandarizados

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RESUMEN

Debido al número de enfermedades asociadas con el mal funcionamiento del sistema hematopoyético, como son leucemias, linfomas, mielomas, anemias, entre otras, existe el interés de conocer los mecanismos moleculares que controlan la diferenciación de los linajes de células sanguíneas. En particular, el estudio de la diferenciación de los linfocitos B ha permitido identificar un creciente número de moléculas involucradas en el control de este proceso para dar lugar a la formación de células efectoras especializadas en respuesta a diversas señales microambientales.

La diferenciación de los linfocitos B está regulada por una compleja red de regulación que promueve el compromiso con el linaje y el establecimiento de programas específicos de expresión genética que confieren identidad celular hasta completar su diferenciación terminal a células productoras de anticuerpos, todo esto a partir de una población de progenitores multipotenciales. Sin embargo, no existe un consenso acerca de la estructura de esta red de regulación. El conocer la arquitectura de dicha red, así como su comportamiento dinámico, tanto a nivel molecular como celular, ayuda a mejorar nuestro entendimiento sobre la formación de diversos tipos celulares. Además, permite estudiar sistemáticamente el efecto de diversas perturbaciones para dilucidar el papel de las moléculas que integran a la red.

En el presente trabajo se propone estudiar el comportamiento de la red de regulación que controla la diferenciación de los linfocitos B desde la perspectiva de los sistemas dinámicos, teniendo en cuenta múltiples escalas de regulación. Para ello, se desarrollaron distintos modelos enfocados a entender la manera en que la red de regulación controla la diferenciación de los linfocitos B.

Como primera aproximación, se infirió la arquitectura de la red de la diferenciación terminal de los linfocitos B a partir de la información experimental reportada en la literatura, particularmente de modelos murinos y humano. Esta red se implementó en la forma de un modelo matemático con ecuaciones lógicas y continuas, y se identificaron los estados estacionarios del sistema, los cuales describen de manera cualitativa los patrones de activación de las moléculas que regulan el proceso de diferenciación. Posteriormente, se corroboró la correspondencia entre los patrones obtenidos con los patrones de expre-

sión reportados para distintos tipos de linfocitos B. La comparación se hizo tanto para condiciones normales (*i.e.* tipo silvestre), como con los patrones de activación de algunas mutantes, así como bajo la presencia de señales extracelulares específicas que dirigen el proceso de diferenciación.

Con base en los análisis realizados se encontró que la red de regulación reconstruida es capaz de dirigir la formación de al menos cuatro tipos celulares que son: Naive, GC, Mem y PC. Cabe señalar que con base en estos resultados se propusieron algunos circuitos de retroalimentación necesarios para el establecimiento de estos destinos. Este modelo nos permitió evaluar el proceso de diferenciación terminal de los linfocitos B simulando la respuesta de la red ante señales externas. Específicamente, se encontró que la red dirige el proceso de diferenciación de manera plástica en estadios precursores, mientras que asegura la diferenciación de manera irreversible hacia el estadio de células efectoras.

Por otra parte, la red de regulación de la diferenciación de las células B se comporta como un módulo funcional. Por ello, fue posible integrarlo con la red de regulación que controla la diferenciación de células T junto con otros reguladores tempranos del linaje linfoide. En conjunto, esta red conforma el modelo dinámico más complejo que se ha estudiado para la diferenciación de células linfoides. A dicha red se le estudió su comportamiento dinámico en respuesta a señales extracelulares. El modelo extendido recapitula eventos clave de diferenciación celular que ocurren durante la linfopoyesis que dan lugar a la formación de por lo menos seis tipos celulares maduros pertenecientes a los linajes de linfocitos T y B.

Finalmente, el modelo predice interacciones necesarias para mantener niveles constantes de activación entre los factores que regulan el compromiso con los linajes linfoides así como para controlar la exclusión mutua entre los programas de diferenciación de linfocitos T y B. Se espera que los presentes modelos sirvan como un punto de partida para el estudio de enfermedades asociadas con los linajes linfoides.

ABSTRACT

Due to the large number of diseases associated with the malfunction of the hematopoietic system, such as leukemias, lymphomas, myelomas, anemias, among others, there is an interest in knowing the molecular mechanisms that control the differentiation of blood cell lineages. In particular, the study of the differentiation of B lymphocytes has allowed to identify a growing number of molecules involved in the control of this process to give rise to the formation of specialized effector cells in response to various microenvironmental cues.

B lymphocyte differentiation is regulated by a complex regulatory network that promotes lineage commitment and the establishment of specific gene expression programs that confer cellular identity to complete their terminal differentiation to antibody producing cells, all from a population of multipotential progenitors. However, there is no consensus on the structure of this regulatory network. Knowing the architecture of such network, as well as its dynamical behavior, both at molecular and cellular levels, helps to improve our understanding of the formation of various cell types. In addition, it allows to systematically study the effect of diverse perturbations to elucidate the role of the molecules in the network.

In the present work, we propose to study the behavior of the regulation network that controls the differentiation of B lymphocytes from the dynamical systems perspective, taking into account multiple scales of regulation. To do this, different models were developed to understand how the regulatory network controls the differentiation of B lymphocytes.

As a first approximation, the architecture of the network controlling terminal differentiatio of B lymphocytes was inferred from the experimental information reported in the literature, particularly from murine and human models. This network was translated into a formal mathematical model with the use of logical and continuous equations, and the stationary states of the system were identified which qualitatively describe the activation patterns of the molecules that regulate the differentiation process. Subsequently, the correspondence between the patterns obtained with the reported expression patterns for different types of B lymphocytes was corroborated. The comparison was made for both normal (*i.e.* wild type) and for the activation patterns of some mutants, as well as under

the presence of specific extracellular signals known to direct the differentiation process.

Based on the analyses, it was found that the reconstructed regulatory network is able to direct the formation of at least four cell types that are: Naive, GC, Mem and PC. It should be noted that based on these results some newly proposed feedback circuits were found to be necessary for the establishment of these cell fates. This model allowed us to evaluate the process of terminal differentiation of B lymphocytes by simulating the response of the network to extracellular signals. Specifically, it was found that the network directs the process of differentiation in a plastic way in precursor stages, while irreversibly ensuring differentiation towards the stage of effector cells.

Furthermore, the regulatory network of B cell differentiation behaves as a functional module. Therefore, it was possible to integrate it with the regulatory network that controls the differentiation of T cells along with other early regulators of the lymphoid lineage. Together, this enlarged network forms the most complex dynamical model that has been studied for the differentiation of lymphoid cells. The dynamical behavior of this network in response to extracellular signals was studied and found that the extended model recapitulates key events of cell differentiation that occur during lymphopoiesis leading to the formation of at least six mature cell types belonging to the T and B lymphocyte lineages.

Finally, the model predicts interactions necessary to maintain constant levels of activation between the factors regulating the commitment to lymphoid lineages as well as for the control of mutual exclusion of T and B lymphocyte differentiation programs. The present models are expected to serve as a starting point for the study of diseases associated with lymphoid lineages.

1 INTRODUCCIÓN

1.1 DIFERENCIACIÓN CELULAR Y HEMATOPOYESIS

La diferenciación celular es un fenómeno complejo en el que las células de un organismo adquieren características estructurales y funcionales específicas. Este proceso involucra la pérdida gradual del potencial de desarrollo hacia múltiples tipos celulares. Esto se da además de manera paralela a la adquisición del compromiso con un linaje celular y la maduración para dar lugar a la formación de células especializadas (Graf and Enver, 2009; Orkin and Zon, 2008).

Se han realizado esfuerzos en diversos sistemas biológicos para dilucidar la regulación molecular que subyace a los procesos de diferenciación celular. Estos esfuerzos se han enfocado principalmente en el estudio de diferentes genes cuyas mutaciones de ganancia o perdida de función presentan un efecto fenotípico en estadios críticos de diferenciación, lo que ayuda a inferir el papel de estos genes en múltiples procesos de diferenciación celular. El sistema hematopoyético representa un sistema experimental adecuado para el estudio de la diferenciación celular y los procesos que subyacen a las decisiones de destino celular como son la especificación de linaje y el compromiso para dar lugar a la generación de células especializadas terminalmente diferenciadas. En particular, el descubrimiento de los linfocitos B como un linaje independiente del sistema inmune hace poco más de cincuenta años, ha permitido el estudio de algunos de los mecanismos que subyacen a la diferenciación y la regulación de múltiples procesos celulares para llevar a cabo su papel como los principales efectores de la respuesta humoral inmune en vertebrados.

La descripción de un número de moléculas involucradas en el control de la diferenciación celular durante la formación de los linfocitos B ha puesto de manifiesto la complejidad que subyace a la regulación de dicho proceso. Por ejemplo, la regulación de cada una de estas moléculas puede llevarse a cabo por una o más moléculas diferentes. A su vez, un gen puede intervenir en la regulación de múltiples genes y regular diferentes procesos celulares, lo que resulta en la formación de complejas redes de regulación. Sin embargo, se desconoce la estructura dichas redes y más aún, su comportamiento dinámico, por lo que resulta necesario el análisis de las redes de regulación de manera que podamos inte-

grar la información existente y generar predicciones valiosas que guíen el planteamiento de nuevas hipótesis y experimentos para el estudio de la diferenciación celular.

Las interacciones entre las moléculas que conforman a las redes pueden tener un efecto no lineal, por lo que su comportamiento dinámico no puede ser fácilmente estudiado de manera intuitiva. Por ejemplo, las modificaciones a una o varias moléculas que regulan a un gen pueden tener un efecto fenotípico que no corresponda en magnitud al efecto de sus reguladores. Este efecto no puede ser inferido por medio de una inspección visual de las interacciones, por lo que es necesario utilizar modelos computacionales que nos ayuden a analizar esta información para generar predicciones útiles en el planteamiento de nuevas hipótesis y experimentos. Los modelos computacionales nos han permitido entender mejor diversas propiedades dinámicas de las redes de regulación, como son su estabilidad, redundancia, homeostasis y multiestacionareidad (Furusawa and Kaneko, 2012; Le Novère, 2015; Thieffry, 2007).

1.1.1 HEMATOPOYESIS

Los linfocitos B forman parte del sistema hematopoyético, un sistema encargado de la producción de todas las células sanguíneas y del sistema inmune del organismo. Las subpoblaciones que componen a este sistema pueden clasificarse en al menos cinco grupos de acuerdo con su grado de madurez y capacidad para diferenciarse hacia múltiples linajes celulares (Seita and Weissman, 2010). El primer grupo corresponde a las células troncales hematopoyéticas (HSC, *hematopoietic stem cells*), que se caracterizan por su capacidad para autorrenovarse y su potencial para dar lugar a la formación de todas las células sanguíneas. Las células HSC dan origen a las células progenitoras multipotentes (MPP, *multipotent progenitor* y LMPP, *lympoid-primed multipotent progenitor*) que, a diferencia de sus progenitores, pierden la capacidad de autorrenovación y poseen un menor potencial para diferenciarse dando lugar a progenitores oligopotentes (CMP, *common myeloid progenitor*, ELP *early lymphoid progenitor*) y CLP (*common lymphoid progenitor*), (**Figura 1**).

Las células CMP y CLP poseen una mayor capacidad proliferativa pero un potencial de diferenciación restringido que da lugar a la formación de progenitores restringidos a

un linaje celular. Estos progenitores dan lugar a la formación de células maduras terminalmente diferenciadas con características morfológicas y funcionales específicas que pueden dividirse en dos grupos: el de células mieloides y linfoides. Las células mieloides comprenden a los monocitos, macrófagos, eritrocitos, megacariocitos, granulocitos y células dendríticas, mientras que las células linfoides comprenden a las células NK (*natural killer*), linfocitos innatos, linfocitos T y linfocitos B. La producción de ambos grupos de células se lleva cabo por medio de un proceso dinámico conocido como mielopoiesis y linfopoiesis, respectivamente.

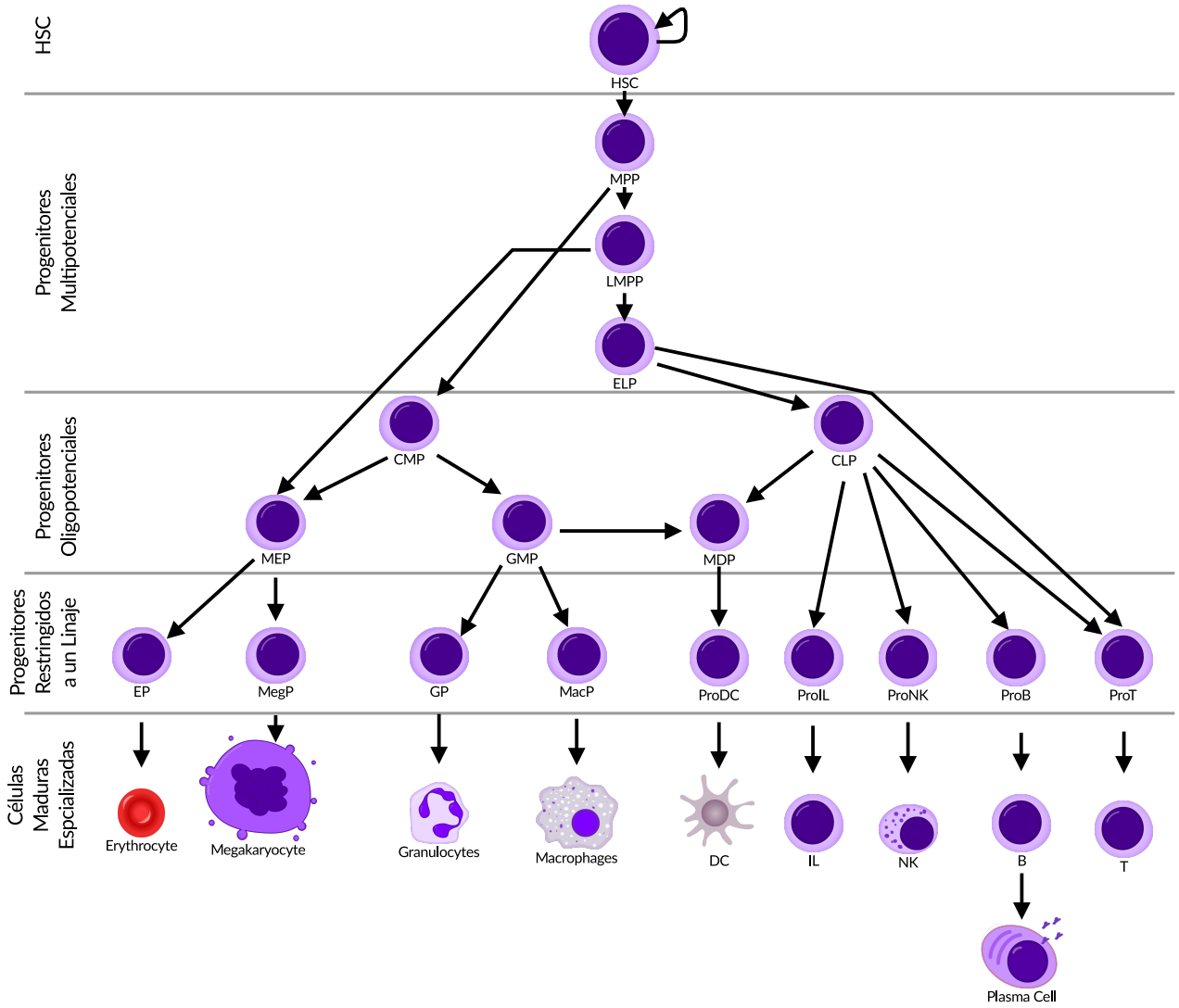

Figura 1: Las células hematopoyéticas se originan de manera progresiva a partir de un progenitor troncal pluripotente, dando lugar a múltiples linajes que originan células especializadas. Se muestran los compartimentos del sistema hematopoyético. Las diferentes poblaciones celulares se etiquetan por sus siglas en inglés; HSC: Célula troncal hematopoyética, MPP: Progenitor multipotencial, LMPP: Progenitor multipotente tendente al linaje linfoide, CMP: Progenitor mieloide común, GMP: Progenitor granulocítico y monocítico, MDP: Progenitor de macrófagos y células dendríticas, CLP: Progenitor linfoide común, EP: Progenitor eritroide, MegP: Progenitor de megacariocitos, GP: Progenitor de granulocitos, DC: Células dendríticas, NK: Natural killer, IL: Linfocitos innatos. Adaptado de Seita *et al.,* 2010 y Moignard *et al.*, 2013

1.2 LINFOPOYESIS

La diferenciación de las células linfoides (linfocitos T, células NK, linfocitos innatos y linfocitos B) a partir de células progenitoras hematopoyéticas conlleva la adquisición de patrones de expresión genética particulares que confieren especificidad hacia un linaje junto con la pérdida gradual de la capacidad de diferenciación hacia otros tipos celulares (Welinder *et al.*, 2011b). Este proceso es guiado por la acción concertada de distintos genes y señales microambientales que promueven la adquisición de funciones especializadas y la exclusión de otros programas de diferenciación (Kondo *et al.*, 2003).

La especificación del linaje linfoide comienza en la médula ósea con la expresión del receptor del factor de crecimiento Flt3 y los factores transcripcionales Ikaros y PU.1 en las células LMPP. Estas células tienen un potencial combinado para dar lugar a linfocitos T, NK, B y células del linaje mieloide (Ichii *et al.*, 2010). Posteriormente se pierde el potencial hacia el linaje mieloide, dando lugar a la formación de los progenitores ELP. Esta es la población celular más temprana con el potencial para generar células del linaje linfoide y dan lugar a los progenitores linfoides comunes (CLP), que se caracterizan por la expresión del receptor de IL-7 (IL-7R) y su capacidad, similar a los ELP, de generar a las células T, B y NK. Adicionalmente, los CLPs son los precursores más eficientes que dan lugar a la formación de linfocitos B (Grosschedl, 2014).

Los CLPs pueden dividirse a su vez en dos subpoblaciones de acuerdo con la expresión del marcador Ly6d. Los CLPs que no expresan ($Ly6d^{-}$) mantienen su potencial para diferenciarse a células NK y linfocitos T, mientras que los CLPs $Ly6d^{+}$ adquieren un compromiso hacia el linaje de las células B y pierden la capacidad de diferenciarse en otros tipos celulares (Inlay *et al.*, 2009), (**Figura 2**). De manera paralela, los CLPs también dan lugar a la formación de los precursores de los linfocitos T que comienzan como células $CD4^{-}$ $CD8^{-}$ dobles negativas debido a la ausencia de los marcadores de superficie CD4 y CD8. La maduración y diferenciación de estos progenitores se lleva a cabo en el timo donde expresan la cadena TCRα y los correceptores CD4 y CD8, dando lugar a las células $CD4^{+}$ $CD8^{+}$ doble positivas (DP) (Naito *et al.*, 2011), (**Figura 2**).

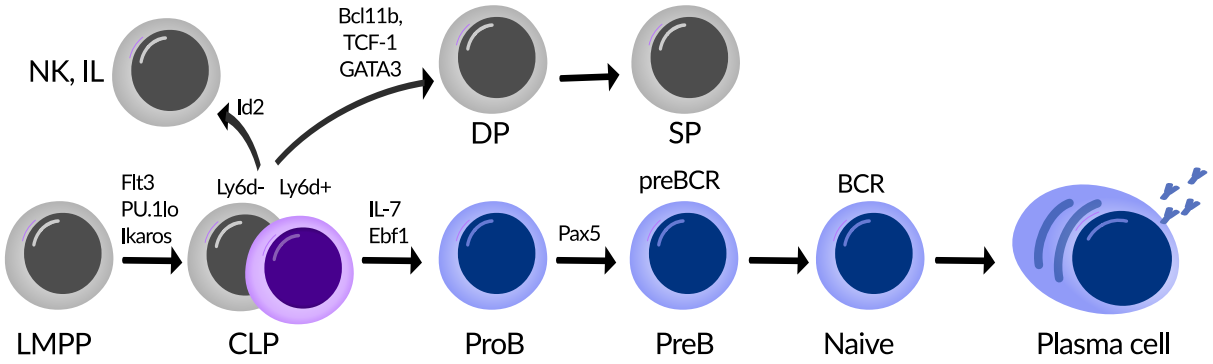

Figura 2: Linfopoyesis y desarrollo del linaje de células B. Se muestran las diferentes etapas de la generación de células linfoides, a partir de una población progenitora LMPP Flt3$^+$ los factores PU.1 e Ikaros marcan los primeros pasos para la formación de los linajes linfoides dando lugar a los progenitores CLP que originan a las células NK, IL, linfocitos T doble positivos (DP), simples positivos (SP), marcados en color gris, así como linfocitos B (ProB, PreB, Naive y Plasma cells), color azul. La subpoblación Ly6d$^+$ de CLPs (color morado) adquiere una mayor propensión para dar lugar al linaje de células B. El compromiso y la especificación con el linaje de los linfocitos B es controlado por Ebf1 y Pax5 en respuesta a la señalización por IL-7. Adaptada de (Grosschedl, 2014).

Posteriormente, las células DP se comprometen con un linaje y expresan exclusivamente uno de los dos receptores CD4 o CD8, convirtiéndose en células CD4$^+$ o CD8$^+$ simples positivas (SP), que se caracterizan por poseer distinta especificidad para el reconocimiento de antígenos, las células CD4$^+$ están restringidas al reconocimiento de MHC-II, mientras que las células CD8$^+$ están restringidas al reconocimiento por MHC-I (Xiong and Bosselut, 2012). A su vez, las células CD4$^+$ pueden dividirse en linfocitos T cooperadores (Th): Th1, Th2, Th17 y linfocitos T reguladores (Treg). Los linfocitos Th1 se caracterizan por la producción IFN-γ y la expresión de T-bet, y participan en la respuesta ante infecciones virales intracelulares y bacterianas (Szabo *et al.*, 2003). Las células Th2 expresan GATA3 y producen interleucina IL-4 y están involucradas en la respuesta contra patógenos extracelulares (Murphy and Reiner, 2002). Las células Th17 producen IL-17 y expresan RORγt y son necesarias para la defensa contra patógenos extracelulares (Ivanov *et al.*, 2006), las células Treg se caracterizan por la expresión de Foxp3 y poseen una actividad inmunosupresora (Gavin *et al.*, 2007).

Por otra parte, las células $CD8^+$ dan lugar a las células T efectoras citotóxicas (CTL), las cuales matan a células infectadas y se caracterizan por la producción de perforina (Prf1) y granzyma B (Gzmb) así como de IFN-γ, (Morishima *et al.*, 2010).

1.2.1 DESARROLLO DE LOS LINFOCITOS B

La estimulación de los CLPs por Flt3L e IL-7 marca los primeros estadios de desarrollo de los linfocitos B una vez que los progenitores han adquirido un compromiso con este linaje. La activación del programa que confiere especificidad hacia el linaje de los linfocitos B en células CLP depende de la expresión de los factores transcripcionales Ebf1 y Pax5. La activación de *Ebf1* promueve la transición de CLPs a células progenitoras de los linfocitos B (ProB) y su expresión requiere de la señalización por IL-7 (Roessler *et al.*, 2007).

Por otra parte, Pax5 promueve la activación de genes característicos de los linfocitos B y reprime genes de programas alternos de diferenciación celular (Carotta *et al.*, 2006; Schebesta *et al.*, 2007), por lo que además de ser necesario para la especificación del linaje, Pax5 es un factor clave en el compromiso y mantenimiento de la identidad de los linfocitos B (Cobaleda *et al.*, 2007; Medina *et al.*, 2004). Durante el estadio de diferenciación ProB, los linfocitos B generan un amplio repertorio de anticuerpos por medio de la recombinación de las cadenas pesadas *IgH* de los genes de inmunoglobulina por la acción de las recombinasas Rag1 y Rag2. El correcto ensamble de las cadenas pesadas con las cadenas ligeras *VpreB* y $\lambda 5$ determina la formación del receptor primario de las células B (pre-BCR), lo que promueve la formación de células PreB (Boller and Grosschedl, 2014), (**Figura 2**).

1.2.2 DIFERENCIACIÓN TERMINAL DE LOS LINFOCITOS B

Las señales intracelulares producidas por la activación del pre-BCR promueven la recombinación de las cadenas ligeras de inmunoglobulina que serán ensambladas para dar lugar a la expresión de un receptor de células B maduro (BCR). Una vez que las células B que expresan un receptor BCR maduro migran fuera de la médula ósea hacia los órganos linfáticos secundarios donde completarán su diferenciación de células maduras o naive a uno de al menos tres destinos celulares: células de los centros germinales (GC), células de

memoria (Mem) y células plasmáticas (PC).

Los linfocitos B son los principales efectores de la respuesta humoral adaptativa del sistema inmune en vertebrados encargados de la generación y producción de anticuerpos específicos que promueven el reconocimiento y la atenuación de moléculas ajenas al organismo por medio de la unión del BCR a antígenos específicos (LeBien and Tedder, 2008). El reconocimiento de un antígeno promueve la formación de los centros germinales y la diferenciación a células plasmáticas. Durante la etapa de los centros germinales, los linfocitos B llevan a cabo una segunda edición de los genes de inmunoglobulina y sufren un proceso de selección que promueve el mejoramiento de la afinidad los anticuerpos por su antígeno mediante la hipermutación somática (SHM) y la recombinación de cambio de clase (CSR) de los genes de inmunoglobulina (Igarashi *et al.*, 2014). De manera paralela, tanto las células Naive como las GC pueden diferenciarse en células Mem. Este proceso puede llevarse a cabo de manera dependiente o independiente del contacto con linfocitos T cooperadores foliculares (Tfh) que producen moléculas importantes para el mantenimiento y la diferenciación de los linfocitos B. Finalmente, la estimulación de las células Naive, GC o Mem por medio de la unión del BCR a un antígeno determina la diferenciación a células efectoras productoras de anticuerpos (PC) (Grosschedl, 2014; Johnson *et al.*, 2005), (**Figura 3**).

1.3 FACTORES MOLECULARES INVOLUCRADOS EN LA DIFERENCIACIÓN DE LOS LINFOCITOS B

Se han identificado diversas moléculas involucradas en el control de la diferenciación de los linfocitos B, entre ellas figuran algunos factores transcripcionales como Ikaros, Ebf1, E2A, Pax5, entre otros, necesarios para el establecimiento de programas específicos a lo largo del desarrollo de estas células (Nutt and Kee, 2007). De igual manera, durante este proceso es importante también la activación de receptores, como Flt3, IL-7R y CXCR4, que capacitan a los linfocitos B para percibir señales microambientales (Grosschedl, 2014). Estas señales dirigen su diferenciación o controlan la migración de estas células hacia los órganos linfáticos secundarios y los centros germinales, que es donde completaran su diferenciación a células efectoras. Todas las moléculas involucradas en es-

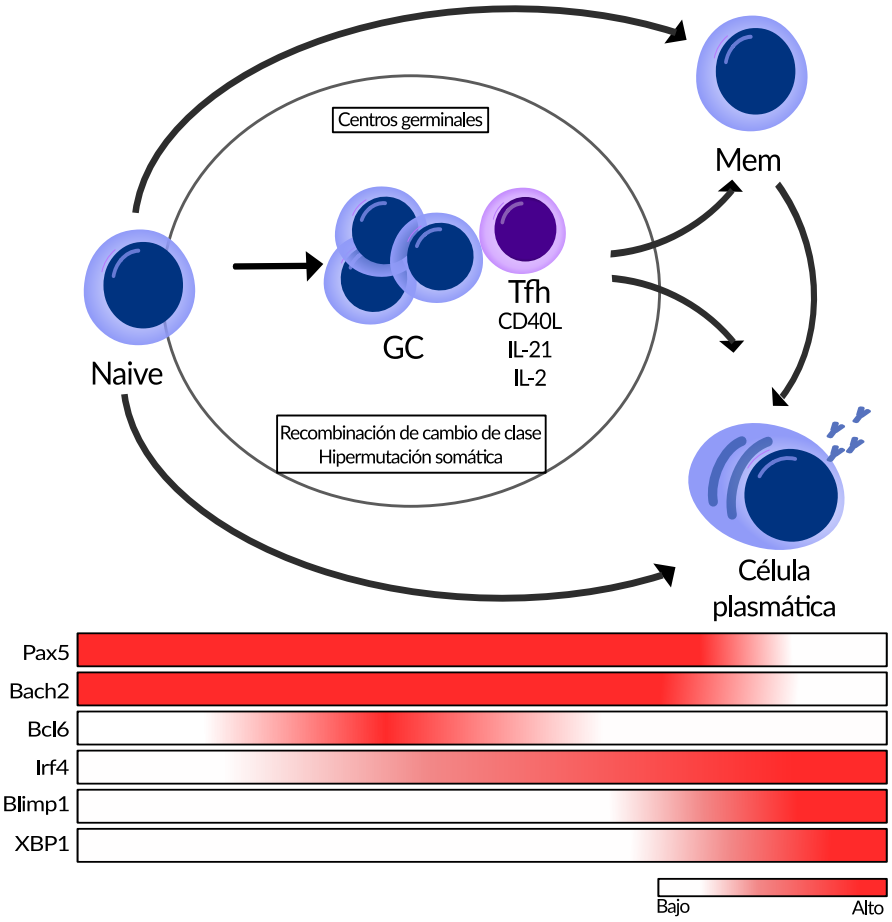

Figura 3: Diferenciación terminal de los linfocitos B. La diferenciación de linfocitos B a partir de un progenitor Naive (Bach2$^+$, Pax5$^+$) está controlada por la acción concertada de diferentes moléculas, como factores de transcripción (panel inferior) y citocinas, que confieren especificidad a los programas de células B e inhiben programa programas celulares alternativos. La interacción de los linfocitos B con los linfocitos Tfh (panel superior) promueve la formación y el mantenimiento de las células GC (Bcl6$^+$, Bach2$^+$, Pax5$^+$) así como su diferenciación a células Mem (Irf4$^+$, Bach2$^+$, Pax5$^+$) que al contacto con un antígeno se diferencian de manera terminal a células PC (Blimp1, Irf4, XBP1) productoras de anticuerpos. Adaptada de (Basso and Dalla-favera, 2015).

tos procesos actúan de manera concertada formando una compleja red de regulación (Nutt and Tarlinton, 2011).

Los factores y moléculas de señalización que se han caracterizado para la formación de

los linfocitos B, pueden agruparse dependiendo de su papel en los diferentes estadios del desarrollo de estas células. Por ejemplo, la activación del receptor Flt3 en los progenitores CLP por su ligando Flt3L, producido por las células estromales de la médula ósea, es necesaria para el comienzo del desarrollo de los linfocitos B, ya que la deficiencia de Flt3L afecta severamente la formación de estos progenitores (Tsapogas *et al.*, 2014). Los factores de transcripción Ikaros y PU.1 participan a partir de las primeras etapas de la formación del linaje B. Ikaros induce la expresión de Gfi1 que a su vez inhibe la expresión de PU.1 (Spooner *et al.*, 2009). Puesto que PU.1 participa también en la regulación de programas de otros linajes, como en el caso del linaje mieloide, la represión de este factor mediada por Gfi1 favorece el compromiso hacia el linaje de las células B (Dahl *et al.*, 2007). Además, la deficiencia de Runx1 causa una severa reducción de los progenitores de células B debido a que la expresión de Ebf1 no se induce en estos progenitores (Guo *et al.*, 2012; Seo *et al.*, 2012), por lo tanto, la activación de Ebf1 por Runx1 es necesaria para la diferenciación de linfocitos B.

Por su parte, la expresión de Ebf1 requiere de la expresión de E2A y Foxo1 (Welinder *et al.*, 2011a). La activación de estos factores promueve la expresión de Pax5 que a su vez regula los programas de identidad de las células B. La deficiencia de E2A y Ebf1 afecta severamente la formación de células del linaje B (Inlay *et al.*, 2009). Una vez activo, Pax5 participa en el establecimiento de programas celulares que promueven la maduración de los linfocitos B a células maduras (Nera and Lassila, 2006). Estas células posteriormente migran fuera de la médula ósea hacia los órganos linfáticos secundarios en la forma de células naive, donde entran en contacto con linfocitos T, células presentadoras de antígeno, entre otras, y llevan a cabo los procesos de CSR y SHM para una segunda edición y mejora de los anticuerpos. Estos procesos se llevan a cabo dentro de los centros germinales, cuyo establecimiento está regulado por la expresión de Bcl6 (Basso *et al.*, 2012; McHeyzer-Williams *et al.*, 2006).

Finalmente, los linfocitos B se diferencian en células de memoria (Mem) que son capaces de proliferar y renovarse por largos periodo de tiempo y desencadenar respuestas humorales ante posteriores encuentros con un antígeno, o bien, diferenciarse a células plasmáticas productoras de anticuerpos una vez que son estimuladas por el contacto con su antígeno. La transición a células Mem y PC está marcada por la inducción del factor

Irf4 (Saito *et al.*, 2007), el cual juega un papel dual en la diferenciación de los linfocitos B, ya que por un lado promueve los procesos de CSR y SHM en células B maduras e inhibe la expresión de *Bcl6* y activa a *prdm1* para promover la diferenciación terminal a células PC cuando alcanza un nivel mayor de expresión (Klein *et al.*, 2006; Sciammas *et al.*, 2006).

La diferenciación de células PC está controlada por el factor Blimp1, ya que la falta de este factor evita la diferenciación terminal de células PC (Shapiro-Shelef *et al.*, 2003). A su vez, Blimp1 inhibe la expresión de Pax5 y también programas específicos de las células B (Shaffer *et al.*, 2002b), (**Figura 3**).

1.4 MODELOS COMPUTACIONALES Y REDES DE REGULACIÓN

Los avances en distintas tecnologías de biología molecular han permitido la descripción de un creciente número de moléculas, así como de las interacciones entre estas, involucradas en la regulación de diferentes procesos biológicos. Integrar esta información puede ayudarnos a entender propiedades de este sistema biológico que no podrían ser comprendidas estudiando sus partes por separado. Para ello, se ha vuelto necesario utilizar herramientas matemáticas y computacionales para construir modelos que integren de manera formal y coherente la información disponible y nos permitan analizar el complejo comportamiento de los sistemas biológicos (Le Novère, 2015).

Una de las herramientas comúnmente utilizadas para estudiar estos procesos son las redes de regulación. Las redes están formadas por nodos y aristas que describen las interacciones entre los nodos. Los nodos pueden representar las diferentes moléculas (genes, mRNAs, proteínas, etc.) que están involucradas en la regulación de un proceso biológico, mientras que las aristas representan distintos tipos de interacciones entre las moléculas de la red (Albert, 2007). Por ejemplo, en una red metabólica los nodos pueden representar diferentes sustratos y productos que interaccionan por medio de una o más reacciones químicas, mientras que en una red de regulación transcripcional, las interacciones pueden representar el efecto, de activación o inhibición, de una o varias moléculas sobre la expresión de un gen (de~Jong and Liebert, 2002).

A su vez, los nodos pueden tomar diferentes valores que pueden representarse por variables *discretas* o *continuas*. Dichas variables pueden utilizarse para simular la presencia

o ausencia de una molécula en el caso de los valores discretos, o un diferentes niveles de activación dependiendo de la concentración de dicha molécula por medio de valores continuos.

Además, dependiendo del tipo de interacción, las conexiones entre los nodos pueden ser dirigidas o no dirigidas, es decir, pueden poseer una dirección, magnitud y signo. Por ejemplo, las interacciones físicas entre los componentes de un complejo proteico pueden representarse por medio de aristas que sin dirección, mientras que la regulación transcripcional de una proteína sobre un gen puede describirse utilizando flechas dirigidas cuyo signo puede ser positivo o negativo si la influencia de un elemento de la red sobre otro es la de aumentar el nivel de activación de una molécula o reducirla, respectivamente.

El conocimiento de las interacciones entre diferentes moléculas, aunque valioso, ofrece una visión estática del comportamiento de diferentes procesos biológicos, por lo que para entender mejor la funcionalidad de las redes de regulación, es necesario desarrollar modelos que permitan analizar las propiedades dinámicas de las redes, tales como su robustez, redundancia, homeostasis y multiestacionareidad. Dichas propiedades nos ayudan a entender cuestiones relacionadas con el número, tipos y estabilidad de los posibles patrones de activación que emergen del comportamiento dinámico una red de manera que podamos obtener información sobre el papel de moléculas e interacciones específicas en la regulación de un proceso biológico. Además, los modelos dinámicos permiten simular el efecto de diversos estímulos o perturbaciones permanentes, como mutaciones de ganancia o pérdida de función de las moléculas de la red y generar información que puede ser usada eventualmente para entender mecanismos de control con el propósito de dirigir el comportamiento del sistema hacia un estado determinado. Por lo tanto, los modelos dinámicos son una herramienta útil para el estudio de las redes de regulación ya que permiten integrar la información disponible en una estructura formal y concisa de elementos que interaccionan entre sí (Albert, 2007).

Los modelos de redes de regulación pueden representarse como una abstracción matemática de los elementos que participan en un proceso biológico. Una red puede visualizarse como un conjunto de nodos y aristas. Los nodos representan moléculas, tales como genes, proteínas, mRNAs, etc. o complejos moleculares, mientras que las aristas representan las interacciones entre ellos, que pueden ser de activación o inhibición (de~Jong and

Liebert, 2002). Para capturar los cambios en las concentraciones de las moléculas involucradas, las redes pueden ser modeladas como un sistema dinámico, los cuales describen el comportamiento del sistema a través del tiempo.

En este tipo de modelos, el valor de los nodos de la red cambia dependiendo de sus interacciones con los nodos que lo regulan (Albert, 2007). La dinámica de una red de regulación genética está relacionada con los estados de expresión de los genes a lo largo del tiempo. La expresión o inhibición de grandes conjuntos de genes determina características fenotípicas importantes, como el destino celular o en general el "estado funcional" de la célula (Huang *et al.*, 2005). El comportamiento de las redes de regulación depende de las conexiones entre los nodos de la red y el tipo de interacciones (activación o inhibición), así como las señales externas que la célula recibe de su entorno (Karlebach and Shamir, 2008).

No obstante, debido a la gran cantidad de elementos e interacciones que intervienen en un proceso biológico, resulta difícil la descripción exacta del sistema por lo que comúnmente se hacen simplificaciones que ayuden a capturar la esencia cualitativa del proceso. Por ejemplo, puede considerarse que los genes actúan activando o inhibiendo la transcripción de otros genes, no importando si estas interacciones son directas, por medio del contacto físico entre moléculas, o indirectas (Shmulevich and Aitchison, 2009). Por otra parte, la información experimental disponible generalmente es de naturaleza cualitativa, por lo que puede considerarse que la expresión de un gen puede tener dos estados: *activo* o *inactivo*, es decir, posee un número finito o discreto de estados de activación (Shmulevich and Aitchison, 2009).

Existen dos aproximaciones para la construcción de modelos dinámicos de las redes de regulación, dividiéndose en modelos discretos y continuos. Los modelos discretos o lógicos son usados para describir sistemas con un número finito de estados y hacen uso de la información cualitativa (Abou-Jaoudé *et al.*, 2016). El caso más simple de los modelos discretos se denominan modelos Booleanos. En estos modelos, los nodos en la red pueden tener dos estados de activación, 1 (activo) o 0 (inactivo) en un tiempo determinado y un conjunto de reglas lógicas de regulación que pueden inferirse a partir de resultados experimentales (Bornholdt, 2008). Estas reglas determinan la transición entre los estados de activación para cada nodo de la red. Los modelos continuos pueden utilizarse para describir

sistemas con número infinito de posibles estados, por lo cual son adecuados para incorporar información cuantitativa referente al comportamiento de las moléculas que componen a la red. Sin embargo, los modelos cuantitativos requieren información detallada acerca de la estequiometría y parámetros cinéticos de diferentes reacciones bioquímicas que generalmente es escasa, por lo que se han desarrollado métodos que aprovechan también la información cualitativa utilizada en los modelos discretos, trasladándolos a sistemas dinámicos continuos. Esto permite analizar el comportamiento de las redes en ausencia de parámetros cinéticos detallados (Mendoza and Xenarios, 2006; Wittmann *et al.*, 2009). Además, los modelos continuos permiten estudiar el comportamiento dinámico de una red ante distintos umbrales de activación de sus nodos y estudiar su sensibilidad bajo gradientes de señales externas.

1.5 ANTECEDENTES DE OTROS MODELOS

Se han desarrollado diversos modelos que capturan la dinámica de diferentes procesos biológicos, como la progresión del ciclo celular en levaduras (Davidich and Bornholdt, 2008), la formación de los órganos florales en *Arabidopsis thaliana* (Alvarez-Buylla *et al.*, 2010; Espinosa-Soto *et al.*, 2004), y la diferenciación de los linfocitos T (Martinez-Sanchez *et al.*, 2015; Martínez-Sosa and Mendoza, 2013; Mendoza, 2006; Mendoza and Pardo, 2010).

Además, se han generado modelos que exploran el comportamiento de un proceso biológico tomando en cuenta diferentes escalas de organización para estudiar el efecto de diversos factores ambientales, la interacción entre distintas poblaciones de células, división celular, migración, quimiotaxis, entre otros. Ejemplo de ello es el caso de los modelos basados en agentes, donde el comportamiento de cada elemento del sistema esta determinado por un conjunto de reglas básicas que determinan la manera en que cada agente interacciona con otros en el espacio, dando lugar a la emergencia de comportamientos complejos (Chavali *et al.*, 2008; Krinner *et al.*, 2013). Los modelos antes mencionados han sido útiles para predecir el comportamiento de estos sistemas bajo condiciones que no han sido probadas experimentalmente, ofreciendo la oportunidad de medir el efecto de diferentes perturbaciones, como mutaciones de ganancia o perdida de función, o cambios

en las interacciones de sus componentes, lo que también ha permitido la generación de nuevas hipótesis y contribuido a nuestro entendimiento sobre estos procesos.

Para el caso particular de los linfocitos B se han determinado experimentalmente algunos de los reguladores transcripcionales necesarios para la especificación de múltiples tipos de células B y las señales que dirigen el desarrollo y diferenciación de estas células para la formación de células especializadas. Existe suficiente información tanto a nivel celular como molecular referente de los factores que intervienen en este proceso, sin embargo no existe un consenso acerca de la arquitectura de la red de regulación que controla la diferenciación de los linfocitos B a partir de un precursor troncal a células efectoras especializadas. Más aún, se desconoce la manera en que esta red responde a señales extracelulares, brindadas por los linfocitos T y otras células, para dirigir la formación de los distintos tipos celulares y cómo la desregulación de los componentes de esta red puede llevar a desbalances que repercutan en el correcto establecimiento del proceso de diferenciación necesarios para la respuesta humoral adaptativa.

En el presente proyecto nos planteamos estudiar de manera sistemática el comportamiento dinámico de la red de regulación que controla la diferenciación de los linfocitos B. Se desarrollaron dos modelos computacionales. En primer lugar, se construyó un modelo para estudiar la diferenciación terminal de los linfocitos B para la producción de células efectoras productoras de anticuerpos y en segundo lugar, se infirió y analizó el comportamiento dinámico de la red que controla la formación de los linajes linfoides a partir de sus precursores hematopoyéticos. Estos modelos ayudaron a investigar la manera en que esta red integra distintas señales que dirigen la formación de distintos tipos celulares.

2 HIPÓTESIS

Los patrones de activación que definen los estadios de diferenciación de los linfocitos B emergen del comportamiento dinámico de una red de regulación molecular que incluye factores transcripcionales, moléculas señalizadoras y citocinas. La dinámica de la red será capaz de describir el proceso de diferenciación bajo la presencia de señales extracelulares específicas y diferentes condiciones mutantes.

3 OBJETIVOS

3.1 OBJETIVO GENERAL

Estudiar los mecanismos que sustentan el proceso de diferenciación de los linfocitos B por medio de la reconstrucción y simulación del comportamiento dinámico de redes de regulación.

3.2 OBJETIVOS PARTICULARES

- Inferir la red de regulación que controla la diferenciación de los linfocitos B.
- Identificar los patrones estacionarios de activación de las moléculas de la red por medio de simulaciones de su comportamiento dinámico.
- Estudiar la relevancia funcional de las moléculas de la red por medio de simulaciones de ganancia y pérdida de función de cada uno de sus nodos.
- Estudiar la diferenciación de los linfocitos B a partir de un estadio progenitor simulando la presencia de señales extracelulares que dirigen este proceso y evaluando su efecto sobre el comportamiento dinámico del sistema.
- Validar las simulaciones computacionales por medio de la comparación con la información experimental reportada en la literatura.

- Generar predicciones acerca de posibles interacciones que contribuyan a entender el comportamiento dinámico de la red de regulación y al estudio de la diferenciación de los linfocitos B.

4 METODOLOGÍA

En la presente sección se describen los pasos generales para la construcción de modelos de las redes de regulación y el estudio de su comportamiento desde la perspectiva de los sistemas dinámicos. En la **Figura 4** se presenta un resumen general de la metodología empleada.

La descripción detallada sobre la construcción de las redes de regulación a partir de la información experimental disponible en la literatura y el uso de diferentes aproximaciones para estudiar su comportamiento dinámico se aborda en el **Apéndice I**: *"The SQUAD method for the qualitative modeling of regulatory networks"* que se presenta más adelante en la sección **8.1**.

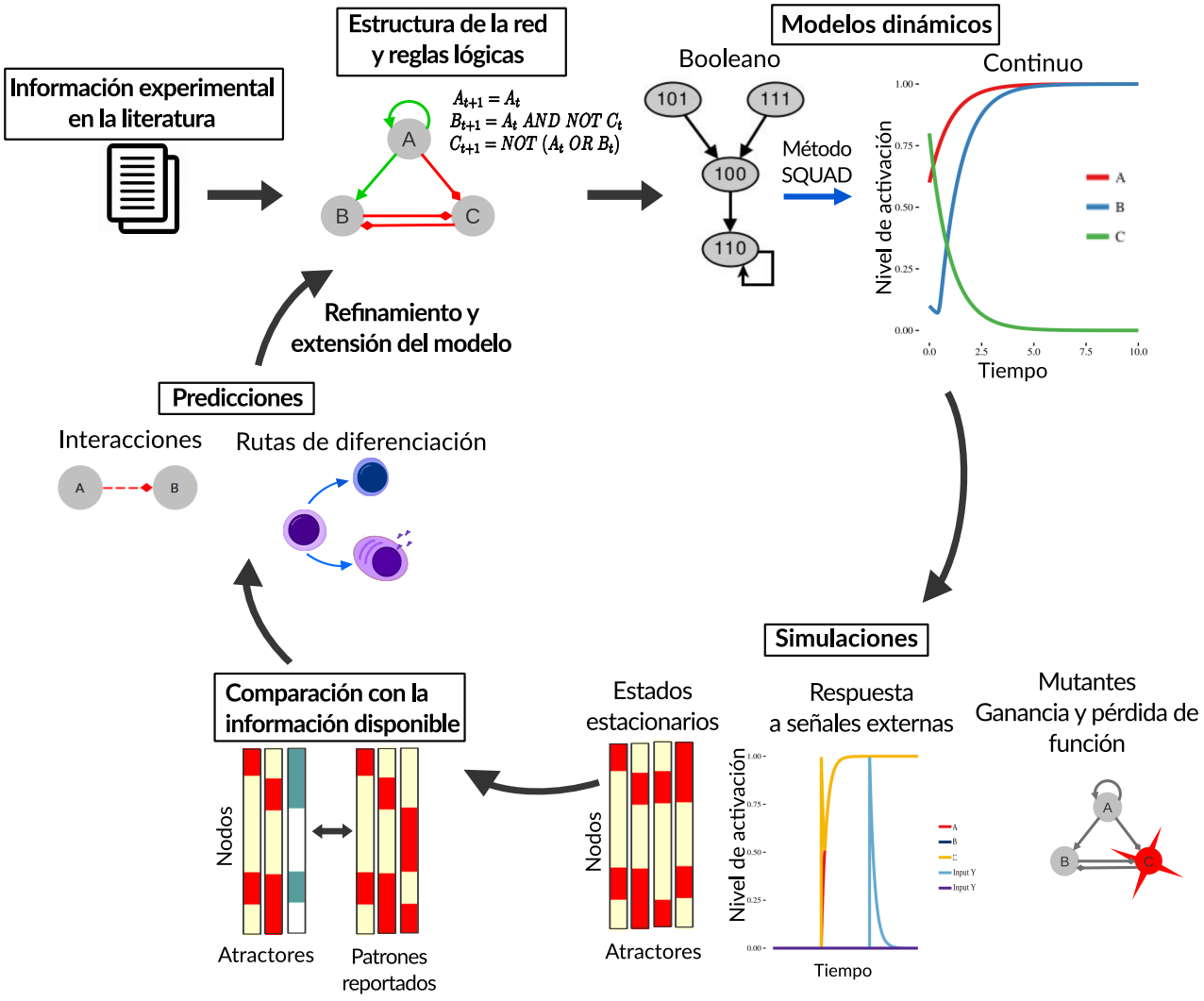

Figura 4: Metodología general. Se muestran los pasos generales para la construcción de los modelos dinámicos de redes de regulación desarrollados en este trabajo.

4.1 RECONSTRUCCIÓN DE REDES DE REGULACIÓN

4.1.1 Inferencia de la red que controla la diferenciación de los linfocitos B

Para entender los mecanismos que controlan la diferenciación de los linfocitos B se determinó la arquitectura de la red de regulación con base en la información experimental

reportada en la literatura referente a las moléculas involucradas en el control de este proceso. La revisión extensa de la literatura nos permitió identificar moléculas involucradas en el establecimiento de estadios críticos de diferenciación de los linfocitos B así como sus patrones estables de expresión y las interacciones entre dichas moléculas.

De especial interés fue la inclusión de factores transcripcionales involucrados en la especificación de los linfocitos B, así como factores que controlan la diferenciación de células plasmáticas, moléculas señalizadoras y citocinas. Los trabajos experimentales considerados para construir modelos de redes de regulación se enfocan en el estudio de la relación física o funcional entre moléculas que son relevantes para la regulación de un proceso biológico de interés. Estos experimentos pueden dividirse en distintos tipos como se describe a continuación:

- Experimentos *in vivo* o *in vitro* que prueben la interacción directa entre moléculas (interacciones proteína-proteína, unión a sitios de regulación de DNA. etc.) en el contexto del proceso biológico de interés.

- Experimentos que prueben cómo la concentración de una molécula (proteína, metabolito, RNA, etc.) cambia como consecuencia de una perturbación o por la modulación en la actividad de moléculas asociadas con dicha molécula.

- Experimentos que describan la relación funcional entre las moléculas de la red (epistasis, mutaciones de ganancia y pérdida de función, regulación transcripcional, etc.).

- Experimentos que prueben el efecto fenotípico en estadios críticos de diferenciación como consecuencia de la adición de un fármaco, factores de crecimiento, citocinas, etc.

Con base en la información experimental recabada, se construyó una tabla de interacciones y se infirió el flujo de información entre las moléculas que conforman a la red, es decir, considerando la naturaleza activadora o inhibidora de las interacciones entre las moléculas se construyó una red de interacciones dirigidas, lo que nos ofreció una idea acerca de la complejidad, arquitectura y circuitos que existen en la red de regulación. Los diagramas de red para este trabajo se construyeron con el software *yEd* (http://www.yworks.com/products/yed).

4.1.2 Planteamiento de reglas lógicas

Una vez inferida la arquitectura de la red de regulación, se propusieron las reglas lógicas necesarias para determinar las condiciones de activación de cada nodo y simular el comportamiento dinámico de la red. Las reglas lógicas representan de una manera formal y concisa los mecanismos moleculares de regulación que controlan la actividad de cada nodo. Para cada nodo se postuló una regla lógica, estas reglas se construyeron utilizando distintas combinaciones de los operadores lógicos *AND*, *OR* y *NOT*.

Por ejemplo, en la **Figura 4** se presenta una pequeña red de regulación donde el nodo *B* (que puede representar un gen, proteína, mRNA, etc.) está controlado de manera positiva por el nodo *A* y es inhibido por *C*. La regulación del nodo *B* puede resumirse de la siguiente manera: *"la molécula B ejerce su función si su regulador positivo A está presente y siempre y cuando su inhibidor C esté ausente"*. A partir de la información anterior puede plantearse una regla lógica de la regulación del nodo *B* expresada en términos de operadores lógicos como se muestra a continuación:

$$B_{t+1} = A_t \; AND \; NOT \; C_t \tag{1}$$

La complejidad las reglas lógicas depende del número y el tipo de reguladores de cada nodo así como de la información experimental disponible. Las reglas lógicas postuladas para los modelos desarrollados en este proyecto se presentan en los **Apéndices II y III**, respectivamente.

4.2 MODELADO DE LA RED COMO UN SISTEMA DINÁMICO

El comportamiento dinámico de la red de regulación se estudió por medio del enfoque de los sistemas dinámicos. Como primer aproximación se construyó un sistema dinámico basado en lógica Booleana, este modelo se utilizó para identificar los estados estacionarios de la red de regulación y para refinar y extender el modelo. En segundo lugar, se tomó el modelo Booleano como punto de partida para la construcción de un modelo dinámico continuo por medio del uso de un sistema de ecuaciones diferenciales ordinarias (ODEs), lo que sirvió para simular la presencia de señales extracelulares con diferentes valores de

activación y evaluar el comportamiento dinámico de la red en un intervalo más amplio de condiciones. A continuación se describen las aproximaciones utilizadas para estudiar el comportamiento dinámico de las redes de regulación.

4.2.1 Construcción de un sistema dinámico discreto

Entre los métodos existentes para analizar la dinámica de las redes el más simple es el de los modelos Booleanos, donde el valor de cada nodo se representa por una variable binaria (que puede tomar dos valores: 0 o 1) que simula la presencia/ausencia de una molécula o su estado activo/inactivo. En los modelos Booleanos el comportamiento de la red cambia de acuerdo con la siguiente ecuación:

$$x_i(t+1) = F_i(x_{i_1}(t), x_{i_2}(t), \ldots, x_{i_n}(t)) \tag{2}$$

Donde el estado de activación de cada nodo x_i en el tiempo $t+1$ depende de una función F_i que determina las condiciones para que un nodo se active en función de su interacción con otros nodos de la red. Esta función se plantea en la forma de reglas lógicas, lo que permite hacer simulaciones para estudiar la acción concertada entre los genes y moléculas que conforman a la red en términos de sus reguladores.

Con base en la arquitectura de la red inferida y de el planteamiento de las reglas lógicas para cada nodo, se analizó el comportamiento del modelo Booleano por medio de simular todas las posibles combinaciones de estados iniciales de activación de los nodos de la red y se identificaron todos los estados estacionarios o atractores a los que el sistema convergió después de varios pasos de tiempo. Los atractores obtenidos se compararon con los patrones de activación reportados experimentalmente referentes a los estadios de diferenciación de los linfocitos B. Los análisis del modelo Booleano se realizaron con el paquete *BoolNet* del lenguaje de programación R (Müssel *et al.*, 2010).

4.2.2 Construcción de un sistema dinámico continuo

La red de regulación se trasladó a la forma de un sistema dinámico continuo por medio del Método Cualitativo Estandarizado de los Sistemas Dinámicos (SQUAD) (Mendoza and Xenarios, 2006; Weinstein and Mendoza, 2012) con las modificaciones incluidas

en (Sánchez-Corrales *et al.*, 2010). Este método permite aprovechar las reglas lógicas planteadas en un sistema Booleano y convertirlo a un sistema de ODEs donde la activación de los nodos de la red puede representarse por variables continuas que pueden tomar valores normalizados de concentración en un intervalo [0,1]. El cambio en la concentración de un nodo x_i de la red está determinado por la acción concertada de un término de activación y otro de decaimiento de la siguiente manera:

$$\frac{dx_i}{dt} = \frac{-e^{0,5h_i} + e^{-h_i(\omega_i - 0,5)}}{(1 - e^{0,5h_i})(1 + e^{-h_i(\omega_i - 0,5)})} - \gamma_i x_i \tag{3}$$

Donde el parámetro ω_i es la forma continua de las reglas lógicas que describen la respuesta del nodo x_i a sus reguladores. Los parámetros h_i y γ_i controlan la ganancia de la función y la tasa de decaimiento, respectivamente.

Las reglas lógicas planteadas por los operadores lógicos *AND*, *OR* y *NOT* se convierten respectivamente a una forma continua por medio de operadores de lógica difusa $min()$, $max()$ y $1 - x$, (Zadeh, 1965). Las reglas lógicas pueden entonces representarse no sólo como verdaderas o falsas, sino que pueden cumplirse en un amplio intervalo de valores.

Por ejemplo, la regla lógica descrita en la **Ecuación 1** puede entonces convertirse en una expresión continua como:

$$B = min(A, 1 - C) \tag{4}$$

Así, la ecuación diferencial que describe la activación del nodo B a través del tiempo en este ejemplo es:

$$\frac{dB}{dt} = \frac{-e^{0,5h_i} + e^{-h_B(min(A,1-C)-0,5)}}{(1 - e^{0,5h_i})(1 + e^{-h_i(min(A,1-C)-0,5)})} - \gamma_i B \tag{5}$$

Para las redes analizadas en este trabajo se propuso una ecuación para cada nodo y se construyó un sistema de ODEs. Este sistema de ecuaciones se resolvió numéricamente por medio del paquete *deSolve* del lenguaje de programación R (Soetaert *et al.*, 2010).

Para identificar los estados estacionarios del sistema, se evaluó el comportamiento a largo plazo de la red a partir de un gran número de estados iniciales elegidos al azar en el intervalo [0,1]. Además, se simuló la presencia o ausencia de señales externas por medio

de fijar el valor del parámetro ω a 0 o 1 para simular la inactivación permanente o la activación constitutiva de los nodos, respectivamente.

4.2.3 Simulación del proceso de diferenciación

Para entender mejor la funcionalidad de la red de regulación y la manera en que diferentes señales extracelulares dirigen el proceso de diferenciación de los linfocitos B, se utilizó el modelo continuo antes mencionado para simular el comportamiento de la red ante la presencia de señales externas, ya que dichas señales se presentan en forma de gradientes que pueden tener distinta duración e intensidad. Para ello, se tomó cada uno de los estados estacionarios de la red obtenidos previamente como estados iniciales para cada simulación. A partir de estos estados se simuló la presencia de un estímulo durante uno o más pasos de tiempo por medio de una señal de alta intensidad (con un valor saturado de 1) de alguna de las señales externas (IL-2, IL-4, IL-21, CD40L, Ag) y se evaluó su efecto sobre la dinámica de la red hasta que el sistema convergiera en un estado estacionario.

Posteriormente, se evaluó el efecto de perturbaciones transitorias para cada uno de los nodos de la red. Dichas perturbaciones se simularon intercambiando el valor de un nodo a la vez y tomando como punto de partida alguno de los estados estacionarios de los linfocitos B previamente identificados.

Por ejemplo, si el nodo Pax5 posee un valor de activación de 1 en el estado Naive, su valor se intercambió de $1 \rightarrow 0$ o de $1 \rightarrow 0.5$ durante uno o más pasos de tiempo y se evaluó en cada caso si dichas perturbaciones ocasionaban la transición de un tipo celular a otro. El sistema se resolvió numéricamente como se explica previamente en el apartado **4.2.2** de esta **Sección**. Esta operación se repitió para cada nodo de la red y para cada atractor. Finalmente, con base en los resultados de las simulaciones se construyó un mapa de destinos entre atractores que representa las posibles transiciones entre destinos celulares.

4.3 VALIDACIÓN COMPUTACIONAL DEL MODELO

4.3.1 Simulación de mutantes de ganancia y pérdida de función

Para validar este modelo se estudió el efecto de todas las posibles mutantes de ganancia y pérdida de función de cada nodo de la red. Este análisis brinda información importante sobre la confiabilidad del modelo, tanto para comparar el modelo con fenotipos reportados experimentalmente como para predecir estados aún no probados o estudiar la contribución de una molécula de la red en el establecimiento de múltiples estados celulares.

Las mutantes de ganancia de función se simularon fijando a un valor constante de 1 el parámetro ω y la activación de un nodo, mientras que las mutantes de pérdida de función se simularon fijando los respectivos valores a 0.

Para cada modelo mutante, se identificaron sus atractores utilizando el mismo método descrito previamente para la red de tipo silvestre, como se explica en el apartado **4.2.2** de esta **Sección**. Los estados de activación obtenidos para cada mutante se compararon con la información experimental reportada en la literatura.

5 RESULTADOS Y DISCUSIÓN

Durante el desarrollo de este proyecto se propusieron diferentes modelos para analizar el comportamiento dinámico de la red de regulación que controla la diferenciación de los linfocitos B. Dichos modelos se enfocaron en el estudio de este proceso desde un enfoque de abajo hacia arriba, es decir, comenzando por un modelo simple e ir extendiendo dicho modelo paso a paso para describir diferentes aspectos de la diferenciación de estas células, por lo que en primer lugar se desarrolló un modelo para estudiar la etapa terminal de la diferenciación de los linfocitos B. Posteriormente, la red de regulación de los linfocitos B se extendió y se integró con modelos de la red de regulación de los linfocitos T para describir la diferenciación de los linfocitos T y B durante el proceso de la linfopoyesis.

A continuación se presentan y discuten los resultados de ambos modelos. Los detalles de cada modelo pueden consultarse en las publicaciones correspondientes que se incluyen en las secciones **8.2** y **8.3** de los **Apéndices**.

5.1 Artículo: A Network Model to Describe the Terminal Differentiation of B Cells

5.1.1 Las interacciones reportadas en la literatura son necesarias mas no suficientes para describir la diferenciación de los linfocitos B

Como primer objetivo de este proyecto nos propusimos determinar si la información experimental reportada en la literatura era suficiente para describir el proceso de diferenciación terminal de los linfocitos B. Para ello, se realizó una revisión exhaustiva de la literatura científica referente a las moléculas que participan en la regulación de este proceso y se infirió una red de regulación formada por un módulo de factores transcripcionales (Bach2, Bcl6, Blimp1, Irf4, Pax5 y XBP1), moléculas señalizadoras (ERK, NF-κB, STAT3, STAT5 y STAT6) y citocinas (IL-2, IL-4, IL-21 y CD40L), como se muestra en la **Tabla 1**. Esta información se integró en un modelo de red con lógica Booleana como se explica en la sección **4.1.1** de la **Metodología**, ver **Figura 5**.

Tabla 1: Tabla de interacciones Referencias que soportan a las interacciones de la red. Se muestran las interacciones entre cada regulador (izquierda) y su molécula blanco (derecha). Las interacciones positivas y negativas se representan por los símbolos $\rightarrow$ y $\dashv$, respectivamente.

Interacción	Referencias	Interacción	Referencias
Ag $\rightarrow$ BCR	(Schmidt *et al.*, 2009)	Bach2 $\dashv$ Blimp1	(Muto *et al.*, 2010; Ochiai *et al.*, 2006, 2008)
Bcl6 $\rightarrow$ Bcl6	Predicha en este trabajo	Bcl6 $\dashv$ Blimp1	(Reljic *et al.*, 2000; Shaffer *et al.*, 2000; Tunyaplin *et al.*, 2004; Vasanwala *et al.*, 2002)
Bcl6 $\dashv$ Irf4	(Alinikula and Lassila, 2011; Basso *et al.*, 2010; Ci *et al.*, 2009; Gupta *et al.*, 1999)	BCR $\rightarrow$ ERK	(Niu *et al.*, 1998; Yasuda *et al.*, 2011, 2012)
Blimp1 $\dashv$ AID	(Shaffer *et al.*, 2002b)	Blimp1 $\dashv$ Bach2	(Muto *et al.*, 2010)
Blimp1 $\dashv$ Bcl6	(Kuo *et al.*, 2007; Sciammas and Davis, 2004; Shaffer *et al.*, 2002b)	Blimp1 $\rightarrow$ Irf4	(Kallies *et al.*, 2004; Sciammas and Davis, 2004)
Blimp1 $\dashv$ Pax5	(Lin *et al.*, 2002; Mora-lópez *et al.*, 2007; Shaffer *et al.*, 2002b)	Blimp1 $\rightarrow$ XBP1	(Klein *et al.*, 2006; Shaffer *et al.*, 2004; Teng *et al.*, 2007)

Tabla de interacciones (Continuación).

Interacción	Referencias	Interacción	Referencias
CD40 → NFκB	(Basso *et al.*, 2004; Saito *et al.*, 2007)	CD40L → CD40	(Saito *et al.*, 2007)
ERK ⊣ Bcl6	(Niu *et al.*, 1998)	ERK → Blimp1	(Yasuda *et al.*, 2011)
ERK ⊣ Pax5	(Yasuda *et al.*, 2012)	IL-2 → IL-2R	(Linterman *et al.*, 2010; Rankin *et al.*, 2011; Zotos *et al.*, 2010)
IL-2R → STAT5	(Scheeren *et al.*, 2005)	IL-4 → IL-4R	(Linterman *et al.*, 2010; Rankin *et al.*, 2011; Zotos *et al.*, 2010)
IL-4R → STAT6	(Dedeoglu, 2004; Scheeren *et al.*, 2005)	IL-21 → IL-21R	(Linterman *et al.*, 2010; Rankin *et al.*, 2011; Zotos *et al.*, 2010)
IL-21R → STAT3	(Kwon *et al.*, 2009; Linterman *et al.*, 2010; Rankin *et al.*, 2011; Zotos *et al.*, 2010)	Irf4 ⊣ Bcl6	(Kuo *et al.*, 2007; Saito *et al.*, 2007)
Irf4 → Blimp1	(Kwon *et al.*, 2009; Sciammas *et al.*, 2006; Shaffer *et al.*, 2008)	Irf4 → Irf4	(Shaffer *et al.*, 2008)

Tabla de interacciones (Continuación).

Interacción	Referencias	Interacción	Referencias
Irf4 ⊣ Pax5	Predicha en este trabajo	NFκB → AID	(Klein *et al.*, 2006; Sciammas *et al.*, 2006; Tran *et al.*, 2010)
NFκB → Irf4	(Saito *et al.*, 2007; Sciammas *et al.*, 2006)	Pax5 → AID	(Gonda *et al.*, 2003; Tran *et al.*, 2010)
Pax5 → Bach2	(Basso *et al.*, 2004; Saito *et al.*, 2007)	Pax5 → Bcl6	Predicha en este trabajo
Pax5 ⊣ Blimp1	(Delogu *et al.*, 2006; Mora-lópez *et al.*, 2007; Nera *et al.*, 2006)	Pax5 → Pax5	Predicha en este trabajo
Pax5 ⊣ XBP1	(M *et al.*, 1996; Todd *et al.*, 2009)	STAT3 → Blimp1	(Diehl *et al.*, 2008; Kwon *et al.*, 2009)
STAT5 → Bcl6	(Scheeren *et al.*, 2005)	STAT6 → AID	(Dedeoglu, 2004; Scheeren *et al.*, 2005)
STAT6 → Bcl6	(Arguni *et al.*, 2006; Scheeren *et al.*, 2005)		

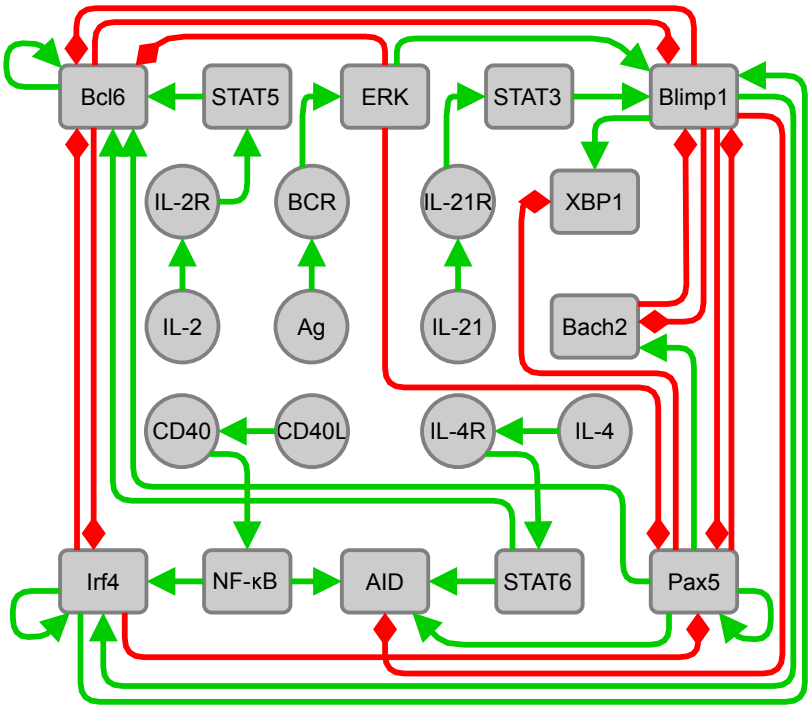

Figura 5: La red de regulación que controla la diferenciación terminal de los linfocitos B. Las interacciones de regulación positiva y negativa se muestran en flechas de color verde y flechas con extremos romos de color rojo respectivamente. La red está compuesta por 22 nodos y 39 interacciones entre ellos, los nodos representan moléculas o complejos moleculares entre los que se incluyen factores transcripcionales: Bach2, Bcl6, Blimp1, Irf4, Pax5 y XBP1, moléculas señalizadoras (STAT3, STAT5, STAT6, NF-κB, ERK), receptores y citocinas (IL-2, IL-4, IL-21, CD40L) así como la regulación mediada por un antígeno (BCR).

El estudio de la primera red inferida con base en interacciones reportadas en la literatura mostró que los datos existentes eran insuficientes para describir las principales etapas del proceso de diferenciación terminal de los linfocitos B (Naive, GC, Mem y PC), ya que los atractores obtenidos para esta red no recuperan el estado correspondiente al progenitor Naive, como se muestra en la **Figura 6A**.

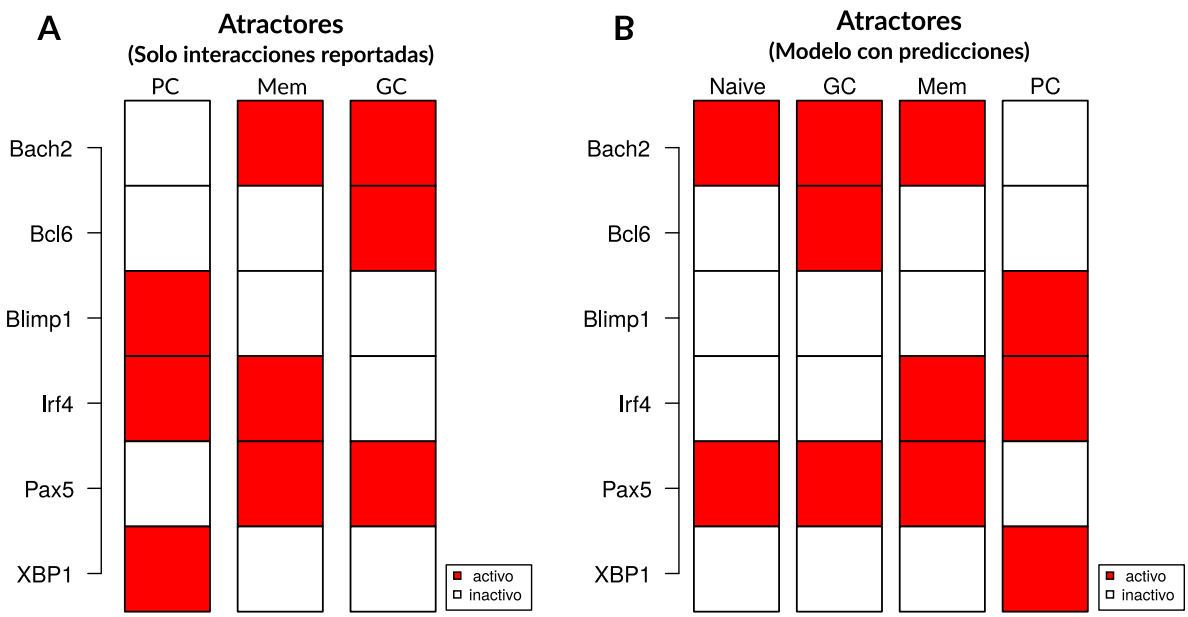

Figura 6: Comparación de patrones de expresión obtenidos para el modelo con y sin predicciones. **(A)** Se muestran los patrones de activación obtenidos para la red construida únicamente con interacciones reportadas en la literatura. **(B)** Atractores obtenidos para el modelo una vez incluidas las interacciones propuestas. El modelo con predicciones recupera un estado de activación adicional correspondiente a los progenitores Naive.

5.1.2 La arquitectura de red propuesta recupera los patrones de activación de tipo silvestre característicos de los linfocitos B

El refinamiento y la extensión del modelo permitió generar algunas predicciones acerca de posibles interacciones necesarias para el funcionamiento de la red. Dichas interacciones constituyen predicciones del modelo y sugieren la existencia de mecanismos directos o indirectos de regulación que son necesarios para mantener la activación sostenida de los factores Pax5 y Bcl6 durante los estadios no diferenciados de los linfocitos B. También se propuso una interacción entre Irf4 y Pax5 como una interacción necesaria para el mantenimiento de la expresión de ambos factores en estadios previos de la diferenciación terminal a células plasmáticas. La descripción detallada acerca de las razones para proponer estas interacciones se presenta en el artículo. La red de regulación que incluye las interacciones

inferidas de la literatura experimental así como las predicciones generadas se presenta en la **Figura 5**.

La inclusión de las interacciones propuestas en el modelo permitió definir con mayor detalle los estados de los progenitores Naive y GC así como los patrones de activación de diferentes mutantes reportadas como se presenta más adelante en el apartado **5.1.4** de esta sección. El comportamiento dinámico de de la red de regulación propuesta recupera los patrones de expresión que caracterizan a los diferentes tipos de células B durante las etapas de diferenciación terminal de tipo silvestre, como se muestra en la **Figura 6B**, así como el comportamiento de diferentes mutantes como se explica más adelante.

5.1.3 El comportamiento dinámico de la red describe el proceso de diferenciación de los linfocitos B ante la presencia de señales específicas

Este modelo fue trasladado a una versión continua como se explica en la sección de **Metodología**, lo que permitió estudiar el proceso de diferenciación terminal de las células B como resultado de la acción concertada de un pequeño módulo de factores transcripcionales que responden ante la presencia de múltiples señales extracelulares.

Al simular la presencia de un estímulo de alta intensidad sobre los progenitores Naive, se promueve la transición de estos al estado de las células GC, que al entrar en contacto con los linfocitos T por medio de simular la presencia del ligando CD40L, se diferencian en células Mem, que a su vez se diferencian terminalmente en células PC al ser estimuladas por un antígeno, que se representa como un pulso de alta intensidad del nodo Ag en las simulaciones, (**Figura 7**).

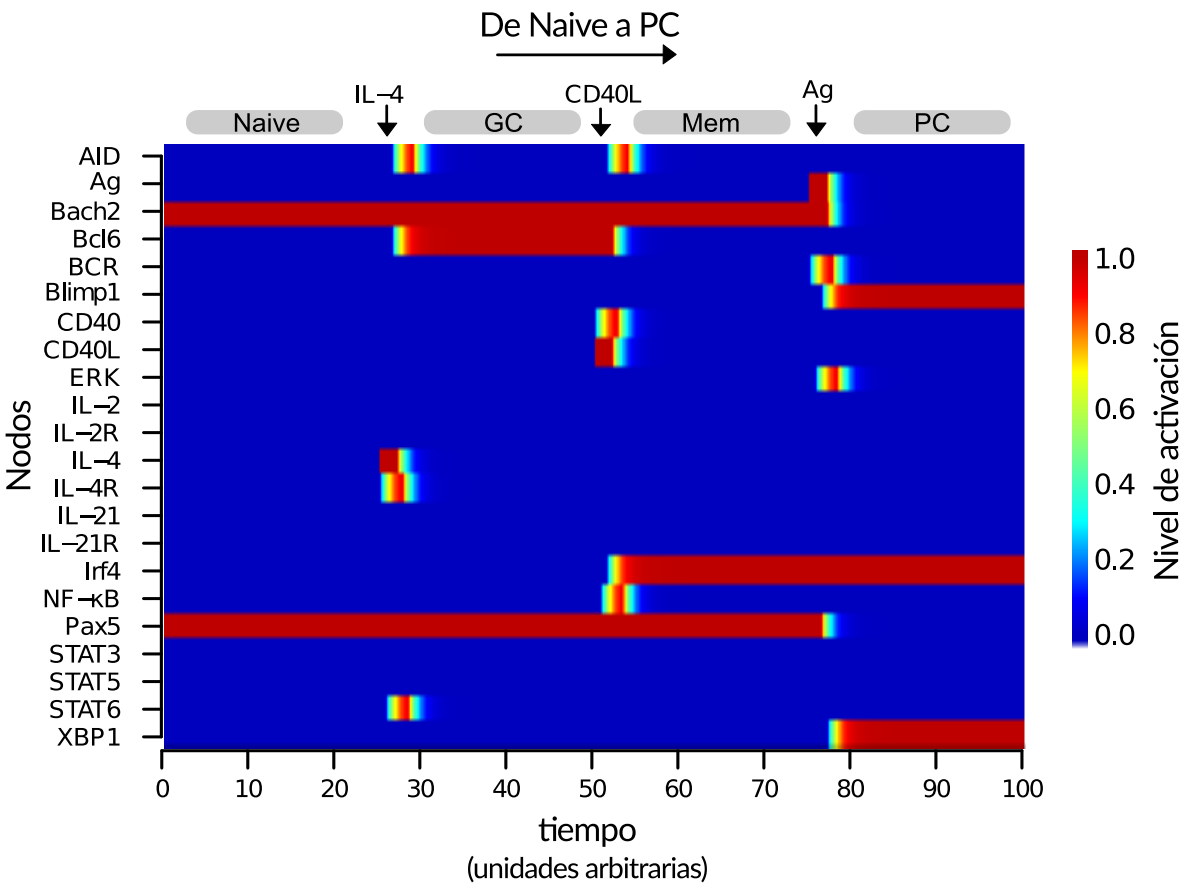

Figura 7: Simulación del proceso de diferenciación terminal de los linfocitos B. Se muestra la diferenciación a partir de un progenitor Naive a células PC terminalmente diferenciadas. Los cambios en la activación de los nodos va de azul a rojo conforme alcanzan un nivel de 0 a 1, respectivamente. La presencia de señales externas (IL-4, CD40L o Ag) se simula por perturbaciones de alta intensidad durante uno o más pasos de tiempo (flechas negras). Comenzando por el estado estacionario Naive (Bach2^+, Pax5^+), el sistema transita a un estado GC (Bach2^+, Bcl6^+, Pax5^+) debido a la presencia de IL-4 ($t \approx 25$) que a su vez transita al estado Mem (Bach2^+, Irf4^+, Pax5^+) por la acción de CD40L ($t \approx 55$) que finalmente transita al estado de células PC (Blimp1^+, Irf4^+) por la presencia de Ag ($t \approx 75$).

Es importante notar, que una vez que una señal transitoria induce la transición de un tipo celular a otro, el sistema permanece en un estado estacionario de manera que el tipo celular se mantiene a pesar de la desaparición de una señal inductiva, lo que siguiere que

la red asegura que la diferenciación hacia uno u otro tipo celular ocurra de manera estable.

Las transiciones entre los estados estacionarios del sistema describen el comportamiento dinámico del proceso de diferenciación terminal de los linfocitos B y predicen rutas alternativas que podrían ocurrir durante este proceso.

Finalmente, se exploró el comportamiento dinámico del proceso de diferenciación por medio de la simulación de perturbaciones transitorias, con las que pudimos confirmar el comportamiento ramificado y dirigido del proceso de diferenciación a partir de un tipo celular progenitor a células terminalmente diferenciadas y pudimos proponer diferentes rutas de diferenciación, extendiendo la visión canónica de cómo se lleva a cabo este proceso, (**Figura 8**).

5.1.4 Las mutantes de ganancia y pérdida de función de los nodos de la red corresponden con algunos de los patrones de expresión para las mutantes reportadas experimentalmente

Para validar el modelo, se compararon los patrones de activación obtenidos de simulaciones computacionales de las mutantes de ganancia y pérdida de función para cada nodo de la red. Los resultados para cada simulación se compararon con los patrones de expresión reportados experimentalmente. Como se indica en la **Figura 9**, la pérdida o ganancia de función de cada nodo resulta en la pérdida o la adquisición de varios atractores con respecto a los patrones obtenidos para el modelo de tipo silvestre.

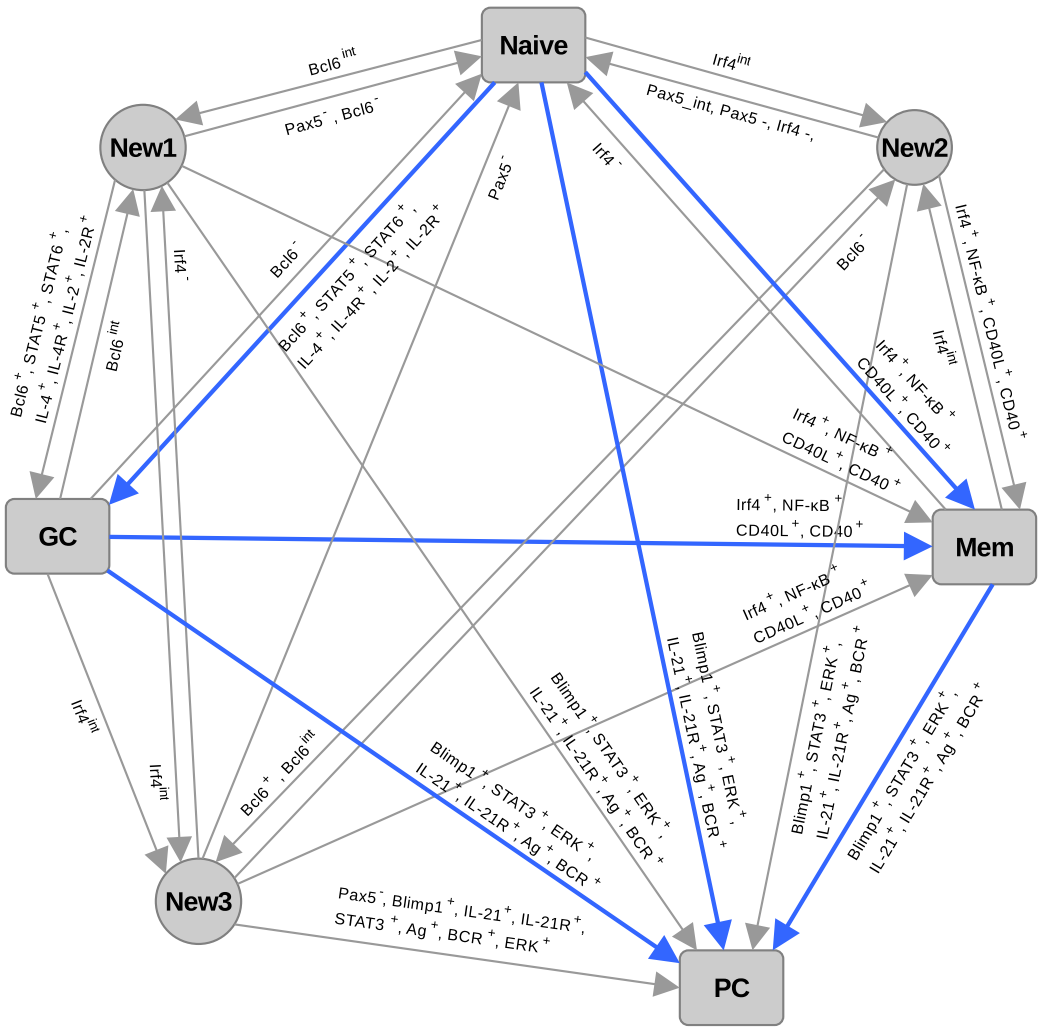

Figura 8: Mapa de posibles rutas de diferenciación de los linfocitos B. Se muestran los destinos celulares entre los que puede transitar el sistema bajo la presencia de distintas perturbaciones transitorias de alta intensidad. En flechas de color azul se indican las rutas de diferenciación terminal canónicas de los linfocitos B, mientras que en flechas grises se indican rutas alternativas de diferenciación. Las señales que llevan de un estado estacionario a otro se indican en las etiquetas de las interacciones con el nodo de la red y los símbolos marcan el tipo de perturbación (+: Activación del nodo, *int*: Valores intermedios, −: Inactivación o ausencia de la señal).

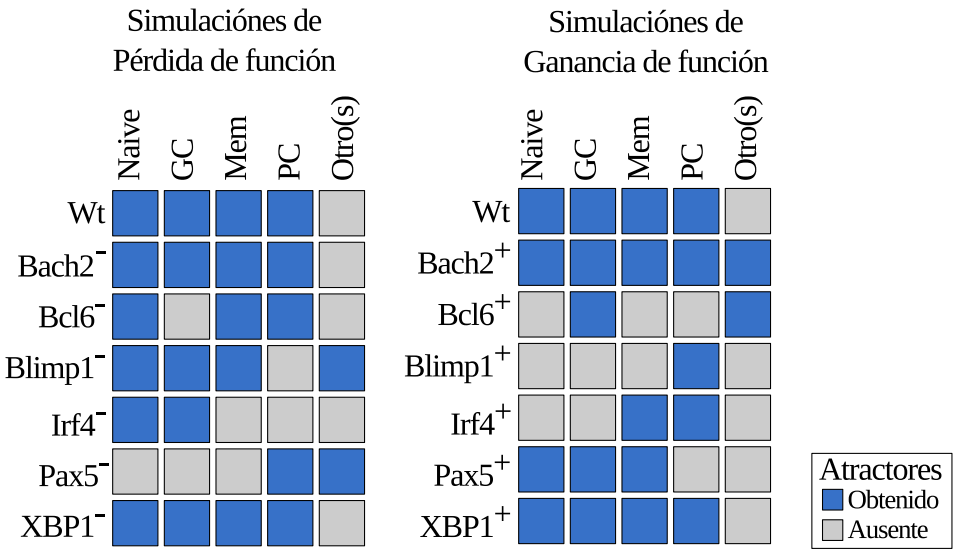

Figura 9: Simulaciones de mutantes de ganancia y pérdida de función. Se muestran los atractores obtenidos para cada uno de los modelos mutantes. Las simulaciones de la deleción o expresión constitutiva de los nodos de la red se comparan con los patrones de activación de la red de tipo silvestre (Wt). Los cuadros en color azul indican que dicho atractor se obtuvo para ambos modelos (Wt y mutante), o bien, se obtuvo uno o más un patrones de activación durante las simulaciones (marcados con la etiqueta .ºtro(s)"), mientras que los cuadros grises indican la ausencia de un atractor.

Los resultados para las simulaciones de los modelos mutantes mostraron que algunos de los nodos de la red tienen un efecto más drástico para el establecimiento de los tipos celulares, ya que la inactivación de dichos factores resulta en la pérdida de estados asociados con diferentes tipos celulares o bien, su activación constitutiva promueve que el sistema se mantenga en uno de los cuatro estados con respecto a los atractores obtenidos para el modelo de tipo silvestre. Este efecto coincide con el comportamiento reportado para el establecimiento de estados críticos de diferenciación en mutantes reportadas en la literatura, como se muestra en la **Tabla 2**.

Tabla 2: Comparación de los resultados de la simulación de modelos mutantes con reportes experimentales.

Nodo	Simulación de Pérdida de función	Simulación de Ganancia de función	Observaciones experimentales	Referencias
Bach2	Se obtienen únicamente atractores similares a los patrones de tipo silvestre.	Pérdida del estado PC. Se encuentra un atractor con niveles altos de Bach2 e Irf4.	Células B deficientes de *Bach2* no llevan a cabo SHM y CSR pero pueden diferenciarse de manera terminal a células PC. La ausencia de *Bach2* en ratón muestra un número reducido de células B maduras. La sobre-expresión de *Bach2* reprime la expresión de *Prdm1* e inhibe la diferenciación a células PC.	(Muto *et al.*, 2004, 2010; Ochiai *et al.*, 2006)
Bcl6	Pérdida del estado GC.	Se alcanza únicamente el atractor GC.	Las formación de células GC se abate en células B y ratones deficientes de *Bcl6*. Células B deficientes en *Bcl6* pueden dar lugar a células Mem. La expresión constitutiva de *Bcl6* en ratón muestra un incremento en la formación de células PC y evita la formación de células PC.	(Baron *et al.*, 2004; Cattoretti *et al.*, 2005; Dent *et al.*, 1997; Fukuda *et al.*, 1997; Pasqualucci *et al.*, 2003; Toyama *et al.*, 2002)
Blimp1	Pérdida del estado PC, se encuentra un estado adicional con altos niveles de Irf4.	El sistema alcanza únicamente el atractor PC. Pérdida de los atractores Naive, GC y Mem.	Las células PC se abaten en ratones con deleción del gen *prdm1*$^{-}$. La deleción condicional de este gen lleva a la pérdida de células PC en condiciones *in vitro* e *in vivo*. La expresión ectópica de *prdm1* en células B maduras promueve la diferenciación a células PC.	(Lin, 1997; Piskurich *et al.*, 2000; Shaffer *et al.*, 2002a; Shapiro-Shelef *et al.*, 2003, 2005; Turner *et al.*, 2010)

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Continuación

Nodo	Simulación de Pérdida de función	Simulación de Ganancia de función	Observaciones experimentales	Referencias
Irf4	Pérdida de los atractores Mem y PC.	Se encuentran sólo los atractores Mem y PC.	La inducción de *Irf4* en células B maduras induce la diferenciación a células PC. La ausencia de *Irf4* evita la formación de células Mem y PC.	(Klein *et al.*, 2006; Mittrucker, 1997; Ochiai *et al.*, 2013; Sciammas *et al.*, 2006; Shaffer *et al.*, 2008; Teng *et al.*, 2007)
Pax5	Se encuentra un atractor de tipo PC y otro caracterizado por la inactivación de todos los factores.	Pérdida del atractor PC.	Ratones deficientes de *Pax5* muestran un bloqueo del desarrollo de células B desde etapas tempranas a células PC. La expresión forzada de Pax5 inhibe la diferenciación terminal a células PC, mientras que la pérdida de Pax5 promueve la diferenciación a células PC.	(Lin *et al.*, 2002; Morrison *et al.*, 1998; Nera *et al.*, 2006; Usui *et al.*, 1997)
XBP1	Se encuentran sólo atractores similares a los de tipo silvestre.	Se encuentran sólo atractores similares a los de tipo silvestre.	La deleción condicional de XBP1 en ratón presenta una disminución de la capacidad secretora de las células B pero no afecta la formación de células Mem o PC. La expresión forzada de XBP1 en células B deficientes de *Prdm1* no restablece la diferenciación a células PC.	(Shaffer *et al.*, 2004; Shapiro-Shelef *et al.*, 2003; Todd *et al.*, 2009)

La comparación de las mutantes simuladas con el efecto fenotípico de las mutantes reportadas experimentalmente brindó información útil para identificar el papel de cada nodo en el establecimiento de tipos celulares específicos de los linfocitos B, ya que el número y tipo de atractores que pueden emerger de la dinámica la red depende únicamente de la función de algunos de los factores del módulo que controla el proceso de diferenciación, como son Bcl6, Blimp1, Irf4 y Pax5, mientras que los factores Bach2 y XBP1 juegan un papel importante mas no necesario para controlar el establecimiento de los diferentes estadios de diferenciación de linfocitos B, lo que concuerda con el efecto reportado para las mutantes de estos factores, ver sección **8.2** de los **Apéndices**.

5.2 Artículo: A dynamical model of the regulatory network controlling lymphopoiesis

El modelo de la red de los linfocitos B descrito en la sección anterior sirvió como parte de una colaboración para ser integrado con otros modelos de redes involucradas en la regulación de la diferenciación de los linfocitos T. Con base en estos modelos y en la incorporación de nuevos reguladores, se construyó un modelo extendido con el objetivo de estudiar las decisiones que dirigen el compromiso de los progenitores linfoides comunes para dar lugar a los linajes de los linfocitos T y B durante la linfopoyesis. Este modelo recupera de manera cualitativa los eventos clave de diferenciación celular de la linfopoyesis así como la manera ramificada y direccional como se lleva a cabo este proceso a partir de un progenitor multipotente hacia por lo menos seis tipos de células terminalmente diferenciadas. Los detalles de este modelo pueden consultarse en el apartado **8.3** de la sección de **Apéndices**.

5.2.1 La red de regulación propuesta describe los patrones de activación característicos de los linajes de linfocitos T y B

Con base en la información disponible en la literatura se reconstruyó la red de regulación de la linfopoyesis. Esta red consiste en 81 nodos y 159 interacciones, de las cuales 144 están soportadas por la información experimental disponible y 15 constituyen predicciones del modelo, (**Figura 10**). La incorporación de dichas interacciones dio como resultado un modelo que recapitula el comportamiento dinámico de la linfopoyesis, tanto a nivel molecular como celular, al describir tanto los patrones estables de activación observados experimentalmente para los diferentes programas de los linfocitos T y B, como también las decisiones ramificadas que dan lugar a la formación de ambos linajes.

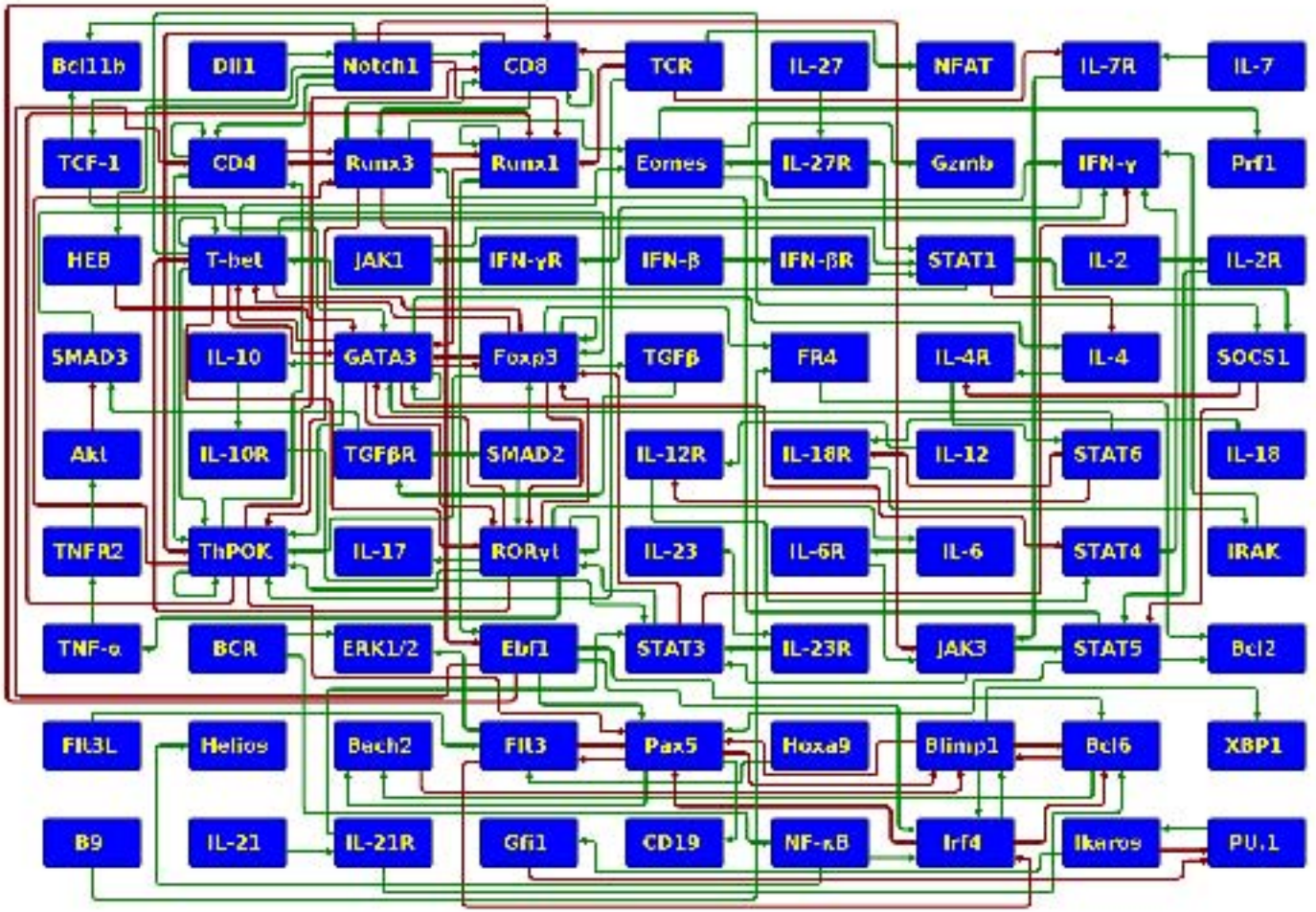

Figura 10: La red de regulación de la Linfopoyesis. Se muestran las moléculas involucradas en la regulación de la linfopoyesis (rectángulos azules) y las interacciones de activación o inhibición entre dichas moléculas (flechas verdes y flechas rojas con extremos romos, respectivamente).

El comportamiento dinámico de la red de regulación de la linfopoyesis se estudió como se explica en la sección **4.2.2** de la **Metodología**. Para ello, se evaluaron los estados estacionarios a los que el sistema convergía tomando como punto de partida 100,000 estados iniciales elegidos al azar. El sistema convergió a 10 atractores, los cuales se identificaron como patrones que corresponden a los tipos celulares DP, $CD4^+$ naive, Th1, Th2, Th17, Treg, $CD8^+$ naive, CTL, B naive, y PC.

Por ejemplo, el estado de las células B naive se identificó por la presencia de CD19, Pax5, Bach2 y la ausencia de Bcl6, Irf4 y Blimp1 (Cobaleda *et al.*, 2007; Muto *et al.*, 2010; Nutt and Tarlinton, 2011). Asimismo, el estado de las células PC se identificó por la resencia de Irf4 y Blimp1, así como por la ausencia de Pax5, Bcl6 y Bach2 (Cobaleda *et al.*, 2007; Igarashi *et al.*, 2007). A su vez, los patrones moleculares de expresión que caracterizan a las células del linaje T como son: DP, $CD4^+$ naive, $CD8^+$ naive, Th1, Th2, Th17, Treg y CTL, se identificaron como se ha definido en modelos previos desarrollados en nuestro grupo (Martínez-Sosa and Mendoza, 2013; Mendoza, 2006, 2013; Mendoza and Pardo, 2010; Mendoza and Xenarios, 2006), ver **Figura 11** para un resumen de los atractores obtenidos en este modelo.

Cabe mencionar que si bien se pudieron identificar 10 atractores a partir de la búsqueda aleatoria, el conjunto de atractores obtenidos carecía de los estados correspondientes tanto al progenitor CLP como al estado de las células pro-B. Puesto que la diferenciación temprana de las células linfoides a partir de las células troncales hematopoyéticas se lleva a cabo en la médula ósea, el siguiente paso fue evaluar si la simulación de señales extracelulares presentes en la médula ósea era suficiente para recuperar estos dos atractores restantes.

De esta manera, se estudió el comportamiento dinámico de la red simulando la presencia continua del ligando Flt3L (con un valor constante de 1 en las simulaciones) a partir de los estados definidos como CLP y pro-B. En ambos casos se encontró que dichos estados eran estacionarios, es decir, por medio de fijar el valor del nodo Flt3L que representa una señal externa producida en el microambiente de la médula ósea, fue posible recuperar dos atractores adicionales; uno que corresponde al progenitor CLP y otro correspondiente a las células pro-B, (**Figura 11**).

Por lo tanto, el modelo sugiere que Flt3L actúa como una señal instructiva requerida

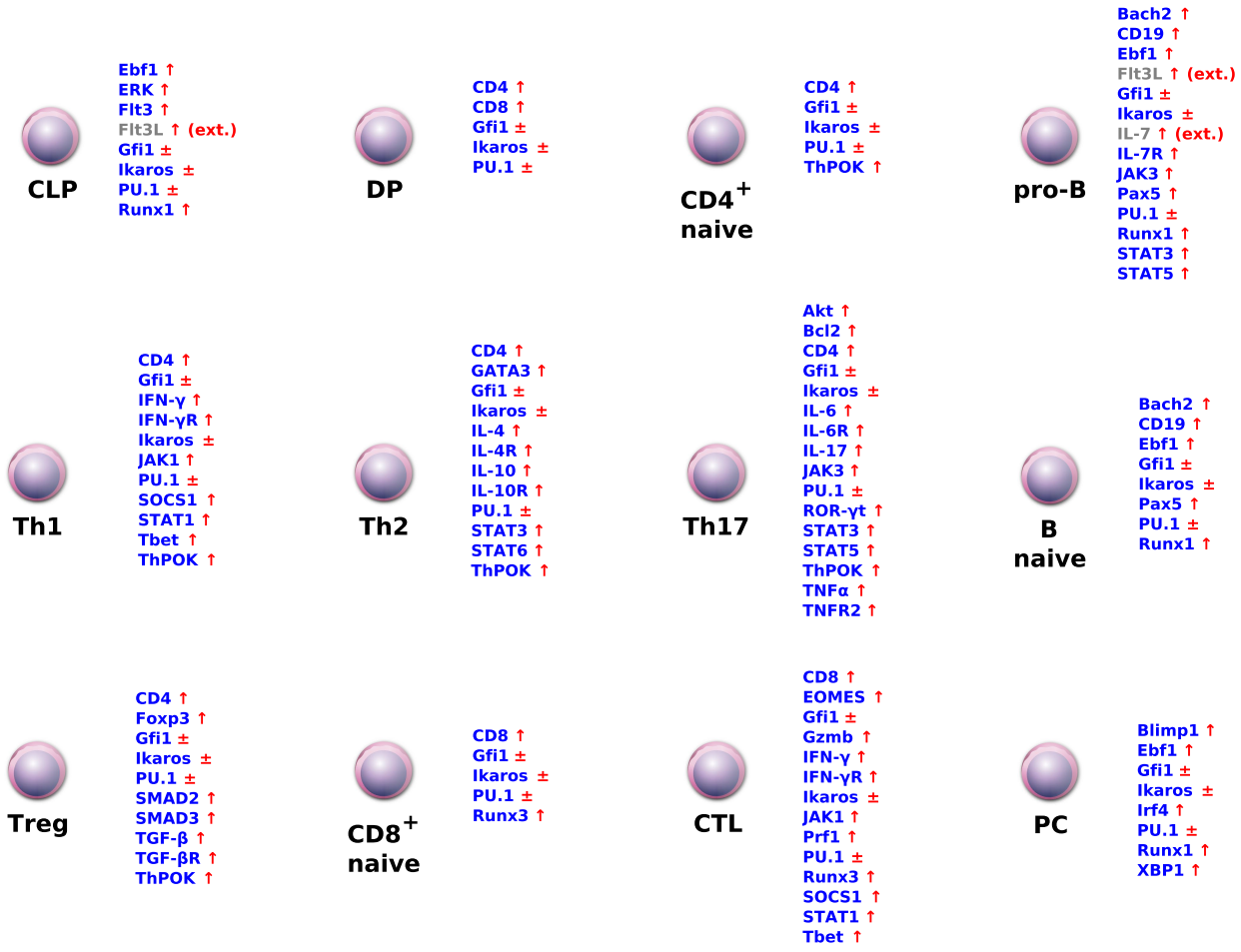

Figura 11: Atractores obtenidos de la red de regulación de la linfopoyesis. Se muestran los patrones de activación de la red de la linfopoyesis interpretados como tipos celulares. Por simplicidad, solo se muestran los nodos con un valor de activación alto (↑) o intermedio (±). Las moléculas Flt3L e IL-7 que representan las señales externas requeridas para la diferenciación de CLPs y células pro-B se muestran en color gris con la etiqueta "*ext.*".

para el mantenimiento de los fenotipos de células CLP y pro-B, lo que concuerda con las observaciones experimentales donde la deficiencia de Flt3L en ratón afecta la producción de células CLP así como de precursores de células T y B (Buza-Vidas *et al.*, 2007;

McKenna *et al.*, 2000; Sitnicka *et al.*, 2002, 2007) .

5.2.2 El comportamiento de la red describe los procesos de diferenciación durante la linfopoyesis

El presente modelo no solo describe los patrones de expresión característicos de las células linfoides, sino que además describe cualitativamente la diferenciación a partir del progenitor multipotente CLP hacia seis tipos celulares maduros: Th1, Th2, Th17, Treg, CTL y células PC.

Como se muestra en la **Figura 12**, para estudiar la diferenciación del progenitor CLP a células PC, se evaluó el comportamiento de la red tomando como punto de partida el estado de células CLP, el cual es estable bajo la presencia de Flt3L. Una vez en este estado, al simular una perturbación transitoria de altos niveles de IL-7 durante 5 unidades de tiempo, el sistema se mueve del tipo celular CLP al estado de células pro-B, lo que concuerda con el comportamiento observado experimentalmente donde IL-7 es requerida para la diferenciación de células pro-B a partir de CLPs así como para la expansión en el estadio pre-pro-B (Miller *et al.*, 2002).

Después, en la simulación es suficiente con eliminar la señal de Flt3L (fijando su valor a 0), para promover la transición hacia el atractor de células B Naive, ver $t \approx 35 - 50$ en la **Figura 12**. La eliminación de la señal de Flt3L puede interpretarse como el proceso mediante el cual los linfocitos B migran fuera de la médula ósea hacia los órganos linfáticos, donde la estimulación del receptor de BCR, simulada como una perturbación de alta intensidad del nodo BCR, es suficiente para mover finalmente el sistema hacia el atractor de células PC. Esta simulación describe el proceso de diferenciación terminal de los linfocitos B donde la estimulación del BCR mediada por el reconocimiento de un antígeno promueve la diferenciación a células plasmáticas (Shapiro-Shelef and Calame, 2005).

Además, el modelo es capaz de describir la diferenciación de CLPs a CTL. Para ello, en las simulaciones se tomó como estado inicial el atractor de CLPs y al eliminar la señal de Flt3L conjuntamente con la adición de una señal saturante de Dll1 (con un valor de activación de 1 del nodo Dll1) por 5 o más pasos de tiempo, el sistema transita hacia el atractor de las células DP ($CD4^+$ $CD8^+$), ver **Figura A.3** en el apartado **8.3** de los

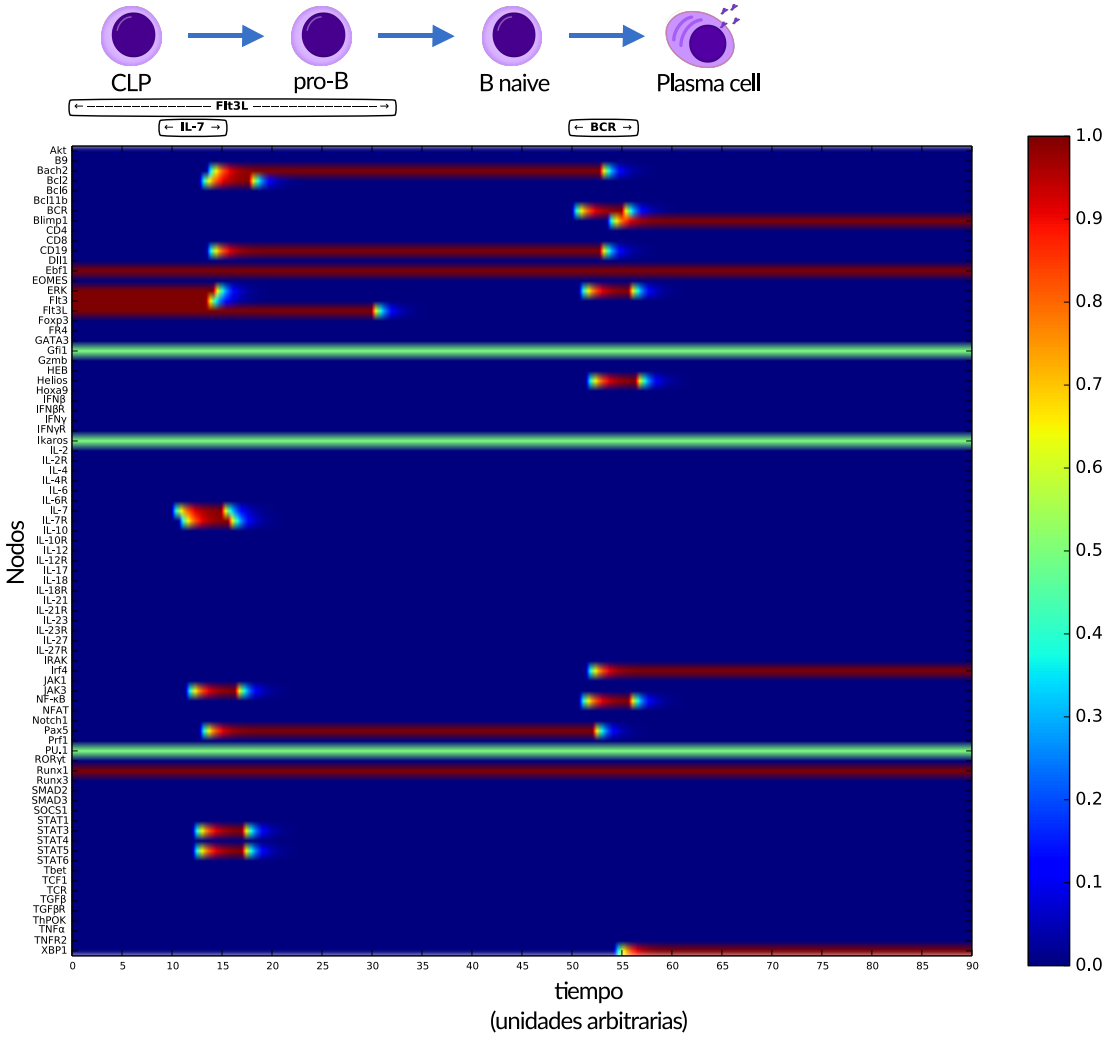

Figura 12: Diferenciación de CLP a célula plasmática. Se muestra el proceso de diferenciación a partir de un progenitor multipotente CLP a células plasmáticas terminalmente diferenciadas. Los valores de activación de los nodos de la red que van de 0 a 1 se muestran como un gradiente de color azul a rojo, respectivamente. Las señales extracelulares, marcadas como rectángulos en la parte superior, se simulan como perturbaciones transitorias y se evalúa la transición de un atractor a otro. Se alcanzan cuatro atractores, que corresponden a los tipos celulares CLP ($t \approx 0-10$), pro-B ($t \approx 25-30$), B Naive ($t \approx 35-50$) y células plasmáticas ($t \approx 65$ en adelante). Adaptada de (Mendoza and Méndez, 2015).

La combinación de estas señales simula el movimiento de los linfocitos fuera de la médula ósea hacia el timo, donde los ligandos de Notch como Dll1, promueven la diferenciación a células DP (Ciofani *et al.*, 2006; Michie *et al.*, 2007). El estado DP es estable, pero una vez que se simula una perturbación como una señal de IL-7 en combinación con una señal intermedia de TCR (simulada con un valor de 0.5) por algunos pasos de tiempo, el sistema transita hacia el estado de células $CD8^+$ naive. Finalmente, la presencia de IL-27 simulada como una señal de alta intensidad, mueve el sistema del estado $CD8^+$ naive hacia el estado CTL.

De igual manera, el modelo describe la diferenciación de las células CLP hacia el linaje de las células T cooperadoras, ya que una vez que el sistema alcanza el estado DP como se explicó anteriormente, la presencia de una señal saturante de TCR mueve el sistema hacia el atractor de las células $CD4^+$ naive. Finalmente, el sistema transita del estado estacionario $CD4^+$ naive hacia los destinos de células Th1, Th2, Th17 o Treg, dependiendo de la simulación de señales como IFN-γ, IL-4, TGF-β+ IL-6 o TGF-β, respectivamente (ver **Figuras A.4-A.7** en la sección **8.3** de **Apéndices**). Estos resultados concuerdan con versiones previas del modelo de la red de diferenciación de los linfocitos T (Martínez-Sosa and Mendoza, 2013; Mendoza, 2013; Mendoza and Pardo, 2010).

En conjunto, los resultados de las simulaciones sugieren que el comportamiento de la red de regulación de la linfopoyesis, al responder ante señales externas específicas, es capaz de describir el proceso de diferenciación de los linfocitos T y B de manera ramificada a partir del progenitor multipotente CLP hacia diferentes progenitores celulares (pro-B, DP, B naive, $CD4^+$ y $CD8^+$ naive) para dar lugar a la formación de seis tipos celulares maduros, (**Figura 13**).

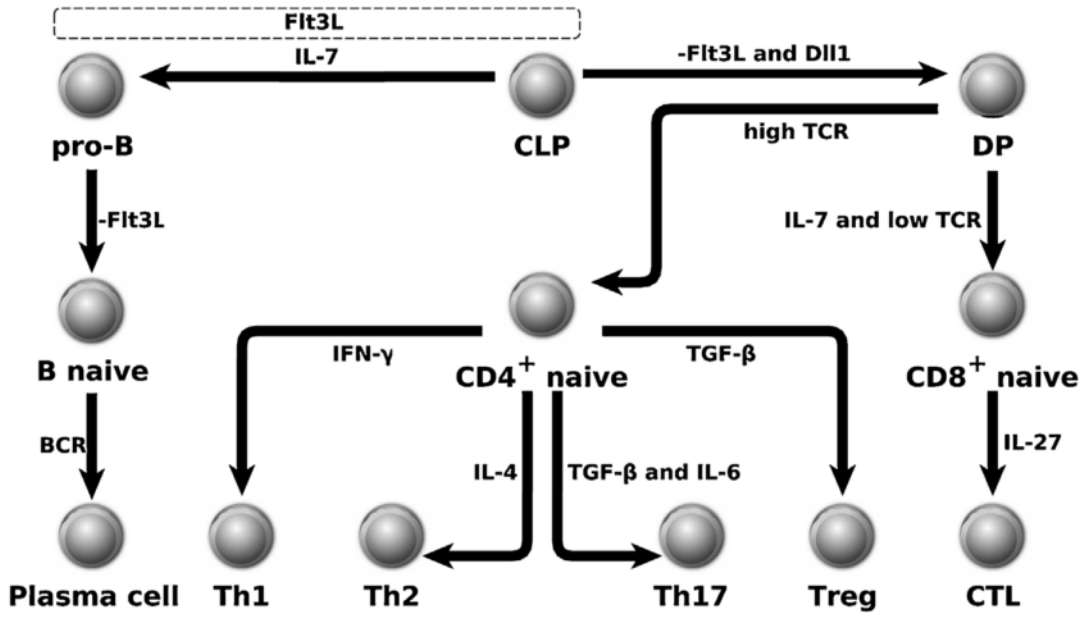

Figura 13: Simulaciones de la diferenciación linfoide. Se muestran las transiciones entre los diferentes estados estacionarios que emergen del comportamiento dinámico de la red. Dichos atractores son interpretados como tipos celulares. La presencia de señales específicas (etiquetas sobre las flechas) promueve la transición de un estado estacionario a otro, recapitulando los procesos de diferenciación celular que ocurren durante la linfopoyesis.

6 CONCLUSIONES

A partir de los resultados obtenidos durante el desarrollo de este trabajo se pudieron derivar las siguientes conclusiones:

Los modelos desarrollados describen de manera cualitativa los patrones de activación característicos de los linfocitos B tanto en condiciones de tipo silvestre como para un amplio conjunto de mutantes de ganancia y pérdida de función de los factores que componen a la red.

El comportamiento dinámico de estos modelos recapitula eventos clave de diferenciación celular descritos experimentalmente y predice rutas alternativas para la formación de células maduras terminalmente diferenciadas a partir de progenitores celulares hematopo-

yéticos.

La red de regulación de los linfocitos B asegura que la diferenciación terminal se lleve a cabo de manera flexible pero dirigida de células progenitoras a células plasmáticas.

Para guiar la transición de las células B Naive a células GC es necesaria la acción de circuitos de retroalimentación positivos que controlen la activación sostenida de los factores Bcl6 y Pax5.

La interacción entre los factores Irf4 y Pax5 es necesaria para promover la formación de células Mem y facilitar el establecimiento del programa de células PC en etapas previas a la diferenciación terminal.

La red de regulación de la linfopoyesis recapitula la manera dirigida y ramificada de la diferenciación a partir de un progenitor linfoide común a células maduras pertenecientes a los linajes de linfocitos T y B.

El modelo de red de la linfopoyesis predice interacciones necesarias para controlar la exclusión mutua entre los programas de diferenciación de los linfocitos T y B, así como para inhibir dichos programas durante las etapas tempranas del desarrollo linfoide.

Es necesaria la acción de un mecanismo de regulación homeostático para mantener niveles constantes de activación entre los factores PU.1, Gfi1 e Ikaros durante la formación de los linajes linfoides.

7 REFERENCIAS

Abou-Jaoudé, W., Thieffry, D., and Feret, J. (2016). Formal Derivation of Qualitative Dynamical Models from Biochemical Networks. *Biosystems*.

Albert, R. (2007). Network inference, analysis, and modeling in systems biology. *Plant Cell*, **19**(11), 3327–38.

Alinikula, J. and Lassila, O. (2011). Gene interaction network regulates plasma cell differentiation. *Scand. J. Immunol.*, **73**(6), 512–9.

Alvarez-Buylla, E. R., Azpeitia, E., Barrio, R., Benítez, M., and Padilla-Longoria, P. (2010). From ABC genes to regulatory networks, epigenetic landscapes and flower morphogenesis: making biological sense of theoretical approaches. *Semin. Cell Dev. Biol.*, **21**(1), 108–17.

Arguni, E., Arima, M., Tsuruoka, N., Sakamoto, A., Hatano, M., and Tokuhisa, T. (2006). JunD/AP-1 and STAT3 are the major enhancer molecules for high Bcl6 expression in germinal center B cells. *Int. Immunol.*, **18**(7), 1079–89.

Baron, B. W., Anastasi, J., Montag, A., Huo, D., Baron, R. M., Karrison, T., Thirman, M. J., Subudhi, S. K., Chin, R. K., Felsher, D. W., Fu, Y.-X., McKeithan, T. W., and Baron, J. M. (2004). The human BCL6 transgene promotes the development of lymphomas in the mouse. *Proc. Natl. Acad. Sci. U. S. A.*, **101**(39), 14198–203.

Basso, K. and Dalla-favera, R. (2015). Germinal centres and B cell lymphomagenesis. *Nat. Rev. Immunol.*, **15**(3), 172–184.

Basso, K., Klein, U., Niu, H., Stolovitzky, G. a., Tu, Y., Califano, A., Cattoretti, G., and Dalla-Favera, R. (2004). Tracking CD40 signaling during germinal center development. *Blood*, **104**(13), 4088–96.

Basso, K., Saito, M., Sumazin, P., Margolin, A. a., Wang, K., Lim, W.-k., Kitagawa, Y., Schneider, C., Alvarez, M. J., Califano, A., and Dalla-Favera, R. (2010). Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. *Blood*, **115**(5), 975–84.

Basso, K., Schneider, C., Shen, Q., Holmes, A. B., Setty, M., Leslie, C., and Dalla-Favera, R. (2012). BCL6 positively regulates AID and germinal center gene expression via repression of miR-155. *J. Exp. Med.*

Boller, S. and Grosschedl, R. (2014). The regulatory network of B-cell differentiation: a focused view of early B-cell factor 1 function. *Immunol. Rev.*, **261**(1), 102–115.

Bornholdt, S. (2008). Boolean network models of cellular regulation: prospects and limitations. *J. R. Soc. Interface*, **5 Suppl 1**(May), S85–94.

Buza-Vidas, N., Cheng, M., Duarte, S., Nozad, H., Jacobsen, S. E. W., and Sitnicka, E. (2007). Crucial role of flt3 ligand in immune reconstitution after bone marrow transplantation and high-dose chemotherapy. *Blood*, **110**(1), 424–432.

Carotta, S., Holmes, M. L., Pridans, C., and Nutt, S. L. (2006). Pax5 maintains cellular identity by repressing gene expression throughout B cell differentiation. *Cell Cycle*, **5**(21), 2452–2456.

Cattoretti, G., Pasqualucci, L., Ballon, G., Tam, W., Nandula, S. V., Shen, Q., Mo, T., Murty, V. V., and Dalla-Favera, R. (2005). Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. *Cancer Cell*, **7**(5), 445–55.

Chavali, A. K., Gianchandani, E. P., Tung, K. S., Lawrence, M. B., Peirce, S. M., and Papin, J. a. (2008). Characterizing emergent properties of immunological systems with multi-cellular rule-based computational modeling. *Trends Immunol.*, **29**(12), 589–99.

Ci, W., Polo, J. M., Cerchietti, L., Shaknovich, R., Wang, L., Yang, S. N., Ye, K., Farinha, P., Horsman, D. E., Gascoyne, R. D., Elemento, O., and Melnick, A. (2009). The BCL6 transcriptional program features repression of multiple oncogenes in primary B cells and is deregulated in DLBCL. *Blood*, **113**(22), 5536–48.

Ciofani, M., Knowles, G. C., Wiest, D. L., von Boehmer, H., and Zúñiga-Pflücker, J. C. (2006). Stage-specific and differential notch dependency at the $\alpha\beta$ and $\gamma\delta$ t lineage bifurcation. *Immunity*, **25**(1), 105–116.

Cobaleda, C., Jochum, W., and Busslinger, M. (2007). Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. *Nature*, **449**(7161), 473–7.

Dahl, R., Iyer, S. R., Owens, K. S., Cuylear, D. D., and Simon, M. C. (2007). The transcriptional repressor GFI-1 antagonizes PU.1 activity through protein-protein interaction. *J. Biol. Chem.*, **282**(9), 6473–6483.

Davidich, M. and Bornholdt, S. (2008). The transition from differential equations to Boolean networks: a case study in simplifying a regulatory network model. *J. Theor. Biol.*, **255**(3), 269–77.

de Jong, H. and Liebert, M. A. (2002). Modeling and simulation of genetic regulatory systems: a literature review. *J. Comput. Biol.*, **9**(1), 67–103.

Dedeoglu, F. (2004). Induction of activation-induced cytidine deaminase gene expression by IL-4 and CD40 ligation is dependent on STAT6 and NF B. *Int. Immunol.*, **16**(3), 395–404.

Delogu, A., Schebesta, A., Sun, Q., Aschenbrenner, K., Perlot, T., and Busslinger, M. (2006). Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. *Immunity*, **24**(3), 269–81.

Dent, a. L., Shaffer, A. L., Yu, X., Allman, D., and Staudt, L. M. (1997). Control of inflammation, cytokine expression, and germinal center formation by BCL-6. *Science*, **276**(5312), 589–92.

Diehl, S. A., Schmidlin, H., Nagasawa, M., van Haren, S. D., Kwakkenbos, M. J., Yasuda, E., Beaumont, T., Scheeren, F. A., and Spits, H. (2008). STAT3-mediated up-regulation of BLIMP1 Is coordinated with BCL6 down-regulation to control human plasma cell differentiation. *J. Immunol.*, **180**(7), 4805–15.

Espinosa-Soto, C., Espinosa-Soto, C., Padilla-longoria, P., Padilla-longoria, P., Alvarez-Buylla, E. R., and 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 Pro les. *Society*, **16**(November), 2923–2939.

Fukuda, T., Yoshida, T., Okada, S., Hatano, M., Miki, T., Ishibashi, K., Okabe, S., Koseki, H., Hirosawa, S., Taniguchi, M., Miyasaka, N., and Tokuhisa, T. (1997). Disruption of the Bcl6 gene results in an impaired germinal center formation. *J. Exp. Med.*, **186**(3), 439–48.

Furusawa, C. and Kaneko, K. (2012). A dynamical-systems view of stem cell biology. *Science*, **338**(6104), 215–7.

Gavin, M. A., Rasmussen, J. P., Fontenot, J. D., Vasta, V., Manganiello, V. C., Beavo, J. A., and Rudensky, A. Y. (2007). Foxp3-dependent programme of regulatory t-cell differentiation. *Nature*, **445**(7129), 771–775.

Gonda, H., Sugai, M., Nambu, Y., Katakai, T., Agata, Y., Mori, K. J., Yokota, Y., and Shimizu, A. (2003). The balance between Pax5 and Id2 activities is the key to AID gene expression. *J. Exp. Med.*, **198**(9), 1427–37.

Graf, T. and Enver, T. (2009). Forcing cells to change lineages. *Nature*, **462**(7273), 587–94.

Grosschedl, R. (2014). Establishment and Maintenance of B Cell Identity. *Cold Spring Harb. Symp. Quant. Biol.*, **LXXVIII**, sqb.2013.78.020057–.

Guo, H., Ma, O., Speck, N. a., and Friedman, A. D. (2012). Runx1 deletion or dominant inhibition reduces Cebpa transcription via conserved promoter and distal enhancer sites to favor monopoiesis over granulopoiesis. *Blood*, **119**(19), 4408–4418.

Gupta, S., Jiang, M., Anthony, A., and Pernis, A. B. (1999). Lineage-specific modulation of interleukin 4 signaling by interferon regulatory factor 4. *J. Exp. Med.*, **190**(12), 1837–1848.

Huang, S., Eichler, G., Bar-Yam, Y., and Ingber, D. E. (2005). Cell fates as high-dimensional attractor states of a complex gene regulatory network. *Phys. Rev. Lett.*, **94**(12), 1–4.

Ichii, M., Shimazu, T., Welner, R. S., Garrett, K. P., Zhang, Q., Esplin, B. L., and Kincade, P. W. (2010). Functional diversity of stem and progenitor cells with B-lymphopoietic potential. *Immunol. Rev.*, **237**(1), 10–21.

Igarashi, K., Ochiai, K., and Muto, A. (2007). Architecture and dynamics of the transcription factor network that regulates B-to-plasma cell differentiation. *J. Biochem.*, **141**(6), 783–9.

Igarashi, K., Ochiai, K., Itoh-Nakadai, A., and Muto, A. (2014). Orchestration of plasma cell differentiation by Bach2 and its gene regulatory network. *Immunol. Rev.*, **261**(1), 116–25.

Inlay, M. A., Bhattacharya, D., Sahoo, D., Serwold, T., Seita, J., Karsunky, H., Plevritis, S. K., Dill, D. L., and Weissman, I. L. (2009). Ly6d marks the earliest stage of B-cell specification and identifies the branchpoint between B-cell and T-cell development. *Genes Dev.*, **23**(20), 2376–2381.

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., Cua, D. J., and Littman, D. R. (2006). The Orphan Nuclear Receptor RORγt Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. *Cell*, **126**(6), 1121–1133.

Johnson, K., Shapiro-Shelef, M., Tunyaplin, C., and Calame, K. (2005). Regulatory events in early and late B-cell differentiation. *Mol. Immunol.*, **42**(7), 749–61.

Kallies, A., Hasbold, J., Tarlinton, D. M., Dietrich, W., Corcoran, L. M., Hodgkin, P. D., and Nutt, S. L. (2004). Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. *J. Exp. Med.*, **200**(8), 967–77.

Karlebach, G. and Shamir, R. (2008). Modelling and analysis of gene regulatory networks. *Nat. Rev. Mol. Cell Biol.*, **9**(10), 770–80.

Klein, U., Casola, S., Cattoretti, G., Shen, Q., Lia, M., Mo, T., Ludwig, T., Rajewsky, K., and Dalla-Favera, R. (2006). Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. *Nat. Immunol.*, **7**(7), 773–82.

Kondo, M., Wagers, A. J., Manz, M. G., Prohaska, S. S., Scherer, D. C., Beilhack, G. F., Shizuru, J. a., and Weissman, I. L. (2003). B <scp>IOLOGY OF</scp> H <scp>EMATOPOIETIC</scp> S <scp>TEM</scp> C <scp>ELLS AND</scp> P <scp>ROGENITORS</scp> : Implications for Clinical Application. *Annu. Rev. Immunol.*, **21**(1), 759–806.

Krinner, A., Roeder, I., Loeffler, M., and Scholz, M. (2013). Merging concepts - coupling an agent-based model of hematopoietic stem cells with an ODE model of granulopoiesis. *BMC Syst. Biol.*, **7**, 117.

Kuo, T. C., Shaffer, A. L., Haddad, J., Choi, Y. S., Staudt, L. M., and Calame, K. (2007). Repression of BCL-6 is required for the formation of human memory B cells in vitro. *J. Exp. Med.*, **204**(4), 819–30.

Kwon, H., Thierry-Mieg, D., Thierry-Mieg, J., Kim, H.-P., Oh, J., Tunyaplin, C., Carotta, S., Donovan, C. E., Goldman, M. L., Tailor, P., Ozato, K., Levy, D. E., Nutt, S. L., Calame, K., and Leonard, W. J. (2009). Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors. *Immunity*, **31**(6), 941–52.

Le Novère, N. (2015). Quantitative and logic modelling of molecular and gene networks. *Nat. Rev. Genet.*, **16**(3), 146–158.

LeBien, T. W. and Tedder, T. F. (2008). B lymphocytes: how they develop and function. *Blood*, **112**(5), 1570–80.

Lin, K.-I., Angelin-Duclos, C., Kuo, T. C., and Calame, K. (2002). Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. *Mol. Cell. Biol.*, **22**(13), 4771–80.

Lin, Y. (1997). Repression of c-myc Transcription by Blimp-1, an Inducer of Terminal B Cell Differentiation. *Science (80-.).*, **276**(5312), 596–599.

Linterman, M. a., Beaton, L., Yu, D., Ramiscal, R. R., Srivastava, M., Hogan, J. J., Verma, N. K., Smyth, M. J., Rigby, R. J., and Vinuesa, C. G. (2010). IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J. Exp. Med.*, **207**(2), 353–63.

M, B. A., Ponath, P. D., Lift, Y.-s., Hardy, R. R., David, C. S., Strominger, I. I. J. L., Glimcher, L. H., Reimold, a. M., Li, Y. S., and Strominger, J. L. (1996). Transcription factor B cell lineage-specific activator protein regulates the gene for human X-box binding protein 1. *J. Exp. Med.*, **183**(2), 393–401.

Martinez-Sanchez, M. E., Mendoza, L., Villarreal, C., and Alvarez-Buylla, E. R. (2015). A Minimal Regulatory Network of Extrinsic and Intrinsic Factors Recovers Observed Patterns of CD4+ T Cell Differentiation and Plasticity. *PLOS Comput. Biol.*, **11**(6), e1004324.

Martínez-Sosa, P. and Mendoza, L. (2013). The regulatory network that controls the differentiation of T lymphocytes. *BioSystems*, **113**(2), 96–103.

McHeyzer-Williams, L. J., Malherbe, L. P., and McHeyzer-Williams, M. G. (2006). Checkpoints in memory B-cell evolution. *Immunol. Rev.*, **211**, 255–68.

McKenna, H. J., Stocking, K. L., Miller, R. E., Brasel, K., De Smedt, T., Maraskovsky, E., Maliszewski, C. R., Lynch, D. H., Smith, J., Pulendran, B., *et al.* (2000). Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood*, **95**(11), 3489–3497.

Medina, K. L., Pongubala, J. M. R., Reddy, K. L., Lancki, D. W., Dekoter, R., Kieslinger, M., Grosschedl, R., and Singh, H. (2004). Assembling a gene regulatory network for specification of the B cell fate. *Dev. Cell*, **7**(4), 607–17.

Mendoza, L. (2006). A network model for the control of the differentiation process in Th cells. *Biosystems.*, **84**(2), 101–14.

Mendoza, L. (2013). A Virtual Culture of CD4+ T Lymphocytes. *Bull. Math. Biol.*

Mendoza, L. and Méndez, A. (2015). A dynamical model of the regulatory network controlling lymphopoiesis. *BioSystems*, **137**, 26–33.

Mendoza, L. and Pardo, F. (2010). A robust model to describe the differentiation of T-helper cells. *Theory Biosci.*, **129**, 283–293.

Mendoza, L. and Xenarios, I. (2006). A method for the generation of standardized qualitative dynamical systems of regulatory networks. *Theor. Biol. Med. Model.*, **3**, 13.

Michie, A. M., Chan, A. C., Ciofani, M., Carleton, M., Lefebvre, J. M., He, Y., Allman, D. M., Wiest, D. L., Zúñiga-Pflücker, J. C., and Izon, D. J. (2007). Constitutive notch signalling promotes cd4- cd8- thymocyte differentiation in the absence of the pre-tcr complex, by mimicking pre-tcr signals. *International immunology*, **19**(12), 1421–1430.

Miller, J. P., Izon, D., DeMuth, W., Gerstein, R., Bhandoola, A., and Allman, D. (2002). The earliest step in b lineage differentiation from common lymphoid progenitors is critically dependent upon interleukin 7. *Journal of Experimental Medicine*, **196**(5), 705–711.

Mittrucker, H.-W. (1997). Requirement for the Transcription Factor LSIRF/IRF4 for Mature B and T Lymphocyte Function. *Science (80-.).*, **275**(5299), 540–543.

Mora-lópez, F., Reales, E., Brieva, J. A., Campos-Caro, A., Dc, W., Mora-lo, F., and Brieva, A. (2007). Human BSAP and BLIMP1 conform an autoregulatory feedback loop. *Blood*, **110**(9), 3150–7.

Morishima, N., Mizoguchi, I., Okumura, M., Chiba, Y., Xu, M., Shimizu, M., Matsui, M., Mizuguchi, J., and Yoshimoto, T. (2010). A pivotal role for interleukin-27 in CD8+ T cell functions and generation of cytotoxic T lymphocytes. *J. Biomed. Biotechnol.*, **2010**, 605483.

Morrison, a. M., Nutt, S. L., Thévenin, C., Rolink, a., and Busslinger, M. (1998). Loss- and gain-of-function mutations reveal an important role of BSAP (Pax-5) at the start and end of B cell differentiation. *Semin. Immunol.*, **10**(2), 133–42.

Murphy, K. M. and Reiner, S. L. (2002). The lineage decisions of helper T cells. *Nat. Rev. Immunol.*, **2**(12), 933–944.

Müssel, C., Hopfensitz, M., and Kestler, H. a. (2010). BoolNet - an R package for generation, reconstruction, and analysis of Boolean networks. *Bioinformatics*, **26**(10), 1378–1380.

Muto, A., Tashiro, S., Nakajima, O., Hoshino, H., Takahashi, S., Sakoda, E., Ikebe, D., Yamamoto, M., and Igarashi, K. (2004). The transcriptional programme of antibody class switching involves the repressor Bach2. *Nature*, **429**(6991), 566–71.

Muto, A., Ochiai, K., Kimura, Y., Itoh-Nakadai, A., Calame, K. L., Ikebe, D., Tashiro, S., and Igarashi, K. (2010). Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch. *EMBO J.*, **29**(23), 4048–61.

Naito, T., Tanaka, H., Naoe, Y., and Taniuchi, I. (2011). Transcriptional control of T-cell development. *Int. Immunol.*, **23**(11), 661–668.

Nera, K.-P. and Lassila, O. (2006). Pax5–a critical inhibitor of plasma cell fate. *Scand. J. Immunol.*, **64**(3), 190–9.

Nera, K.-P., Kohonen, P., Narvi, E., Peippo, A., Mustonen, L., Terho, P., Koskela, K., Buerstedde, J.-M., and Lassila, O. (2006). Loss of Pax5 promotes plasma cell differentiation. *Immunity*, **24**(3), 283–93.

Niu, H., Ye, B. H., and Dalla-Favera, R. (1998). Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. *Genes Dev.*, **12**(13), 1953–61.

Nutt, S. L. and Kee, B. L. (2007). The transcriptional regulation of B cell lineage commitment. *Immunity*, **26**(6), 715–25.

Nutt, S. L. and Tarlinton, D. M. (2011). Germinal center B and follicular helper T cells: siblings, cousins or just good friends? *Nat. Immunol.*, **131**(6), 472–477.

Ochiai, K., Katoh, Y., Ikura, T., Hoshikawa, Y., Noda, T., Karasuyama, H., Tashiro, S., Muto, A., and Igarashi, K. (2006). Plasmacytic transcription factor Blimp-1 is repressed by Bach2 in B cells. *J. Biol. Chem.*, **281**(50), 38226–34.

Ochiai, K., Muto, A., Tanaka, H., Takahashi, S., and Igarashi, K. (2008). Regulation of the plasma cell transcription factor Blimp-1 gene by Bach2 and Bcl6. *Int. Immunol.*, **20**(3), 453–60.

Ochiai, K., Maienschein-Cline, M., Simonetti, G., Chen, J., Rosenthal, R., Brink, R., Chong, A. S., Klein, U., Dinner, A. R., Singh, H., and Sciammas, R. (2013). Transcriptional Regulation of Germinal Center B and Plasma Cell Fates by Dynamical Control of IRF4. *Immunity*, **38**(5), 918–929.

Orkin, S. and Zon, L. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. *Cell*, **132**(4), 631–44.

Pasqualucci, L., Migliazza, A., Basso, K., Houldsworth, J., Chaganti, R. S. K., and Dalla-Favera, R. (2003). Mutations of the BCL6 proto-oncogene disrupt its negative autoregulation in diffuse large B-cell lymphoma. *Blood*, **101**(8), 2914–23.

Piskurich, J. F., Lin, K. I., Lin, Y., Wang, Y., Ting, J. P., and Calame, K. (2000). BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. *Nat. Immunol.*, **1**(6), 526–32.

Rankin, A. L., MacLeod, H., Keegan, S., Andreyeva, T., Lowe, L., Bloom, L., Collins, M., Nickerson-Nutter, C., Young, D., and Guay, H. (2011). IL-21 Receptor Is Critical for the Development of Memory B Cell Responses. *J. Immunol.*, **186**(2), 667–74.

Reljic, R., Wagner, S. D., Peakman, L. J., and Fearon, D. T. (2000). Suppression of Signal Transducer and Activator of Transcription 3-Dependent B Lymphocyte Terminal Differentiation by Bcl-6. *J. Exp. Med.*, **192**(12), 1841–1848.

Roessler, S., György, I., Imhof, S., Spivakov, M., Williams, R. R., Busslinger, M., Fisher, A. G., and Grosschedl, R. (2007). Distinct promoters mediate the regulation of Ebf1 gene expression by interleukin-7 and Pax5. *Mol. Cell. Biol.*, **27**(2), 579–594.

Saito, M., Gao, J., Basso, K., Kitagawa, Y., Smith, P. M., Bhagat, G., Pernis, A., Pasqualucci, L., and Dalla-Favera, R. (2007). A Signaling Pathway Mediating Downregulation of BCL6 in Germinal Center B Cells Is Blocked by BCL6 Gene Alterations in B Cell Lymphoma. *Cancer Cell*, **12**(3), 280–292.

Sánchez-Corrales, Y.-E., Alvarez-Buylla, E. R., and Mendoza, L. (2010). The Arabidopsis thaliana flower organ specification gene regulatory network determines a robust differentiation process. *J. Theor. Biol.*, **264**(3), 971–83.

Schebesta, A., McManus, S., Salvagiotto, G., Delogu, A., Busslinger, G. A., and Busslinger, M. (2007). Transcription Factor Pax5 Activates the Chromatin of Key Genes Involved in B Cell Signaling, Adhesion, Migration, and Immune Function. *Immunity*, **27**(1), 49–63.

Scheeren, F. a., Naspetti, M., Diehl, S., Schotte, R., Nagasawa, M., Wijnands, E., Gimeno, R., Vyth-Dreese, F. a., Blom, B., and Spits, H. (2005). STAT5 regulates the self-renewal capacity and differentiation of human memory B cells and controls Bcl-6 expression. *Nat. Immunol.*, **6**(3), 303–13.

Schmidt, C., Kim, D., Ippolito, G. C., Naqvi, H. R., Probst, L., Mathur, S., Rosas-Acosta, G., Wilson, V. G., Oldham, A. L., Poenie, M., Webb, C. F., and Tucker, P. W. (2009). Signalling of the BCR is regulated by a lipid rafts-localised transcription factor, Bright. *EMBO J.*, **28**(6), 711–24.

Sciammas, R. and Davis, M. M. (2004). Modular nature of Blimp-1 in the regulation of gene expression during B cell maturation. *J. Immunol.*, **172**(9), 5427–40.

Sciammas, R., Shaffer, a. L., Schatz, J. H., Zhao, H., Staudt, L. M., and Singh, H. (2006). Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. *Immunity*, **25**(2), 225–36.

Seita, J. and Weissman, I. L. (2010). Hematopoietic stem cell: Self-renewal versus differentiation. *Wiley Interdiscip. Rev. Syst. Biol. Med.*, **2**(6), 640–653.

Seo, W., Ikawa, T., Kawamoto, H., and Taniuchi, I. (2012). Runx1-Cbfβ facilitates early B lymphocyte development by regulating expression of Ebf1. *J. Exp. Med.*, **209**(7), 1255–62.

Shaffer, A., Yu, X., He, Y., Boldrick, J., Chan, E. P., and Staudt, L. M. (2000). BCL-6 Represses Genes that Function in Lymphocyte Differentiation, Inflammation, and Cell Cycle Control. *Immunity*, **13**(2), 199–212.

Shaffer, A. L., Lin, K. I., Kuo, T. C., Yu, X., Hurt, E. M., Rosenwald, A., Giltnane, J. M., Yang, L., Zhao, H., Calame, K., and Staudt, L. M. (2002a). Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. *Immunity*, **17**(1), 51–62.

Shaffer, a. L., Rosenwald, A., and Staudt, L. M. (2002b). Decision making in the immune system: Lymphoid Malignancies: the dark side of B-cell differentiation. *Nat. Rev. Immunol.*, **2**(12), 920–933.

Shaffer, A. L., Shapiro-Shelef, M., Iwakoshi, N. N., Lee, A.-H., Qian, S.-B., Zhao, H., Yu, X., Yang, L., Tan, B. K., Rosenwald, A., Hurt, E. M., Petroulakis, E., Sonenberg, N., Yewdell, J. W., Calame, K., Glimcher, L. H., and Staudt, L. M. (2004). XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity*, **21**(1), 81–93.

Shaffer, A. L., Emre, N. C. T., Lamy, L., Ngo, V. N., Wright, G., Xiao, W., Powell, J., Dave, S., Yu, X., Zhao, H., Zeng, Y., Chen, B., Epstein, J., and Staudt, L. M. (2008). IRF4 addiction in multiple myeloma. *Nature*, **454**(7201), 226–231.

Shapiro-Shelef, M. and Calame, K. (2005). Regulation of plasma-cell development. *Nat. Rev. Immunol.*, **5**(3), 230–42.

Shapiro-Shelef, M., Lin, K. I., McHeyzer-Williams, L. J., Liao, J., McHeyzer-Williams, M. G., and Calame, K. (2003). Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. *Immunity*, **19**(4), 607–620.

Shapiro-Shelef, M., Lin, K.-I., Savitsky, D., Liao, J., and Calame, K. (2005). Blimp-1 is required for maintenance of long-lived plasma cells in the bone marrow. *J. Exp. Med.*, **202**(11), 1471–6.

Shmulevich, I. and Aitchison, J. D. (2009). *Deterministic and stochastic models of genetic regulatory networks.*, volume 467. Elsevier Inc., 1 edition.

Sitnicka, E., Bryder, D., Theilgaard-Mönch, K., Buza-Vidas, N., Adolfsson, J., and Jacobsen, S. E. W. (2002). Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool. *Immunity*, **17**(4), 463–472.

Sitnicka, E., Buza-Vidas, N., Ahlenius, H., Cilio, C. M., Gekas, C., Nygren, J. M., Månsson, R., Cheng, M., Jensen, C. T., Svensson, M., *et al.* (2007). Critical role of flt3 ligand in il-7 receptor–independent t lymphopoiesis and regulation of lymphoid-primed multipotent progenitors. *Blood*, **110**(8), 2955–2964.

Soetaert, K., Petzoldt, T., and Setzer, R. (2010). Solving Differential Equations in R. *R J.*, **2**(December), 5–15.

Spooner, C. J., Cheng, J. X., Pujadas, E., Laslo, P., and Singh, H. (2009). A recurrent network involving the transcription factors PU.1 and Gfi1 orchestrates innate and adaptive immune cell fates. *Immunity*, **31**(4), 576–86.

Szabo, S. J., Sullivan, B. M., Peng, S. L., and Glimcher, L. H. (2003). Molecular mechanisms regulating th1 immune responses. *Annual review of immunology*, **21**(1), 713–758.

Teng, Y., Takahashi, Y., Yamada, M., Kurosu, T., Koyama, T., Miura, O., and Miki, T. (2007). IRF4 negatively regulates proliferation of germinal center B cell-derived Burkitt's lymphoma cell lines and induces differentiation toward plasma cells. *Eur. J. Cell Biol.*, **86**(10), 581–9.

Thieffry, D. (2007). Dynamical roles of biological regulatory circuits. *Brief. Bioinform.*, **8**(4), 220–225.

Todd, D. J., McHeyzer-Williams, L. J., Kowal, C., Lee, A.-H., Volpe, B. T., Diamond, B., McHeyzer-Williams, M. G., and Glimcher, L. H. (2009). XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development. *J. Exp. Med.*, **206**(10), 2151–9.

Toyama, H., Okada, S., Hatano, M., Takahashi, Y., Takeda, N., Ichii, H., Takemori, T., Kuroda, Y., and Tokuhisa, T. (2002). Memory B Cells without Somatic Hypermutation Are Generated from Bcl6-Deficient B Cells. *Immunity*, **17**(3), 329–339.

Tran, T. H., Nakata, M., Suzuki, K., Begum, N. A., Shinkura, R., Fagarasan, S., Honjo, T., and Nagaoka, H. (2010). B cell-specific and stimulation-responsive enhancers derepress Aicda by overcoming the effects of silencers. *Nat. Immunol.*, **11**(2), 148–54.

Tsapogas, P., Swee, L. K., Nusser, A., Nuber, N., Kreuzaler, M., Capoferri, G., Rolink, H., Ceredig, R., and Rolink, A. (2014). In vivo evidence for an instructive role of fms-like tyrosine kinase-3 (FLT3) ligand in hematopoietic development. *Haematologica*, **99**, 638–646.

Tunyaplin, C., Shaffer, a. L., Angelin-Duclos, C. D., Yu, X., Staudt, L. M., and Calame, K. L. (2004). Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. *J. Immunol.*, **173**(2), 1158–65.

Turner, C. A., Mack, D. H., and Davis, M. M. (2010). Pillars article: Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. 1994. *J. Immunol.*, **185**(1), 5–14.

Usui, T., Wakatsuki, Y., Matsunaga, Y., Kaneko, S., Koseki, H., Kita, T., and Kosek, H. (1997). Overexpression of B cell-specific activator protein (BSAP/Pax-5) in a late B cell is sufficient to suppress differentiation to an Ig high producer cell with plasma cell phenotype. *J. Immunol.*, **158**(7), 3197–204.

Vasanwala, F. H., Kusam, S., Toney, L. M., and Dent, A. L. (2002). Repression of AP-1 function: a mechanism for the regulation of Blimp-1 expression and B lymphocyte differentiation by the B cell lymphoma-6 protooncogene. *J. Immunol.*, **169**(4), 1922–9.

Weinstein, N. and Mendoza, L. (2012). Building Qualitative Models of Plant Regulatory Networks with SQUAD. *Front. Plant Sci.*, **3**(April), 72.

Welinder, E., Ahsberg, J., and Sigvardsson, M. (2011a). B-lymphocyte commitment: identifying the point of no return. *Semin. Immunol.*, **23**(5), 335–40.

Welinder, E., Mansson, R., Mercer, E. M., Bryder, D., Sigvardsson, M., and Murre, C. (2011b). The transcription factors E2A and HEB act in concert to induce the expression of FOXO1 in the common lymphoid progenitor. *Proc. Natl. Acad. Sci. U. S. A.*, **108**(42), 17402–7.

Wittmann, D. M., Krumsiek, J., Saez-Rodriguez, J., Lauffenburger, D. a., Klamt, S., and Theis, F. J. (2009). Transforming Boolean models to continuous models: methodology and application to T-cell receptor signaling. *BMC Syst. Biol.*, **3**, 98.

Xiong, Y. and Bosselut, R. (2012). CD4-CD8 differentiation in the thymus: Connecting circuits and building memories. *Curr. Opin. Immunol.*, **24**(2), 139–145.

Yasuda, T., Kometani, K., Takahashi, N., Imai, Y., Aiba, Y., and Kurosaki, T. (2011). ERKs induce expression of the transcriptional repressor Blimp-1 and subsequent plasma cell differentiation. *Sci. Signal.*, **4**(169), ra25.

Yasuda, T., Hayakawa, F., Kurahashi, S., Sugimoto, K., Minami, Y., Tomita, A., and Naoe, T. (2012). B cell receptor-ERK1/2 signal cancels PAX5-dependent repression of BLIMP1 through PAX5 phosphorylation: a mechanism of antigen-triggering plasma cell differentiation. *J. Immunol.*, **188**(12), 6127–34.

Zadeh, L. (1965). Fuzzy sets. *Inf. Control*, **8**(3), 338–353.

Zotos, D., Coquet, J. M., Zhang, Y., Light, A., D'Costa, K., Kallies, A., Corcoran, L. M., Godfrey, D. I., Toellner, K.-M., Smyth, M. J., Nutt, S. L., Tarlinton, D. M., and Costa, K. D. (2010). IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J. Exp. Med.*, **207**(2), 365–78.

8 APÉNDICES

8.1 APÉNDICE I: The SQUAD method for the qualitative modeling of regulatory networks

The SQUAD method for the qualitative modeling of regulatory networks

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Abstract

The wealth of molecular information provided by high-throughput technologies has enhanced the efforts dedicated to the reconstruction of regulatory networks in diverse biological systems. This information, however, has proven to be insufficient for the construction of quantitative models due to the absence of sufficiently accurate measurements of kinetic constants. As a result, there have been efforts to develop methodologies that permit the use of qualitative information about patterns of expression to infer the regulatory networks that generate such patterns. One of these approaches is the SQUAD method, which approximates a Boolean network with the use of a set of ordinary differential equations. The main benefit of the SQUAD method over purely Boolean approaches is the possibility of evaluating the effect of continuous external signals, which are pervasive in biological phenomena. A brief description and code on how to implement this method can be found at the following link:
https://github.com/caramirezal/SQUADBookChapter.

Keywords: Regulatory networks, Network modeling, Cell fate, Expression pattern

1 Introduction

One of the challenges in biology is to understand the relationship between genotype and phenotype. The phenotype of a cell emerges from the complex interactions between molecules, the genome, and environmental cues that act in a nonlinear manner. Understanding how these interactions act in concert to regulate cellular phenotypes requires an integrative view of the processes that control cell fate decisions. The advent of the ”omics” era has contributed to the study of the genotype-phenotype relationship, as new molecular biology methods and high-throughput technologies allow the identification and mapping of an increasing number of molecules and their interactions, thus offering valuable information regarding the regulatory networks involved in biological processes.

Knowledge of the connectivity of regulatory networks, while valuable, only provides a static view of the complex spatio-temporal behavior observed in any biological phenomenon [1]. Therefore, it is necessary to develop and analyze mathematical models to understand the functionality and dynamical properties of regulatory networks. This allows us to understand questions related to the number, nature and stability of the possible patterns of activation, the role of specific molecules and interactions in the establishment of such patterns, as well as the effects of external stimuli or permanent perturbations such as gain- and loss-of-function mutations. This information could eventually be used to devise control mechanisms with the aim of driving the system to a desired state [2, 3].

Network-based modeling approaches have been shown to be valuable tools to integrate biological information to understand the dynamical behavior observed in multiple cellular processes [4, 5, 6, 7, 8, 9, 10]. For example, the establishment of molecular patterns in floral morphogenesis of both wild type and mutants in *Arabidopsis thaliana* [11, 12], the phenotype stability and plasticity of T helper lymphocytes and other blood cell subpopulations in mammals [13, 6, 14], the cell cycle in yeast or mammals [15, 16, 7], and apoptosis [17].

There are several approaches for modeling regulatory networks (for reviews on

qualitative modeling approaches, see [18, 19, 20, 21]). In particular, logical models are used to describe systems with a finite number of possible states. In contrast, ODE-based models are able to describe systems with a possibly infinite number of states. [22, 23]. As a result, logical approaches are well suited for qualitative descriptions, while ODE-based models are more appropriate for quantitative descriptions.

When dealing with biological problems it is often convenient to start with a simple model describing a few basic characteristics, and then gradually transform the model into a more refined and complex one. In the case of a regulatory network, one basic characteristic is the number and nature of the steady states of activation or expression generated by the system. In some cases, steady states have been shown to be largely insensitive to the precise values of parameters [24], or even the formalism chosen [5] to find them. In such cases, qualitative modeling can be implemented as a first approximation to model regulatory networks [19].

One approach to make a qualitative model more realistic, is to incorporate continuous variables, so as to provide the model with the capacity of describing graded variations. There are several methods to transform Boolean networks into continuous dynamical systems. All of them rely of the general principle of interpolating or replacing the discrete functions by continuous mappings defined in the $[0, 1]$ closed interval [25]. Interpolation functions can be linear [26], continuous logical extensions [27], polynomial approximations [28], or polynomial composed with Hill or exponential functions [3, 1] (see [25] for a comparative study). In this chapter, we describe the Standardized Qualitative Dynamical Systems (SQUAD) method, which is used to formalize regulatory networks as a continuous dynamical system focusing on their qualitative behavior rather than in their detailed kinetic parameters [1, 12].

The SQUAD method can be used to either automatically transform a Boolean network model to a continuous system of ordinary differential equations (ODEs), or to directly define a continuous model from the available information regarding the interactions among the components of a regulatory network. In this way, the modeler can simulate a regulatory network based mostly on the network architecture by making a few basic assumptions regarding the response of the nodes to their regulators, or alter-

natively, to construct more elaborated models based on known regulatory mechanisms obtained from experimental data.

In the next section we briefly describe how to define a Boolean model, and then we show how to transform it into a continuous model using the SQUAD method. Finally, we show how to analyze the dynamical behavior of regulatory networks to identify its steady states, as well as how to study the effect of diverse perturbations.

2 Methods

2.1 Reconstruction of the regulatory network

To construct a regulatory network model, it is necessary to identify the key molecules involved in the control of a biological process of interest (genes, proteins, molecular complexes, etc.), as well as the regulatory relationships among them (activating or inhibitory). This is done by integrating available experimental data regarding the function of the components of the network under different experimental conditions, such as gain- or loss-of-function mutants, epistasis analysis, and known expression patterns, for instance [18, 21]. While it is very important to have the most comprehensive data possible on the list of molecular markers that are present or absent under a given condition, it is also absolutely necessary to have a notion about the flux of information among the nodes to be included into the network model. This is, besides knowing that markers A and B are co-expressed, it is necessary to know if A regulates B, B regulates A, another node regulates both A and B, or even if there is no regulatory relationship between A and B.

While a detailed discussion on the different network inference methods is beyond the scope of this work, we refer the interested reader to some relevant literature on the topic [29, 30, 31]. Most of these methods relies on the estimation of the probability of dependency measures between gene expression values (Pearson correlation, mutual information and Bayesian) [29]. In general, these methods are not well suited to infer the specific combinations of regulators that turn a particular gene *ON* or *OFF*. And more importantly most of such methodologies are very inefficient at inferring the presence

of regulatory circuits [29]. As a result, most of the published Boolean models were inferred manually, using carefully selected experiments reported in the literature.

The proposed molecules and interactions conforming a regulatory network can be incorporated into a table of interactions summarizing its architecture. Once we have a static representation of the regulatory network it is necessary to postulate a set of logical rules or functions controlling the activation of the nodes of the network and simulate its dynamical behavior to qualitatively analyze the temporal expression profiles that can be attained under multiple conditions.

2.2 Definition of the discrete model

A deterministic Boolean model is the simplest formalism that can be used to study regulatory networks as dynamical systems. Despite their conceptual simplicity, Boolean networks show a non-linear behavior resulting in interesting and non-intuitive dynamical properties such as multistability, cyclic trajectories, and robustness against perturbations [32, 33, 13, 34]. In Boolean models the nodes of a regulatory network are represented by binary variables, i.e, they can attain one of two possible levels of activity (*ON/OFF*) at any given time (**Fig. 1A**). Also, regulatory interactions are formalized by means of Boolean functions that can be described by logic rules or truth tables (**Fig. 1B**). The Boolean function assigned to a node determines its value of activity at the next time step depending on the values of their regulators at a given time. Formally,

$$x_k(t+1) = f_k(x_{k,1}(t),\ x_{k,2}(t),\ x_{k,3}(t),\ \ldots,\ x_{k,r}(t)) \tag{1}$$

Where k represents the index of a node with r input regulators. The variables $x_{k,1}(t),\ldots,x_{k,r}(t)$ represent the set of values of the regulators of the node x_k at time t, and f_k is the Boolean function defining the activation of the node at time $t+1$. For example, the node B in **Fig. 1A** has two regulators, given that nodes A and C activate and inhibit B, respectively. These interactions can be translated into a logical form to represent the regulatory mechanisms that control the activation levels of node B. Thus, node B becomes active only when its activator (node A) is present and if its inhibitor (node

Boolean operator	Fuzzy logic function
A $\wedge$ B	$min(A,B)$
A $\vee$ B	$max(A,B)$
$\neg$ A	$1-A$

Table 1: The logic rules describing the response of a node to its regulators expressed with the logic operators $\wedge$ (AND), $\vee$ (OR) and $\neg$ (NOT) can be transformed into a continuous form with the use of their equivalent Fuzzy logic functions.

C) is absent (**Fig. 1B**). For each node, representing molecules or molecular complexes in the network, it is possible to formulate a logical rule summarizing the regulatory mechanisms behind each interaction, which can then be used to construct a dynamical model of the regulatory network. In the network shown in **Fig. 1** the Boolean function for the node *B* is given by:

$$B(t+1) = f_B(A(t),\, C(t)) = A(t) \wedge \neg\, C(t) \tag{2}$$

Where the symbols $\wedge$ and $\neg$ represent the logic functions AND and NOT, respectively. It is important to note that any logical rule can be expressed in terms of the Boolean operators AND, OR and NOT, see **Table 1.**

If n is the number of nodes of the regulatory network, we call any binary vector $\hat{x} = (x_1,\, x_2,\, x_3, ...,\, x_n)$ a state of the network. In a Boolean system, there exist 2^n possible states, this set of configurations is called the phase space [18]. **Eq. 1** determines the transition of the network through the phase space, where successive states of the network are determined by the present state of the system. Therefore, we can rewrite the system of Boolean equations, if $F = (f_1,\, f_2,\, f_3, ...,\, f_n)$, then **Eq. 1** becomes:

$$\hat{x}_{t+1} = F(\hat{x}_t) \tag{3}$$

Where $\hat{x}_t$ and $\hat{x}_{t+1}$ are the predecessor and successor states in the dynamics of the network.

The updating of the values of the nodes can be done in different ways, the sim-

plest of which is the *synchronous* updating scheme, in which all nodes are updated at the same time. Otherwise, if nodes of the network are updated in different order, the Boolean network is said to be *asynchronous*[35]. We will focus for the rest of the Chapter to the synchronous updating [35, 36]. The dynamics of the Boolean network can be represented as a transition diagram (**Fig. 1C**), in which the phase space is drawn along with all the possible transitions between network states. The dynamical behavior of a Boolean network starting from an initial state x_0 can be easily seen by just following the arrows until the system converges to a closed cycle, i.e., a set of states that repeats consecutively for more than one time step during a trajectory.

Cycles are called *attractors* (i.e. solutions of the network) and capture the long-term behavior of the system [21, 18, 37]. If an attractor contains only one network state it is called a *fixed point* attractor, otherwise, it is referred as a *cyclic* attractor [37].

The network in **Fig. 1** possesses two fixed point attractors. There are two sets of network states, shown as red and green nodes, each set of states converges to one of the fixed point attractors (**Fig. 1C**). The set of all network states that converge to a specific attractor is known as its basin of attraction. In the example of **Fig. 1**, the set of states $S = \{010, 011, 000, 001\}$, shown in red, correspond to the basin of the attractor 001. Because the system once in an attractor will remain in it, attractors are interpreted as patterns of activation associated to specific stable phenotypes [38, 32].

Boolean networks have the advantage that the entire phase space can be explored, so as to find all the possible attractors of the system. This is very useful because it permits to understand all the possible phenotypes allowed by a given network model. Although the computational time of search scales very fast with the number of nodes of the network, there are algorithms that search for attractors in large networks comprising hundreds to several thousands of nodes [1, 39].

2.3 Construction of a continuous model using SQUAD

For most biological systems, relatively few quantitative data is available regarding the kinetics and stoichiometry of biochemical reactions. Nonetheless, there is a wealth of qualitative data regarding the molecular interactions, and the effect of genetic mutations

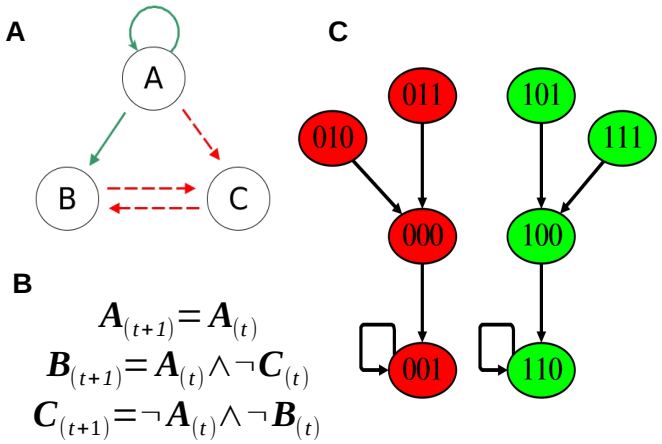

Fig. 1: Boolean networks. **A**. A regulatory network comprising three factors A, B, and C, depicted as nodes in the graph. Activations and inhibitions are represented as green and red arrows, respectively. Every node has an associated state, which can be either 0/OFF/false or 1/ON/true. **B**. Logic rules can be used to represent regulatory interactions in the regulatory network. **C**. Dynamical behavior of the synchronous Boolean network model. In this case, nodes represent states of the network which are labeled by the binary values of the nodes A, B and C, in that order. States are colored according to the attractor they converge to.

on the establishment of particular cell phenotypes. Qualitative data also offer valuable functional information about the regulation of some biological processes. This imbalance in the kind of available information has limited the development of dynamical models of regulatory networks to only a small number of well-characterized systems [19].

To overcome these restrictions, and thus facilitate the systematic construction of regulatory network models, the SQUAD method was developed to simulate a dynamical system without the need for detailed kinetic parameters [40]. Instead, the method relies mostly on the connectivity of the regulatory network, i.e, the flow of information among the nodes, as well as on the rules describing the regulatory mechanisms among the molecules they represent. The method approximates a Boolean network with the use of a set of ordinary differential equations. Importantly, a continuous approximation of a Boolean system allows the construction of complex dynamical models even

in the absence of quantitative information regarding the precise molecular regulatory mechanisms of the biological system.

The continuous system describes the rate of change of activation of a node x_k with the following ODE:

$$\frac{dx_k}{dt} = \frac{-e^{0.5h_k} + e^{-h_k(\omega_k - 0.5)}}{(1 - e^{0.5h_k})(1 + e^{-h_k(\omega_k - 0.5)})} - \gamma_k x_k \tag{4}$$

Where $0 < h_k$, γ_k and $0 \leq x_k$, $\omega_k \leq 1$. The non-linear function on the right hand side of the differential equation defines a sigmoid curve constrained in the interval $[0, 1]$. The parameter h_k determines the steepness of the sigmoid, for high h_k values the sigmoid function approaches the step function characteristic of Boolean models **(Fig. 2)**.

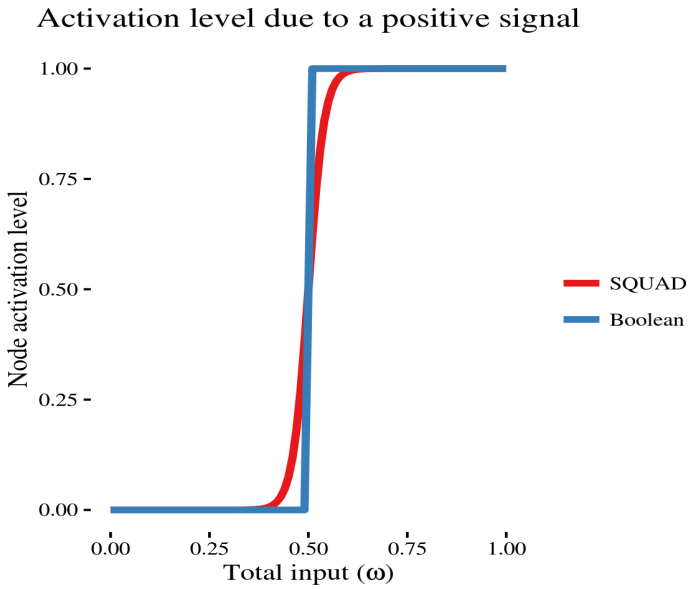

Fig. 2: Discrete versus continuous models. Response to a positive input in a discrete Boolean context (blue), and the continuous SQUAD version (red). The parameters h in SQUAD can be modified resulting in a steeper or shallower sigmoid.

The parameter γ_k is the decay rate for the node x_k. Although its specific numerical value may vary according to the necessities of the modeler and depends on the biologi-

cal system under study, it has been found that the attractors of a model are rather robust to variations in the variation of this parameter [4, 41].

The total regulatory input of a node is represented by the variable ω_k. In the first implementation of the SQUAD method, ω_k was defined as a weighted sum of positive regulations multiplied by a weights sum of negative regulators [40, 42]. This approach permitted the automatic creation of a dynamical system based exclusively on the topology of the network. While the instant transformation of a diagrammatic representation into a dynamical system was a very convenient feature, the chosen definition of ω_k had two assumptions: First, that any positive regulator is strong enough to activate its target in the absence of inhibitors; and second, that any negative regulator is stronger than any combination of positive regulators. These assumptions, however, are very strong and they are not necessarily true for most biological systems.

A new SQUAD version was proposed in [12] with the aim to relax the assumptions mentioned above. In this second version, ω_k is defined in terms of an interpolated Boolean function with the use of fuzzy logic operators [43], (**Table 1**). The first thing to do is to define the regulatory input of a node x_k as a Boolean function f_k of its regulators, just as in the case of the Boolean networks described in the previous section. It can be shown that any f_k can be rewritten in the following form:

$$f_k(x_1,...,x_r) = \bigvee_{i=1}^{n} (x_1 \wedge x_2 \ldots \wedge x_l) \wedge (\neg x_{l+1} \wedge \neg x_{l+2} \wedge \ldots \wedge \neg x_r) \tag{5}$$

Where r denotes the total number of regulators of the node x_k and l represents the positive regulators of the node x_k whereas the remaining $r-l$ nodes indicate negative regulators of such node. This equation states that any Boolean function can be represented as a disjunction of n Boolean input configurations (2^r in total), conforming the set of states of the regulators that result in a value of f_k of $1/ON$. Then, the Boolean function can be interpolated by using fuzzy logic operators [1, 40], see **Table 1**, thus defining ω_k as:

$$\omega_k(x_1,...,x_r) = max_{i=1}^{n}(min(x_1,...,x_l, 1-x_{l+1},...,1-x_r)) \tag{6}$$

Where, as in **Eq. 5**, the fuzzy logic rule for each x_k node is constructed according to its x_r regulators, of which l input nodes are positive regulators and the remaining $r - l$ nodes correspond to negative regulators. An example of how to translate a Boolean model into its continuous fuzzy logic form is shown in **Fig. 3**. This methodology thus provides a straightforward manner to translate a Boolean model into a continuous model in the form of a set of ODEs.

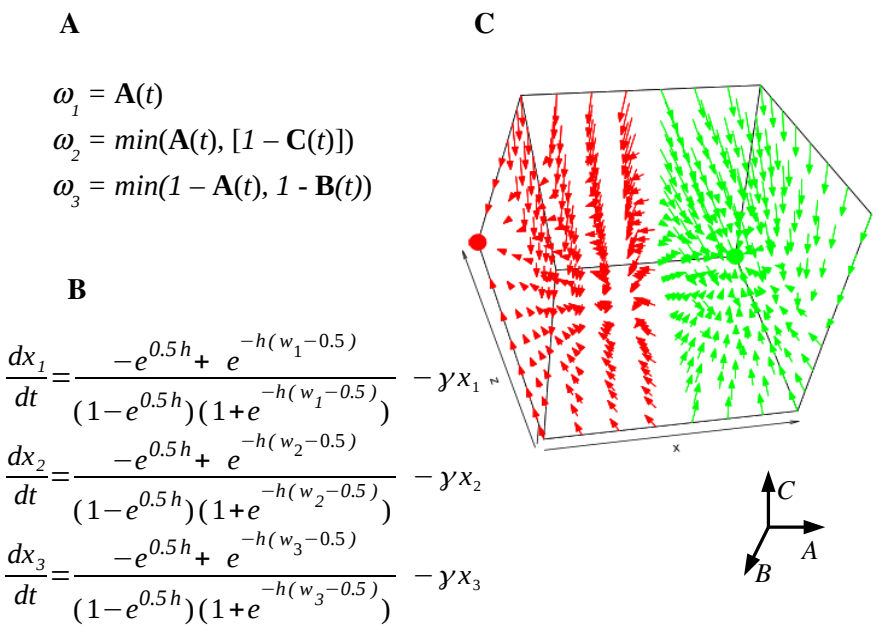

Fig. 3: SQUAD Method. Example of the SQUAD methodology applied to the regulatory network given in **Fig. 1**. **A**. Determination of the parameter ω_k summarizing the regulation for each node in terms of fuzzy logic operators. **B**. Definition of the continuous dynamical system in the form of a set of ODEs. The parameters h and γ are set to 50 and 1, respectively. **C**. Dynamical behavior of the regulatory network as simulated by the continuous model. Attractors are shown as red and green dots.

Given that the SQUAD method approximates a Boolean model, there is a close correspondence between the steady states found in Boolean discrete models and the steady states found in the continuous model. Notice, however, that SQUAD may find (usually unstable) steady states, or cyclic behaviors not recovered in the Boolean system [44, 12]. As in the discrete Boolean model the phase space is split into attractor

basins. This can be seen in the **Fig. 3C**, where the dynamics of the continuous model analogous to the regulatory network given in the **Fig. 1** are shown. The phase space is split into two basins, observed as green and red partitions, according to the convergence to the corresponding attractors $(A,B,C) = (0,0,1)$ and $(1,1,0)$, that coincides with that of the discrete model.

The ability of SQUAD to incorporate graded signals makes it a suitable tool to study the effect of extracellular signals on the establishment of expression patterns during differentiation processes [45, 12]. **Fig. 4** shows the difference in effect between small and large perturbations on the steady state configurations attained by a regulatory network. Also, it is possible to observe the effect of the order of such external signals. In this example, when the regulatory network represented in **Fig. 4A** is perturbed by a small increase of inputs X and Y the perturbations are absorbed by the system (**Fig. 4B**). However, with stronger perturbations there is a transition to another network state, and thus the effect on the system is permanent (**Fig. 4C**). Moreover, the long term behavior of the network depends on the order in which the perturbations are given (**Fig. 4C to D**). The interested reader may find in https://github.com/caramirezal/SQUADBookChapter the implementation of this example in the programming language R.

3 Modeling biological networks using SQUAD

In this section we describe the construction of a model of a particular biological process, so as to give a clearer view of the steps needed to make use of SQUAD. Specifically, we will focus on the regulatory network controlling terminal B cell differentiation, see [46]. A brief description of the model is provided in R language at https://github.com/caramirezal/SQUADBookChapter/. The model is also available in a standard SBMLqual format in The Cell Collective platform, see "B cell differentiation" model at https://cellcollective.org/.

B cells are the main effectors of humoral immune response in vertebrates, which is responsible for the recognition of foreign agents by the production of highly specific antibodies. Terminal B cell differentiation is achieved by the concerted action of

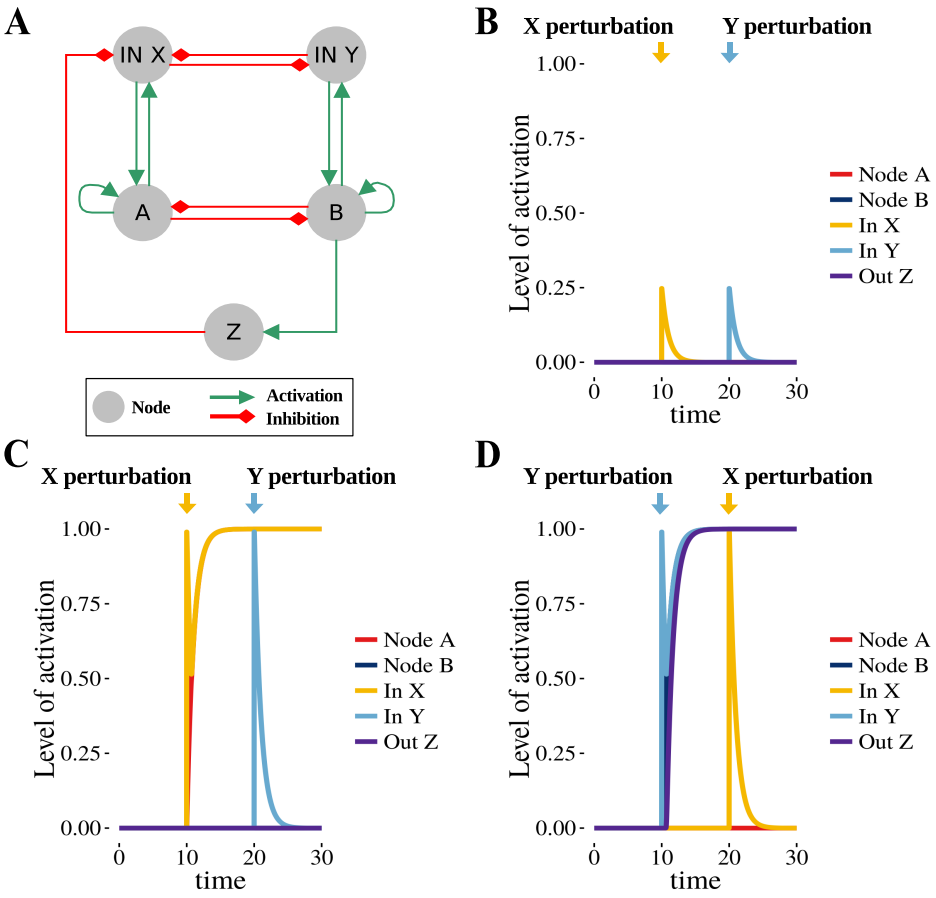

Fig. 4: Modeling B cell differentiation using SQUAD. Based on available information from a biological process, it is possible to infer a regulatory network taking into account the information flow among molecules (nodes), by activations (green arrows) and inhibitions (red arrows) between them. The regulatory mechanisms are incorporated to the network as logical rules. The system is translated into a set of ODEs and the dynamical behavior is analyzed. The basic analysis consists in the identification of steady states, under wild-type and simulated mutations, as well as the analysis of the response of the system to certain signals.

several transcription factors in response to antigen recognition and extracellular signals provided by other blood cell types, like T-helper cells, dendritic cells, etc. This process of cell differentiation is characterized by the transition from a progenitor cell type (Naive B cell) into specialized cell types such as germinal center B cells (GC) and memory B cells (Mem), responsible for the editing and improvement of antibodies and its subsequent differentiation into antibody producing plasma cells (PC) in response to antigen recognition [47].

The wealth of published experimental data regarding the molecules and signals involved in the control of B cell differentiation allowed us to reconstruct a regulatory network that incorporates several molecules known to be necessary for the control of the differentiation process, namely, Bach2, Bcl6, Blimp1, Irf4, and Pax5, see **Fig. 5**.

The information regarding the regulatory mechanisms controlling the activation of each node can be expressed in the form of logical rules. For example, it is known that Pax5 is regulated by itself and by low levels of Irf4 [48, 49], and also that Pax5 is negatively regulated by Blimp1 [50, 51]. This information can be expressed in the form of a logical rules as follows: $Pax5(t+1) = (Pax5(t) \vee \neg\, Irf4(t)) \wedge \neg\, Blimp1(t))$. This expression, in turn, can be expressed in fuzzy logic terms as $Pax5(t+1) = min(max(Pax5(t), 1 - Irf4(t)), 1 - Blimp1(t))$. The complete set of logical rules summarizing the regulatory mechanisms controlling the activation of each node are presented in the **Table 2**.

Once the complete set of fuzzy logic rules is established, they can be inserted as the corresponding ωs in the skeleton **Eq. 4**. This is, for the equation $dPax5/dt$ the corresponding ω_{Pax5} is $min(max(Pax5(t), 1 - Irf4(t)), 1 - Blimp1(t))$. Then, once the complete set of ODEs is determined, the resulting system is numerically integrated by sampling a large number of random initial states. The asymptotic solutions of the system of ODEs can then be compared with the known activation patterns characterizing each of the aforementioned B cell phenotypes.

Specifically, the method allowed the identification of attractors corresponding to the reported activation patterns of Naive, GC, Mem and PC B cell phenotypes. Additionally, the SQUAD method was used to study the temporal response of the regulatory network to external stimuli with varying levels of intensity to simulate the effect of

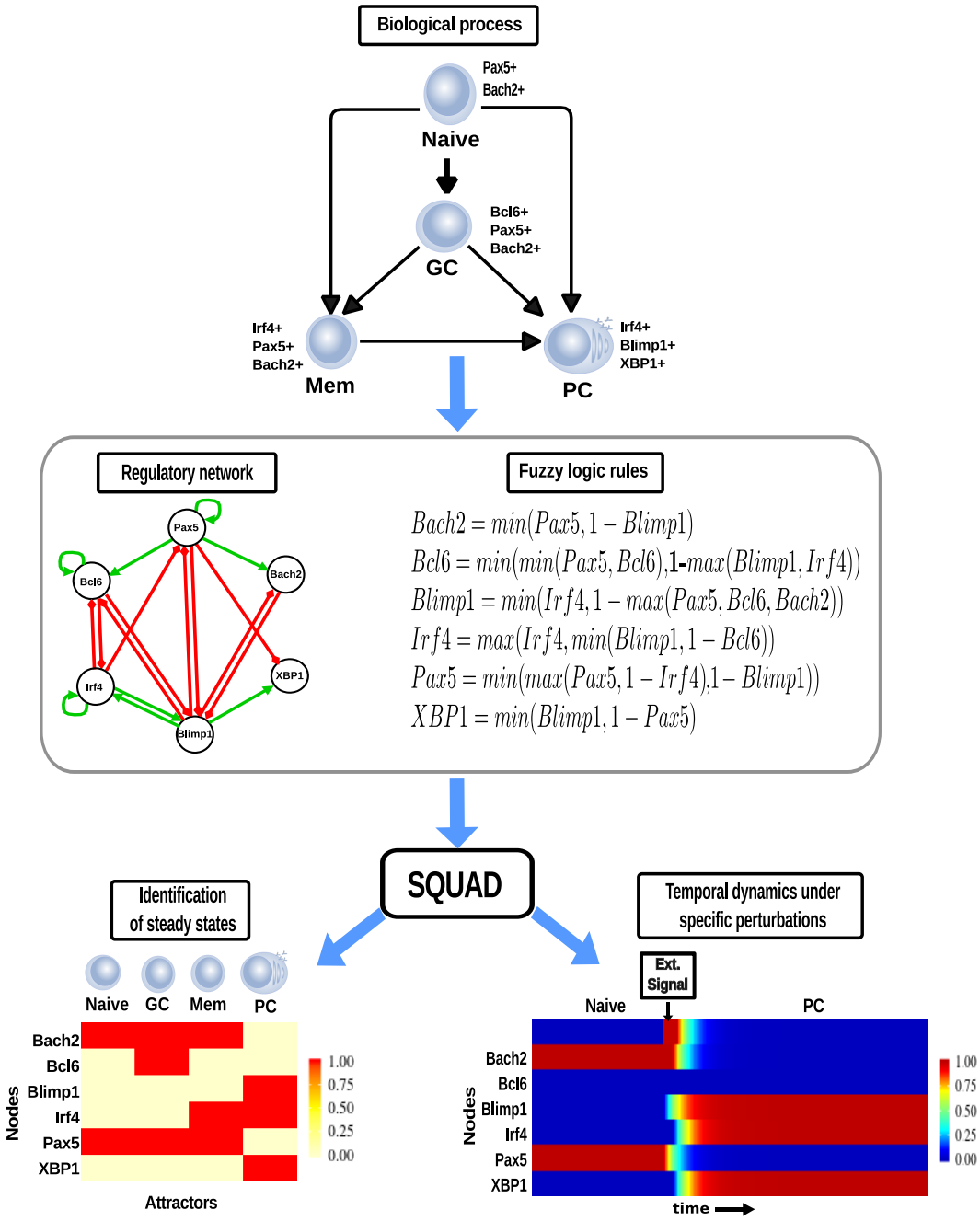

Fig. 5: Effects of node perturbations on the dynamics of a regulatory network. **A**. Regulatory network module. **B**. Small perturbations are given to node X and Y, they are absorbed by the system an vanish. **C-D**. Larger perturbations cause permanent network state transitions. **C**. If X is perturbed first, the network transits to a state in which node Z is inactive. **D**. If the order of stimuli is inverted, the node Z becomes active

Node	Description	Logical Rule	Fuzzy logic function
Bach2	Bach2 is activated by Pax5 if the supressor Blimp1 is absent.	Pax5 AND NOT Blimp1	min(Pax5, 1- Blimp1)
Bcl6	Bcl6 regulates its own expression and is activated by Pax5 only if their repressors Blimp1 and Irf4 are inactive.	Pax5 AND Bcl6 AND NOT (Blimp1 OR Irf4)	min(Pax5, Bcl6, 1-max(Blimp1, Irf4))
Blimp1	Blimp1 is activated by Irf4 if all its inhibitors, Pax5, Bcl6, and Bach2 are absent.	Irf4 AND NOT (Pax5 OR Bcl6 OR Bach2)	min(Irf4, 1 - max(Pax5, Bcl6, Bach2))
Irf4	Irf4 regulates its own activation and is positively regulated by Blimp1 if its inhibitor Bcl6 is inactive.	Irf4 OR (Blimp1 AND NOT Bcl6)	max(Irf4, min(Blimp1, 1 - Bcl6))
Pax5	Pax5 positively regulates its own expression and is maintained by low levels of Irf4. Pax5 is repressed by Blimp1.	(Pax5 OR NOT Irf4) AND NOT Blimp1	min(max(Pax5, 1 - Irf4), 1 - Blimp1)
XBP1	XBP1 is activated by Blimp1 and repressed by Pax5	Blimp1 AND NOT Pax5	min(Blimp1, 1 - Pax5)

Table 2: The logic rules summarizing the regulatory interactions in the B cell differentiation network expressed by the use of the logic operators and their translation into fuzzy logic functions.

relevant biological signals and to evaluate the effect of multiple perturbations such as gain and loss-of function mutations.

The model was able to recapitulate the dynamical behavior of the set of key molecules involved in the differentiation of B cells [46]. Moreover, the model allowed the prediction of regulatory interactions that are necessary for the correct specification of multiple cell types during terminal B cell differentiation. Furthermore, the model gave theoretical support for the instructive role of cytokines involved in this differentiation process. And finally, the model explained the mechanism underlying the dynamical robustness of the PC attractor, a property that closely resembles the stability of the terminally differentiated plasma cells.

4 Summary and outlook

There is a large variety of network modeling approaches, the selection of a particular methodology mostly depends on the available information and the kind of question

one is seeking to answer. Specifically, the SQUAD method allows the construction of qualitative dynamical models of regulatory networks by making use of available experimental information where there is a lack of kinetic parameters. This method was developed to analyze the nature and number of the stationary states found in a regulatory network under different scenarios, such as gain- and loss-of-function mutants, or the presence of external stimuli.

Despite the usefulness of the SQUAD method, as evidenced by the number of networks analyzed with it, there is ample room for the improvement. Fore example, some future refinements might incorporate stochastic effects on the concentration of molecules by adding noise to the nodes. Also, it would be desirable to include modifications that permit the transformation of multivalued discrete systems into ODE systems. These and other modifications might be re-implemented in the form of a user-friendly software package to facilitate the construction and exchange of models. Moreover, a long-term desirable goal would be to seek integration of SQUAD with other methods and platforms that complement it, such as GINsim [52], BoolNet [53], MaBoSS [54], JIMENA [55] and The Cell Collective [56].

5 Conclusions

SQUAD was developed to provide a flexible methodology to develop continuous models of regulatory networks, whenever only qualitative information is available. It can also be used as an initial tool to understand the qualitative behavior of a network before embarking in the use of more refined, complex, and time-consuming modeling techniques. The successful use of SQUAD to understand the dynamical behavior of different biological systems shows its value as a tool of first choice for modelers.

In agreement with this, a comparative study carried out in [57] found SQUAD to be computationally more efficient than Odefy and CellNetAnalyzer tools [28, 58]. Recently, the group of Thomas Dandekar and colleagues implemented a optimization algorithm using RBDD Boolean representation methods and developed a tool called JIMENA to simulate regulatory networks. They found that with their optimization

procedure the method performs as efficiently as SQUAD (old version) [55]. Although comparisons with the new version of SQUAD has not been carried out, in our hands we have been able to analyze regulatory networks comprising more than 80 nodes efficiently [14].

SQUAD was developed for qualitative modeling, thus it would not be an ideal tool for use in those systems with extensive presence of cooperative binding or allosteric regulation, where the kinetic details are of importance to determine the behavior of the system. Only very general molecular mechanisms are able to be included in the SQUAD equations details of such mechanisms cannot be incorporated into the equations. The SQUAD method focuses in the modeling on system characteristics derived by the flux of information of the regulatory circuits, rather than in the details of molecular mechanisms.

SQUAD was developed to better understand the role of regulatory networks in the process of cellular differentiation. The flexibility and performance of the methodology, however, has allowed it to be used as a general tool in the analysis of the dynamical properties of regulatory networks. Its main strength is its capacity to analyze graded signals whenever the available information allows only for the construction of Boolean regulatory network models. Due to the large diversity of biological systems, modeling methods must be continuously created and modified to make the best possible use of all the available experimental information.

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References

[1] Abhishek Garg, Ioannis Xenarios, Luis Mendoza, and Giovanni DeMicheli. An efficient method for dynamic analysis of gene regulatory networks and in silico gene perturbation experiments. pages 62–76, 2007.

[2] Jorge GT Zañudo and Réka Albert. Cell fate reprogramming by control of intracellular network dynamics. *PLoS Comput Biol*, 11(4):e1004193, 2015.

[3] Stefan Karl and Thomas Dandekar. Convergence behaviour and control in non-linear biological networks. *Scientific reports*, 5, 2015.

[4] Pablo Martínez-Sosa and Luis Mendoza. The regulatory network that controls the differentiation of t lymphocytes. *Biosystems*, 113(2):96–103, 2013.

[5] Elena R Álvarez-Buylla, Álvaro Chaos, Maximino Aldana, Mariana Benítez, Yuriria Cortes-Poza, Carlos Espinosa-Soto, Diego A Hartasánchez, R Beau Lotto, David Malkin, Gerardo J Escalera Santos, et al. Floral morphogenesis: stochastic explorations of a gene network epigenetic landscape. *Plos one*, 3(11):e3626, 2008.

[6] Mariana Esther Martinez-Sanchez, Luis Mendoza, Carlos Villarreal, and Elena R Alvarez-Buylla. A minimal regulatory network of extrinsic and intrinsic factors recovers observed patterns of cd4+ t cell differentiation and plasticity. *PLoS Comput Biol*, 11(6):e1004324, 2015.

[7] Adrien Fauré, Aurélien Naldi, Claudine Chaouiya, and Denis Thieffry. Dynamical analysis of a generic boolean model for the control of the mammalian cell cycle. *Bioinformatics*, 22(14):e124–e131, 2006.

[8] Elisabeth Remy, Sandra Rebouissou, Claudine Chaouiya, Andrei Zinovyev, François Radvanyi, and Laurence Calzone. A modeling approach to explain mutually exclusive and co-occurring genetic alterations in bladder tumorigenesis. *Cancer research*, 75(19):4042–4052, 2015.

[9] Aurélien Naldi, Jorge Carneiro, Claudine Chaouiya, and Denis Thieffry. Diversity and plasticity of th cell types predicted from regulatory network modelling. *PLoS Comput Biol*, 6(9):e1000912, 2010.

[10] Laurence Calzone, Laurent Tournier, Simon Fourquet, Denis Thieffry, Boris Zhivotovsky, Emmanuel Barillot, and Andrei Zinovyev. Mathematical modelling of cell-fate decision in response to death receptor engagement. *PLoS Comput Biol*, 6(3):e1000702, 2010.

[11] Luis Mendoza and Elena R Alvarez-Buylla. Dynamics of the genetic regulatory network for arabidopsis thaliana flower morphogenesis. *Journal of theoretical biology*, 193(2):307–319, 1998.

[12] Yara-Elena Sanchez-Corrales, Elena R Alvarez-Buylla, and Luis Mendoza. The arabidopsis thaliana flower organ specification gene regulatory network determines a robust differentiation process. *Journal of theoretical biology*, 264(3):971–983, 2010.

[13] Luis Mendoza. A network model for the control of the differentiation process in th cells. *Biosystems*, 84(2):101–114, 2006.

[14] Luis Mendoza and Akram Méndez. A dynamical model of the regulatory network controlling lymphopoiesis. *Biosystems*, 137:26–33, 2015.

[15] Fangting Li, Tao Long, Ying Lu, Qi Ouyang, and Chao Tang. The yeast cell-cycle network is robustly designed. *Proceedings of the National Academy of Sciences of the United States of America*, 101(14):4781–4786, 2004.

[16] Maria I Davidich and Stefan Bornholdt. Boolean network model predicts cell cycle sequence of fission yeast. *PloS one*, 3(2):e1672, 2008.

[17] Rebekka Schlatter, Kathrin Schmich, Ima Avalos Vizcarra, Peter Scheurich, Thomas Sauter, Christoph Borner, Michael Ederer, Irmgard Merfort, and Oliver Sawodny. On/off and beyond-a boolean model of apoptosis. *PLoS Comput Biol*, 5(12):e1000595, 2009.

[18] Guy Karlebach and Ron Shamir. Modelling and analysis of gene regulatory networks. *Nature Reviews Molecular Cell Biology*, 9(10):770–780, 2008.

[19] Nicolas Le Novère. Quantitative and logic modelling of molecular and gene networks. *Nature Reviews Genetics*, 16 (3):146–158, 2015.

[20] Wassim Abou-Jaoudé, Pauline Traynard, Pedro Tiago Monteiro, Julio Saez Rodriguez, Tomáš Helikar, Denis Thieffry, and Claudine Chaouiya. Logical modeling and dynamical analysis of cellular networks. *Frontiers in Genetics*, 7:94, 2016.

[21] Reka Albert and Juilee Thakar. Boolean modeling: a logic-based dynamic approach for understanding signaling and regulatory networks and for making useful predictions. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 6(5):353–369, 2014.

[22] Hidde De Jong. Modeling and simulation of genetic regulatory systems: a literature review. *Journal of computational biology*, 9(1):67–103, 2002.

[23] Nedumparambathmarath Vijesh, Swarup Kumar Chakrabarti, Janardanan Sreekumar, et al. Modeling of gene regulatory networks: A review. *Journal of Biomedical Science and Engineering*, 6(02):223, 2013.

[24] Ryan N Gutenkunst, Joshua J Waterfall, Fergal P Casey, Kevin S Brown, Christopher R Myers, and James P Sethna. Universally sloppy parameter sensitivities in systems biology models. *PLoS Comput Biol*, 3(10):e189, 2007.

[25] Dominik M Wittmann, Jan Krumsiek, Julio Saez-Rodriguez, Douglas A Lauffenburger, Steffen Klamt, and Fabian J Theis. Transforming boolean models to continuous models: methodology and application to t-cell receptor signaling. *BMC systems biology*, 3(1):1, 2009.

[26] Leon Glass and Stuart A Kauffman. The logical analysis of continuous, non-linear biochemical control networks. *Journal of theoretical Biology*, 39(1):103–129, 1973.

[27] Lotfi A Zadeh. Fuzzy sets. *Information and control*, 8(3):338–353, 1965.

[28] Jan Krumsiek, Sebastian Pölsterl, Dominik M Wittmann, and Fabian J Theis. Odefy-from discrete to continuous models. *BMC bioinformatics*, 11(1):1, 2010.

[29] Alejandro F Villaverde and Julio R Banga. Reverse engineering and identification in systems biology: strategies, perspectives and challenges. *Journal of The Royal Society Interface*, 11(91):20130505, 2014.

[30] Katia Basso, Adam A Margolin, Gustavo Stolovitzky, Ulf Klein, Riccardo Dalla-Favera, and Andrea Califano. Reverse engineering of regulatory networks in human b cells. *Nature genetics*, 37(4):382–390, 2005.

[31] Michael Hecker, Sandro Lambeck, Susanne Toepfer, Eugene Van Someren, and Reinhard Guthke. Gene regulatory network inference: data integration in dynamic models—a review. *Biosystems*, 96(1):86–103, 2009.

[32] Stuart A Kauffman. Metabolic stability and epigenesis in randomly constructed genetic nets. *Journal of theoretical biology*, 22(3):437–467, 1969.

[33] Bernard Derrida and Yves Pomeau. Random networks of automata: a simple annealed approximation. *EPL (Europhysics Letters)*, 1(2):45, 1986.

[34] Denis Thieffry. Dynamical roles of biological regulatory circuits. *Briefings in bioinformatics*, 8(4):220–225, 2007.

[35] Abhishek Garg, Alessandro Di Cara, Ioannis Xenarios, Luis Mendoza, and Giovanni De Micheli. Synchronous versus asynchronous modeling of gene regulatory networks. *Bioinformatics*, 24(17):1917–1925, 2008.

[36] Stefan Bornholdt. Boolean network models of cellular regulation: prospects and limitations. *Journal of the Royal Society Interface*, 5(Suppl 1):S85–S94, 2008.

[37] Maximino Aldana. Boolean dynamics of networks with scale-free topology. *Physica D: Nonlinear Phenomena*, 185 (1):45–66, 2003.

[38] Sui Huang, Gabriel Eichler, Yaneer Bar-Yam, and Donald E Ingber. Cell fates as high-dimensional attractor states of a complex gene regulatory network. *Physical review letters*, 94(12):128701, 2005.

[39] Elena Dubrova and Maxim Teslenko. A sat-based algorithm for finding attractors in synchronous boolean networks. *IEEE/ACM Transactions on Computational Biology and Bioinformatics (TCBB)*, 8(5):1393–1399, 2011.

[40] Luis Mendoza and Ioannis Xenarios. A method for the generation of standardized qualitative dynamical systems of regulatory networks. *Theoretical Biology and Medical Modelling*, 3(1):1, 2006.

[41] Martial Sankar, Karen S Osmont, Jakub Rolcik, Bojan Gujas, Danuse Tarkowska, Miroslav Strnad, Ioannis Xenarios, and Christian S Hardtke. A qualitative continuous model of cellular auxin and brassinosteroid signaling and their crosstalk. *Bioinformatics*, 27(10):1404–1412, 2011.

[42] Alessandro Di Cara, Abhishek Garg, Giovanni De Micheli, Ioannis Xenarios, and Luis Mendoza. Dynamic simulation of regulatory networks using squad. *BMC bioinformatics*, 8(1):1, 2007.

[43] L.A. Zadeh. Fuzzy sets. *Inf. Control*, 8(3):338–353, jun 1965.

[44] Elizabeth Ortiz-Gutiérrez, Karla García-Cruz, Eugenio Azpeitia, Aaron Castillo, María de la Paz Sánchez, and Elena R Álvarez-Buylla. A dynamic gene regulatory network model that recovers the cyclic behavior of arabidopsis thaliana cell cycle. *PLoS Comput Biol*, 11(9):e1004486, 2015.

[45] Luis Mendoza and Fátima Pardo. A robust model to describe the differentiation of t-helper cells. *Theory in biosciences*, 129(4):283–293, 2010.

[46] Akram Méndez and Luis Mendoza. A network model to describe the terminal differentiation of b cells. *PLoS Comput Biol*, 12(1):e1004696, 2016.

[47] Stephen L Nutt, Philip D Hodgkin, David M Tarlinton, and Lynn M Corcoran. The generation of antibody-secreting plasma cells. *Nature Reviews Immunology*, 15(3):160–171, 2015.

[48] Mary O'Riordan and Rudolf Grosschedl. Coordinate regulation of b cell differentiation by the transcription factors ebf and e2a. *Immunity*, 11(1):21–31, 1999.

[49] Thorsten Decker, Marina Pasca di Magliano, Shane McManus, Qiong Sun, Constanze Bonifer, Hiromi Tagoh, and Meinrad Busslinger. Stepwise activation of enhancer and promoter regions of the b cell commitment gene pax5 in early lymphopoiesis. *Immunity*, 30(4):508–520, 2009.

[50] Francisco Mora-López, Elena Reales, José A Brieva, and Antonio Campos-Caro. Human bsap and blimp1 conform an autoregulatory feedback loop. *Blood*, 110(9):3150–3157, 2007.

[51] Kuo-I Lin, Cristina Angelin-Duclos, Tracy C Kuo, and Kathryn Calame. Blimp-1-dependent repression of pax-5 is required for differentiation of b cells to immunoglobulin m-secreting plasma cells. *Molecular and cellular biology*, 22(13):4771–4780, 2002.

[52] Claudine Chaouiya, Aurelien Naldi, and Denis Thieffry. Logical modelling of gene regulatory networks with ginsim. *Bacterial Molecular Networks: Methods and Protocols*, pages 463–479, 2012.

[53] Christoph Müssel, Martin Hopfensitz, and Hans A Kestler. Boolnet—an r package for generation, reconstruction and analysis of boolean networks. *Bioinformatics*, 26(10):1378–1380, 2010.

[54] Gautier Stoll, Eric Viara, Emmanuel Barillot, and Laurence Calzone. Continuous time boolean modeling for biological signaling: application of gillespie algorithm. *BMC systems biology*, 6(1):116, 2012.

[55] Stefan Karl and Thomas Dandekar. Jimena: efficient computing and system state identification for genetic regulatory networks. *BMC bioinformatics*, 14(1):1, 2013.

[56] Tomáš Helikar, Bryan Kowal, Sean McClenathan, Mitchell Bruckner, Thaine Rowley, Alex Madrahimov, Ben Wicks, Manish Shrestha, Kahani Limbu, and Jim A Rogers. The cell collective: toward an open and collaborative approach to systems biology. *BMC systems biology*, 6(1):96, 2012.

[57] Rebekka Schlatter, Nicole Philippi, Gaby Wangorsch, Robert Pick, Oliver Sawodny, Christoph Borner, Jens Timmer, Michael Ederer, and Thomas Dandekar. Integration of boolean models exemplified on hepatocyte signal transduction. *Briefings in bioinformatics*, 13(3):365–376, 2012.

[58] Steffen Klamt, Julio Saez-Rodriguez, and Ernst D Gilles. Structural and functional analysis of cellular networks with cellnetanalyzer. *BMC systems biology*, 1(1):1, 2007.

8.2 APÉNDICE II: A Network Model to Describe the Terminal Differentiation of B Cells

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RESEARCH ARTICLE

A Network Model to Describe the Terminal Differentiation of B Cells

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Abstract

Terminal differentiation of B cells is an essential process for the humoral immune response in vertebrates and is achieved by the concerted action of several transcription factors in response to antigen recognition and extracellular signals provided by T-helper cells. While there is a wealth of experimental data regarding the molecular and cellular signals involved in this process, there is no general consensus regarding the structure and dynamical properties of the underlying regulatory network controlling this process. We developed a dynamical model of the regulatory network controlling terminal differentiation of B cells. The structure of the network was inferred from experimental data available in the literature, and its dynamical behavior was analyzed by modeling the network both as a discrete and a continuous dynamical systems. The steady states of these models are consistent with the patterns of activation reported for the Naive, GC, Mem, and PC cell types. Moreover, the models are able to describe the patterns of differentiation from the precursor Naive to any of the GC, Mem, or PC cell types in response to a specific set of extracellular signals. We simulated all possible single loss- and gain-of-function mutants, corroborating the importance of Pax5, Bcl6, Bach2, Irf4, and Blimp1 as key regulators of B cell differentiation process. The model is able to represent the directional nature of terminal B cell differentiation and qualitatively describes key differentiation events from a precursor cell to terminally differentiated B cells.

Author Summary

Generation of antibody-producing cells through terminal B cell differentiation represents a good model to study the formation of multiple effector cells from a progenitor cell type. This process is controlled by the action of several molecules that maintain cell type specific programs in response to cytokines, antigen recognition and the direct contact with T helper cells, forming a complex regulatory network. While there is a large body of experimental data regarding some of the key molecules involved in this process and there have been several efforts to reconstruct the underlying regulatory network, a general consensus

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about the structure and dynamical behavior of this network is lacking. Moreover, it is not well understood how this network controls the establishment of specific B cell expression patterns and how it responds to specific external signals. We present a model of the regulatory network controlling terminal B cell differentiation and analyze its dynamical behavior under normal and mutant conditions. The model recovers the patterns of differentiation of B cells and describes a large set of gain- and loss-of-function mutants. This model provides an unified framework to generate qualitative descriptions to interpret the role of intra- and extracellular regulators of B cell differentiation.

Introduction

Adaptive immunity in vertebrates depends on the rapid maturation and differentiation of T and B cells. While T cells originate cell-mediated immune responses, B cells are responsible for the humoral response of the organism by means of the production of high-affinity antibodies. B cells develop in the bone marrow from hematopoietic progenitors, and migrate as mature B cells (Naive) to the germinal centers (GCs), which are highly specialized environments of the secondary lymphoid organs [1]. There, B cells are activated by antigens (Ag) and undergo diversification of the B cell receptor (BCR) genes by somatic hypermutation (SHM), as well as the subsequent expression of distinct isotypes by class switch recombination (CSR) [2]. After the activation due to Ag recognition, Naive and GC B cells differentiate into antibody-producing plasma cells (PC), as well as memory cells (Mem) [3]. Cytokines secreted by T-helper cells, such as IL-2, IL-4 and IL-21 as well as the direct contact with these cells, mediated by the union CD40 receptor on B cells with its ligand CD40L, play a key role in the determination of B cell fate [4], since these external signals act as instructive cues that promote the differentiation from a cell progenitor to multiple cell types (Fig 1).

Terminal differentiation of B cells is controlled by the concerted action of multiple transcription factors that integrate physiologic signals in response to BCR cross-linking, extracellular cytokines, and the direct interaction with T cells, thus creating a complex regulatory network. These factors appear to regulate mutually antagonistic programs and can be divided into those that promote and maintain B cell identity, such as Pax5, Bcl6 and Bach2, and those that control differentiation into memory cells or plasma cells, i.e., Irf4, Blimp1 and XBP1, as has been shown by multiple functional, biochemical and gene expression analysis [5–7].

A type is characterized by the expression of a specific set of master transcriptional regulators. Naive B cells express Pax5 and Bach2, which are induced at the onset of B cell development, and are maintained through all developmental stages upon plasma cell differentiation [8, 9]. Furthermore, Pax5 is essential for the maintenance of B cell identity, since Pax5 deficiency results in the acquisition of multilineage potential [10]. Both Pax5 and Bach2 are required to inhibit PC differentiation [11, 12]. In addition to Pax5 and Bach2, GC cells express Bcl6, a transcription factor necessary for germinal center formation that allows the SHM and CSR processes to occur [13–15]. Development of B cells toward Mem cells requires Bcl6 downregulation and the induction of Irf4 [16, 17]. Conversely, PCs are characterized by the expression of Blimp1 and XBP1 that along with Irf4, inhibit the B cell identity program [5, 18].

Although a number of molecules that play a key role in the process of the terminal differentiation of B cells are known, it is not completely clear how such molecules regulate each other to ensure the proper appearance of GC, Mem, and PC from progenitor Naive B cells. There exist models describing several aspects of the differentiation of B cells such as the decisions promoting the developmental processes of CSR and SHM [19, 20], the response to environmental

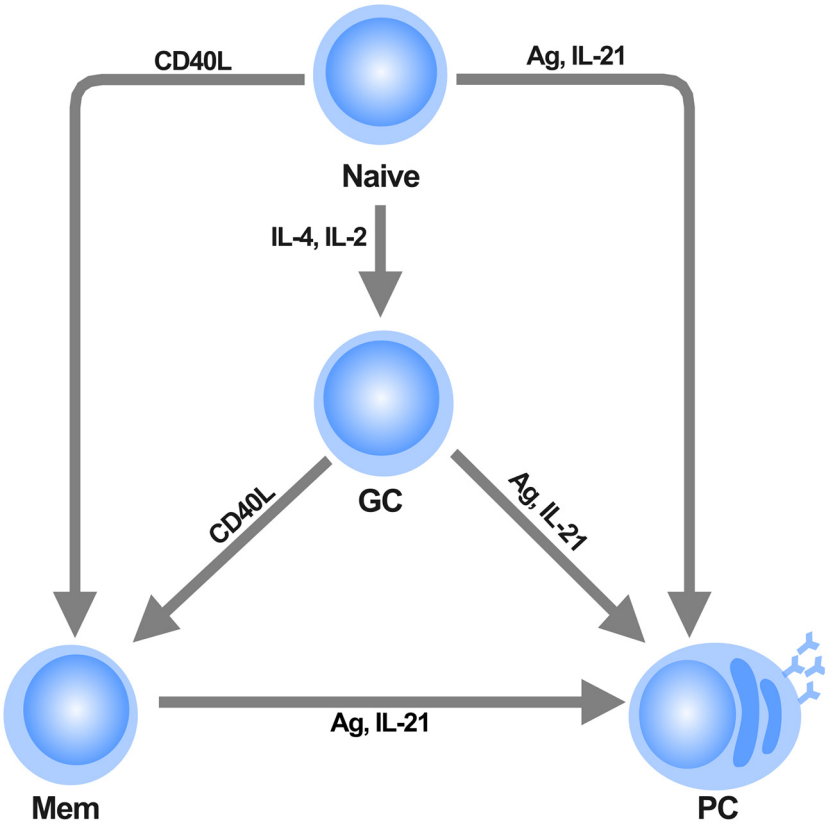

Fig 1. Terminal B cell differentiation. Precursor Naive B cells can differentiate into three possible cell types depending on proper molecular stimuli. Cytokines secreted by T-helper cells play a central role in the determination of B cell fate. IL-2 and IL-4 are required for the transition of Naive to GC cells. Direct contact of B cells with T cells by means of the CD40L receptor promote the differentiation of Naive or GC cells toward the Mem cell type. Antigen (Ag) activation drives terminal differentiation toward the PC cells, a process that is favored by the presence of IL-21.

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contaminants that disrupt B cell differentiation [21, 22], the B cell exit from the GC phase for the differentiation into plasma or memory cells [23], as well as the dynamics of B cell differentiation inside the complex microenvironment of germinal centers [24, 25]. Nonetheless, a general consensus about the regulatory network controlling cell fate decisions of B lymphocytes is lacking.

The modeling of regulatory networks has been shown to be a valuable approach to understand the way cells integrate several signals that control the differentiation process [26, 27]. In particular, the logical modeling approach has been useful to qualitatively describe biological processes for which detailed kinetic information is lacking [28]. This type of modeling usually focus on the nature and number of steady states reached by the network, which are often interpreted as stable patterns of gene expression that characterize multiple cell fates [29]. In this paradigm, the transit from one steady state to another occurs when cells receive a specific external stimuli, such as hormones, cytokines, changes in osmolarity, etc. These external stimuli are sensed and integrated to create an intracellular response that may trigger a global response such as cell growth, division, differentiation, etc. External signals are usually continuous in nature, i.e., they are present as concentration gradients of external molecules that may attain different values of strength and duration. Therefore it becomes desirable to develop models

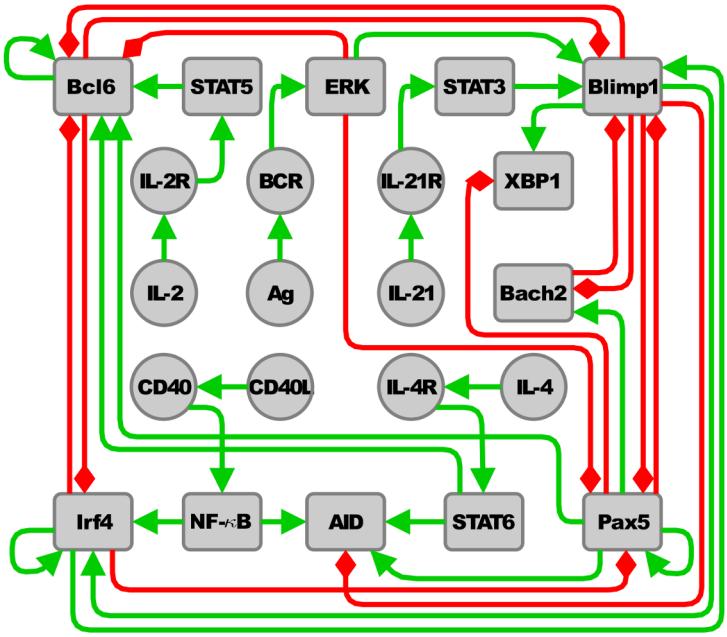

Fig 2. The regulatory network of B cells. Nodes represent molecules or molecular complexes. Positive and negative regulatory interactions among molecules are represented as green continuous arrows and red blunt arrows respectively.

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that incorporate the possibility of following the response of the network to continuous signals while at the same time, describe qualitatively the directional and branched nature of cell differentiation processes.

In this work we infer the regulatory network that controls the terminal differentiation of B cells. We then construct two dynamical systems, one discrete and one continuous, to analyze the dynamical properties of the regulatory network. Specifically, we find the stationary states of the models, and compare them against the known stationary molecular patterns observed in Naive, GC, Mem, and PC cells, under wild type and mutant backgrounds. Finally, we show that the dynamical models are able to describe the cellular differentiation pattern under a variety of external signals. Importantly, the models predict the existence of several interactions necessary for the network to ensure the proper pattern of terminal differentiation of B cells. Furthermore, the continuous model predicts the existence of intermediary states that could be reached by the network, but that have not been reported experimentally.

Results

We inferred the regulatory network that controls the terminal differentiation of B cells from experimental data available in the literature referring to the key molecules involved in the control of terminal B cell differentiation from the precursor B cell (Naive) to GC, Mem or PC cell types (Fig 2). The network contains 22 nodes representing functional molecules or molecular complexes, namely AID, Ag, Bach2, Bcl6, BCR, Blimp1, CD40, CD40L, ERK, IL-2, IL-2R, IL-4, IL-4R, IL-21, IL-21R, Irf4, NF-κB, Pax5, STAT3, STAT5, STAT6 and XBP1. These nodes have 39 regulatory interactions among them, being either positive or negative. S1 Table contains the set of key references used to infer the regulatory network depicted in Fig 2.

The regulatory network consists of two sets of nodes, i.e., those pertaining to a core module integrated by the master transcriptional regulators of terminal B cell differentiation (Bach2, Bcl6, Blimp1, Irf4, Pax5 and XBP1) and a set of nodes representing several signal transduction cascades (Ag/BCR/ERK, CD40L/CD40NF-κB, IL-2/IL-2R/STAT5, IL-4/IL-4R/STAT6 and IL-21/IL21R/STAT3) representing key external signals required for the control of the differentiation process. The nodes corresponding to these signaling pathways are active if a external stimuli is present, i.e., if a extracellular molecule is available, it is recognized by a specific receptor that transduce the signal by a messenger molecule, which in turn regulates the expression of the transcription factors in the core regulatory network. Most interactions among the nodes in Fig 2 were inferred from the literature. However, we found necessary to incorporate to the network the interactions Pax5 → Bcl6, Irf4 ⊣ Pax5 and the self-regulatory interactions Bcl6 → Bcl6, and Pax5 → Pax5 so as to obtain attractors with biological significance. Therefore, these four regulatory interactions constitute predictions of our model. The following paragraphs resume the reasons to incorporate such unreported interactions into our regulatory network model.

B cells develop in the bone marrow from hematopoietic progenitors that progressively lose its multipotent potential as they commit with the B cell lineage. This process strictly depends on the expression of Pax5, which induces chromatin changes of B cell specific genes and restricts the developmental potential of lymphoid progenitors by repressing genes associated with other cell type programs [30, 31]. Pax5 is upregulated at the onset of B cell development until differentiation to plasma cells [7]. During early stages of B cell development, Pax5 expression is positively controlled by the transcription factor Ebf1 [32], which in turn is activated by Pax5 [33], thus conforming a mutually activatory regulatory circuit that controls B cell identity. However, the signals that maintain Pax5 expression throughout late stages of B cell differentiation are not well understood. Therefore, a positive autoregulatory interaction for Pax5 was included in order to account for the direct mechanisms, possibly via the positive regulatory circuit between Ebf1 and Pax5, or indirect mechanisms, via other signals, that might sustain high Pax5 expression during late B cell differentiation.

Once B cells have completed their development in the bone marrow, they migrate to the bloodstream into the secondary lymphoid organs where they complete maturation throughout the germinal center reaction. The transcription factor Bcl6 is essential for germinal center formation, since Bcl6 deficiency results in the absence of germinal centers in mice [9, 15]. Given that Pax5 is required from the beginning of B cell development [10], it was necessary to include a positive regulatory signal from Pax5 to Bcl6 to keep Bcl6 in an active state when the Pax5 node is active.

A high expression of Bcl6 is required during the GC phase where it controls the expression of genes necessary for the germinal center program, such as DNA damage response and apoptosis, thus promoting the processes of SHM and CSR and cell proliferation [6]. It has been shown that mutations that disrupt a negative autoregulatory circuit deregulate Bcl6 expression and contribute to extensive proliferation in dense large B cell lymphoma (DLBCL) [34]. Moreover, it has been reported that in normal conditions there exist epigenetic mechanisms associated with positive regulation of Bcl6 expression during the GC phase that overcome its negative autoregulation [35, 36]. However, the precise mechanisms and signals that maintain high levels of Bcl6 in GCs are not fully understood. Therefore, we found necessary to include a positive autoregulatory interaction for Bcl6 in order to account for the possible role of these mechanisms in GC cell differentiation.

Direct contact of B cells with T cells mediated by the union of the CD40 receptor with its ligand CD40L induce the expression of Irf4 [16]. It has been shown that low levels of Irf4 promote the early B cell program, while high Irf4 levels inhibit GC program and promote differentiation toward the Mem or PCs in later stages of B cell differentiation [18, 37]. Given that Pax5

Table 1. Attractors of the discrete and continuous models of the B cell regulatory network.

	Naive		GC		Mem		PC	
Node	Disc.	Cont.	Disc.	Cont.	Disc.	Cont.	Disc.	Cont.
Bach2	1	1.0*E* + 0	1	1.0*E* + 0	1	1.0*E* + 0	0	1.1*E* – 22
Bcl6	0	1.8*E* – 22	1	1.0*E* + 0	0	1.4*E* – 22	0	1.0*E* – 22
Blimp1	0	2.3*E* – 22	0	1.3*E* – 22	0	1.3*E* – 22	1	1.0*E* + 0
Irf4	0	1.7*E* – 22	0	9.7*E* – 23	1	1.0*E* + 0	1	1.0*E* + 0
Pax5	1	1.0*E* + 0	1	1.0*E* + 0	1	1.0*E* + 0	0	1.0*E* – 22
XBP1	0	2.3*E* – 22	0	1.2*E* – 22	0	9.9*E* – 23	1	1.0*E* + 0

For the continuous system we present averages from a total of 500,000 runs from random initial states. The standard deviation are smaller than $1E^{-22}$ in all cases. For simplicity, only the nodes conforming the network core are shown. The rest of nodes belong to the signal transduction cascades, and all of them are in the inactive state, *i.e.* 0.

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is an essential regulator of the B cell identity program [10], a negative interaction between Pax5 and Irf4 was incorporated to simulate a constant activation of the Pax5 circuit when the Irf4 node is low and to inhibit Pax5 activation when Irf4 present at high levels.

Attractors of the wild type network

We studied the dynamical behavior of the discrete and the continuous systems so as to obtain their attractors. The discrete version of the B cell regulatory network was studied by exhaustively testing the behavior of the network from all possible initial conditions. The system reaches exactly four fixed point attractors, shown in Table 1. Notably, there is a one-to-one relation of these four attractors with the expression patterns of the cell types shown in Fig 1.

We labeled the attractors as Naive, GC, Mem, and PC. It is important to remember that each attractor represents a different configuration pattern of the network at the steady state. Specifically, the first attractor, where the nodes Pax5 and Bach2 are active, can be interpreted as the activation pattern of Naive cells. The second attractor, with high levels of Bcl6, Pax5, and Bach2, corresponds to the GC cell type. The third attractor, with high levels of Irf4, Pax5, and Bach2, along with the absence of Bcl6 can be interpreted as the Mem cell fate. Finally, the fourth attractor, with high Blimp1, Irf4, and XBP1, corresponds to the pattern of the cell type PC (Fig 3).

In the discrete version of the model, the set of initial states draining to the attractors, i.e the basins of attraction, do not partition the state space evenly. The percentage of initial states leading to each of the attractors were as follows: Naive = 56.25%, GC = 6.25%, Mem = 6.25%, PC = 31.25%. The size of the basins reflects how an attractor can be attained from different initial configurations, and may indicate the relative stability of such steady state [38]. It has been suggested that different basins represent stable or semistable cellular differentiation states [29]. Moreover, in order to transit form one steady state to another, a specific external signal would need to trigger a response in order to overcome the basin of attraction such that a different attractor could be reached by the system. Configurations with larger basins can be easily reached from many initial states, therefore, different perturbations could be buffered and canalized by the network towards a particular steady state [39].

Since the Naive and PC states have larger basins than that for GC or Mem attractors, it is possible to suggest that the former are relatively more stable than the later. Importantly, the proportion of basin sizes of the Naive, GC and Mem attractors agree with *in vivo* measures of B

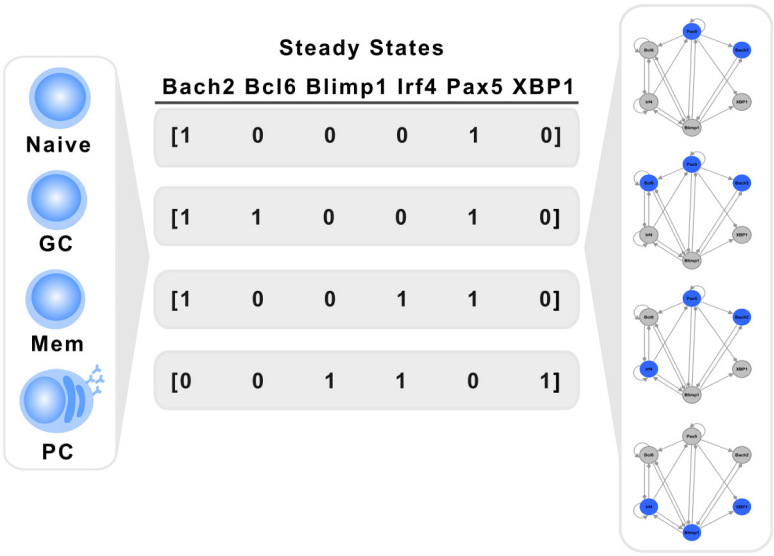

Fig 3. Attractors and cell types The stationary states of the regulatory network correspond to multiple activation patterns that characterize different cell types.

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cells where the Naive progenitor is more abundant in proportion than the other three cell types [40, 41]. However in spite of the low abundance of PC cells *in vivo* as a result of a selection process of B lymphocytes during the germinal center reaction, the largest basins corresponding to the progenitor Naive and terminally differentiated PC cells suggest that the regulatory network assures the formation of these cell types in a robust manner.

Contrary to discrete systems, continuous dynamical systems have an infinite number of possible initial states so that the search for attractors by sampling a large number of random initial states can lead to the possibility to miss attractors with small basins of attraction. Indeed, the sampling of initial states resulted in the finding of only four attractors for the continuous model, which resulted identical to the attractors of the discrete model, see Table 1. Therefore, to find possible missing attractors we made an exhaustive perturbation study by temporarily modifying the activation state of each node in the four attractors found by random sampling [42]. With this approach we found three more fixed point attractors in the continuous model. These extra attractors are characterized by intermediate values of activation of the nodes conforming the network core and do not have a counterpart in the discrete model, since the discrete model can attain only 0 or 1 activation values (Table 2).

These attractors with intermediate values may represent possible unstable activation states that can be reached by the system but have not been yet experimentally observed or may correspond to transient differentiation states. Indeed, one of the attractors ("New3" attractor) found in Table 2 shows intermediate levels of Bcl6 and Irf4, in spite of the antagonistic role of these two factors, suggesting that low levels of Irf4 controls the establishment of stationary states prior to Bcl6 downregulation. This attractor may correspond to the known activation pattern of centrocytes, which are $Irf4^{int}$, $Bcl6^{hi}$ B cells exiting the GC reaction that represent an intermediate cellular state between GC and PC cells [43]. This result supports the role of Irf4 as a regulator of the differentiation process prior the terminal differentiation to PCs since it has been observed that intermediate levels of Irf4 promote the GC program, whereas high levels of Irf4 promote Bcl6 downregulation and further PC differentiation as B cells exit the germinal center [44].

Table 2. Fixed point attractors of the continuous system not found in the random search.

	Attractor		
Node	New1	New2	New3
AID	0	0	0
Ag	0	0	0
Bach2	1	1	1
Bcl6	0.5	0	0.5
BCR	0	0	0
Blimp1	0	0	0
CD40	0	0	0
CD40L	0	0	0
ERK	0	0	0
IL-2	0	0	0
IL-2R	0	0	0
IL-4	0	0	0
IL-4R	0	0	0
IL-21	0	0	0
IL-21R	0	0	0
Irf4	0	0.5	0.5
NF-κB	0	0	0
Pax5	1	1	1
STAT3	0	0	0
STAT5	0	0	0
STAT6	0	0	0
XBP1	0	0	0

Three fixed point attractors were found with the perturbation analysis that has not been found in the random search. These attractors are characterized by intermediate values of the nodes.

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The differentiation process

The B cell regulatory network is able to describe the differentiation process outlined in Fig 1, from the Naive precursor to any of the GC, Mem, or PC cell types by means of sequential pulses of extracellular signals known to direct terminal B cell differentiation (Fig 4). The system is initialized starting from the Naive attractor, and the system is perturbed at a time $t \approx 25$ with a single high pulse of IL-2 or IL-4 for 2 or more time units. Computationally, this is achieved by fixing the variable $IL-4 = 1$ and the equation $\frac{dIL-4}{dt} = 0$ for the indicated period of time. This signal was intended to mimic the effect of subjecting the Naive cell to a saturating extracellular concentration of IL-2 or IL-4 for a brief incubation time. After the pulse, the entire system was left to evolve until it converged. This perturbation is sufficient to move the dynamical system to the GC attractor which is in agreement with the observations that IL-2 and IL-4 promote B cell proliferation and germinal center formation, and are also necessary signals for the transition of Naive B cells to GC B cells [45–47].

Differentiation of either Naive or GC cells to Mem cells is mediated by the activation of the CD40 receptor by its ligand CD40L [48], which leads to Irf4 induction and to the repression of Bcl6 [16]. Our model recovers these differentiation routes with a saturating activation of CD40L for ≈2 or more abitrary time units, which leads to the activation of Irf4 node when the

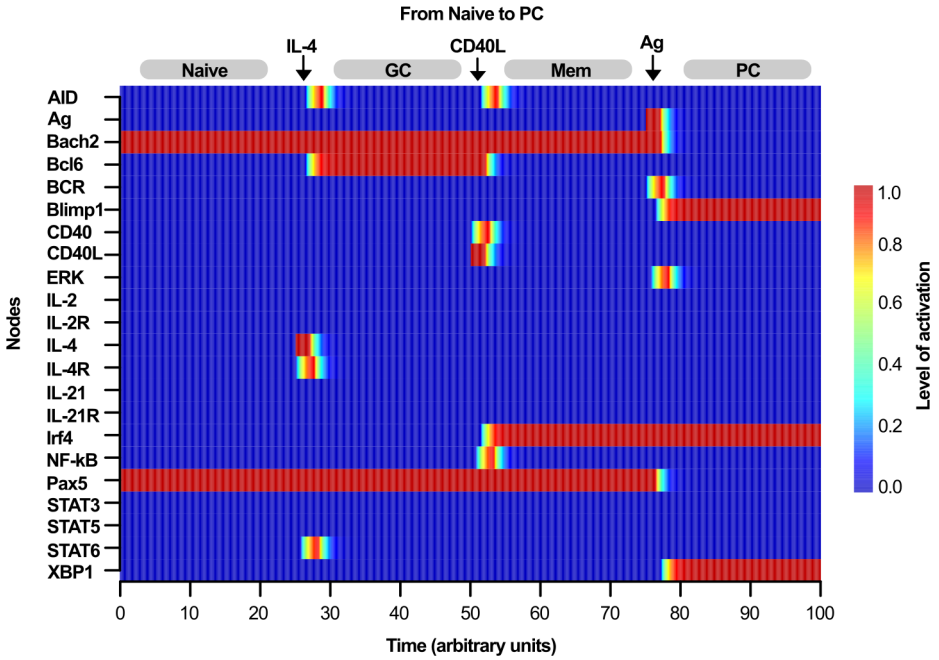

Fig 4. Differentiation from Naive to the PC cell type. The changes in the activation of all nodes of the network are shown as a heatmap which scales from blue to red as the activation level goes from 0 to 1, respectively. Extracellular signals are simulated as a burst for two or more units of time (arrows). Starting from the Naive ($Bach2^+$, $Pax5^+$) stationary state ($t = 0$ to $t \approx 25$), the system moves to the GC attractor ($Bach2^+$, $Bcl6^+$, $Pax5^+$) due to the presence of a simulated pulse of IL-4 ($t \approx 25$) which in turn transit to the Mem attractor ($Bach2^+$, $Irf4^+$, $Pax5^+$) due to the action of CD40L ($t \approx 55$) and finally, Mem attractor moves to the PC state ($Blimp1^+$, $Irf4^+$) by the presence of Ag signal ($t \approx 75$).

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Pax5 node is active and Blimp1 is not present. Activation of Irf4 downregulates Bcl6 and directs the transition from the GC to the Mem attractor of the dynamical system, see Fig 4.

Similarly, starting from any of the Naive, GC, or Mem attractors, the system is able to move to the PC attractor by applying a saturating signal of either IL-21 or Ag. This is consistent with the experimental reports where BCR activation by Ag induce Blimp1 upregulation, as well as Pax5 and Bcl6 downregulation thus promoting plasma cell differentiation from either Naive, GC, or Mem cell types [49–51]. This process is facilitated by the presence of IL-21 which is transduced by STAT3 [52, 53].

For both the discrete and continuous models we obtained the same biological relevant transition paths that describe the wild type differentiation pattern outlined in Fig 1. However, given that the continuous model has 7 fixed-point attractors, its complete fate map is larger than that for the discrete model (S1 Fig). Nonetheless, the continuous model also presents the known biologically relevant transitions.

It has been suggested that progression toward a terminal differentiated state involves several epigenetic changes that reduce the options of a cell to differentiate to other cell types, possibly by several mechanisms that constraint the function of the components of a regulatory network thus reducing the dimensionality of the state space and controlling the compartmentalization of this space into basins of attraction with different sizes [29]. Therefore, the presence of external signals could affect the way the nodes of the network activate in response to these signals which in turn regulate the activation of multiple parts of the network to control the establishment of stationary states of the system and the transitions between these states. Interestingly,

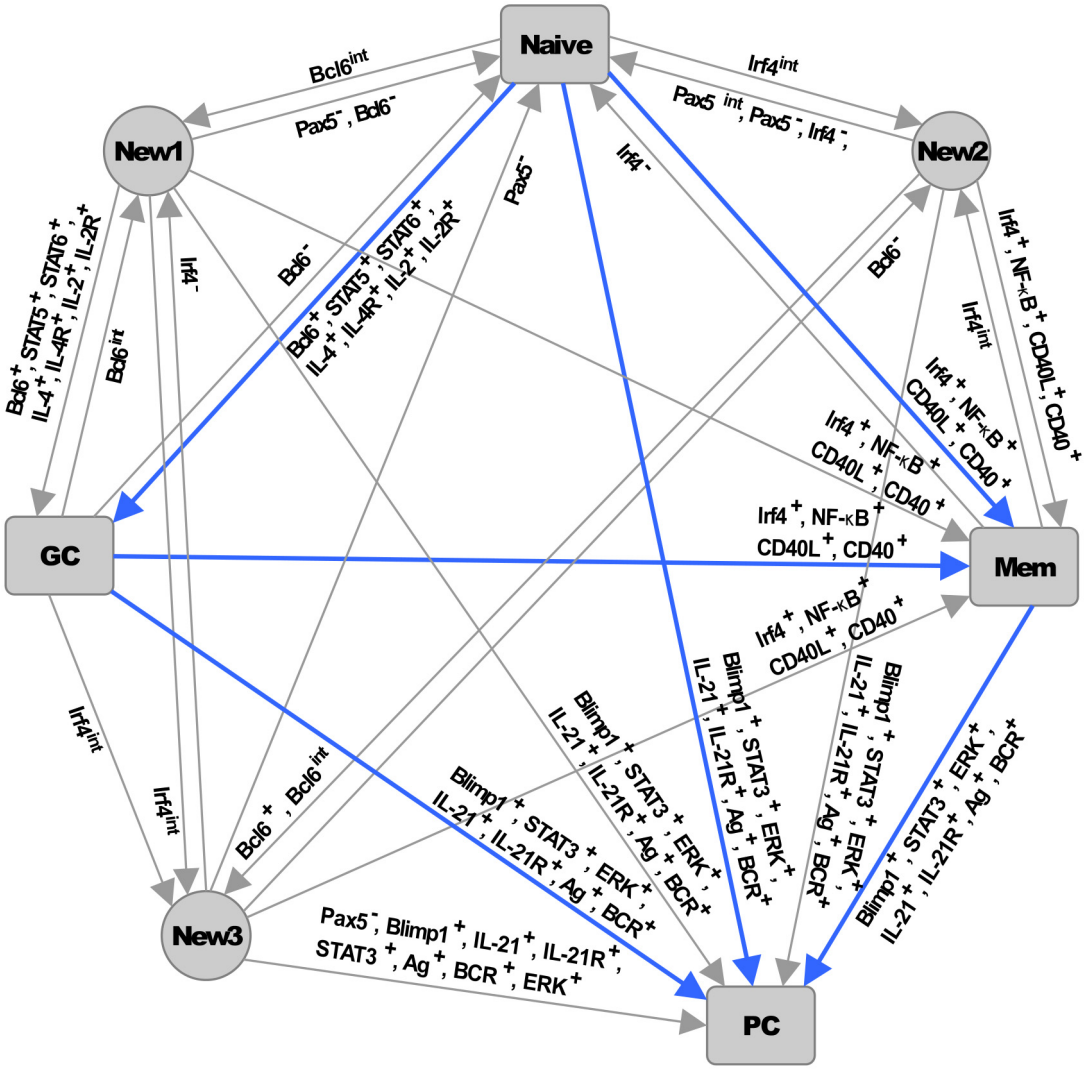

Fig 5. Complete fate map. Nodes represent the fixed point attractors, and the edges correspond to all the possible single-node perturbations able to move the system from one attractor to another. For the continuous model, perturbations are simulated by temporarily change the value of a single node to 0, 1 or 0.5, represented by the symbols "–", "+" and "*int*", respectively. For example, IL-2^{+} means that a temporal activation of IL-2 is able to cause the system to move from the Naive attractor to the GC attractor. Biologically relevant differentiation routes are represented as blue arrows.

doi:10.1371/journal.pcbi.1004696.g005

no transitions from the PC state to other attractors were obtained in any of the two models, suggesting that the network controls B cell differentiation towards an effector cell fate in an irreversible manner while allowing the transition between precursor cell fates (Fig 5).

Simulation of mutants

To gain further insight of the dynamical behavior of the B cell regulatory network we systematically simulated all possible single loss- and gain-of-function mutants and evaluated the severity of each mutation by comparing the resulting attractors with those of the wild type model. Loss-of-function mutations were simulated by fixing at 0 the value of a node, whereas gain-of-function was simulated by fixing at 1 the same activation state of a node. For each mutant, its attractors were found, exhaustively in the case of the discrete model, and for the continuous

Table 3. Simulated null mutant attractors.

Mutant model	Obtained pattern	Effect	References
Bach2	[0, 0, 0, 0, 1, 0] Naive-like	Only similar attractors to the wild type fates were found.	[9, 12, 19]
	[0, 1, 0, 0, 1, 0] GC-like		
	[0, 0, 0, 1, 1, 0] Mem-like		
	[0, 0, 1, 1, 0, 1] PC		
Bcl6	[1, 0, 0, 0, 1, 0] Naive	Loss of GC attractor.	[13–15, 57, 58]
	[1, 0, 0, 1, 1, 0] Mem		
	[0, 0, 1, 1, 0, 1] PC		
Blimp1	[1, 0, 0, 0, 1, 0] Naive	Loss of PC attractor. A distinct attractor with high Irf4 levels found.	[54, 56, 59]
	[1, 1, 0, 0, 1, 0] GC		
	[1, 0, 0, 1, 1, 0] Mem		
	[0, 0, 0, 1, 0, 0] Other		
Irf4	[1, 0, 0, 0, 1, 0] Naive	Only Naive and GC attractors are reached by the network. Loss of Mem and PC attractors.	[18, 37, 60, 61]
	[1, 1, 0, 0, 1, 0] GC		
Pax5	[0, 0, 1, 1, 0, 1] PC	Inactivation of Pax5 drives the system to the PC state. An attractor not reported in literature was found.	[8, 30, 62, 63]
	[0, 0, 0, 0, 0, 0] Other		
XBP1	[1, 0, 0, 0, 1, 0] Naive	Mild effect over the PC attractor. Naive, GC and Mem attractors are not affected	[64, 65]
	[1, 1, 0, 0, 1, 0] GC		
	[1, 0, 0, 1, 1, 0] Mem		
	[0, 0, 1, 1, 0, 0] PC-like		

Null mutant attractors. The attractors found for each null mutant model and the literature supporting its effect are summarized, for simplicity, only the patterns of activation for the nodes that conform the core of the network, namely Bach2, Bcl6, Blimp1, Irf4, Pax5 and XBP1 are shown. The steady state pattern for each mutant is shown in the following order: [Bach2, Bcl6, Blimp1, Irf4, Pax5, XBP1].

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version, by running the dynamical system from 5000 random initial states and solving the equations numerically until the system converged. Tables 3, 4 and 5 shows that the mutants can be grouped according to whether its effect results in the loss of one or more attractors with respect to the wild type model or if it results in the appearance of atypical attractors not found in the wild type model.

Importantly, both the discrete and continuous versions of the model were able to describe most of the reported mutants for the six master regulators that conform the core of the network. For instance, the simulated loss-of-function of the Blimp1 node results in the disappearance of the PC attractor, which is in accordance with the experimentally acknowledged role of Blimp1 as an essential regulator for PC differentiation [54]. Although absence of Blimp1 in B cells impedes PC differentiation, it does not affect the establishment of Naive, GC or Mem cell types [54–56], which is in turn reflected by the model since the network reaches all the Naive, GC and Mem attractors in spite of the loss-of-function of the Blimp1 node (Table 3).

Additionally, for Blimp1 null mutant a distinct attractor was found showing low Pax5 and high Irf4 levels. It has been reported that Pax5 inactivation along with Irf4 induction precedes Blimp1 expression and while Irf4 activation is not sufficient to rescue PC differentiation in the absence of Blimp1, the coordinate expression of both factors is necessary for complete terminal

Table 4. Simulated constitutive mutant attractors.

Mutant model	Obtained pattern	Effect	References
Bach2	[1, 0, 0, 0, 1, 0] Naive	Loss of PC attractor. An attractor with active Bach2 and Irf4 was found.	[12]
	[1, 1, 0, 0, 1, 0] GC		
	[1, 0, 0, 1, 1, 0] Mem		
	[1, 0, 0, 1, 0, 0] Other		
Bcl6	[1, 1, 0, 0, 1, 0] GC	Only GC and similar attractor are reached.	[34, 66, 69–71]
	[1, 1, 0, 1, 1, 0] Other		
	[0, 1, 0, 1, 0, 0] Other		
Blimp1	[0, 0, 1, 1, 0, 1] PC	The system stays in the PC state. Loss of Naive, GC and Mem attractors.	[5, 72–76]
Irf4	[1, 0, 0, 1, 1, 0] Mem	The system reaches only the Mem and PC attractors.	[18, 44, 70]
	[0, 0, 1, 1, 0, 1] PC		
Pax5	[1, 0, 0, 0, 1, 0] Naive	Loss of PC attractor, the other three wild type activation patterns are reached by the network.	[63, 67, 77, 78]
	[1, 1, 0, 0, 1, 0] GC		
	[1, 0, 0, 1, 1, 0] Mem		
XBP1	[1, 0, 0, 0, 1, 1] Naive-like	Activation of XBP1 node does not affects the establishment of any of the Naive, GC, Mem or PC attractors. Only similar attractors to the wild type patterns were found.	[54]
	[1, 1, 0, 0, 1, 1] GC-like		
	[1, 0, 0, 1, 1, 1] Mem-like		
	[0, 0, 1, 1, 0, 1] PC		

Constitutive mutant attractors. The attractors found for each mutant model and the literature supporting its effect are summarized, for simplicity, only the patterns of activation for the nodes that conform the core of the network, namely Bach2, Bcl6, Blimp1, Irf4, Pax5 and XBP1 are shown. The steady state pattern for each mutant is shown in the following order: [Bach2, Bcl6, Blimp1, Irf4, Pax5, XBP1].

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B cell differentiation [56]. Therefore, this attractor may represent a cellular state prior to the PC state.

Similarly to the Blimp1 null mutation, the simulated gain-of-function mutants for the Pax5, Bcl6 or Bach2 nodes also result in the loss of the PC attractor but the other three wild type activation patterns are still reached by the network (Table 4), the constitutive activation of any of these nodes maintains the system in attractors corresponding to precursor B cell fates, in accordance with the observations showing that forced expression of Pax5 or Bach2 in mature B cells inhibit terminal differentiation to PCs and are required to maintain the B cell identity program [12, 66, 67]. Moreover, for the Bach2 gain-of-function model an additional attractor was found. This attractor is characterized by high levels of Bach2 and Irf4 and low Pax5 in a pattern similar to the Mem attractor, this attractor may correspond to a state previous to PC differentiation where Bach2 avoids Blimp1 activation when Pax5 is inactive [56, 68].

The simulated Irf4 loss-of-function results in the loss of PC and Mem cell attractors (See Table 3). Since Irf4 deficient B cells are unable to differentiate into Mem and PCs, the attractors

Table 5. Summary of the simulated mutants and external signals.

Mutant models and simulated signals	Resulting attractors with respect to the wild type model
$Bach2^+$, $Bcl6^+$, $Blimp1^-$, $Irf4^-$, $Pax5^+$	Loss of PC attractor
$Bcl6^+$, $IL\text{-}2^+$, $IL\text{-}2R^+$, $STAT5^+$, $IL\text{-}4^+$, $IL\text{-}4R^+$, $STAT6^+$, $Irf4^+$, $CD40L^+$, $CD40^+$, $NF\text{-}\kappa B^+$, $Blimp1^+$, Ag^+, BCR^+, ERK^+, $IL\text{-}21^+$, $IL\text{-}21R^+$, $STAT3^+$	Loss of Naive attractor
$Bach2^+$, $Bach2^-$, $XBP1^+$	Replaced Naive, Mem and GC attractor by similar ones
$Bcl6^-$, $IL\text{-}21^+$, $IL\text{-}21R^+$, $STAT3^+$, Ag^+, BCR^+, ERK^+, $CD40L^+$, $CD40^+$, $NF\text{-}\kappa B^+$	Loss of GC attractor
$Bcl6^+$, $IL\text{-}4^+$, $IL\text{-}4R^+$, $STAT6^+$	Only the GC and GC-like attractors are found
$Blimp1^-$, $Pax5^-$	Atypical attractor found
$Blimp1^+$, Ag^+, BCR^+, ERK^+, $IL\text{-}21^+$, $IL\text{-}21R^+$, $STAT3^+$	Only PC attractor is found
$Irf4^+$, $CD40L^+$, $CD40^+$, $NF\text{-}\kappa B^+$	Only Mem and PC attractors are found
$Irf4^-$	Loss of Mem and PC attractors
$Irf4^-$, Ag^+, BCR^+, ERK^+	Loss of Mem attractor
$XBP1^+$	Replaced PC attractor by a similar one

Effect of all possible single gain- and loss-of-function mutants of the B cell model with respect to wild type, as reflected by their type of attractors. Symbols "+" and "–" after a node name denote gain-of-function and loss-of-function mutations, respectively. The effect of the continued activation of the nodes pertaining to signaling pathways is also indicated with the symbol "+" and summarized in the table.

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found for this mutant support the role of Irf4 in the formation of PC and Mem cell types [18, 37, 60, 61]. Induction of Irf4 promotes the formation of Mem cells and PC differentiation [18, 44, 70], which is also described by the model as simulated gain-of-function of the Irf4 recovers only two attractors corresponding to the Mem and PC states. Therefore, constitutive activation of the Irf4 node drives the system to the Mem and PC cell fate states.

Conversely, constitutive activation of the Bcl6 node results into three attractors, one of them corresponds to the GC cell pattern, the other two attractors correspond to patterns where Bcl6 is active along with Irf4. These activation patterns coincide with the expression patterns observed for centrocytes, which are $Bcl6^+$ $Irf4^+$ B cells exiting from the GC reaction [79]. This result suggest that sustained activation of the Bcl6 node drives the system to a GC or GC-like state, in accordance with the reported observations where Bcl6 enforced expression in B cells blocks terminal differentiation and regulates GC formation [34, 66, 71, 80].

Bach2 null mutation does not affects the formation of any of the Naive, GC, Mem or PC cell types, thus confirming its role as a dispensable regulator of B cell terminal differentiation, but a necessary negative regulator for Blimp1 expression and PC formation. Only similar attractors to the wild type fates were found [9, 12, 19].

Bcl6 null mutant mice does not form GC cells but differentiation to Naive, Mem or PC cell types is not affected. Also, Bcl6-deficient B cells can differentiate into Mem cells or PC independently of germinal center reactions. Accordingly the GC attractor is lost in the simulated Bcl6 loss-of-function mutant [13–15, 57, 58].

Deletion of Pax5 in mice results in the loss of B cells from early pro-B stage. Inactivation of Pax5 in mature B cells results in the repression of genes necessary for B cell identity. Pax5 deficient B cells differentiate towards the PC cell fate and show Blimp1 up-regulation. Conditional inactivation of Pax5 in mice mature B cells promotes differentiation toward PCs, in line with

the PC attractor found for this mutant. An attractor not reported in literature was found which may correspond to the total loss of expression of the B cell lineage factors [8, 30, 62, 63].

XBP1 is not strictly required for initiation of PC cell differentiation or for previous differentiation stages of terminal B cell differentiation. The network reaches all the wild type attractors [64, 65].

Forced expression of Blimp1 promotes terminal differentiation to PC cells. Only the PC attractor was found for this simulated mutant [5, 72–76].

Loss-of-function of XBP1 affects subsequent PC development but it does not impairs B cell differentiation or the establishment of any of the Naive, GC, Mem and PC cell types [54]. Accordingly, similar attractors to the wild type patterns were found.

It is important to note that not all single loss- or gain-of-function mutants have a severe effect on the dynamics of our B cell differentiation model, since simulated Bach2 and XBP1 constitutive and null mutations result in attractors similar to the wild type, suggesting that these nodes have only a mild effect on the global behavior of the network. However, the Bach2 node is not dispensable since the constitutive activation of this node avoided the network for reach the PC attractor, in accordance with its biological role as an inhibitor of PC differentiation [12]. These results show the contribution of each node to the dynamics of network and therefore indicate the importance of these factors as regulators of the differentiation process.

Given that the expression patterns defining each cell type are controlled by the core module of the regulatory network, the attractors found for the wild-type models as well as for the single loss- and gain-of-function mutants persist even in the absence of external signals. However, as mentioned in the above paragraphs, external stimuli can drive the system from one steady state to another, thus affecting the way the network controls the establishment of different expression patterns. Therefore, we simulated the continuous presence of external signals by fixing the activation value of the nodes representing signaling pathways, namely Ag, BCR, CD40, CD40L, ERK, IL-2, IL-2R, IL-4, IL-4R, IL-21, IL-21R, NF-κB, STAT3, STAT5, and STAT6, in order to analyze how its continued activation influences the behavior of the core regulatory network affecting the appearance and maintenance of multiple cell fates. For clarity, the effect of the continued stimulation by external signals and the effect of the simulated mutants on the stationary patterns reached by the network is summarized in Table 5.

Discussion

The hematopoietic system is well characterized at the cellular level, and there exist several efforts to reconstruct and analyze parts of its underlying molecular regulatory network to understand the differentiation process of multiple cell types. Network modeling has become an appropriate tool for the systematic study of the dynamical properties of specific regulatory networks and signaling pathways. The dynamic behavior of even relatively simple networks is neither trivial nor intuitive. Moreover, experimental information about the kinetic parameters of the molecules conforming such networks is generally lacking. However, the use of qualitative methods shows that it is possible to predict the existence of expression patterns or pointing at missing regulatory interactions.

The model presented in this work describes the activation states observed experimentally for Naive, GC, Mem and PC cell types. This model is also able to describe the differentiation pattern from Naive B cells to GC, Mem and PC subsets in response to specific external signals. Despite the lack of qualitative information it was possible to reconstruct the regulatory network of B cells and propose a basic regulatory architecture. This model propose the existence of some missing regulatory interactions and activation states not documented in the literature that might play an important role in the context of terminal B cell differentiation. Importantly,

these interactions constitute specific predictions that can be tested experimentally. It is also relevant to stress that the proposed regulatory interactions might be attained by way of intermediary molecules not included in the regulatory network. This is so because the whole network modeling approach is based upon the net effect of one node over another, focusing on whether the flow of information is known, rather than relying on the direct physical contact between molecules. Furthermore, the results suggest that the dynamical behavior of the B cell regulatory network is to a large extent determined by the structure of the network rather than the detail of the kinetic parameters, in accordance to analyses of related models [42, 81, 82].

While Boolean networks constitute a valuable modeling approach of choice whenever there is only qualitative data available, for this biological system we wanted to incorporate qualitative continue variables that in addition to the identification of the stationary states as in the discrete model, allows for the analysis of the effect of gradients of external signals. The dynamical behavior of the model resembles the qualitative behavior of the differentiation process by recovering the transition of the system from a Naive state to the terminally differentiated PC state under the presence of external signals. This result recapitulates the directional and branched nature of B cell differentiation events and supports the key role of extracellular signals in the maintenance and instruction of the differentiation process. Importantly, the model allows the exploration of system transitions that describe the differentiation form one cell type to another, it is interesting to note that no transitions from the PC state to other attractors were obtained, suggesting that the B cell regulatory network assures the differentiation towards an effector cell fate in an irreversible manner whereas allowing plasticity of the precursor cell fates.

There are several ways in which our model could be improved in future versions. One general change may be the implementation of the model as a stochastic dynamical system. Although both the stochastic and deterministic models retain the same steady states, the implementation as a stochastic system could be useful to generate information about the probability of the cells to transit from one state to another.

Another possible route of refinement of the models would be the inclusion of a specific time scale. Both the discrete and continuous models presented here use qualitative modeling frameworks, with results having arbitrary time units. In order to incorporate phenomena with specific timescales, it will be necessary either to calibrate the continuous dynamical system by scanning for appropriate values for the parameters, or alternatively make use of a quantitative modeling framework. Also, it possible to add other layers of regulation to the model, for example by incorporating the effect of chromatin remodeling on the availability of some genes. However, given that we were able to recover with a small qualitative network the basic patterns of activation, it is possible that the role played by the levels of regulation not included in the present model may significantly reduce the number of possible transitory trajectories of the system, instead of determining nature and number of the stationary states themselves.

Finally, despite the qualitative nature of the model presented here, we believe it might be used as seed to analyze important biological and clinical phenomena, given that deregulation of the master regulators included in the network are known to be involved in oncogenic events occurring in multiple lymphomas. For instance, aberrant expression of Bcl6 may lead to constitutive repression of genes necessary for exit of the GC program and normal differentiation, therefore contributing to lymphomagenesis [83]. In addition, activation of Irf4 leads to extensive cell proliferation and survival [84]. The present model could serve as a starting framework to test different hypothesis regarding the possible routes by which the expression of the aforementioned factors and other components of the network could be regulated in order to find therapeutic intervention strategies or to test how deregulation of the known mechanisms could lead to pathological conditions, thus contributing to our knowledge on the development of lymphomas.

Materials and Methods

Molecular basis of the B cell regulatory network

We inferred the regulatory network controlling terminal B cell differentiation from experimental data available in literature. The evidence used to recover the nodes and interactions of the B cell regulatory network (Fig 2) is summarized in the following paragraphs. The transition from Naive B cells to GC, Mem, and antibody-secreting PCs is regulated by the coordinated activity of transcription factors that act as key regulators of the differentiation process. These factors appear to regulate mutually antagonistic genetic programs and can be divided into those that promote and maintain the B cell program, such as Pax5, Bcl6, and Bach2, and those that control terminal differentiation into memory cells or plasma cells, such as Irf4 and Blimp1 and XBP1 [7].

Pax5 functions as the master regulator of B cell identity, it is expressed at the onset of B cell differentiation and is maintained in all developmental stages of B cells upon commitment to plasma cells. Pax5-deficiency results in the loss of B cell identity and the acquisition of multilineage potential [10]. Pax5 directly inhibits Blimp1 transcription by binding to the promoter of *Prdm1* the gene encoding Blimp1 [11]. In turn, Blimp1 represses Pax5 [78], thus conforming a mutually exclusive regulatory circuit. Along with Pax5, Bach2 avoids PC differentiation and promotes class switch recombination by repressing Blimp1 through binding to a regulatory element on the *Prdm1* gene [12]. Bach2 is positively regulated by Pax5 [31], while being repressed by Blimp1 in PCs, thus creating a mutual inhibition feedback loop [19].

Bcl6 expression is induced upon arrival of Naive B cells into the germinal centers. Bcl6 is a transcription factor essential for germinal center formation, since deficiency of Bcl6 results in the absence of germinal centers in mice [14, 15]. The signals that promote high Bcl6 expression in GC cells are not fully understood. However, it has been shown that mutations that disrupt a negative autoregulatory circuit of Bcl6 deregulate its expression and promote the proliferation of GC cells in dense large B cell lymphomas (DLBCL) [34]. Moreover, it has been reported that there exists a positive regulatory mechanism controlling high Bcl6 expression during the GC phase that overcome its negative autoregulation [35, 36]. In accordance with these data, we found necessary to include in our model a positive autoregulatory interaction for Bcl6 (Bcl6 → Bcl6) in order to account for the required signals that maintain high Bcl6 activation levels in GC cells.

Additionally, the presence of IL-2 and IL-4 produced by follicular T helper cells play an important role in the transition from Naive to GC cells, as these signals are required for the maintenance and proliferation of GC cells. IL-2/IL-2R and IL-4/IL-4R signals are transduced by STAT5 and STAT6, respectively, thus positively regulating the expression of Bcl6 [46]. Bcl6 binds directly to the *Prdm1* promoter and down-regulates the expression of Blimp1 in GC cells, thus preventing the terminal differentiation to PCs [85]. Conversely, Bcl6 is a direct target of Blimp1. This creates a mutual inhibition circuit among Bcl6 and Blimp1 [86]. Maturation of GC cells towards the Mem or PC cell fates requires the downregulation of Bcl6 [17]. This process also depends on the activation of BCR by Ag recognition, as well as on the direct contact of B cells with T helper cells which leads to BCR activation and the proteosomal degradation of Bcl6, mediated by ERK [49].

The direct contact between B and T cells is mediated by the union of CD40 with its ligand CD40L, which in turn activates NF-κB, a positive regulator of Irf4 [16]. Irf4 is a key regulator required for the development of Mem cells from Naive and GC cells, and is involved in the control of CSR and PC differentiation [18, 37]. It has been shown that low levels of Irf4 promote CSR while high Irf4 levels promote PC differentiation. Irf4 inhibits Bcl6 by binding to a regulatory site in the *Bcl6* gene promoter in response to the direct contact of B and T cells [16].

Conversely, Bcl6 is a direct negative regulator of Irf4 in GC cells [87, 88], thus generating a mutual inhibition circuit between Bcl6 and Irf4. Moreover, high Irf4 expression is maintained through direct binding of Irf4 to its own promoter creating a positive autoregulatory circuit [61].

Irf4 also plays an important role in early stages of B cell development where it regulates Pax5 expression through the formation of molecular complexes in the *Pax5* enhancer region [89]. Similarly, Pax5 activation during B cell development is maintained by the transcription factor Ebf1 [33] which in turn is activated by Pax5 [32], therefore conforming a mutually positive regulatory circuit. However, the role of the regulatory circuits between Pax5, Irf4 and Ebf1 during terminal B cell differentiation is not clearly understood. Nevertheless, we found necessary to include these interactions in our model (Pax5 → Pax5 and Irf4 ⊣ Pax5) in order to account for the known activation patterns for these two regulators. Therefore, these interactions constitute predictions of the model that may support an important role of these regulatory interactions during the late stages of B cell differentiation.

The processes of CSR and SHM are controlled by the action of AID [90] which is regulated by the direct binding of Pax5, NF-κB and STAT6 to its regulatory regions in response to IL-4 and CD40 signals [91–93]. AID expression is inhibited in PCs by Blimp1 [5].

Finally, PC differentiation program is regulated by the coordinated activity of Blimp1, Irf4 and XBP1. Blimp1 is specifically expressed in PCs and its activation is sufficient to drive mature B cell differentiation towards the PC fate [56]. Blimp1 is induced by the direct binding of Irf4 to an intronic region of the *Prdm1* gene [18, 61]. Also Blimp1 is involved in Irf4 activation conforming a double positive regulatory circuit. Deficient B cells do not express Irf4 and fail to differentiate into PCs [94, 95]. In turn, Blimp1 activates XBP1 [64] which is normally repressed by Pax5 in mature B cells [65].

The regulatory network as a discrete dynamical system

Boolean networks constitute the simplest approach to modeling the dynamics of regulatory networks. A Boolean network consists of a set of nodes, each of which may attain only one of two states: 0 if the node is OFF, or 1 if the node is ON [96, 97]. The level of activation for the *i*-th node is represented by a discrete variable x_i, which is updated at discrete time steps according to a Boolean function F_i such that $x_i(t+1) = F_i[x_1(t), x_2(t), \ldots, x_n(t)]$, where $[x_1(t), x_2(t), \ldots, x_n(t)]$ is the activation state of the regulators of the node x_i at time t. The Boolean function F_i is expressed using the logic operators $\wedge$ (*AND*), $\vee$ (*OR*), and $\neg$ (*NOT*). In our model, all F_is are updated simultaneously, which is known as the synchronous approach. The resulting set of F_is is shown in Table 6.

We obtained all the attractors of the Boolean model by testing all possible initial states under a synchronous updating scheme using the R package BoolNet [99]. Moreover, we simulated all possible single loss- and gain-of-function mutants by fixing the value of each node to 0 or 1, respectively.

The complete discrete model is available for testing in The Cell Collective (http://www.thecellcollective.org/) model *B cell differentiation* [98]. Furthermore, the model is available as the accompanying file S1 File (*Bcells_model.xml*) in SBMLqual format.

The regulatory network as a continuous dynamical system

The B cell regulatory network was converted into a continuous dynamical system by using the standardized qualitative dynamical systems method (SQUAD) [104, 105] with the modification by Sánchez-Corrales and colaborators [42] to include into the equations a version of the regulatory logic rule for each node. This methodology offers two main advantages, first, it allows to construct a qualitative model in spite of the lack of kinetic information, making use

Table 6. Logical rules. The set of Boolean rules defining the regulatory network of the terminal differentiation of B cells.

Logic rule	Description	References
AID ← (STAT6 ∨ (NF-κB ∧ Pax5)) ∧¬ Blimp1	AID node is positively regulated by the presence of Pax5 in response to CD40 and IL-4 signals, transduced by NF-κB and STAT6 respectively. AID is active only if its inhibitor Blimp1 is absent.	[91–93]
Bach2 ← Pax5 ∧¬ Blimp1	Bach2 node is activated if its positive regulator Pax5 is active and the suppressor Blimp1 is absent.	[19, 31]
Bcl6 ← (STAT5 ∨ STAT6 ∨ (Pax5 ∧Bcl6)) ∧¬ (Blimp1 ∨ Irf4 ∨ ERK)	The node Bcl6 is induced in response to IL-2 and IL-4, transduced by STAT5 and STAT6 respectively. Its activation depends on the presence of Pax5 (proposed as a positive interaction), and on the mechanisms maintaining its own expression (proposed as a positive autoregulation). Bcl6 node is repressed if either the nodes Blimp1, Irf4 or ERK are active.	[35, 36, 46]
BCR ← Ag	BCR node is activated by the input node Ag, simulating the presence of extracellular antigen.	[100]
Blimp1 ← (ERK ∨ STAT3) ∨ (Irf4 ∧¬ (Pax5 ∨ Bcl6 ∨ Bach2))	Blimp1 is activated by Irf4 if all its inhibitors, Pax5, Bcl6 and Bach2 are inactive. Blimp1 is induced by Ag and IL-21 which are transduced by ERK and STAT3, respectively.	[49, 50, 52–54]
CD40 ← CD40L	The CD40 node is activated by the input node CD40L simulating the direct contact of B with T cells mediated by the union of the CD40 receptor with its ligand.	[16]
ERK ← BCR	BCR cross-linking promotes ERK activation after Ag stimulation	[49, 51, 76]
IL-2R ← IL-2	The IL-2R node is induced by the input node IL-2, simulating the activation of the IL-2R receptor by IL-2 stimulation, a signal involved in GC differentiation	[4]
IL-4R ← IL-4	The IL-4 input node induces the IL-4R node simulating the activation of the IL-4R receptor activation by the cytokine IL-4 required for GC differentiation.	[4]
IL-21R ← IL-21	The IL-21R receptor is induced by IL-21, a signal required for differentiation toward PCs	[101–103]
Irf4 ← (NF-κB ∨ Irf4) ∨ (Blimp1 ∧¬ Bcl6)	Irf4 is induced in response to CD40L signals, transduced by the node NF-κB. Irf4 regulates its own activation and is positively regulated by Blimp1 if its inhibitor Bcl6 is off.	[16, 18, 61, 89]
NF-κB ← CD40	Activation of the CD40 receptor promotes the activation of the transcription factor NF-κB in response to CD40L stimulation	[16]
Pax5 ← (Pax5 ∨ ¬ Irf4) ∧¬(Blimp1 ∨ ERK)	Pax5 is maintained active by low levels of Irf4, proposed as a negative interaction, and possibly by a positive regulatory circuit with Ebf1 that plays a key role during early B cell differentiation, included as a positive autoregulatory interaction. Pax5 is inhibited if Blimp1 or ERK are present.	[11, 51, 78]
STAT3 ← IL-21R	IL-21 signals are transduced by STAT3, represented in the model as a positive interaction of the IL-21R receptor with STAT3.	[101–103]
STAT5 ← IL-2R	Activation of the IL-2R receptor by IL-2 induces STAT5 activation	[46]
STAT6 ← IL-4R	Activation of the IL-4R receptor induces STAT6 in response to IL-4 stimulation	[91]
XBP1 ← Blimp1 ∧¬ Pax5	XBP1 is activated by Blimp1 if the suppressor Pax5 is absent	[65]

The rules determining the state of activation of each node as a function of its regulatory inputs are expressed by the use of the logic operators ∧ (AND), ∨ (OR), and ¬ (NOT).

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only of the regulatory interactions of the network, and second, since the external signals are continuous in nature, this methodology permit to study the response of the network to such signals while at the same time allowing a direct comparison with the Boolean model. Moreover, due to its formulation as a set of ordinary differential equations, it may find additional unstable steady states, cyclic behavior, or attraction basins with respect to Boolean approaches [105].

The SQUAD method approximate a Boolean system with the use of a set of ordinary differential equations, where the activation level of a node is represented by a variable x_i which is normalized in the range [0, 1]. This is a dimensionless variable since it represents the functional activation level of a node, but it may be used to represent the normalized concentration of the active form of a molecule or a macromolecular complex. The change of the x_i node over time is

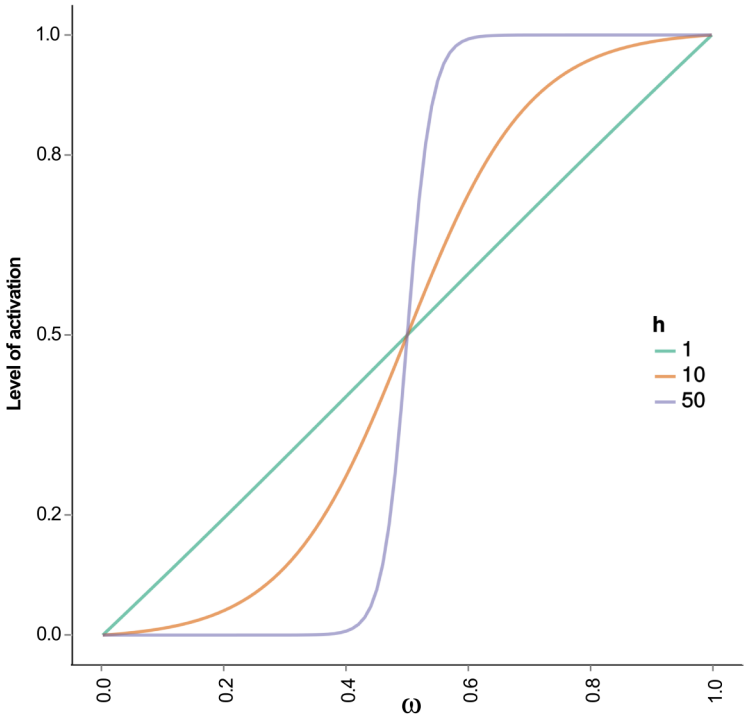

Fig 6. The activation part of Eq (1) is a sigmoid function of the total input of the node (ω_i) Regardless of the value of *h*, the sigmoid touches the points (0,0), (0.5,0.5) and (1,1). For values of $h \geq 50$ the curve resembles a step function.

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controlled by an activation term and a decay term as described by:

$$\frac{dx_i}{dt} = \frac{-e^{0.5h_i} + e^{-h_i(\omega_i - 0.5)}}{(1 - e^{0.5h_i})(1 + e^{-h_i(\omega_i - 0.5)})} - \gamma_i x_i \quad (1)$$

In Eq (1) parameters h_i and γ_i are the gain of the input of the node and the decaying rate, respectively. The term ω_i is the continuous form of the logical rule describing the response of the node x_i to its regulatory inputs, as defined for the discrete dynamical system in the previous section. The logical statements defined for the discrete model are converted into their continuous equivalent by changing $A \wedge B$, $A \vee B$, and $\neg A$ in an expression of classic logic into min(A, B), max(A, B), and $1-A$, respectively, thus creating a fuzzy-logic expression. Note that the term ω_i cannot be applied to all nodes of Fig 2, because there are five of them that do not have any regulatory inputs, therefore equations representing these nodes contain only the term for the decaying rate.

The activation term for Eq (1) has the form of a sigmoid as a function of the total input to a node ω_i, and was constructed so as to pass through the points (0,0), (0.5,0.5), (1,1) for any positive value of h. We found that for values of $h \geq 50$, the curve is very close to a step function; for intermediate values of h the function is similar to a logistic curve and as h approaches 0 the function is almost a straight line (Fig 6). This characteristic allows the study of different qualitative response curves on the overall behavior of the regulatory network, while at the same time conserving the direct comparison against a Boolean model due to the three fixed points mentioned above. Since there is a lack of published quantitative data that could be used to estimate the values of either of the h_i and γ_i parameters to solve the system of equations, we decided to

use a set of default values. Therefore, all h's were set to 50 and $\gamma = 1$ so as to obtain steep response curves, thus making an easy comparison of the discrete model against the current continuous model and/or forthcoming models.

We found that values $h \neq \{4,8\}$ and $\gamma = 1$ recover the experimentally observed patterns of expression S2 Fig. In contrast to the relative insensitivity of changes in the strength of interactions h, the attractors are highly sensitive to changes in values of the decay rate γ. Eq (1) is constructed in such a way that γ has to have a value equal to 1 in order for x_i's to lie in the closed interval [0, 1]. Now, values of γ different than 1 make all attractors to disappear S3 Fig. The attractors of the B cell regulatory network model, therefore, are highly dependent on the value used in the parameter specifying the decaying rate.

The resulting dynamical system in shown as S2 Table in the Supporting Information, and available as the supplementary S1 File. Due to the high non-linearity of the continuous system of equations, we located the steady states of this model by numerically solving the system of equations from 500,000 random initial states and letting it converge, with the use of the R package deSolve [106], the detailed attractors found for both the wild type and the mutant models are shown in S2 File.

Supporting Information

S1 Table. Table of interactions. Key references supporting network interactions.
(PDF)

S2 Table. The B cell network as a continuous dynamical system. The set of ordinary differential equations conforming the continuous version of the B cell regulatory network model.
(PDF)

S1 Fig. Complete fate map for the discrete model.
(TIFF)

S2 Fig. Location of the fixed-point attractors as a function of the parameter *h*.
(TIFF)

S3 Fig. Location of the fixed-point attractors as a function of the parameter γ.
(TIFF)

S1 File. SBMLqual format version of the B cell model. Complete model for testing in the The Cell Collective platform (http://www.thecellcollective.org/), model *B cell differentiation* and file Bcell_model.xml.
(XML)

S2 File. Detailed attractors of wild type and simulated mutants.
(XLSX)

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Author Contributions

Conceived and designed the experiments: LM. Performed the experiments: AM. Analyzed the data: AM LM. Contributed reagents/materials/analysis tools: AM. Wrote the paper: AM LM.

References

1. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 2008; 112(5):1570–80. doi: 10.1182/blood-2008-02-078071 PMID: 18725575
2. Gatto D, Brink R. The germinal center reaction. J Allergy Clin Immunol. 2010; 126(5):898–907; quiz 908–9. doi: 10.1016/j.jaci.2010.09.007 PMID: 21050940
3. Victora GD, Nussenzweig MC. Germinal centers. Annu Rev Immunol. 2012; 30:429–57. doi: 10.1146/annurev-immunol-020711-075032 PMID: 22224772
4. Moens L, Tangye SG. Cytokine-Mediated Regulation of Plasma Cell Generation: IL-21 Takes Center Stage. Front Immunol. 2014 jan; 5(February):65. doi: 10.3389/fimmu.2014.00065 PMID: 24600453
5. Shaffer AL, Lin KI, Kuo TC, Yu X, Hurt EM, Rosenwald A, et al. Blimp-1 Orchestrates Plasma Cell Differentiation by Extinguishing the Mature B Cell Gene Expression Program. Immunity. 2002 Jul; 17 (1):51–62. doi: 10.1016/S1074-7613(02)00335-7 PMID: 12150891
6. Basso K, Saito M, Sumazin P, Margolin Aa, Wang K, Lim Wk, et al. Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. Blood. 2010 feb; 115(5):975–84. doi: 10.1182/blood-2009-06-227017 PMID: 19965633
7. Nutt SL, Taubenheim N, Hasbold J, Corcoran LM, Hodgkin PD. The genetic network controlling plasma cell differentiation. Semin Immunol. 2011; 23(5):341–9. doi: 10.1016/j.smim.2011.08.010 PMID: 21924923
8. Delogu A, Schebesta A, Sun Q, Aschenbrenner K, Perlot T, Busslinger M. Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. Immunity. 2006 Mar; 24(3):269–81. doi: 10.1016/j.immuni.2006.01.012 PMID: 16546096
9. Muto A, Tashiro S, Nakajima O, Hoshino H, Takahashi S, Sakoda E, et al. The transcriptional programme of antibody class switching involves the repressor Bach2. Nature. 2004 Jun; 429(6991):566–71. doi: 10.1038/nature02596 PMID: 15152264
10. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of B cell identity and function. Nat Immunol. 2007; 8(5):463–70. doi: 10.1038/ni1454 PMID: 17440452
11. Mora-López F, Reales E, Brieva JA, Campos-Caro A, Dc W, Mora-lo F, et al. Human BSAP and BLIMP1 conform an autoregulatory feedback loop. Blood. 2007; 110(9):3150–7. doi: 10.1182/blood-2007-05-092262 PMID: 17682124
12. Ochiai K, Katoh Y, Ikura T, Hoshikawa Y, Noda T, Karasuyama H, et al. Plasmacytic transcription factor Blimp-1 is repressed by Bach2 in B cells. J Biol Chem. 2006; 281(50):38226–34. doi: 10.1074/jbc.M607592200 PMID: 17046816
13. Toyama H, Okada S, Hatano M, Takahashi Y, Takeda N, Ichii H, et al. Memory B Cells without Somatic Hypermutation Are Generated from Bcl6-Deficient B Cells. Immunity. 2002 Sep; 17(3):329–339. doi: 10.1016/S1074-7613(02)00387-4 PMID: 12354385
14. Fukuda T, Yoshida T, Okada S, Hatano M, Miki T, Ishibashi K, et al. Disruption of the Bcl6 gene results in an impaired germinal center formation. J Exp Med. 1997; 186(3):439–48. doi: 10.1084/jem.186.3.439 PMID: 9236196
15. Dent aL, Shaffer AL, Yu X, Allman D, Staudt LM. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. Science. 1997; 276(5312):589–92. doi: 10.1126/science.276.5312.589 PMID: 9110977
16. Saito M, Gao J, Basso K, Kitagawa Y, Smith PM, Bhagat G, et al. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. Cancer Cell. 2007; 12(3):280–92. doi: 10.1016/j.ccr.2007.08.011 PMID: 17785208
17. Kuo TC, Shaffer AL, Haddad J, Choi YS, Staudt LM, Calame K. Repression of BCL-6 is required for the formation of human memory B cells in vitro. J Exp Med. 2007; 204(4):819–30. doi: 10.1084/jem.20062104 PMID: 17403935
18. Sciammas R, Shaffer aL, Schatz JH, Zhao H, Staudt LM, Singh H. Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. Immunity. 2006; 25 (2):225–36. doi: 10.1016/j.immuni.2006.07.009 PMID: 16919487
19. Muto A, Ochiai K, Kimura Y, Itoh-Nakadai A, Calame KL, Ikebe D, et al. Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch. EMBO J. 2010; 29(23):4048–61. doi: 10.1038/emboj.2010.257 PMID: 20953163
20. Sciammas R, Li Y, Warmflash A, Song Y, Dinner AR, Singh H. An incoherent regulatory network architecture that orchestrates B cell diversification in response to antigen signaling. Mol Syst Biol. 2011; 7(495):495. doi: 10.1038/msb.2011.25 PMID: 21613984

21. Bhattacharya S, Conolly RB, Kaminski NE, Thomas RS, Andersen ME, Zhang Q. A bistable switch underlying B-cell differentiation and its disruption by the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Sci. 2010; 115(1):51–65. doi: 10.1093/toxsci/kfq035 PMID: 20123757

22. Zhang Q, Kline DE, Bhattacharya S, Crawford RB, Conolly RB, Thomas RS, et al. All-or-none suppression of B cell terminal differentiation by environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol. 2013; 268(1):17–26. doi: 10.1016/j.taap.2013.01.015 PMID: 23357550

23. Martínez MR, Corradin A, Klein U, Álvarez MJ, Toffolo GM, di Camillo B, et al. Quantitative modeling of the terminal differentiation of B cells and mechanisms of lymphomagenesis. Proc Natl Acad Sci U S A. 2012; 109(7):2672–7. doi: 10.1073/pnas.1113019109 PMID: 22308355

24. Meyer-Hermann M. A mathematical model for the germinal center morphology and affinity maturation. J Theor Biol. 2002;p. 273–300.

25. Hawkins JB, Jones MT, Plassmann PE, Thorley-Lawson Da. Chemotaxis in densely populated tissue determines germinal center anatomy and cell motility: a new paradigm for the development of complex tissues. PLoS One. 2011; 6(12):e27650. doi: 10.1371/journal.pone.0027650 PMID: 22145018

26. Kestler Ha, Wawra C, Kracher B, Kühl M. Network modeling of signal transduction: Establishing the global view; 2008.

27. Morris MK, Saez-Rodriguez J, Sorger PK, Lauffenburger Da. Logic-based models for the analysis of cell signaling networks; 2010.

28. Albert R, Thakar J. Boolean modeling: A logic-based dynamic approach for understanding signaling and regulatory networks and for making useful predictions; 2014.

29. 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 Apr; 94(12):1–4. doi: 10.1103/PhysRevLett.94.128701

30. Horcher M, Souabni A, Busslinger M. Pax5/BSAP maintains the identity of B cells in late B lymphopoiesis. Immunity. 2001 Jun; 14(6):779–90. doi: 10.1016/S1074-7613(01)00153-4 PMID: 11420047

31. Schebesta A, Mcmanus S, Salvagiotto G, Delogu A, Busslinger GA, Busslinger M. Transcription factor Pax5 activates the chromatin of key genes involved in B cell signaling, adhesion, migration, and immune function. Immunity. 2007; 27(1):49–63. doi: 10.1016/j.immuni.2007.05.019 PMID: 17658281

32. Roessler S, Györy I, Imhof S, Spivakov M, Williams RR, Busslinger M, et al. Distinct promoters mediate the regulation of Ebf1 gene expression by interleukin-7 and Pax5. Mol Cell Biol. 2007 Jan; 27(2):579–594. doi: 10.1128/MCB.01192-06 PMID: 17101802

33. O'Riordan M, Grosschedl R. Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. Immunity. 1999 Jul; 11(1):21–31. doi: 10.1016/S1074-7613(00)80078-3 PMID: 10435576

34. Pasqualucci L, Migliazza A, Basso K, Houldsworth J, Chaganti RSK, Dalla-Favera R. Mutations of the BCL6 proto-oncogene disrupt its negative autoregulation in diffuse large B-cell lymphoma. Blood. 2003; 101(8):2914–23. doi: 10.1182/blood-2002-11-3387 PMID: 12515714

35. Ramachandrareddy H, Bouska A, Shen Y, Ji M, Rizzino A, Chan WC, et al. BCL6 promoter interacts with far upstream sequences with greatly enhanced activating histone modifications in germinal center B cells. Proc Natl Acad Sci U S A. 2010; 107(26):11930–5. doi: 10.1073/pnas.1004962107 PMID: 20547840

36. Batlle-López A, Cortiguera MG, Rosa-Garrido M, Blanco R, Del Cerro E, Torrano V, et al. Novel CTCF binding at a site in exon1A of BCL6 is associated with active histone marks and a transcriptionally active locus. Oncogene. 2013;(April:):1–11.

37. Klein U, Casola S, Cattoretti G, Shen Q, Lia M, Mo T, et al. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. Nature immunology. 2006; 7(7):773–782. doi: 10.1038/ni1357 PMID: 16767092

38. Demongeot J, Goles E, Morvan M, Noual M, Sené S. Attraction basins as gauges of robustness against boundary conditions in biological complex systems. PLoS One. 2010 Jan; 5(8):e11793. doi: 10.1371/journal.pone.0011793 PMID: 20700525

39. Huang S. The molecular and mathematical basis of Waddington's epigenetic landscape: A framework for post-Darwinian biology? BioEssays. 2012 Feb; 34(2):149–157. doi: 10.1002/bies.201100031 PMID: 22102361

40. Kjeldsen MK, Perez-Andres M, Schmitz A, Johansen P, Boegsted M, Nyegaard M, et al. Multiparametric flow cytometry for identification and fluorescence activated cell sorting of five distinct B-cell subpopulations in normal tonsil tissue. American Journal of Clinical Pathology. 2011; 136(6):960–969. doi: 10.1309/AJCPDQNP2U5DZHVV PMID: 22095383

41. Perez-Andres M, Paiva B, Nieto WG, Caraux a, Schmitz a, Almeida J, et al. Human peripheral blood B-Cell compartments: A crossroad in B-cell traffic. Cytometry Part B—Clinical Cytometry. 2010; 78 (SUPPL. 1):47–60. doi: 10.1002/cyto.b.20547

42. Sánchez-Corrales YE, Alvarez-Buylla ER, Mendoza L. The Arabidopsis thaliana flower organ specification gene regulatory network determines a robust differentiation process. J Theor Biol. 2010; 264 (3):971–83. doi: 10.1016/j.jtbi.2010.03.006 PMID: 20303988

43. Cattoretti G, Shaknovich R, Smith PM, Jäck Hm, Murty VV, Alobeid B. Stages of germinal center transit are defined by B cell transcription factor coexpression and relative abundance. Journal of immunology (Baltimore, Md: 1950). 2006 Nov; 177(10):6930–9. doi: 10.4049/jimmunol.177.10.6930

44. Ochiai K, Maienschein-Cline M, Simonetti G, Chen J, Rosenthal R, Brink R, et al. Transcriptional Regulation of Germinal Center B and Plasma Cell Fates by Dynamical Control of IRF4. Immunity. 2013 May; 38(5):918–929. doi: 10.1016/j.immuni.2013.04.009 PMID: 23684984

45. Holder MJ, Liu YJ, Defrance T, Flores-Romo L, MacLennan IC, Gordon J. Growth factor requirements for the stimulation of germinal center B cells: evidence for an IL-2-dependent pathway of development. Int Immunol. 1991; 3(12):1243–51. doi: 10.1093/intimm/3.12.1243 PMID: 1723294

46. Scheeren Fa, Naspetti M, Diehl S, Schotte R, Nagasawa M, Wijnands E, et al. STAT5 regulates the self-renewal capacity and differentiation of human memory B cells and controls Bcl-6 expression. Nat Immunol. 2005; 6(3):303–13. doi: 10.1038/ni1172 PMID: 15711548

47. Nojima T, Haniuda K, Moutai T, Matsudaira M, Mizokawa S, Shiratori I, et al. In-vitro derived germinal centre B cells differentially generate memory B or plasma cells in vivo. Nat Commun. 2011; 2:465. doi: 10.1038/ncomms1475 PMID: 21897376

48. Arpin C, Déchanet J, Van Kooten C, Merville P, Grouard G, Brière F, et al. Generation of memory B cells and plasma cells in vitro. Science. 1995; 268(5211):720–2. doi: 10.1126/science.7537388 PMID: 7537388

49. Niu H, Ye BH, Dalla-Favera R. Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcriptionÃ? factor. Genes Dev. 1998; 12(13):1953–1961. doi: 10.1101/gad.12.13.1953 PMID: 9649500

50. Kwon H, Thierry-Mieg D, Thierry-Mieg J, Kim HP, Oh J, Tunyaplin C, et al. Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors. Immunity. 2009; 31(6):941–52. doi: 10.1016/j.immuni.2009.10.008 PMID: 20064451

51. Yasuda T, Hayakawa F, Kurahashi S, Sugimoto K, Minami Y, Tomita A, et al. B cell receptor-ERK1/2 signal cancels PAX5-dependent repression of BLIMP1 through PAX5 phosphorylation: a mechanism of antigen-triggering plasma cell differentiation. J Immunol. 2012; 188(12):6127–34. doi: 10.4049/jimmunol.1103039 PMID: 22593617

52. Ettinger R, Sims GP, Fairhurst AM, Robbins R, da Silva YS, Spolski R, et al. IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. J Immunol. 2005; 175 (12):7867–79. doi: 10.4049/jimmunol.175.12.7867 PMID: 16339522

53. Berglund LJ, Avery DT, Ma CS, Moens L, Deenick EK, Bustamante J, et al. IL-21 signalling via STAT3 primes human naïve B cells to respond to IL-2 to enhance their differentiation into plasmablasts. Blood. 2013; 122(24):3940–3950. doi: 10.1182/blood-2013-06-506865 PMID: 24159173

54. Shapiro-Shelef M, Lin KI, McHeyzer-Williams LJ, Liao J, McHeyzer-Williams MG, Calame K. Blimp-1 Is Required for the Formation of Immunoglobulin Secreting Plasma Cells and Pre-Plasma Memory B Cells. Immunity. 2003; 19(4):607–620. doi: 10.1016/S1074-7613(03)00267-X PMID: 14563324

55. Shapiro-Shelef M, Lin KI, Savitsky D, Liao J, Calame K. Blimp-1 is required for maintenance of long-lived plasma cells in the bone marrow. J Exp Med. 2005; 202(11):1471–6. doi: 10.1084/jem.20051611 PMID: 16314438

56. Kallies A, Nutt SL. Terminal differentiation of lymphocytes depends on Blimp-1. Current opinion in immunology. 2007; 19(2):156–62. doi: 10.1016/j.coi.2007.01.003 PMID: 17291741

57. Ye BH, Cattoretti G, Shen Q, Zhang J, Hawe N, de Waard R, et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nat Genet. 1997 Jun; 16(2):161–70. doi: 10.1038/ng0697-161 PMID: 9171827

58. Huang C, Hatzi K, Melnick A. Lineage-specific functions of Bcl-6 in immunity and inflammation are mediated by distinct biochemical mechanisms. Nat Immunol. 2013 Mar; 14(4). doi: 10.1038/ni.2543

59. Shapiro-Shelef M, Lin KI, Savitsky D, Liao J, Calame K. Blimp-1 is required for maintenance of long-lived plasma cells in the bone marrow. J Exp Med. 2005 Dec; 202(11):1471–6. doi: 10.1084/jem.20051611 PMID: 16314438

60. Mittrucker HW. Requirement for the Transcription Factor LSIRF/IRF4 for Mature B and T Lymphocyte Function. Science. 1997; 275(5299):540–543. doi: 10.1126/science.275.5299.540 PMID: 8999800

61. Shaffer AL, Emre NCT, Lamy L, Ngo VN, Wright G, Xiao W, et al. IRF4 addiction in multiple myeloma. Nature. 2008; 454(7201):226–31. doi: 10.1038/nature07064 PMID: 18568025

62. Urbánek P, Wang ZQ, Fetka I, Wagner EF, Busslinger M. Complete block of early B cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP. Cell. 1994 Dec; 79 (5):901–12. doi: 10.1016/0092-8674(94)90079-5 PMID: 8001127

63. Nera KP, Kohonen P, Narvi E, Peippo A, Mustonen L, Terho P, et al. Loss of Pax5 promotes plasma cell differentiation. Immunity. 2006 Mar; 24(3):283–93. doi: 10.1016/j.immuni.2006.02.003 PMID: 16546097

64. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, Lee AH, Qian SB, Zhao H, et al. XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. Immunity. 2004; 21(1):81–93. doi: 10.1016/j.immuni.2004.06.010 PMID: 15345222

65. Todd DJ, McHeyzer-Williams LJ, Kowal C, Lee AH, Volpe BT, Diamond B, et al. XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development. The Journal of experimental medicine. 2009; 206(10):2151–9. doi: 10.1084/jem.20090738 PMID: 19752183

66. Baron BW, Anastasi J, Montag A, Huo D, Baron RM, Karrison T, et al. The human BCL6 transgene promotes the development of lymphomas in the mouse. Proc Natl Acad Sci U S A. 2004; 101 (39):14198–203. doi: 10.1073/pnas.0406138101 PMID: 15375218

67. Usui T, Wakatsuki Y, Matsunaga Y, Kaneko S, Koseki H, Kita T, et al. Overexpression of B cell-specific activator protein (BSAP/Pax-5) in a late B cell is sufficient to suppress differentiation to an Ig high producer cell with plasma cell phenotype. J Immunol. 1997; 158(7):3197–204. PMID: 9120274

68. Kurosaki T, Kometani K, Ise W. Memory B cells. Nature Reviews Immunology. 2015; 15(3):149–159. doi: 10.1038/nri3802 PMID: 25677494

69. Cattoretti G, Angelin-Duclos C, Shaknovich R, Zhou H, Wang D, Alobeid B. PRDM1/Blimp-1 is expressed in human B-lymphocytes committed to the plasma cell lineage. J Pathol. 2005; 206(1):76–86. doi: 10.1002/path.1752 PMID: 15772984

70. Teng Y, Takahashi Y, Yamada M, Kurosu T, Koyama T, Miura O, et al. IRF4 negatively regulates proliferation of germinal center B cell-derived Burkitt's lymphoma cell lines and induces differentiation toward plasma cells. Eur J Cell Biol. 2007 Oct; 86(10):581–9. doi: 10.1016/j.ejcb.2007.05.006 PMID: 17651861

71. Reljic R, Wagner SD, Peakman LJ, Fearon DT. Suppression of Signal Transducer and Activator of Transcription 3-Dependent B Lymphocyte Terminal Differentiation by Bcl-6. J Exp Med. 2000; 192 (12):1841–1848. doi: 10.1084/jem.192.12.1841 PMID: 11120780

72. Horikawa K, Takatsu K. Interleukin-5 regulates genes involved in B-cell terminal maturation. Immunology. 2006 Aug; 118(4):497–508. doi: 10.1111/j.1365-2567.2006.02382.x PMID: 16895557

73. Turner CA, Mack DH, Davis MM. Pillars article: Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. 1994. J Immunol. 2010 Jul; 185(1):5–14. doi: 10.4049/jimmunol.1090043 PMID: 20562267

74. Lin Y. Repression of c-myc Transcription by Blimp-1, an Inducer of Terminal B Cell Differentiation. Science. 1997 Apr; 276(5312):596–599. doi: 10.1126/science.276.5312.596 PMID: 9110979

75. Piskurich JF, Lin KI, Lin Y, Wang Y, Ting JP, Calame K. BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. Nat Immunol. 2000 Dec; 1(6):526–32. doi: 10.1038/82788 PMID: 11101876

76. Yasuda T, Kometani K, Takahashi N, Imai Y, Aiba Y, Kurosaki T. ERKs induce expression of the transcriptional repressor Blimp-1 and subsequent plasma cell differentiation. Sci Signal. 2011 Jan; 4 (169):ra25. doi: 10.1126/scisignal.2001592 PMID: 21505187

77. Morrison aM, Nutt SL, Thévenin C, Rolink a, Busslinger M. Loss- and gain-of-function mutations reveal an important role of BSAP (Pax-5) at the start and end of B cell differentiation. Semin Immunol. 1998 Apr; 10(2):133–42. doi: 10.1006/smim.1998.0115 PMID: 9618759

78. Lin KI, Angelin-Duclos C, Kuo TC, Calame K. Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. Mol Cell Biol. 2002; 22 (13):4771–80. doi: 10.1128/MCB.22.13.4771-4780.2002 PMID: 12052884

79. Falini B, Fizzotti M, Pucciarini A, Bigerna B, Marafioti T, Gambacorta M, et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. Blood. 2000; 95(6):2084–92. PMID: 10706878

80. Cattoretti G, Pasqualucci L, Ballon G, Tam W, Nandula SV, Shen Q, et al. Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. Cancer cell. 2005 May; 7(5):445–55. doi: 10.1016/j.ccr.2005.03.037 PMID: 15894265

81. Martínez-Sosa P, Mendoza L. The regulatory network that controls the differentiation of T lymphocytes. Biosystems. 2013; 113(2):96–103. doi: 10.1016/j.biosystems.2013.05.007 PMID: 23743337

82. Mendoza L, Pardo F. A robust model to describe the differentiation of T-helper cells. Theory Biosci. 2010;p. 283–293.

83. Shaffer aL, Rosenwald A, Staudt LM. Decision making in the immune system: Lymphoid Malignancies: the dark side of B-cell differentiation. Nat Rev Immunol. 2002 dec; 2(12):920–933. doi: 10.1038/nri953 PMID: 12461565

84. Shaffer AL, Young RM, Staudt LM. Pathogenesis of Human B Cell Lymphomas. Annu Rev Immunol. 2012 apr; 30(1):565–610. doi: 10.1146/annurev-immunol-020711-075027 PMID: 22224767

85. Tunyaplin C, Shaffer aL, Angelin-Duclos CD, Yu X, Staudt LM, Calame KL. Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. J Immunol. 2004; 173(2):1158–65. doi: 10.4049/jimmunol.173.2.1158 PMID: 15240705

86. Shaffer AL, Yu X, He Y, Boldrick J, Chan EP, Staudt LM. BCL-6 Represses Genes that Function in Lymphocyte Differentiation, Inflammation, and Cell Cycle Control. Immunity. 2000; 13(2):199–212. doi: 10.1016/S1074-7613(00)00020-0 PMID: 10981963

87. Gupta S, Jiang M, Anthony A, Pernis AB. Lineage-specific modulation of interleukin 4 signaling by interferon regulatory factor 4. The Journal of experimental medicine. 1999; 190(12):1837–1848. doi: 10.1084/jem.190.12.1837 PMID: 10601358

88. Alinikula J, Nera KP, Junttila S, Lassila O. Alternate pathways for Bcl6-mediated regulation of B cell to plasma cell differentiation. European journal of immunology. 2011; 41(8):2404–13. doi: 10.1002/eji.201141553 PMID: 21674482

89. Decker T, Pasca di Magliano M, McManus S, Sun Q, Bonifer C, Tagoh H, et al. Stepwise Activation of Enhancer and Promoter Regions of the B Cell Commitment Gene Pax5 in Early Lymphopoiesis. Immunity. 2009 Apr; 30(4):508–520. doi: 10.1016/j.immuni.2009.01.012 PMID: 19345119

90. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 2000; 102(5):553–63. doi: 10.1016/S0092-8674(00)00078-7 PMID: 11007474

91. Dedeoglu F. Induction of activation-induced cytidine deaminase gene expression by IL-4 and CD40 ligation is dependent on STAT6 and NF B. International Immunology. 2004; 16(3):395–404. doi: 10.1093/intimm/dxh042 PMID: 14978013

92. Gonda H, Sugai M, Nambu Y, Katakai T, Agata Y, Mori KJ, et al. The balance between Pax5 and Id2 activities is the key to AID gene expression. The Journal of experimental medicine. 2003; 198 (9):1427–37. doi: 10.1084/jem.20030802 PMID: 14581609

93. Tran TH, Nakata M, Suzuki K, Begum NA, Shinkura R, Fagarasan S, et al. B cell-specific and stimulation-responsive enhancers derepress Aicda by overcoming the effects of silencers. Nature immunology. 2010; 11(2):148–54. doi: 10.1038/ni.1829 PMID: 19966806

94. Kallies A, Hasbold J, Tarlinton DM, Dietrich W, Corcoran LM, Hodgkin PD, et al. Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. The Journal of experimental medicine. 2004; 200(8):967–77. doi: 10.1084/jem.20040973 PMID: 15492122

95. Sciammas R, Davis MM. Modular nature of Blimp-1 in the regulation of gene expression during B cell maturation. The Journal of Immunology. 2004; 172(9):5427–40. doi: 10.4049/jimmunol.172.9.5427 PMID: 15100284

96. Kauffman SA. Metabolic stability and epigenesis in randomly constructed genetic nets. J Theor Biol. 1969 Mar; 22(3):437–467. doi: 10.1016/0022-5193(69)90015-0 PMID: 5803332

97. Wang RS, Saadatpour A, Albert R. Boolean modeling in systems biology: an overview of methodology and applications; 2012.

98. Helikar T, Kowal B, McClenathan S, Bruckner M, Rowley T, Madrahimov A, et al. The Cell Collective: Toward an open and collaborative approach to systems biology. BMC systems biology. 2012; 6(1):96. doi: 10.1186/1752-0509-6-96 PMID: 22871178

99. Müssel C, Hopfensitz M, Kestler Ha. BoolNet—an R package for generation, reconstruction, and analysis of Boolean networks. Bioinformatics. 2010; 26(10):1378–1380. doi: 10.1093/bioinformatics/btq124 PMID: 20378558

100. Schmidt C, Kim D, Ippolito GC, Naqvi HR, Probst L, Mathur S, et al. Signalling of the BCR is regulated by a lipid rafts-localised transcription factor, Bright. EMBO J. 2009 Mar; 28(6):711–24. doi: 10.1038/emboj.2009.20 PMID: 19214191

101. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010 Feb; 207 (2):365–78. doi: 10.1084/jem.20091777 PMID: 20142430

102. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010 Feb; 207(2):353–63. doi: 10.1084/jem.20091738 PMID: 20142429

103. Rankin AL, MacLeod H, Keegan S, Andreyeva T, Lowe L, Bloom L, et al. IL-21 Receptor Is Critical for the Development of Memory B Cell Responses. J Immunol. 2011 Jan; 186(2):667–74. doi: 10.4049/jimmunol.0903207 PMID: 21169545

104. Mendoza L, Xenarios I. A method for the generation of standardized qualitative dynamical systems of regulatory networks. Theor Biol Med Model. 2006; 3:13. doi: 10.1186/1742-4682-3-13 PMID: 16542429

105. Weinstein N, Mendoza L. Building Qualitative Models of Plant Regulatory Networks with SQUAD. Front Plant Sci. 2012; 3(April):72. doi: 10.3389/fpls.2012.00072 PMID: 22639661

106. Soetaert K, Petzoldt T, Setzer R. Solving Differential Equations in R. R J. 2010; 2(December):5–15.

8.3 APÉNDICE III: A dynamical model of the regulatory network controlling lymphopoiesis

En esta sección se presenta el trabajo de colaboración durante el desarrollo de este proyecto el cual se enfocó al estudio de la red que controla las decisiones de destino que se llevan a cabo durante el proceso de linfopoyesis. Este trabajo integra la información referente a las moléculas que controlan la formación de seis tipos de células efectoras y estadios intermediarios a partir de un progenitor linfoide común. Este modelo recapitula cualitativamente algunos eventos de la linfopoyesis al describir la manera direccional y ramificada del proceso de diferenciación celular desde un progenitor linfoides común hasta el control de la diferenciación de linfocitos T y linfocitos B a células maduras.

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A dynamical model of the regulatory network controlling lymphopoiesis

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ABSTRACT

Due to the large number of diseases associated to a malfunction of the hematopoietic system, there is an interest in knowing the molecular mechanisms controlling the differentiation of blood cell lineages. However, the structure and dynamical properties of the underlying regulatory network controlling this process is not well understood. This manuscript presents a regulatory network of 81 nodes, representing several types of molecules that regulate each other during the process of lymphopoiesis. The regulatory interactions were inferred mostly from published experimental data. However, 15 out of 159 regulatory interactions are predictions arising from the present study. The network is modelled as a continuous dynamical system, in the form of a set of differential equations. The dynamical behaviour of the model describes the differentiation process from the common lymphocyte precursor (CLP) to several mature B and T cell types; namely, plasma cell (PC), cytotoxic T lymphocyte (CTL), T helper 1 (Th1), Th2, Th17, and T regulatory (Treg) cells. The model qualitatively recapitulates key cellular differentiation events, being able to represent the directional and branched nature of lymphopoiesis, going from a multipotent progenitor to fully differentiated cell types.

1. Introduction

Blood cells originate in the bone marrow from hematopoietic stem cells (HSCs), which give origin to the myeloid and lymphoid lineages. Lymphopoiesis is the process of the generation of lymphocytes, which are the main effectors of the adaptive immune response (Bonilla and Oettgen, 2010). Lymphopoiesis starts with a multipotent cell known as the common lymphoid progenitor (CLP) that gives rise to NK, T, and B cells (Bryder and Sigvardsson, 2010). T lymphocytes promote and coordinate several responses by cells of the immune system, while B cells mediate the humoral response by the secretion of antibodies.

T cells precursors initiate as $CD4^-$ $CD8^-$ double negative (DN) cells, lacking CD4 and CD8 surface markers. After a process known as β-selection (Naito et al., 2011), thymocytes express the TCRα chain and the CD4 and CD8 co-receptors, thus becoming $CD4^+$ $CD8^+$ double positive (DP) cells. Then, DP cells commit to the exclusive expression of either CD4 or CD8, becoming either $CD4^+$ or $CD8^+$ single positive (SP) cells. Each of these lineages is characterized by distinct antigen specificities, $CD4^+$ cells are MHC II-restricted, whereas $CD8^+$ cells are MHC I-restricted (Xiong and Bosselut, 2012). $CD4^+$ cells can be divided into T helper 1 (Th1), Th2, Th17, and T regulatory (Treg) types. Th1 cells are characterized by the production of IFN-γ and T-bet (Szabo et al., 2003), and are involved in the protective immune response against intracellular viral and bacterial infections. Th2 cells express GATA3 and produce interleukin 4 (IL-4) (Murphy and Reiner, 2002), helping in the combat against extracellular pathogens. Th17 cells generate IL-17 and RORγt (Ivaylo et al., 2006), and are critical for the defense against extra-cellular pathogens. Treg cells, characterized for the expression of Foxp3 (Gavin et al., 2007), show an immunosuppressor activity. $CD8^+$ cells, in turn, give rise to effector cytotoxic (CTL) cells, which kill infected cells and are characterized by the production of perforin (Prf1), granzyme B (Gzmb), and IFN-γ (Morishima et al., 2010). Although other types of Th cells (Th9, Th22, Tfh) have been discovered (Crotty, 2014), the differentiation of these cell types is not studied in the present model. See, however (Martinez-Sanchez et al., 2015).

CLP cells differentiate towards the B cell lineage by means of Flt3 signalling and the stimulation of IL-7 (Bryder and Sigvardsson, 2010). At their early (pro-B) developmental stage, B cells generate an ample repertoire of antibodies, and later they generate a mature BCR with very high specificity (Johnson et al., 2005). Once B cells have completed their development in the bone marrow, they migrate to the bloodstream and into the secondary lymphatic

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organs, where they complete maturation. In these organs, naive B lymphocytes are activated by means of the recognition of free antigens or antigens presented in the surface of T lymphocytes. This event determines their differentiation towards effector plasma cells (PCs) (LeBien and Tedder, 2008), which secrete soluble antibodies.

While there is a large quantity of molecular information regarding the differentiation of lymphocytes, there is no consensus regarding the structure and dynamical behaviour of the underlying regulatory network. Regulatory networks are constituted by the regulatory interactions among nodes, which usually represent molecules or molecular complexes. These interactions may be either directly conveyed by means of a physical molecular interaction, or may be elicited via (possibly unknown) intermediaries (Albert and Thakar, 2014; Le Novère, 2015). The modelling of regulatory networks as dynamical systems has shown to be an adequate approach to understand the molecular mechanisms integrating several types of signals that control cell differentiation processes (Kestler et al., 2008). Dynamical models of small parts of the network controlling the differentiation of T cells have been published before (Abou-Jaoudé et al., 2015; Martínez-Sosa and Mendoza, 2013; Mendoza and Xenarios, 2006; Mendoza, 2006, 2013; Mendoza and Pardo, 2010; Naldi et al., 2010), with the aim of describing the expression patterns observed in naive, Th1, Th2, Th17, Treg, CTL cell lineages and their precursors, under wild type and mutant backgrounds. The present work introduces an expanded regulatory network that now incorporates for the first time the decision between the T and B cell lineages. The modelling of the regulatory network is able to simulate the expression patterns observed experimentally for CLP, pro-B, B naive, PC, DP, $CD4^+$ naive, Th1, Th2, Th17, Treg, $CD8^+$ naive, and CTL cells. This model recovers the branching process leading from the pluripotent CLP to the fully differentiated effector cells.

2. Methods

2.1. The molecular basis of the regulatory network

The regulatory network presented in this work is an extension of previous models (Martínez-Sosa and Mendoza, 2013; Mendoza and Xenarios, 2006; Mendoza, 2006, 2013; Mendoza and Pardo, 2010). Therefore, several interactions of the network shown in Fig. 1 have been explained in previous works. For brevity, the following paragraphs explain only the interactions not included in the previous versions of the model. Furthermore, Table A.1 contains a set of key references used to infer the regulatory network of Fig. 1.

Flt3L-deficient mice have significantly reduced numbers of CLPs and B-cell progenitors (McKenna et al., 2000; Nutt and Kee, 2007). Flt3L is expressed by bone marrow stroma (Lisovsky et al., 1996) and binds to its receptor, Flt3, which is expressed in CLPs (Sitnicka et al., 2002). Flt3 is upregulated by Hoxa9 (Gwin et al., 2010), and in turn, Flt3 activates ERK via phosphorylation (Åhsberg et al., 2010).

Notch signalling regulates mature T cell activation and differentiation (Amsen et al., 2004). It is known that Bcl11b (Li et al., 2010a,b), HEB (Wang et al., 2006) and TCF-1 (Germar et al., 2011; Weber et al., 2011) are positively regulated by Notch1 in thymic precursors. Also, Notch1 accelerates the degradation of JAK3 (Nie et al., 2008), and inhibits Runx1 (Giambra et al., 2012). Runx1 is a transcription factor that is also inhibited by Runx3 (Giambra et al., 2012) and TCR (Wong et al., 2011).

The process of T-cell specification is conformed by a succession of distinct regulatory states, which start with the activation of TCF-1 that causes the activation of GATA-3 and Bcl11b (Ma et al., 2013; Rothenberg, 2012). However, GATA3 is repressed by HEB factors during T-cell specification (Braunstein and Anderson, 2011), and repressed by Runx1 during Th1 cell differentiation (Wong et al., 2011).

Th17 cells are producers of TNF-α (Annunziato et al., 2013), and since RORγt is the master transcription factor of Th17 cells (Ivaylo et al., 2006), it seems reasonable to propose that RORγt directly or indirectly induces TNF-α. TNF-α binds directly to members of the TNFR family. Specifically, TNRF2 are present at high levels in T cells (Zhang et al., 2013). TNRF2 triggers the activation of Akt (So and Croft, 2013), which in turn inhibits the phosphorylation of SMAD3 (Roffe et al., 2010; Zhang et al., 2013), resulting in a reduction of the activation of Foxp3 (Zhang et al., 2013). Furthermore, SMAD3 is a signal transduction molecule of the TGF-β/TGF-βR pathway (Massagué and Xi, 2012). Natural Treg cells are producers of TGF-β (Zhang et al., 2014), thus there should exist a mechanism by which Foxp3, the master regulator of Treg cells (Fontenot et al., 2003), induce the expression of TGF-β.

Vitamin B9 acts through the folate receptor FR4, which is a marker of Treg cells (Kunisawa et al., 2012; Yamaguchi et al., 2007). Therefore, there should exist a mechanism for Foxp3 to promote the expression of FR4. FR4, in turn, affects the level of the inhibitor of apoptosis Bcl2 (Kunisawa et al., 2012), which is also upregulated by the IL-7R/JAK3/STAT5/ pathway (Jiang et al., 2005; Malin et al., 2010; Möröy and Khandanpour, 2011; Qin et al., 2001).

The transcription factor Ikaros has an important role during the early steps of fate decision leading to B-cell lineage. Ikaros induces Gfi1 expression (Möröy and Khandanpour, 2011; Spooner et al., 2009), which in turn inhibits PU.1 (Möröy and Khandanpour, 2011; Ramírez et al., 2010; Spooner et al., 2009).

Runx1 deficiency causes severe reduction of B cell progenitors because of the resulting lack of Ebf1 (Seo et al., 2012). The B cell lineage can be identified by the expression of CD19, which is regulated by the master regulator Pax5 (Nutt and Kee, 2007). Pax5 establishes a mutual inhibition circuit with Blimp1 (Kikuchi et al., 2012; Lin et al., 2002; Yasuda et al., 2012), and regulates Bach2 positively (Kallies and Nutt, 2010; Kikuchi et al., 2012) and Flt3 negatively (Holmes et al., 2006). In turn, it is known that Pax5 is upregulated by STAT5 and Ebf1 (Hirokawa et al., 2003; O'Riordan and Grosschedl, 1999), and downregulated by Irf4 (Decker et al., 2009; Nutt et al., 2011).

After its activation by antigen, BCR signalling initiates plasma cell differentiation, a step that involves the activation of the NF-κB (De Silva et al., 2012) and ERK1/2 (Yasuda et al., 2012) pathways. NF-κB, in turn, upregulates Irf4 (De Silva et al., 2012; Klein and Dalla-Favera, 2008) and Helios (Serre et al., 2011).

Plasma cell differentiation is inhibited by Bach2, by the direct repression of Blimp1 (Nutt et al., 2011; Ochiai et al., 2006), which in turn activates XBP1 (Kikuchi et al., 2012; Klein et al., 2003). Blimp1 is involved in a mutually activatory regulation with Irf4 (Kallies et al., 2004; De Silva et al., 2012), and a mutually inhibitory regulation with Bcl6 (Cimmino et al., 2008; Klein et al., 2003; Kusam et al., 2004; Tunyaplin et al., 2004). Maturation of plasma cells is accompanied by the abatement of Bcl6. Irf4 is a known repressor of Bcl6 (Alinikula et al., 2011; Saito et al., 2007), while Ebf1 and IL-21R augment its expression (Bouamar et al., 2013; Nutt et al., 2011; Yi et al., 2010). Then, Bcl6 is an activator of Bach2 (Alinikula et al., 2011). Finally, plasma cell differentiation is promoted by IL-21/IL-21R signalling acting through STAT3 (De Silva et al., 2012; Nutt et al., 2011).

The biological information presented within this section was translated into a set of logical expressions, presented in Table A.2.

2.2. Modelling the regulatory network as a continuous dynamical system

The regulatory network controlling lymphopoiesis was implemented as a continuous dynamical system with the use of the

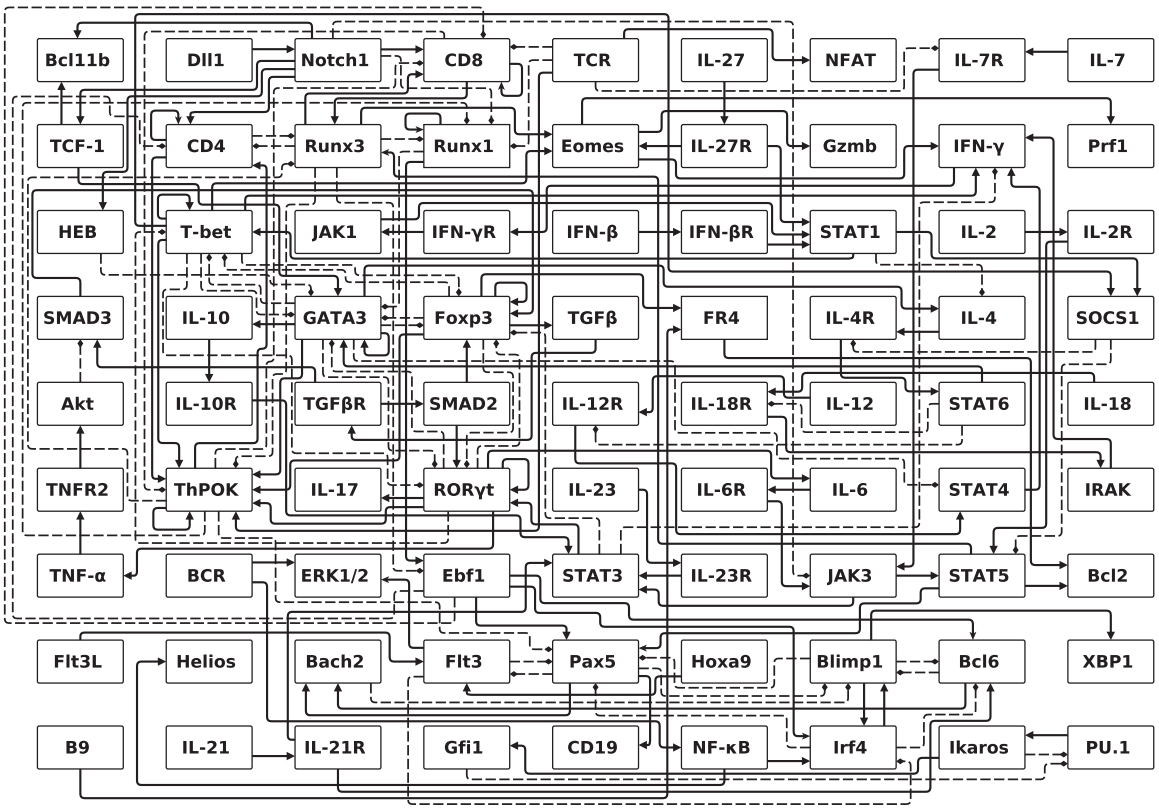

Fig. 1. The regulatory network that controls lymphopoiesis. Positive and negative regulatory interactions among nodes are represented by continuous and discontinuous arrows, respectively.

SQUAD methodology (Mendoza and Xenarios, 2006; Weinstein and Mendoza, 2012). With this approach, a system of Boolean functions can be converted into a system of ordinary differential equations. In the resulting continuous system, the state of activation of a node x is represented by x_i, which is a dimensionless variable. Although x_i represents the level of activation of a node, it may be also be thought of as representing the normalized concentration of the active form of a molecule or a macromolecular complex, since variables representing the activation of the nodes lie in the closed interval [0,1]. Then, the rate of change of x_i, in arbitrary units, is determined by the concerted action of activation and decay terms with the following form:

$$\frac{dx_i}{dt} = \frac{-e^{0.5h_i} + e^{-h_i(\omega_i - 0.5)}}{(1 - e^{0.5h_i})(1 + e^{-h_i(\omega_i - 0.5)})} - \gamma_i x_i \quad (1)$$

In Eq. (1), ω_i is the continuous form of the logical rule describing the response of the node x_i to its regulatory inputs (Table A.2), while parameters h_i and γ_i are the gain of the total input to the node and the decaying rate, respectively.

The continuous form of a logical rule is obtained by replacing the logical operators AND, OR, and NOT by their equivalent fuzzy logic functions min(), max(), and $1-x$, respectively. For example, it is known that the IL-12 receptor is activated by the presence of IL-12, but it is inhibited by STAT6. This statement is translated into the logical rule $IL12R(t+1) = IL12(t)$ *AND NOT* $STAT6(t)$, which in turn can be converted into the continuous expression $\omega_{IL12R} = \min(IL12, 1 - STAT6)$. As a result, the differential equation describing the change of activation of IL-12 receptor over time is:

$$\frac{d\mathrm{IL12}R}{dt} = \frac{-e^{0.5h_i} + e^{-h_i(\min(\mathrm{IL12}, 1-\mathrm{STAT6}) - 0.5)}}{(1 - e^{0.5h_i})(1 + e^{-h_i(\min(\mathrm{IL12}, 1-\mathrm{STAT6}) - 0.5)})} - \gamma_i \mathrm{IL12}R \quad (2)$$

Regarding parameters h_i and γ_i, there is a lack of published quantitative data that could be used to fit either of them. However, it has been found that values $h_i > 14$ and $1 \leq \gamma_i \leq 2$ recover the experimentally observed qualitative stable patterns of expression (Mendoza and Xenarios, 2006). For the results presented in this manuscript, $h_i = 50$ and $\gamma_i = 1$ were used throughout the analysis. The complete set of equations is available as the accompanying runnable R file lymphopoiesis.txt, which requires the deSolve package (Soetaert et al., 2010). For the present study, the system of equations was studied by running the system from 100,000 random initial states within the closed interval [0,1]. The provided R file can be used to model the wild-type system without external stimuli. Also, it is possible to simulate the constant presence of external signals by fixing the relevant ω value to a non-zero value. For example, to simulate a saturating level of IL-7 in the external medium, set w_IL7 to 1.

The SQUAD method approximates a Boolean network with the use of a set of ordinary differential equations. Models that have been studied with this methodology tend to asymptotically reach attractors containing the extreme values 0 and 1 (see for example (Martínez-Sosa and Mendoza, 2013)), but they also may reach attractors with intermediate values (see for example (Sánchez-Corrales et al., 2010)), or even present oscillations (see for example (Sankar et al., 2011)).

3. Results and discussion

3.1. The regulatory network

The reconstructed regulatory network that controls lymphopoiesis consists of 81 nodes and 159 regulatory interactions (Fig. 1). References supporting 144 of these interactions were delineated in the Methods. The dynamical behaviour of a network consisting of only such 144 regulatory interactions does not describe the observed stable patterns of activation. Therefore, it

was necessary to incorporate 15 regulatory interactions for which there are no experimental support. Given that the incorporation of such interactions results in a regulatory network that recovers the known dynamical behaviour, both at the molecular and cellular levels (see ahead), these 15 interactions constitute specific predictions of this work. The following paragraphs resume the reasons to incorporate such interactions into the regulatory network.

CLP cells develop in the bone marrow, under the constant presence of Flt3L/Flt3 signalling (Lisovsky et al., 1996; Sitnicka et al., 2002). Since the B cell program is inactive in CLPs, and Pax5 is generally a positive regulator of B cell specific genes, an inhibition of Flt3 over Pax5 was assumed to exist. Similarly, an inhibition of Flt3 over Irf4 helps in the model to keep the Blimp1/Irf4 circuit off.

Runx1 is negatively regulated by a set of other nodes, but there was no positive regulation over it. Given that Runx1 is needed from the very beginning of lymphopoiesis (Lukin et al., 2010), it was necessary to include a signal to keep it in an active state. Then, Runx1 is essential for B cells development (Seo et al., 2012), but it is downregulated during early stages of T cell development (Wong et al., 2011). Thus, negative signalling from ThPOK and Notch1, which are present during early T cell development, was included.

During the simulations, it was evident the necessity to inhibit the expression of Ebf1 in the CD8 lineage. Given that Runx3 is a key master node in CD8 cells (Naito and Taniuchi, 2010), a negative regulation from Runx3 to Ebf1 was incorporated. Conversely, something in the B cell lineage represses the T cell lineages, therefore inhibitions from Ebf1 to CD4 and CD8 were added. A similar negative effect from the CD4 lineage marker, ThPOK over the activity of Pax5, a main B cell marker, was proposed.

Since ThPOK is a master regulator of CD4 lineage and given that DP cells differentiate into two populations expressing either CD4 or CD8 markers but not both, it was necessary to include a negative interaction of CD8 over ThPOK in order to account for the binary decision between these two cell lineages.

Irf4 expression is induced in activated B cells, while lower concentrations of Irf4 promote germinal centre cells (Nutt et al., 2011). Thus, there could be a mechanism controlling the early activation of Irf4 in the B lineage, the early B cell gene Ebf1 might be such a positive regulator.

Mice lacking Ikaros lack all lymphoid lineages, while Ikaros null mutation results in increase of erythroid and myeloid progenitors (Busslinger, 2004), which is reminiscent of an overexpression of PU.1 (Ramírez et al., 2010), thus suggesting a negative regulation of Ikaros over PU.1. Moreover, by postulating an activation of PU.1 over Ikaros, the resulting negative circuit maintains a constant activation of both Ikaros and PU.1 at intermediate values.

Treg cells are producers of TGFβ (Tran, 2012), therefore, there should be a positive regulation of the master gene Foxp3 over TGFβ. Similarly, Th17 cells produce TNF-α (Annunziato et al., 2013), thus it should be positively regulated by RORγt.

It is important to mention that the predictions regarding the existence of the regulatory interactions mentioned in the above paragraphs do not imply that such interactions should be direct. It is entirely possible that such regulatory interactions might be attained by way of intermediary molecules. The reason is that the whole network modelling approach is based upon the net effect of one node over another, focusing on whether the flow on information is known and not necessarily on the effect of direct physical contact between molecules.

3.2. Attractors

The dynamical system describing the regulatory network that controls lymphopoiesis was studied to analyse its dynamical behaviour. Starting from 100,000 random initial states within the closed interval [0,1], the system converged to 10 fixed point attractors (Table A.3). It was possible to identify all these attractors as molecular patterns observed in specific cells during lymphopoiesis. Specifically, the B naive state was identified by the presence of CD19, Bach2 and Pax5, and the absence of Bcl6, Blimp1, and Irf4 (Cobaleda et al., 2007; Muto et al., 2010; Nutt et al., 2011). The PC cell state, in turn, was identified by the presence of Blimp1 and Irf4, with a concomitant absence of Bach2 and Pax5 (Cobaleda et al., 2007; Igarashi et al., 2007). The molecular profiles used to identify DP, CD4, CD8, Th1, Th2, Th17, Treg, and CTL have been described elsewhere (Martínez-Sosa and Mendoza, 2013; Mendoza and Xenarios, 2006; Mendoza, 2006, 2013; Mendoza and Pardo, 2010) (see also Fig. A.1 for a summary of the resulting patterns of activation).

The case of Gfi1, Ikaros, and PU.1, require special mention since these molecules reach an intermediate level of activation in all attractors. PU.1 is a key regulator of lymphopoiesis, and it is known that it controls the expression of different genes at high and low (but detectable) concentrations. In particular, high levels of PU.1 skew haematopoiesis toward the myeloid lineage (Mak et al., 2011). Thus, an intermediate level of PU.1 in the lymphopoiesis model is adequate. Gfi1 is required for multilineage blood cell development, from stem and progenitor cells to differentiated lymphoid and myeloid cells (van der Meer et al., 2010). Gfi1 is a direct inhibitor of PU.1 (Möröy and Khandanpour, 2011); therefore, high or low levels of the former causes a shift in the required intermediate activation of PU.1, with the consequent malfunction of lymphopoiesis. Finally, Ikaros is a critical transcription factor required for the orderly development of all blood lineages (Davis, 2011). Specifically, Ikaros is required for both early and late events in lymphocyte differentiation (Pérez-Vera et al., 2011), and thus it should be present throughout the process of lymphopoiesis. This molecule is expressed in lymphoid cells, but it reaches higher expression levels in other hematopoietic cells (Dumortier et al., 2013; John and Ward, 2011). It then seems natural to qualitatively represent its expression in lymphocytes with intermediate activation values.

The 10 attractors reached by the dynamical system do not partition the state space equally. The identity and the percentage of random initial states leading to the attractors are as follows: Th17 (58.931%), Th1 (23.915%), Th2 (7.914%), Treg (4.120%), CD4 naive (2.455%), CTL (2.257%), CD8 naive (0.219%), B naive (0.115%), DP (0.056%), and PC (0.018%).

Note that while it was possible to assign specific identities to each of the attractors found by random search, the resulting set was missing attractors representing the CLP and pro-B cells. Early lymphoid differentiation from hematopoietic stem cells takes place in the bone marrow, therefore it was natural to ask if the simulation of the extracellular media present in the bone marrow was sufficient to recover the two missing attractors. This was assessed by directly running the set of differential equations, with the value of Flt3L fixed to 1, from the two states that represent CLP and pro-B cells. In the two cases the system was stationary. This is, by fixing the Flt3L node, which represents a signal that is produced by the bone marrow stroma, and not by the lymphocytes, it was possible to obtain two extra attractors, one representing the CLP, and another the pro-B cells (Table A.3 and Fig. A.1). Therefore, the model indicates that Flt3L functions as an instructive signal for the maintenance of the CLP and the pro-B cell phenotypes.

The specific incorporation of an extracellular signal to maintain an attractor can be interpreted such that the presence of the signal is absolutely necessary to maintain the cell type. Flt3L plays an important role in the differentiation of common lymphoid precursors from hematopoietic stem cells. Indeed, Flt3L-deficient mice display reduced numbers of CLPs, as well as of B and T cell precursors (Buza-Vidas et al., 2007; McKenna et al., 2000; Sitnicka et al., 2002). The model presented here, therefore, indicates that Flt3L has an instructive role in the the maintenance of CLPs. While it was

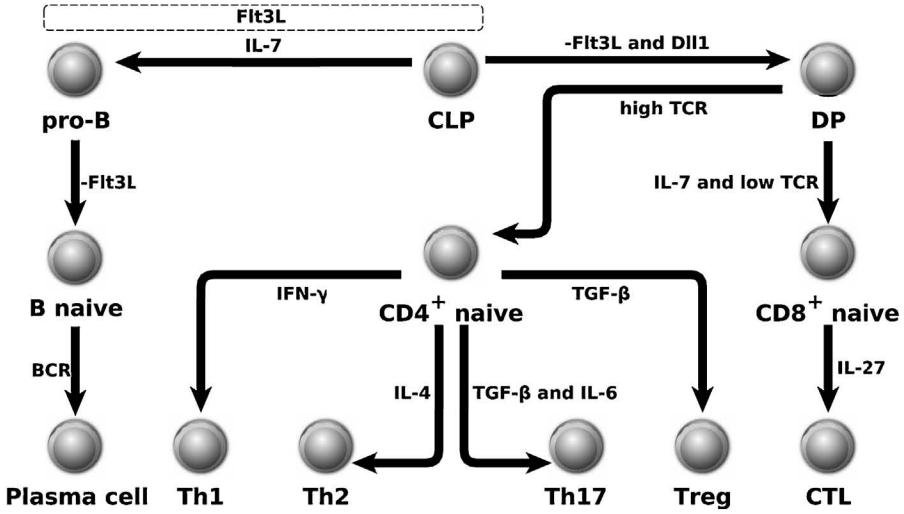

Fig. 2. The attractors of the regulatory network can be unambiguously interpreted as cell types. Furthermore, specific perturbations (names above arrows) are able to move the system between attractors, thus representing the differentiation process observed during lymphopoiesis.

known that this molecule has a critical role in the regulation of the earliest stages of T-cell development (see for example Sitnicka et al., 2007), it was not known that its presence is necessary for the maintenance of an expression pattern non-autonomously.

The dynamical system reached only fixed point attractors, and not cycles, which is in perfect accordance to the experimental data used to infer the regulatory networks. The capacity of a network to present a cyclic behaviour depends upon the presence of functional negative circuits (Thomas et al., 1995). While a formal study of circuits in the network controlling lymphopoiesis was outside the scope of this work, it is important to mention that it has been shown that a central part of the network lacks functional negative circuits (Mendoza, 2006). It is expected, however, that the choice of update rules for the nodes had a contribution on the fixed point attractors presented by the network.

3.3. Differentiation

The model presented here not only recovers the patterns of molecular activation observed in specific lymphocytes, but also is able to qualitatively describe the differentiation process from the CLP to six mature cell types, namely, plasma cell, Th1, Th2, Th17, Treg, and CTL, a process that is resumed in Fig. 2.

Fig. A.2 shows the simulation of the process of differentiation from the CLP to plasma cell. Starting from the CLP, due to a simulated continuous presence of Flt3L, the system is stable. However, a perturbation during 5 time units of saturating levels of IL-7 move the system from the CTL to the pro-B attractor. Experimentally, IL-7 is crucial for the differentiation of CLPs into pro-B cells and for pro-/pre-B-cell expansion (Miller et al., 2002). Then, in the simulation it is sufficient to eliminate the presence of external Flt3L, i.e. to fix the value of the Flt3L to 0, in order to move the system to the attractor representing a B naive cell. The elimination of the Flt3L signal in the simulation can be interpreted as the process during which pro-B cells migrate from the bone marrow into the blood. Then, a simulated saturating activation of the BCR during 5 time units is enough to move the system to the plasma cell attractor. This simulation mimics the experimental results showing that activation of the BCR with an antigen is a key signal for plasma cell differentiation (Shapiro-Shelef and Calame, 2005).

The model is able to describe the differentiation from CLP to CTL (Fig. A.3). The system is stable in the CLP due to a continuous presence of Flt3L. Then, the elimination of the Flt3L altogether with a saturating signal of Dll1 for 5 time units moves the system to the DP attractor. This combination of signals mimics the movement from the developing lymphocyte out of the bone marrow and into the thymus, in agreement with the experimental evidence reporting that Notch ligands (which include Dll1) are sufficient to promote the differentiation to DP cells (Ciofani et al., 2006; Michie et al., 2007). The DP attractor is stable, but when there is a perturbation consisting of saturating IL-7 signal for 5 time units combined with and a weak TCR signal (activation level set to 0.5 during 2.5 time units), the system evolves to the CD8 naive attractor. The role of the duration and strength of the TCR activation in the decision of the $CD4^+$ vs $CD8^+$ lineages has been studied in a previous version of the model, showing that there is a non-linear threshold above which the combination of strength and duration of the TCR signal determines the differentiation towards the $CD4^+$ phenotype (Martínez-Sosa and Mendoza, 2013). Finally, a saturating signal of IL-27 is enough to move the system into the CTL attractor, as it is observed experimentally (Morishima et al., 2010).

The model is able to recapitulate the differentiation of CLP to the T-helper branch of lymphocytes. As explained in the previous paragraph, the CLP attractor can be moved to the DP attractor. Then, a saturating TCR activation moves the system to the $CD4^+$ naive attractor. Finally, like in the previous versions of model (Martínez-Sosa and Mendoza, 2013; Mendoza and Pardo, 2010; Mendoza, 2013), the system moves to either the Th1, Th2, Th17, or Treg attractors, by simulating saturating signals of IFN-γ, IL-4, TGF-β + IL-6, or TGF-β, respectively (Figs. A.4–A.7).

To simulate the differentiation process, it was necessary to incorporate the capacity of responding to external signals. Fig. 2 shows the effect of 10 different signals. Four of them, namely IFN-γ, IL-4, IL-6, and TGF-β, can be produced by the lymphocytes modelled here. However, such signals are also produced by other cells. For example, macrophages secrete IL-4, IL-6, IL-10, and TGF-β (Stow et al., 2009). Therefore, the network in Fig. 1 contains nodes representing molecules that are not necessarily produced by lymphocytes, and thus such nodes are inactive in all the attractors in the Table A.3, but lymphocytes are able to respond to these external signals.

4. Conclusions

This work presents the inferred molecular regulatory network that controls lymphopoiesis. The system recovers 10 steady state

attractors. Moreover, by simulating a fixed external presence of Flt3L, the systems recovers two more fixed point attractors. All these 12 attractors are clearly identifiable with well-characterized lymphocytes. While the network was constructed mostly by incorporating known regulatory interactions, by using only well documented molecules and interactions it was not possible to recover the known dynamical behaviour at the molecular and cellular levels. This suggests that there is still a number of regulatory interactions that are yet to be experimentally documented. Indeed, by incorporating 15 regulatory molecular interactions that are not described in the literature it was possible to obtain a network describing the main observed stable patters of activation during lymphopoiesis as well as the transitions among them.

The interactions proposed in this work do not concentrate in a small set of molecules controlling a specific process. Such interactions involve master genes, surface markers, as well as secreted molecules. These interactions constitute very specific and testable predictions of the model. Given that the identification of regulatory interactions is central to the understanding of gene regulation, the prediction of specific regulatory interactions is a valuable biological result of the modelling effort described in this work. Nonetheless, it is important to stress that these new interactions do not imply the existence of direct physical molecular interactions. These interactions might be mediated by other molecules or factors not included in the regulatory network.

The model is able to recover the qualitative nature of the differentiation process itself, by recovering the response of the system to specific external signals, moving from one steady state to another. This dynamical behaviour clearly recapitulates key cellular differentiation events (Fig. 2), representing the directional and branched nature of lymphopoiesis, going from a multipotent progenitor to fully differentiated cell types. We expect these results to be robust within a large range in the space of parameters, as it has been shown in a previous version of the model (Mendoza and Pardo, 2010). This assumption, however, needs to be be demonstrated with future analyses of the present model. Furthermore, this model is able to reproduce a hierarchical bifurcation pattern triggered by specific sequential external signals representing a specific biological differentiation process. The modelling process presented in this work, therefore, is adequate to integrate in a single framework the molecular mechanisms behind the appearance of specific molecular expression/activation patterns, in a specific order, associated to the cell-fate decisions observed during a differentiation process.

Another important modelling result is that Flt3L has the role of an instructive signal. Specifically, this molecule is produced by the bone marrow stroma, and is necessary to maintain the molecular patterns identified with the CLP and pro-B cells. Previously, experimental results were able to identify master transcription factors (such as T-bet and GATA3) as key components to maintain the identity of lymphoid cells (Szabo et al., 2003; Hosoya et al., 2009). The model presented here indicates that Flt3L, a soluble growth factor, is a central element to the identity maintenance of two cell types during lymphopoiesis.

The differentiation process described by the model appears under different combinations of duration and intensity of the inducing signals. However, there should exist a threshold below which a signal is not able to move the system from one basin of attraction to another and thus unable to elicit differentiation. While we did not explore such thresholds, the network model controlling lymphopoiesis has the overall characteristic of producing a very reliable pattern of cell differentiation in response to specific sets of signals.

The dynamical behaviour of our model closely resembles the qualitative behaviour of the experimental system. While Boolean networks are the modelling framework of choice whenever there is only qualitative data available, for this particular biological system we wanted to incorporate the qualitative intermediate levels of expression reported for key molecules. Furthermore, the incorporation of qualitative continue variables allows for the analysis of the effect of gradients of external signals. For example, we showed in a previous study (Martínez-Sosa and Mendoza, 2013) that the incorporation of a qualitative continuous activation permitted to explain the dual role of TCR activation in the $CD4^+/CD8^+$ fate decision. Indeed, the use of qualitative continuous models has been useful for the analysis of the dynamical behaviour not only on the differentiation of lymphocytes, but in the analysis of other biological systems (see for example, Sánchez-Corrales et al., 2010; Sankar et al., 2011; Rodríguez et al., 2012).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biosystems.2015.09.004.

References

Åhsberg, J., Tsapogas, P., Qian, H., et al., 2010. Interleukin-7-induced Stat-5 acts in synergy with Flt-3 signaling to stimulate expansion of hematopoietic progenitor cells. J. Biol. Chem. 285 (47), 36275–36284.

Abou-Jaoudé, W., Monteiro, P.T., Naldi, A., Grandclaudon, M., Soumelis, V., Chaouiya, C., Thieffry, D., 2015. Model checking to assess T-helper cell plasticity. Front. Bioeng. Biotechnol. 2, 86.

Albert, R., Thakar, J., 2014 September. Boolean modeling: a logic-based dynamic approach for understanding signaling and regulatory networks and for making useful predictions. Wiley Interdiscip. Rev. Syst. Biol. Med. 6 (5), 353–369.

Alinikula, J., Nera, K.-P., Junttila, S., et al., 2011. Alternate pathways for Bcl6-mediated regulation of B cell to plasma cell differentiation. Eur. J. Immunol. 41 (8), 2404–2413.

Amsen, D., Blander, J.M., Lee, G.R., et al., 2004. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. Cell 117 (4), 515–526.

Annunziato, F., Cosmi, L., Liotta, F., et al., 2013. Main features of human T helper 17 cells. Ann. N. Y. Acad. Sci. 1284, 66–70.

Bonilla, F.A., Oettgen, H.C., 2010. Adaptive immunity. J. Allergy Clin. Immunol. 125 (2), 33–40.

Bouamar, H., Abbas, S., Lin, A.-P., et al., 2013. A capture-sequencing strategy identifies IRF8, EBF1, and APRIL as novel IGH fusion partners in b-cell lymphoma. Blood 122 (5), 726–733.

Braunstein, M., Anderson, M.K., 2011. HEB-deficient T-cell precursors lose T-cell potential and adopt an alternative pathway of differentiation. Mol. Cell. Biol. 31 (5), 971–982.

Bryder, D., Sigvardsson, M., 2010. Shaping up a lineage-lessons from B lymphopoesis. Curr. Opin. Immunol. 22 (2), 148–153.

Busslinger, M., 2004. Transcriptional control of early B cell development. Annu. Rev. Immunol. 22, 55–79.

Buza-Vidas, N., Cheng, M., Duarte, S., et al., 2007. Crucial role of FLT3 ligand in immune reconstitution after bone marrow transplantation and high-dose chemotherapy. Blood 110 (1), 424–432.

Cimmino, L., Martins, G.A., Liao, J., et al., 2008. Blimp-1 attenuates Th1 differentiation by repression of ifng, tbx21, and bcl6 gene expression. J. Immunol. 181 (4), 2338–2347.

Ciofani, M., Knowles, G.C., Wiest, D.L., et al., 2006. Stage-specific and differential notch dependency at the $\alpha\beta$ and $\gamma\delta$ T lineage bifurcation. Immunity 25 (1), 105–116.

Cobaleda, C., Schebesta, A., Delogu, A., Busslinger, M., 2007. Pax5: the guardian of B cell identity and function. Nat. Immunol. 8 (5), 463–470.

Crotty, S., October 2014. T follicular helper cell differentiation, function, and roles in disease. Immunity 41 (4), 529–542.

Davis, K.L., 2011. Ikaros: master of hematopoiesis, agent of leukemia. Ther. Adv. Hematol. 2 (6), 359–368.

De Silva, N.S., Simonetti, G., Heise, N., et al., 2012. The diverse roles of IRF4 in late germinal center B-cell differentiation. Immunol. Rev. 247 (1), 73–92.

Decker, T., di Magliano, M.P., McManus, S., et al., 2009. Stepwise activation of enhancer and promoter regions of the B cell commitment gene Pax5 in early lymphopoiesis. Immunity 30 (4), 508–520.
Dumortier, A., Kirstetter, P., Kastner, P., Chan, S., 2013. Ikaros regulates neutrophil differentiation. Blood 2219, 2226.
Fontenot, J.D., Gavin, M.A., Rudensky, A.Y., 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat. Immunol. 4 (4), 330–336.
Gavin, M.A., Rasmussen, J.P., Fontenot, J.D., et al., 2007. Foxp3-dependent programme of regulatory T-cell differentiation. Nature 445 (7129), 771–775.
Germar, K., Dose, M., Konstantinou, T., et al., 2011. T-cell factor 1 is a gatekeeper for T-cell specification in response to notch signaling. Proc. Natl. Acad. Sci. U. S. A. 108 (50), 20060–20065.
Giambra, V., Jenkins, C.R., Wang, H., et al., 2012. Notch1 promotes T cell leukemia-initiating activity by runx-mediated regulation of PKC-θ and reactive oxygen species. Nat. Med. 18 (11), 1693–1698.
Gwin, K., Frank, E., Bossou, A., et al., 2010. Hoxa9 regulates Flt3 in lymphohematopoietic progenitors. J. Immunol. 185 (11), 6572–6583.
Hirokawa, S., Sato, H., Kato, I., et al., 2003. Ebf-regulating Pax5 transcription is enhanced by STAT5 in the early stage of B cells. Eur. J. Immunol. 33 (7), 1824–2182.
Holmes, M.L., Carotta, S., Corcoran, L.M., et al., 2006. Repression of Flt3 by Pax5 is crucial for B-cell lineage commitment. Genes Dev. 20 (8), 933–938.
Hosoya, T., Kuroha, T., Moriguchi, T., Cummings, D., Maillard, I., Lim, K.-C., Engel, J.D., 2009. GATA-3 is required for early T lineage progenitor development. J. Exp. Med. 206 (13), 2987–3000.
Igarashi, K., Ochiai, K., Muto, A., 2007. Architecture and dynamics of the transcription factor network that regulates b-to-plasma cell differentiation. J. Biochem. 141 (6), 783–789.
Ivanov, I.I., McKenzie, B.S., Zhou, L., et al., 2006. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126 (6), 1121–1133.
Jiang, Q., Li, W.Q., Aiello, F.B., et al., 2005. Cell biology of IL-7, a key lymphotrophin. Cytokine Growth Factor Rev. 16 (4-5), 513–533.
John, L.B., Ward, A.C., 2011. The ikaros gene family: transcriptional regulators of hematopoiesis and immunity. Mol. Immunol. 48 (9-10), 1272–1278.
Johnson, K., Shapiro-Shelef, M., Tunyaplin, C., et al., 2005. Regulatory events in early and late B-cell differentiation. Mol. Immunol. 42 (7), 749–761.
Kallies, A., Nutt, S.L., 2010. Bach2: plasma-cell differentiation takes a break. EMBO J. 29 (23), 3896–3897.
Kallies, A., Hasbold, J., Tarlinton, D.M., et al., 2004. Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. J. Exp. Med. 200 (8), 967–977.
Kestler, H.A., Wawra, C., Kracher, B., et al., 2008. Network modeling of signal transduction: establishing the global view. Bioessays 30 (11-12), 1110–1125.
Kikuchi, H., Nakayama, M., Takami, Y., et al., 2012. EBF1 acts as a powerful repressor of Blimp-1 gene expression in immature B cells. Biochem. Biophys. Res. Commun. 422 (4), 780–785.
Klein, U., Dalla-Favera, R., 2008. Germinal centres: role in B-cell physiology and malignancy. Nat. Rev. Immunol. 8 (1), 22–33.
Klein, B., Tarte, K., Jourdan, M., et al., 2003. Survival and proliferation factors of normal and malignant plasma cells. Int. J. Hematol. 78 (2), 106–113.
Kunisawa, J., Hashimoto, E., Ishikawa, I., et al., 2012. A pivotal role of vitamin B9 in the maintenance of regulatory T cells in vitro and in vivo. PLoS ONE 7 (2), e32094.
Kusam, S., Vasanwala, F.H., Dent, A.L., 2004. Transcriptional repressor BCL-6 immortalizes germinal center-like B cells in the absence of p53 function. Oncogene 23 (3), 839–844.
Le Novère, N., 2015 February. Quantitative and logic modelling of molecular and gene networks. Nat. Rev. Genet. 16 (3), 146–158.
LeBien, T.W., Tedder, T.F., 2008. B lymphocytes: how they develop and function. Blood 112 (5), 1570–1580.
Li, L., Leid, M., Rothenberg, E.V., 2010a. An early T cell lineage commitment checkpoint dependent on the transcription factor Bcl11b. Science 329 (5987), 89–93.
Li, P., Burke, S., Wang, J., et al., 2010b. Reprogramming of T cells to natural killerlike cells upon Bcl11b deletion. Science 329 (5987), 85–89.
Lin, K.-I., Angelin-Duclos, C., Kuo, T.C., et al., 2002. Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. Mol. Cell. Biol. 22 (13), 4771–4780.
Lisovsky, M., Braun, S.E., Ge, Y., et al., 1996. Flt3-ligand production by human bone marrow stromal cells. Leukemia 10 (6), 1012–1018.
Lukin, K., Fields, S., Lopez, D., et al., 2010. Compound haploinsufficiencies of Ebf1 and Runx1 genes impede B cell lineage progression. Proc. Natl. Acad. Sci. U. S. A 107 (17), 7869–7874.
Möröy, T., Khandanpour, C., 2011. Growth factor independence 1 (gfi1) as a regulator of lymphocyte development and activation. Semin. Immunol. 23 (5), 368–378.
Ma, D., Wei, Y., Liu, F., 2013. Regulatory mechanisms of thymus and T cell development. Dev. Comp. Immunol. 39 (1-2), 91–102.
Mak, K.S., Funnell, A.P., Pearson, R.C., Crossley, M., 2011. Pu.1 and haematopoietic cell fate: dosage matters. Int. J. Cell Biol. 2011, 808524.
Malin, S., McManus, S., Cobaleda, C., et al., 2010. Role of STAT5 in controlling cell survival and immunoglobulin gene recombination during pro-b cell development. Nat. Immunol. 11 (2), 171–179.
Martínez-Sosa, P., Mendoza, L., 2013. The regulatory network that controls the differentiation of T lymphocytes. BioSystems 113 (2), 96–103.
Martinez-Sanchez, M.E., Mendoza, L., Villarreal, C., Alvarez-Buylla, E.R., 2015. A minimal regulatory network of extrinsic and intrinsic factors recovers observed patterns of CD4+ T cell differentiation and plasticity. PLoS Comput. Biol. 11 (6), e1004324.
Massagué, J., Xi, Q., 2012. TGF-β control of stem cell differentiation genes. FEBS Lett. 586 (14), 1953–1958.
McKenna, H.J., Stocking, K.L., Miller, R.E., et al., 2000. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. Blood 95 (11), 3489–3497.
Mendoza, L., Pardo, F., 2010. A robust model to describe the differentiation of T-helper cells. Theory Biosci. 129 (4), 283–293.
Mendoza, L., Xenarios, I., 2006. A method for the generation of standardized qualitative dynamical systems of regulatory networks. Theor. Biol. Med. Model. 3, 13.
Mendoza, L., 2006. A network model for the control of the differentiation process in Th cells. BioSystems 84 (2), 101–114.
Mendoza, L., 2013. A virtual culture of CD4+ T lymphocytes. Bull. Math. Biol. 75 (6), 1012–1029.
Michie, A.M., Chan, A.C., Ciofani, M., et al., 2007. Constitutive Notch signalling promotes CD4 CD8 thymocyte differentiation in the absence of the pre-TCR complex, by mimicking pre-TCR signals. Int. Immunol. 19 (12), 1421–1430.
Miller, J.P., Izon, D., DeMuth, W., et al., 2002. The earliest step in B lineage differentiation from common lymphoid progenitors is critically dependent upon interleukin 7. J. Exp. Med. 196 (5), 705–711.
Morishima, N., Mizoguchi, I., Okumura, M., Chiba, Y., Xu, M., Shimizu, M., Matsui, M., Mizuguchi, J., Yoshimoto, T., 2010. A pivotal role for interleukin-27 in CD8+ T cell functions and generation of cytotoxic T lymphocytes. J. Biomed. Biotechnol. 605483, 2010.
Murphy, K.M., Reiner, S.L., 2002. The lineage decisions of helper T cells. Nat. Rev. Immunol. 2 (12), 933–944.
Muto, A., Ochiai, K., Kimura, Y., Itoh-Nakadai, A., Calame, K.L., Ikebe, D., Tashiro, S., Igarashi, K., 2010. Bach2 represses plasma cell gene regulatory network in b cells to promote antibody class switch. EMBO J. 29 (23), 4048–4061.
Naito, T., Taniuchi, I., 2010. The network of transcription factors that underlie the CD4 versus CD8 lineage decision. Int. Immunol. 22 (10), 791–796.
Naito, T., Hirokazu, T., Naoe, Y., et al., 2011. Transcriptional control of T-cell development. Int. Immunol. 23 (11), 661–668.
Naldi, A., Carneiro, J., Chaouiya, C., et al., 2010. Diversity and plasticity of Th cell types predicted from regulatory network modelling. PLoS Comput. Biol. 6 (9), e1000912.
Nie, L., Perry, S., Zhao, Y., et al., 2008. Regulation of lymphocyte development by cell-type-specific interpretation of Notch signals. Mol. Cell. Biol. 28 (6), 2078–2090.
Nutt, S.L., Kee, B.L., 2007. The transcriptional regulation of B cell lineage commitment. Immunity 26 (6), 715–725.
Nutt, S.L., Taubenheim, N., Hasbold, J., et al., 2011. The genetic network controlling plasma cell differentiation. Semin. Immunol. 23 (5), 341–349.
Ochiai, K., Katoh, Y., Ikura, T., et al., 2006. Plasmacytic transcription factor Blimp-1 is repressed by Bach2 in B cells. J. Biol. Chem. 281 (50), 38226–38234.
O'Riordan Mary, O., Grosschedl, R., 1999. Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. Immunity 11 (1), 21–31.
Pérez-Vera, P., Reyes-León, A., Fuentes-Pananá, E.M., 2011. Signaling proteins and transcription factors in normal and malignant early B cell development. Bone Marrow Res. 502751, 2011.
Qin, J.-Z., Zhang, C.-L., Kamarashev, J., et al., 2001. Interleukin-7 and interleukin-15 regulate the expression of the bcl-2 and c-myb genes in cutaneous T-cell lymphoma cells. Blood 98 (9), 2778–2783.
Ramírez, J., Lukin, K., Hagman, J., 2010. From hematopoietic progenitors to B cells: mechanisms of lineage restriction and commitment. Curr. Opin. Immunol. 22 (2), 177–184.
Rodríguez, A., Sosa, D., Torres, L., Molina, B., Frías, S., Mendoza, L., 2012. A Boolean network model of the FA/BRCA pathway. Bioinformatics 28, 858–866.
Roffe, S., Hagai, Y., Pines, M., et al., 2010. Halofuginone inhibits Smad3 phosphorylation via the PI3K/Akt and MAPK/ERK pathways in muscle cells: effect on myotube fusion. Exp. Cell Res. 316 (6), 1061–1069.
Rothenberg, E.V., 2012. Transcriptional drivers of the T-cell lineage program. Curr. Opin. Immunol. 24 (2), 132–138.
Sánchez-Corrales, Y.E., Alvarez-Buylla, E.R., Mendoza, L., 2010. The Arabidopsis thaliana flower organ specification gene regulatory network determines a robust differentiation process. J. Theor. Biol. 264 (3), 971–983.
Saito, M., Gao, J., Basso, K., et al., 2007. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. Cancer Cell 12 (3), 280–292.
Sankar, M., Osmont, K.S., Rolcik, J., Gujas, B., Tarkowska, D., Strnad, M., Xenarios, I., C.S., 2011. A qualitative continuous model of cellular auxin and brassinosteroid signaling and their crosstalk. Bioinformatics 27 (10), 1404–1412.
Seo, W., Ikawa, T., Kawamoto, H., et al., 2012. Runx1-Cbfβ facilitates early B lymphocyte development by regulating expression of Ebf1. J. Exp. Med. 209 (7), 1255–1262.
Serre, K., Bénézech, C., Desanti, G., et al., 2011. Helios is associated with CD4 T cells differentiating to T helper 2 and follicular helper T cells in vivo independently of Foxp3 expression. PLoS ONE 6 (6), e20731.
Shapiro-Shelef, M., Calame, K., 2005. Regulation of plasma-cell development. Nat. Rev. Immunol. 5, 230–242.
Sitnicka, E., Bryder, D., Theilgaard-Mönch, K., et al., 2002. Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool. Immunity 17 (4), 463–472.
Sitnicka, E., Buza-Vidas, N., Ahlenius, H., Cilio, C.M., Gekas, C., Nygren, J.M., Mansson, R., Cheng, M., Jensen, C.T., Svensson, M., Leandersson, K., Agace, W.W.,

Sigvardsson, M., Jacobsen, S.E., 2007. Critical role of flt3 ligand in il-7 receptor independent t lymphopoiesis and regulation of lymphoid-primed multipotent progenitors. Blood 110 (8), 2955–2964.
So, T., Croft, M., 2013. Regulation of PI-3-kinase and Akt signaling in T lymphocytes and other cells by TNFR family molecules. Front. Immunol. 4.
Soetaert, K., Petzoldt, T., Setzer, W.R., 2010. Solving differential equations in R. R J. 2 (2), 5–15.
Spooner, C.J., Cheng, J.X., Pujadas, E., et al., 2009. A recurrent network involving the transcription factors PU.1 and Gfi1 orchestrates innate and adaptive immune cell fates. Immunity 31 (4), 576–586.
Stow, J.L., Low, P.C., Offenhäuser, C., Sangermani, D., 2009. Cytokine secretion in macrophages and other cells: pathways and mediators. Immunobiology 214 (7), 601–612.
Szabo, S.J., Sullivan, B.M., Peng, S.L., et al., 2003. Molecular mechanisms regulating Th1 immune responses. Annu. Rev. Immunol. 21, 713–758.
Thomas, R., Thieffry, D., Kaufman, M., 1995. 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. 57 (2), 247–276.
Tran, D.Q., 2012. TGF-β: the sword, the wand, and the shield of FOXP3(+) regulatory T cells. J. Mol. Cell Biol. 4 (1), 29–37.
Tunyaplin, C., Shaffer, A.L., Angelin-Duclos, C.D., et al., 2004. Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. J. Immunol. 173 (2), 1158–1165.
van der Meer, L.T., Jansen, J.H., van der Reijden, B.A., 2010. Gfi1 and Gfi1b: key regulators of hematopoiesis. Leukemia 24 (11), 1834–1843.
Wang, D., Claus, C.L., Vaccarelli, G., et al., 2006. The basic helix-loop-helix transcription factor HEBAlt is expressed in pro-T cells and enhances the generation of T cell precursors. J Immunol 177 (1), 109–119.
Weber, B.N., Chi, A.W.-S., Chavez, A., et al., 2011. A critical role for TCF-1 in T-lineage specification and differentiation. Nature 476 (7358), 63–68.
Weinstein, N., Mendoza, L., 2012. Building qualitative models of plant regulatory networks with SQUAD. Front. Plant Sci. 3, 72.
Wong, W.F., Kurokawa, M., Satake, M., et al., 2011. Down-regulation of Runx1 expression by TCR signal involves an autoregulatory mechanism and contributes to IL-2 production. J. Biol. Chem. 286 (13), 11110–11118.
Xiong, Y., Bosselut, R., 2012. CD4-CD8 differentiation in the thymus: connecting circuits and building memories. Curr. Opin. Immunol. 24 (2), 139–145.
Yamaguchi, T., Hirota, K., Nagahama, K., et al., 2007. Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. Immunity 27 (1), 145–159.
Yasuda, T., Hayakawa, F., Kurahashi, S., et al., 2012. B cell receptor-ERK1/2 signal cancels PAX5-dependent repression of BLIMP1 through PAX5 phosphorylation: a mechanism of antigen-triggering plasma cell differentiation. J. Immunol. 188 (12), 6127–6134.
Yi, J.S., Cox, M.A., Zajac, A.J., 2010. Interleukin-21: a multifunctional regulator of immunity to infections. Microbes Infect. 12 (14-15), 1111–1119.
Zhang, Q., Cui, F., Fang, L., et al., 2013. TNF-α impairs differentiation and function of TGF-β-induced Treg cells in autoimmune diseases through Akt and Smad3 signaling pathway. J. Mol. Cell Biol. 5 (2), 85–98.
Zhang, H., Kong, H., Zeng, X., et al., 2014. Subsets of regulatory T cells and their roles in allergy. J. Transl. Med. 12 (1), 125.

Appendix: A dynamical model of the regulatory network controlling lymphopoiesis

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Table A.1: List of the regulatory interactions of the model and supporting references

Interaction	Description	References
Akt $\dashv$ SMAD3	The protein kinase Akt inhibits the phosphorylation of SMAD3, a transducer of the TGF-β signalling pathway	[1, 2]
Bach2 $\dashv$ Blimp1	The transcription factor Bach2 binds to a Maf recognition element in the promoter of the *Prdm1* gene, coding for Blimp1, inhibiting its expression	[3, 4]
Bcl6 $\rightarrow$ Bach2	The transcription factor Bcl6 binds directly to *Bach2* promoting its expression	[5]
Bcl6 $\dashv$ Blimp1	Bcl6 represses Blimp1 expression by direct binding to the first intron of the *Prdm1* gene	[6, 7]
BCR $\rightarrow$ ERK1\2	BCR signalling activates the protein kinases ERK1\2 via phosphorylation	[8, 9]
BCR $\rightarrow$ NF-κB	BCR signalling promotes the activation and translocation of the transcription factor NF-κB into the nucleus	[10]
Blimp1 $\dashv$ Bcl6	Transcription factor Blimp1 binds directly to the *Bcl6* gene repressing its expression	[11, 12]
Blimp1 $\rightarrow$ Irf4	Blimp1 induces the expression of the *Irf4* gene conforming a positive mutual regulatory loop with the transcription factor Irf4	[10, 13]
Blimp1 $\dashv$ Pax5	Blimp1 directly binds to a site in the *Pax5* promoter repressing its transcription	[14, 15]
Blimp1 $\rightarrow$ XBP1	The transcription factor XBP1 is expressed in a Blimp1-dependent manner	[14, 12]
B9 $\rightarrow$ FR4	Vitamin B9 acts through the folate receptor FR4	[16, 17]
CD4 $\rightarrow$ CD4	The self regulatory interaction of the CD4 receptor represents the epigenetic mechanisms that maintain stable expression patterns of this gene in $CD4^+$ cells	[18]
CD4 $\dashv$ Runx3	Expression of the transcription factor Runx3 is downregulated in $CD4^+CD8^-$ cells	[18]
Continues ...		

Continuation

Interaction	Description	References
CD4 → ThPOK	Transcription factor ThPOK is upregulated in $CD4^+CD8^-$ cells	[18]
CD8 → CD8	The self regulatory interaction of the CD8 receptor represents the epigenetic mechanisms that maintain stable expression patterns of this gene in $CD8^+$ cells	[18]
CD8 → Runx3	Transcription factor Runx3 is upregulated in $CD4^-CD8^+$ cells	[18]
CD8 ⊣ ThPOK	An inhibition of the receptor CD8 over ThPOK, the master regulator of CD4 lineage, was included in order to account for the binary decision between CD8 and CD4 lineages	Predicted in this work
Dll1 → Notch1	Delta-like protein 1 acts as a ligand for the receptor Notch1	[19]
Ebf1 → Bcl6	Transcription factor Ebf1 directly binds to the *Bcl6* promoter and positively regulates its expression	[20]
Ebf1 ⊣ CD4	Inhibition of Ebf1 over the coreceptor CD4 was included in order to account for the inhibition of the $CD8^+$ T cell lineage in B cells	Predicted in this work
Ebf1 ⊣ CD8	Inhibition of Ebf1 over the coreceptor CD8 is meant to represent the inhibition of the $CD8^+$ T cell lineages in the B cell lineage	Predicted in this work
Ebf1 → Irf4	Ebf1 activation over the transcription factor Irf4 was included to represent the mechanisms controlling early Irf4 activation in the B cell lineage	Predicted in this work
Ebf1 → Pax5	Ebf1 directly binds to the promoter of the *Pax5* gene and promotes its expression	[21, 22]
Eomes → Gzmb	The transciption factor Eomes directly regulates the expression of Gzmb enzyme through direct binding to the *Gzmb* promoter	[23, 24]
Eomes → IFN-γ	Eomes directly regulates the expression of the cytokine IFN-γ	[24]

Continues ...

Continuation

Interaction	Description	References
Eomes $\rightarrow$ Prf1	Eomes regulates the expression of cytolitic protein Prf1 through direct binding to the *Prf1* promoter	[23, 24]
Foxp3 $\rightarrow$ FR4	FR4 receptor ir upregulated in Foxp3$^+$Treg cells	[16, 17]
Foxp3 $\rightarrow$ Foxp3	Foxp3 positively regulates its own transcription through an unknown mechanism	[25]
Foxp3 $\dashv$ GATA3	GATA3 transcription is downregulated by Foxp3 through a not described mechanism	[26, 27]
Foxp3 $\dashv$ RORγt	Foxp3 directly interacts with the transcription factor RORγ inhibiting its function	[28]
Foxp3 $\dashv$ Tbet	The abolition of Foxp3 promotes the activation of the transcription factor Tbet	[29, 30]
Foxp3 $\rightarrow$ TGF-β	Activation of Foxp3 over TGF-β was included to represent the induction of TGF-β in Foxp3$^+$Treg cells	Predicted in this work
Foxp3 $\rightarrow$ ThPOK	The activation of Foxp3 over the transcription factor ThPOK accounts for the positive regulation of the two factors in CD4$^+$ cells	[18]
Flt3 $\rightarrow$ ERK1\2	The tyrosine protein kinase Flt3 activates the protein kinases ERK1\2 via phosphorylation	[31]
Flt3 $\dashv$ Irf4	Inhibition of Flt3 over the transcription factor Irf4 was included to represent the inhibition of the PC program in CLP cells	Predicted in this work
Flt3 $\dashv$ Pax5	Inhibition of Flt3 over the transcription factor Pax5 was included to represent the inhibition of the B cell program in CLP cells	Predicted in this work
Flt3L $\rightarrow$ Flt3	The cytokine Flt3L directly binds and activates the tyrosine-protein kinase Flt3	[32]
FR4 $\rightarrow$ Bcl2	FR4 signaling promote the upregulation of the inhibitor of apoptotis Bcl2	[16]
GATA3 $\dashv$ Foxp3	Expression of the transcription factor Foxp3 is downregulated after the induction of the transcription factor GATA3 through a not described mechanism	[33]

Continues ...

Continuation

Interaction	Description	References
GATA3 → GATA3	The transcription factor GATA3 regulates its own transcription through direct binding to the *Gata3* promoter	[34, 35]
GATA3 → IL-10	Production of cytokine IL-10 is induced in cells expressing the transcription factor GATA3, the master regulator of Th2 cells	[36]
GATA3 → IL-4	Expression of GATA3 induces the production of the cytokine IL-4 in Th2 cells	[34]
GATA3 ⊣ RORγt	Expression of the transcription factor RORγt is downregulated in Th2 cells expressing GATA3	[37]
GATA3 ⊣ STAT4	Protein levels of the signal transducer STAT4 are downregulated by GATA3	[38]
GATA3 ⊣ Tbet	Transcription of Tbet is downregulated by virus-induced GATA3 expression through an unknown mechanism	[38]
GATA3 → ThPOK	GATA3 directly binds to a region in the *ThPOK* locus and positively regulates ThPOK expression	[39, 40]
Gfi1 ⊣ PU.1	Transcription factor Gfi1 directly inhibits the binding of the transcription factor PU.1 to its target genes through direct interaction with PU.1 protein or by competition for regulatory sites in different promoters	[41, 42]
HEB ⊣ GATA3	The E-protein transcription factor HEB inhibits GATA3 expression and promotes T-cell specification	[43]
Hoxa9 → Flt3	Transcription factor Hoxa9 directly binds to the *Flt3* promoter and induces its expression	[44]
IFN-γ → IFN-γR	Cytokine IFN-γ directly binds to the IFN-γR membrane receptor	[45, 46]
IFN-γR → JAK1	IFN-γR directly interacts with the tyrosine-protein kinase JAK1 and other members of the JAK family and promotes its activation via phosphorylation	[47, 48]

Continues ...

Continuation

Interaction	Description	References
IFN-β $\rightarrow$ IFN-βR	The cytokine IFN-β directly binds to the receptor IFN-βR	[49]
IFN-βR $\rightarrow$ STAT1	Activation of the receptor IFN-βR promotes the recruitment of the signal transducer and activator of transcription STAT1 and its activation via phosphorylation	[49]
Ikaros $\rightarrow$ Gfi1	Transcription factor Ikaros directly binds to the *Gfi1* promoter and promotes its expression	[41, 42]
Ikaros $\dashv$ PU.1	Ikaros inhibition over the transcription factor PU.1 was postulated to simulate the maintenance of intermediate levels of Ikaros and PU.1 necessary for the development of lymphocyte lineages	Predicted in this work
IL-2 $\rightarrow$ IL-2R	Interleukin IL-2 binds directly to the receptor IL-2R	[50, 51]
IL-2R $\rightarrow$ STAT5	The interleukin receptor IL-2R activates the signal transducer and activator of transcription STAT5	[50, 51]
IL-4 $\rightarrow$ IL-4R	Interleukin IL-4 binds to the receptor IL-4R	[52]
IL-4R $\rightarrow$ STAT6	IL-4R activation induces the activation of the signal transducer and activator of transcription STAT6	[53]
IL-6 $\rightarrow$ IL-6R	Interleukin IL-6 is recognized by the receptor IL-6R	[54, 55]
IL-6R $\rightarrow$ JAK3	IL-6R signalling induces the activation of the tyrosine-protein kinase JAK3	[56]
IL-7 $\rightarrow$ IL-7R	Interleukin IL-7 binds to the receptor IL-7R which is activated along with several components of the cytokine receptor family	[57]
IL-7R $\rightarrow$ JAK3	IL-7R signalling induces JAK3 activation via phosphorylation	[57]
IL-10 $\rightarrow$ IL-10R	Interleukin IL-10 binds directly to the receptor IL-10R	[58]

Continues ...

Continuation

Interaction	Description	References
IL-10R → STAT3	The signal transducer and activator of transcription STAT3 is directly recruited to the IL-10R and is activated via phosphorylation	[58]
IL-12 → IL-12R	Interleukin IL-12 is recognized by the receptor IL-12R	[59]
IL-12R → STAT4	IL-12R signalling promote the activation of the signal transducer and activator of transcription STAT4	[59]
IL-18 → IL-18R	Interleukin IL-18 binds directly to the receptor IL-18R	[60]
IL-18R → IRAK	IL-18R signal is mediated by the activation of the Interleukin-1 receptor associated kinase IRAK	[60]
IL-21R → Bcl6	IL-21R signalling induces Bcl6 expression through JunD/AP-1 activation	[3, 61]
IL-21 → IL-21R	Interleukin IL-21 directly binds to the interleukin receptor IL-21R	[61]
IL-21R → STAT3	IL-21R signalling is mediated by the signal transducer and activator of transcription STAT3	[61]
IL-23 → IL-23R	The heterodimeric cytokine IL-23 binds to the receptor IL-23R	[54, 55]
IL-23R → STAT3	IL-23R directly associates with the signal transducer and activator of transcription STAT3 inducing its activation	[62]
IL-27 → IL-27R	The heterodimeric cytokine IL-27 directly binds to IL-27R	[63]
IL-27R → Eomes	IL-27R signalling induces the upregulation of the transcription factor Eomes possibly through an STAT1-independent pathway	[64, 65]
IL-27R → STAT1	IL-27R directly activates STAT1 via phosphorylation	[63]
Irf4 ⊣ Bcl6	Transcription factor Irf4 directly binds to the promoter of *Bcl6* repressing its expression	[5, 66]

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Continuation

Interaction	Description	References
Irf4 $\rightarrow$ Blimp1	Irf4 binds to the promoter of the *Prdm1* gene positively regulating the expression of the transcription factor Blimp1	[3, 67]
Irf4 $\dashv$ Pax5	Irf4 binds to the enhancer of the *Pax5* gene and regulates its expression	[68, 3]
IRAK $\rightarrow$ IFN-γ	Production of cytokine IFN-γ is abrogated in the absence of the interleukin-1 receptor associated kinase IRAK	[69]
JAK1 $\rightarrow$ STAT1	Activation of the tyrosine kinase JAK1 promotes the phosphorylation and subsequent activation of the signal transducer and activator of transcription STAT1	[47, 70]
JAK3 $\rightarrow$ STAT3	The tyrosine kinase JAK3 activates the signal transducer and activator of transcription STAT3	[71]
JAK3 $\rightarrow$ STAT5	The tyrosine kinase JAK3 positively regulates the activity of signal transducer and activator of transcription STAT5	[41, 72]
NF-κB $\rightarrow$ Helios	Expression of the transcription factor Helios depends on the activation of the transcription factor NF-κB	[73]
NF-κB $\rightarrow$ Irf4	NF-κB directly binds to the *Irf4* promoter and positively regulates its expression	[10]
Notch1 $\rightarrow$ Bcl11b	The transcriptional activator Notch1 associates with the CSL factor and positively regulates Bcl11b expression through binding to the *Bcl11b* promoter	[74, 75]
Notch1 $\rightarrow$ CD4	Notch1 signalling promotes upregulation of the T-cell surface glycoprotein CD4	[76]
Notch1 $\rightarrow$ CD8	otch1 signalling promotes upregulation of the T-cell surface glycoprotein CD8	[76]
Notch1 $\rightarrow$ HEB	Notch1 signalling promotes the activation of HEB, a bHLH class transcription factor	[77]

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Continuation

Interaction	Description	References
Notch1 ⊣ JAK3	Notch1 signalling promotes the ubiquitin-mediated degradation of the tyrosine-protein kinase JAK3	[78]
Notch1 ⊣ Runx1	Inhibition of Notch1 over the transcription factor Runx1 was included to account for Runx1 down-regulation in early stages of T cell development	Predicted in this work
Notch1 → TCF-1	The transcription factor TCF-1 is upregulated upon the activation of Notch1 signalling	[79, 80]
Pax5 → Bach2	The transcription factor Pax5 positively regulates the transcription factor Bach2 by regulating the chromatin accessibility to the *Bach2* promoter	[81, 14]
Pax5 ⊣ Blimp1	Pax5 directly binds to a regulatory site in the first intron of the *Prdm1* gene and represses Blimp1 expression	[9]
Pax5 → CD19	Pax5 directly binds to the *CD19* promoter and positively regulates its expression	[82]
Pax5 ⊣ Flt3	Expression of the cell-surface receptor and tyrosine kinase Flt3 is upregulated upon Pax5 activation	[83]
PU.1 → Ikaros	Activation of the transcription factor PU.1 over the transcription factor Ikaros was postulated in order to maintain constant intermediate expression levels for the two factors due to the existence of a negative regulatory circuit	Predicted in this work
RORγt ⊣ Foxp3	Activation of the nuclear receptor and transcription factor RORγt downregulates the expression of the transcription factor Foxp3	[84]
RORγt ⊣ GATA3	Expression of the transcription factor GATA3 is abrogated in cells expressing RORγt	[84, 29]
RORγt → IL-17	RORγt induces the transcription of *Il17*	[54]
RORγt → IL-6	RORγ upregulation induces IL-6 production	[54]

Continues ...

Continuation

Interaction	Description	References
RORγt → RORγt	Positive self-regulation of the master regulator RORγt maintain stable production of IL-17 in Th17 cells	[85]
RORγt ⊣ Tbet	Expression of the transcription factor Tbet is abrogated in cells expressing RORγt	[54, 50]
RORγt → ThPOK	Activation of RORγt over the transcription factor ThPOK was proposed in previous models in order to account for the positive regulation of ThPOK in the $CD4^+$ lineage by other master genes	[18]
RORγt → TNF-α	Activation of the cytokine TNF-α by the master regulator RORγt was proposed to simulate the regulation of TNF-α production by Th17 cells	Predicted in this work
Runx1 → Ebf1	Transcription factor Runx1 directly binds to the *Ebf1* promoter and positively regulates its expression	[86]
Runx1 ⊣ GATA3	Expression of the transcription factor GATA3 is downregulated upon induction of Runx1	[87]
Runx1 → Runx1	Positive autoregulation of Runx1 was proposed to simulate the constant activation of Runx1 in lymphopoiesis	Predicted in this work
Runx3 ⊣ CD4	Transcription factor Runx3 acts as a transcriptional repressor of CD4 by binding to an intronic region of the *CD4* gene	[88, 89]
Runx3 → CD8	Expression of the CD8 coreceptor is upregulated upon induction of Runx3, a key master regulator of the CD8 lineage	[90, 40]
Runx3 ⊣ Ebf1	Inhibition of Runx3 over the transcription factor Ebf1 was proposed to account for Ebf1 downregulation in the CD8 lineage	Predicted in this work
Runx3 → Eomes	Activation of Runx3 is required for Eomes induction during CTL differentiation	[91]
Runx3 ⊣ Runx1	Expression of the transcription factor Runx1 is downregulated in a Runx3-dependent manner	[92]

Continues ...

Continuation

Interaction	Description	References
Runx3 ⊣ ThPOK	Expression of the transcription factor ThPOK is upregulated in Runx3-deficient cells and Runx3 binds to a transcriptional silencer region in the *ThPOK* gene	[93]
SMAD2 → Foxp3	The signal transducer and transcriptional modulator SMAD2 positively regulates the expression of *Foxp3* upon TGF-β stimulation	[94, 95]
SMAD2 → RORγt	SMAD2 directly interacts with RORγt acting as a coactivator	[96]
SMAD3 → Foxp3	The signal transducer and modulator of transcription SMAD3 positively regulates Foxp3 by binding to the transcription control elements in the *Foxp3* gene	[2]
SOCS1 ⊣ IL-4R	The suppressor of cytokine signalling SOCS1 represses IL-4 signalling by directly inhibiting key components of the IL-4 signalling pathway	[97]
SOCS1 ⊣ STAT5	SOCS1 negatively regulates the signal transducer and activator of transcription STAT5 by inhibiting its phosphorylation	[98, 99]
STAT1 ⊣ IL-4	The signal transducer and activator of transcription STAT1 forms a complex with other proteins of the IFN-γ signalling pathway and inhibits the expression of the *Il4* gene	[100]
STAT1 → SOCS1	STAT1 positively regulates the expression of the suppressor of cytokine signalling STAT1 by binding to regulatory regions in the *Socs1* gene upon IFN-γ stimulation	[101, 102]
STAT1 → Tbet	Expression of the transcription factor Tbet is upregulated by IFN-γ signalling via STAT1 activation	[103]
STAT3 ⊣ Foxp3	Signal transducer and activator of transcription negatively regulates Foxp3 expression by binding to an enhancer region of the *Foxp3* gene competing with other factors for these regulatory sites	[104, 105]

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Continuation

Interaction	Description	References
STAT3 $\dashv$ IFN-γ	IL-10 signalling, transduced via STAT3 inhibits IFN-γ production	[58]
STAT3 $\rightarrow$ RORγt	STAT3 activated by IL-6 and IL-23 signalling promotes upregulation of the orphan nuclear receptor RORγt	[106]
STAT4 $\rightarrow$ IFN-γ	IL-12 signalling transduced via STAT4 induces IFN-γ production	[107, 38]
STAT5 $\rightarrow$ Bcl2	The suppressor of apoptosis Bcl2 is activated by IL-7 signalling mediated by STAT5 activation	[108]
STAT5 $\rightarrow$ Pax5	Expression of the transcription factor Pax5 is induced upon IL-7 stimulation mediated by the signal transducer and activation of transcription STAT5	[21]
STAT5 $\rightarrow$ Runx3	STAT5 activated by IL-7 signalling induces the expression of the transcription factor Runx3	[98, 109]
STAT6 $\rightarrow$ GATA3	Expression of the transcription factor GATA3 is upregulated upon IL-4 signalling mediated by STAT6 activation	[53]
STAT6 $\dashv$ IL-12R	IL-4 signalling transduced via STAT6 inhibits the expression of one subunit of the IL-12 receptor	[110]
STAT6 $\dashv$ IL-18R	IL-4 signalling blocks IL-18 signalling in a STAT6-dependent manner by downregulating a subunit of the IL-18 receptor	[111]
Tbet $\rightarrow$ Eomes	The transcription factor Tbet positively regulates Eomes expression through an IL-27-independent mechanism	[112]
Tbet $\dashv$ Foxp3	Expression of the transcription factor Foxp3 is downregulated in cells expressing Tbet through a mutually inhibitory circuit	[29, 30]
Tbet $\dashv$ GATA3	GATA3 expression is downregulated by Tbet through a mutually inhibitory circuit	[113]
Tbet $\rightarrow$ IFN-γ	IFN-γ production is induced upon Tbet upregulation	[113]

Continues ...

Continuation

Interaction	Description	References
Tbet $\dashv$ RORγt	RORγt expression is downregulated by Tbet through a mutually inhibitory circuit	[54, 50]
Tbet $\rightarrow$ SOCS1	Supressor of cytokine signalling SOCS1 is activated by Tbet via a pathway independent of IFN-γ	[113]
Tbet $\rightarrow$ Tbet	The transcription factor Tbet positively regulates its own expression through an unknown mechanism	[107]
Tbet $\rightarrow$ ThPOK	Activation of ThPOK by Tbet was proposed in a previous model to simulate the regulation of ThPOK by other master regulators in the CD4 lineage	[18]
TCF-1 $\rightarrow$ Bcl11b	Transcription factor TCF-1 directly binds to the *Bcl11b* gene and promotes the expression of the transcription factor Bcl11b	[114]
TCF-1 $\rightarrow$ GATA3	TCF-1 directly binds to the *Gata3* gene positively regulating its expression	[114]
TCR $\dashv$ CD8	TCR signalling downregulates expression of the *Cd8* gene	[115, 116]
TCR $\dashv$ IL-7R	Induction of TCR signalling leads to a decreased expression of the IL-7 receptor	[109, 117]
TCR $\dashv$ Runx1	TCR signalling inhibits Runx1 by negatively regulating the distal promoter of the *Runx1* gene	[87]
TCR $\rightarrow$ ThPOK	TCR signalling induces expression of the transcription factor ThPOK through an unknown mechanism	[90]
TCR $\rightarrow$ NFAT	TCR signalling induces activation of the nuclear factor of activated T cells NFAT by promoting dephosphorylation and translocation of this transcription factor into the nucleus	[118]
TGF-β $\rightarrow$ TGF-βR	The cytokine TGF-β directly binds to the receptor TGF-βR	[119]
TGF-βR $\rightarrow$ SMAD2	The TGF-β receptor activates the cytoplasmic transcription factor SMAD2 by promoting its translocation into the nucleus	[94, 95]

Continues ...

Continuation

Interaction	Description	References
TGF-βR $\rightarrow$ SMAD3	The TGF-β receptor activates the cytoplasmic transcription factor SMAD3 by promoting its translocation into the nucleus	[120]
TNF-α $\rightarrow$ TNFR2	The Cytokine TNF-α directly binds and activates the receptor TNFR2	[2]
TNFR2 $\rightarrow$ Akt	The TNF receptor TNFR2 induces Akt through phosphorylation	[121]
ThPOK $\rightarrow$ CD4	Transcription factor ThPOK positively regulates expression of the coreceptor CD4 through an unknown mechanism	[90]
ThPOK $\dashv$ CD8	Expression of the coreceptor CD8 is inhibited upon ThPOK activation	[122]
ThPOK $\dashv$ Pax5	Negative regulation of ThPOK over Pax5 was proposed in order to account for the inhibition of the B cell program in the CD4 lineage	Predicted in this work
ThPOK $\dashv$ Runx1	Inhibition of the transcription factor Runx1 by the transcription factor ThPOK was included to simulate Runx1 downregulation during early stages of T cell development	Predicted in this work
ThPOK $\dashv$ Runx3	Runx3 expression is downregulated in a ThPOK-dependent manner through a not described mechanism	[123, 40]
ThPOK $\rightarrow$ ThPOK	ThPOK positively regulates its own transcription by inhibiting the binding of antagonistic regulatory factors to a silencer region of the *Zbtb7b* gene coding for ThPOK	[124]

Table A.2: Boolean expressions describing the regulatory interactions among nodes in the network controlling lymphopoiesis.

Node	Regulatory interactions
Akt	TNFR2
B9	is an input
Bach2	Pax5 *OR* Bcl6
Bcl2	FR4 *OR* STAT5
Bcl6	IL21R *AND* Ebf1 *AND NOT* Blimp1 *AND NOT* Irf4
Bcl11b	Notch1 *AND* TCF1
BCR	is an input
Blimp1	Irf4 *AND NOT* Bach2 *AND NOT* Bcl6 *AND NOT* Pax5
CD4	(CD4 *OR* Notch1 *OR* ThPOK) *AND NOT* Ebf1 *AND NOT* Runx3
CD8	(CD8 *OR* Notch1 *OR* Runx3) *AND NOT* Ebf1 *AND NOT* TCR AND NOT ThPOK
CD19	Pax5
Dll1	is an input
Ebf1	Runx1 *AND NOT* Runx3
EOMES	(IL27R OR Tbet) *AND* Runx3
ERK	BCR *OR* Flt3
Flt3	(Flt3L OR Hoxa9) *AND NOT* Pax5
Flt3L	is an input
Foxp3	(SMAD2 *OR* SMAD3 *OR* Foxp3) *AND NOT* GATA3 *AND NOT* RORgt *AND NOT* STAT3 *AND NOT* Tbet
FR4	B9 *AND* Foxp3
GATA3	(GATA3 *OR* STAT6 *OR* TCF1) *AND NOT* Foxp3 *AND NOT* HEB *AND NOT* RORgt *AND NOT* Runx1 *AND NOT* Tbet
Gfi1	Ikaros
Gzmb	EOMES
HEB	Notch1
Helios	NFkB
Hoxa9	is an input
IFNb	is an input
IFNbR	IFNb
IFNg	(EOMES *OR* IRAK *OR* STAT4 *OR* Tbet) *AND NOT* STAT3
IFNgR	IFNg
Continues ...	

Continuation

Node	Regulatory interactions
Ikaros	PU1
IL2	is an input
IL2R	IL2
IL4	GATA3 *AND NOT* STAT1
IL4R	IL4 AND *NOT* SOCS1
IL6	RORgt
IL6R	IL6
IL7	is an input
IL7R	IL7 *AND NOT* TCR
IL10	GATA3
IL10R	IL10
IL12	is an input
IL12R	IL12 *AND NOT* STAT6
IL17	RORgt
IL18	is an input
IL18R	IL18 *AND NOT* STAT6
IL21	is an input
IL21R	IL21
IL23	is an input
IL23R	IL23
IL27	is an input
IL27R	IL27
IRAK	IL18R
Irf4	(Blimp1 *OR* NFkB) *AND* Ebf1 *AND NOT* Flt3
JAK1	IFNgR
JAK3	(IL6R *OR* IL7R) *AND NOT* Notch1
NFkB	BCR
NFAT	TCR
Notch1	Dll1
Pax5	(Ebf1 *AND NOT* Blimp1 *AND NOT* Flt3 *AND NOT* Irf4 *AND NOT* ThPOK) *OR* (Ebf1 *AND* STAT5 *AND NOT* Blimp1 *AND NOT* Irf4 *AND NOT* ThPOK)
Prf1	EOMES
PU1	*NOT* Gfi1 *AND NOT* Ikaros
RORgt	(SMAD2 *AND* STAT3) *OR* (RORgt *AND NOT* Foxp3 *AND NOT* GATA3 *AND NOT* Tbet)
Runx1	Runx1 *AND NOT* Notch1 *AND NOT* Runx3 *AND NOT* TCR *AND NOT* ThPOK

Continues ...

Continuation	
Node	Regulatory interactions
Runx3	(CD8 *AND NOT* CD4 *AND NOT* STAT5 *AND NOT* ThPOK) *OR* (CD8 *AND* STAT5)
SMAD2	TGFbR
SMAD3	TGFbR *AND NOT* Akt
SOCS1	STAT1 *OR* Tbet
STAT1	IFNbR *OR* IL27R *OR* JAK1
STAT3	IL10R *OR* IL21R *OR* IL23R *OR* JAK3
STAT4	IL12R *AND NOT* GATA3
STAT5	(IL2R *OR* JAK3) *AND NOT* SOCS1
STAT6	IL4R
Tbet	(STAT1 *OR* Tbet) *AND NOT* Foxp3 *AND NOT* GATA3 *AND NOT* RORgt
TCF1	Notch1
TCR	is an input
TGFb	Foxp3
TGFbR	TGFb
ThPOK	(CD4 *AND NOT* CD8) *OR* Foxp3 *OR* GATA3 *OR* RORgt *OR* TCR *OR* (Tbet *AND NOT* Runx3) OR ThPOK
TNFa	RORgt
TNFR2	TNFa
XBP1	Blimp1

Table A.3: Attractors of the network.
Results were obtained using 100,000 random initial states within the closed interval [0,1]. Values are averages with a standard deviation smaller than $10E^{-20}$. Only columns "CLP" and "pro-B" were not obtained by random search but by directly assessing the stability of such attractors while fixing the value of Flt3L to 1, see main text for explanation.

Node	CLP	DP	CD4 naive	Th1	Th2	Th17	Treg	CD8 naive	CTL	pro-B	B naive	PC
Akt	0	0	0	0	0	1	0	0	0	0	0	0
B9	0	0	0	0	0	0	0	0	0	0	0	0
Bach2	0	0	0	0	0	0	0	0	0	1	1	0
Bcl2	0	0	0	0	0	1	0	0	0	0	0	0
Bcl6	0	0	0	0	0	0	0	0	0	0	0	0
Bcl11b	0	0	0	0	0	0	0	0	0	0	0	0
BCR	0	0	0	0	0	0	0	0	0	0	0	0
Blimp1	0	0	0	0	0	0	0	0	0	0	0	1
CD4	0	1	1	1	1	1	1	0	0	0	0	0
CD8	0	1	0	0	0	0	0	1	1	0	0	0
CD19	0	0	0	0	0	0	0	0	0	1	1	0
Dll1	0	0	0	0	0	0	0	0	0	0	0	0
Ebf1	1	0	0	0	0	0	0	0	0	1	1	1
EOMES	0	0	0	0	0	0	0	0	1	0	0	0
ERK	1	0	0	0	0	0	0	0	0	0	0	0
Flt3	1	0	0	0	0	0	0	0	0	0	0	0
Flt3L	1	0	0	0	0	0	0	0	0	1	0	0
Foxp3	0	0	0	0	0	0	1	0	0	0	0	0
FR4	0	0	0	0	0	0	0	0	0	0	0	0
GATA3	0	0	0	0	1	0	0	0	0	0	0	0
Gfi1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Gzmb	0	0	0	0	0	0	0	0	1	0	0	0
HEB	0	0	0	0	0	0	0	0	0	0	0	0
Helios	0	0	0	0	0	0	0	0	0	0	0	0
Hoxa9	0	0	0	0	0	0	0	0	0	0	0	0
IFN-β	0	0	0	0	0	0	0	0	0	0	0	0
IFN-βR	0	0	0	0	0	0	0	0	0	0	0	0
IFN-γ	0	0	0	1	0	0	0	0	1	0	0	0
IFN-γR	0	0	0	1	0	0	0	0	1	0	0	0
Ikaros	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
IL-2	0	0	0	0	0	0	0	0	0	0	0	0
IL-2R	0	0	0	0	0	0	0	0	0	0	0	0
...												

...												
Node	CLP	DP	CD4 naive	Th1	Th2	Th17	Treg	CD8 naive	CTL	pro-B	B naive	PC
IL-4	0	0	0	0	1	0	0	0	0	0	0	0
IL-4R	0	0	0	0	1	0	0	0	0	0	0	0
IL-6	0	0	0	0	0	1	0	0	0	0	0	0
IL-6R	0	0	0	0	0	1	0	0	0	0	0	0
IL-7	0	0	0	0	0	0	0	0	0	1	0	0
IL-7R	0	0	0	0	0	0	0	0	0	1	0	0
IL-10	0	0	0	0	1	0	0	0	0	0	0	0
IL-10R	0	0	0	0	1	0	0	0	0	0	0	0
IL-12	0	0	0	0	0	0	0	0	0	0	0	0
IL-12R	0	0	0	0	0	0	0	0	0	0	0	0
IL-17	0	0	0	0	0	1	0	0	0	0	0	0
IL-18	0	0	0	0	0	0	0	0	0	0	0	0
IL-18R	0	0	0	0	0	0	0	0	0	0	0	0
IL-21	0	0	0	0	0	0	0	0	0	0	0	0
IL-21R	0	0	0	0	0	0	0	0	0	0	0	0
IL-23	0	0	0	0	0	0	0	0	0	0	0	0
IL-23R	0	0	0	0	0	0	0	0	0	0	0	0
IL-27	0	0	0	0	0	0	0	0	0	0	0	0
IL-27R	0	0	0	0	0	0	0	0	0	0	0	0
IRAK	0	0	0	0	0	0	0	0	0	0	0	0
Irf4	0	0	0	0	0	0	0	0	0	0	0	1
JAK1	0	0	0	1	0	0	0	0	1	0	0	0
JAK3	0	0	0	0	0	1	0	0	0	1	0	0
NF-κB	0	0	0	0	0	0	0	0	0	0	0	0
NFAT	0	0	0	0	0	0	0	0	0	0	0	0
Notch1	0	0	0	0	0	0	0	0	0	0	0	0
Pax5	0	0	0	0	0	0	0	0	0	1	1	0
Prf1	0	0	0	0	0	0	0	0	1	0	0	0
PU.1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ROR-γt	0	0	0	0	0	1	0	0	0	0	0	0
Runx1	1	0	0	0	0	0	0	0	0	1	1	1
Runx3	0	0	0	0	0	0	0	1	1	0	0	0
SMAD2	0	0	0	0	0	0	1	0	0	0	0	0
SMAD3	0	0	0	0	0	0	1	0	0	0	0	0
SOCS1	0	0	0	1	0	0	0	0	1	0	0	0
STAT1	0	0	0	1	0	0	0	0	1	0	0	0
STAT3	0	0	0	0	1	1	0	0	0	1	0	0
STAT4	0	0	0	0	0	0	0	0	0	0	0	0
STAT5	0	0	0	0	0	1	0	0	0	1	0	0
...												

...												
Node	CLP	DP	CD4 naive	Th1	Th2	Th17	Treg	CD8 naive	CTL	pro-B	B naive	PC
STAT6	0	0	0	0	1	0	0	0	0	0	0	0
Tbet	0	0	0	1	0	0	0	0	1	0	0	0
TCF1	0	0	0	0	0	0	0	0	0	0	0	0
TCR	0	0	0	0	0	0	0	0	0	0	0	0
TGF-β	0	0	0	0	0	0	1	0	0	0	0	0
TGF-βR	0	0	0	0	0	0	1	0	0	0	0	0
ThPOK	0	0	1	1	1	1	1	0	0	0	0	0
TNFα	0	0	0	0	0	1	0	0	0	0	0	0
TNFR2	0	0	0	0	0	1	0	0	0	0	0	0
XBP1	0	0	0	0	0	0	0	0	0	0	0	1

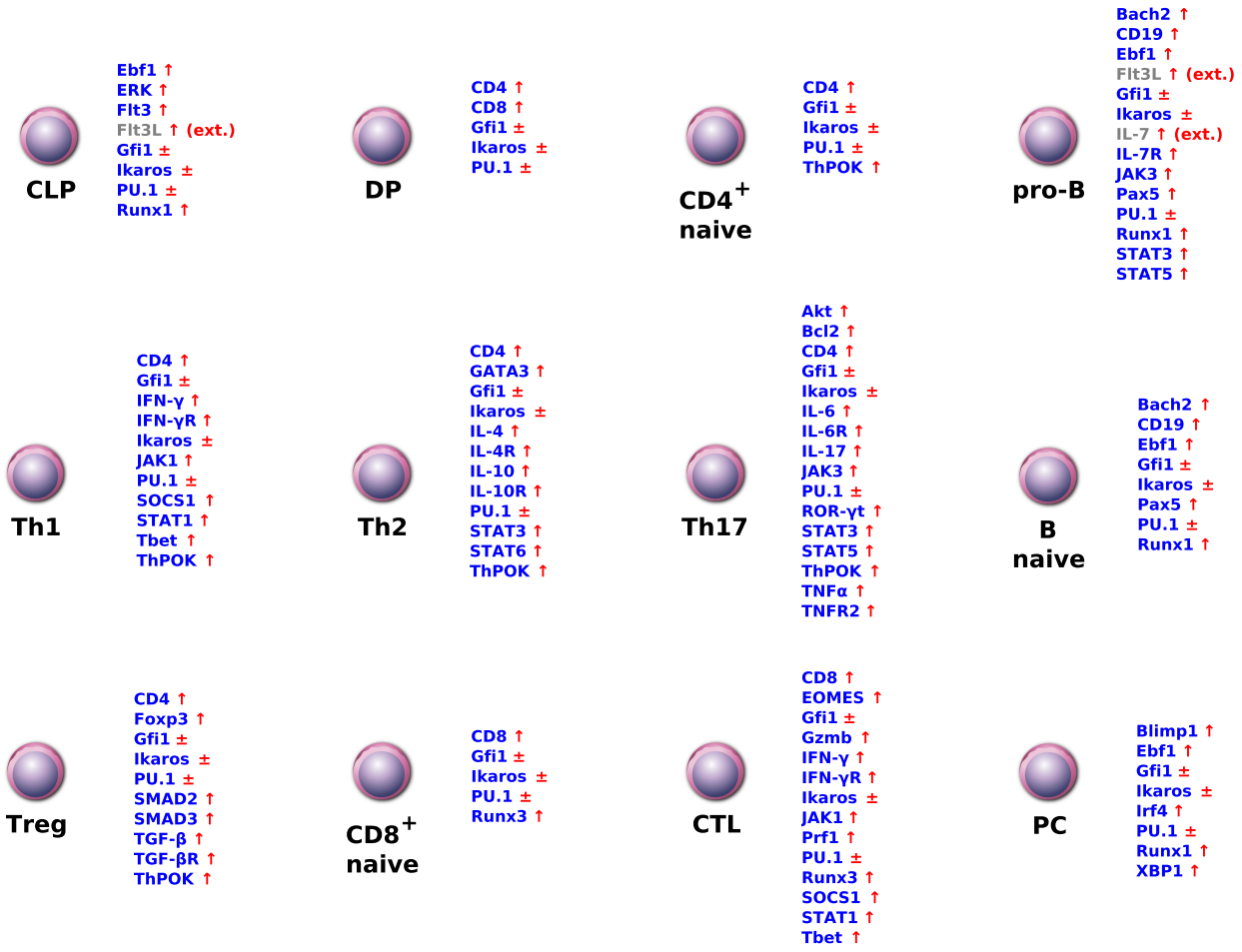

Figure A.1: Schematic summary of the attractors of the regulatory network controlling lymphopoiesis and their interpretation as cell types. For simplicity, only the nodes with high or intermediate steady state activation values are shown (denoted with the symbols "↑" and "±", respectively). The molecules Flt3L and IL-7 representing external signals required for CLP and pro-B cell differentiation are shown in grey and with the label "ext.".

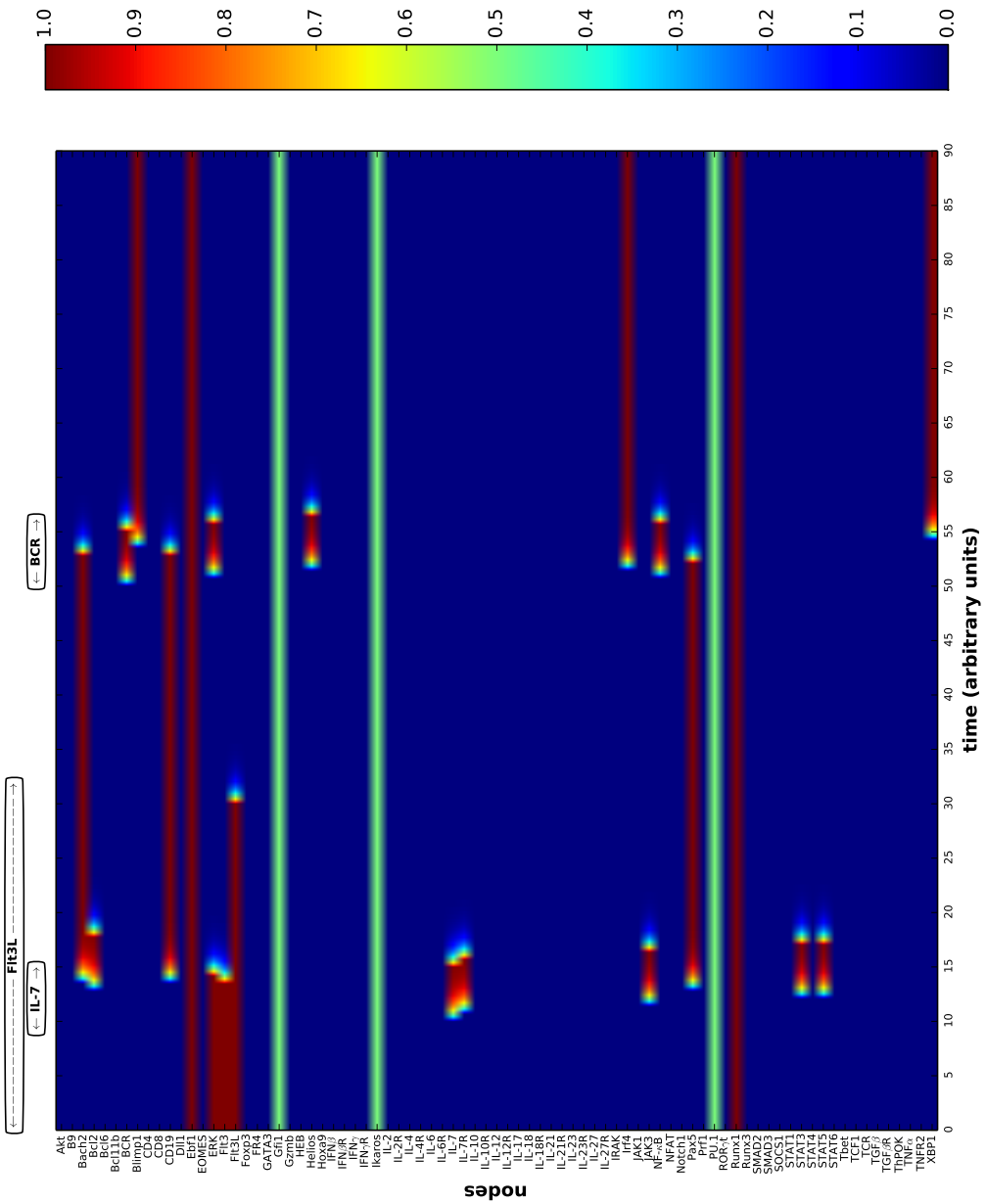

Figure A.2: Simulation of the differentiation process from CLP to plasma cell. The changes in the level of activation of the 81 nodes of the regulatory network are represented as a heatmap. Extracellular signals are simulated as temporal perturbations, indicated as rectangles atop of the heatmap. There are four stationary states, each corresponding to specific cell types: at time 0-10 (CLP), ∼25-30 (pro-B), ∼35-50 (B naive), and ∼65 onward (plasma cell).

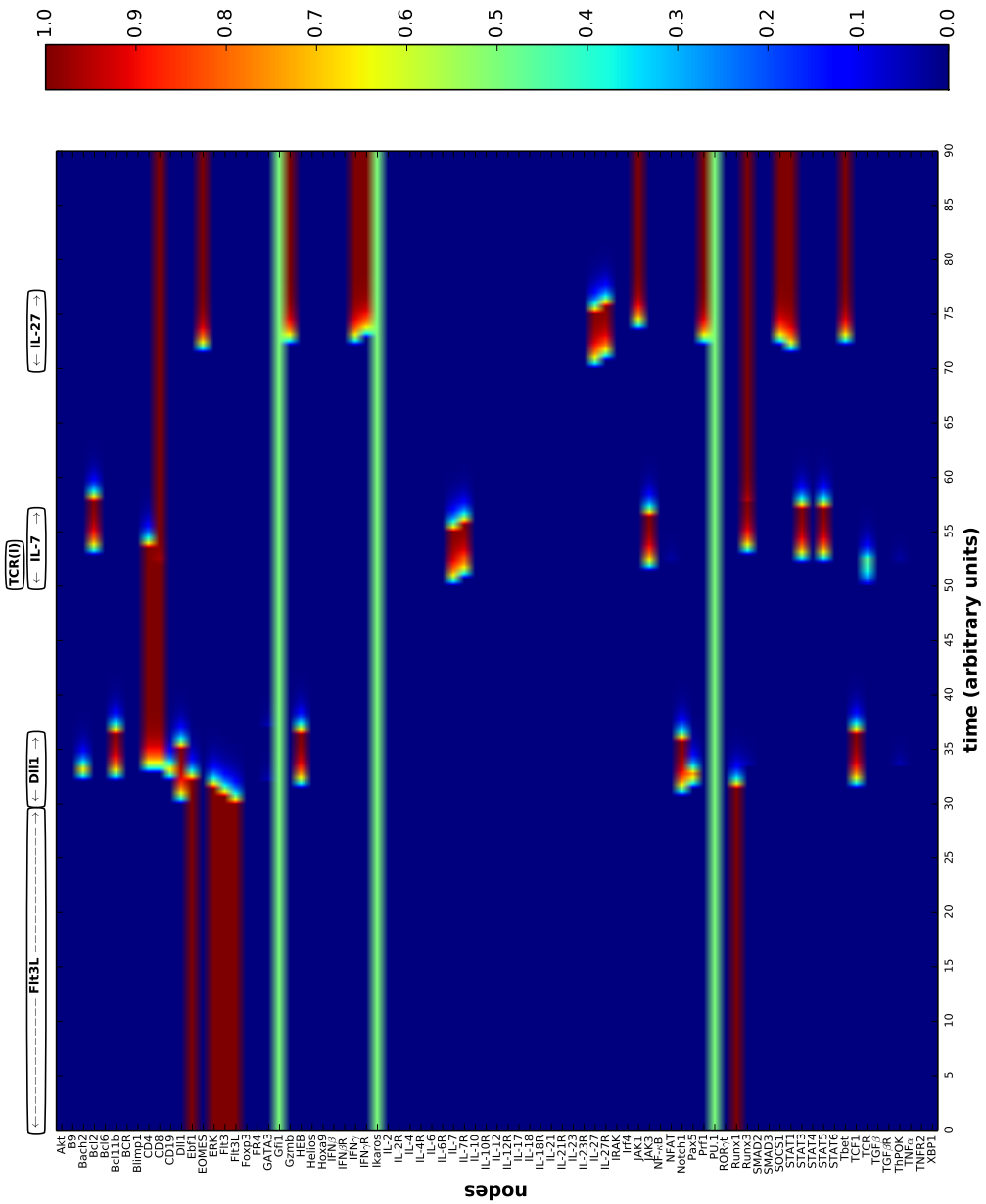

Figure A.3: Simulation of the differentiation process from CLP to CTL. The changes in the level of activation of the 81 nodes of the regulatory network are represented as a heatmap. Extracellular signals are simulated as temporal perturbations, indicated as rectangles atop of the heatmap. There are four stationary states, each corresponding to specific cell types: at time 0-30 (CLP), ∼40-50 (DP), ∼60-70 ($CD8^+$ naive), and ∼80 onward (CTL).

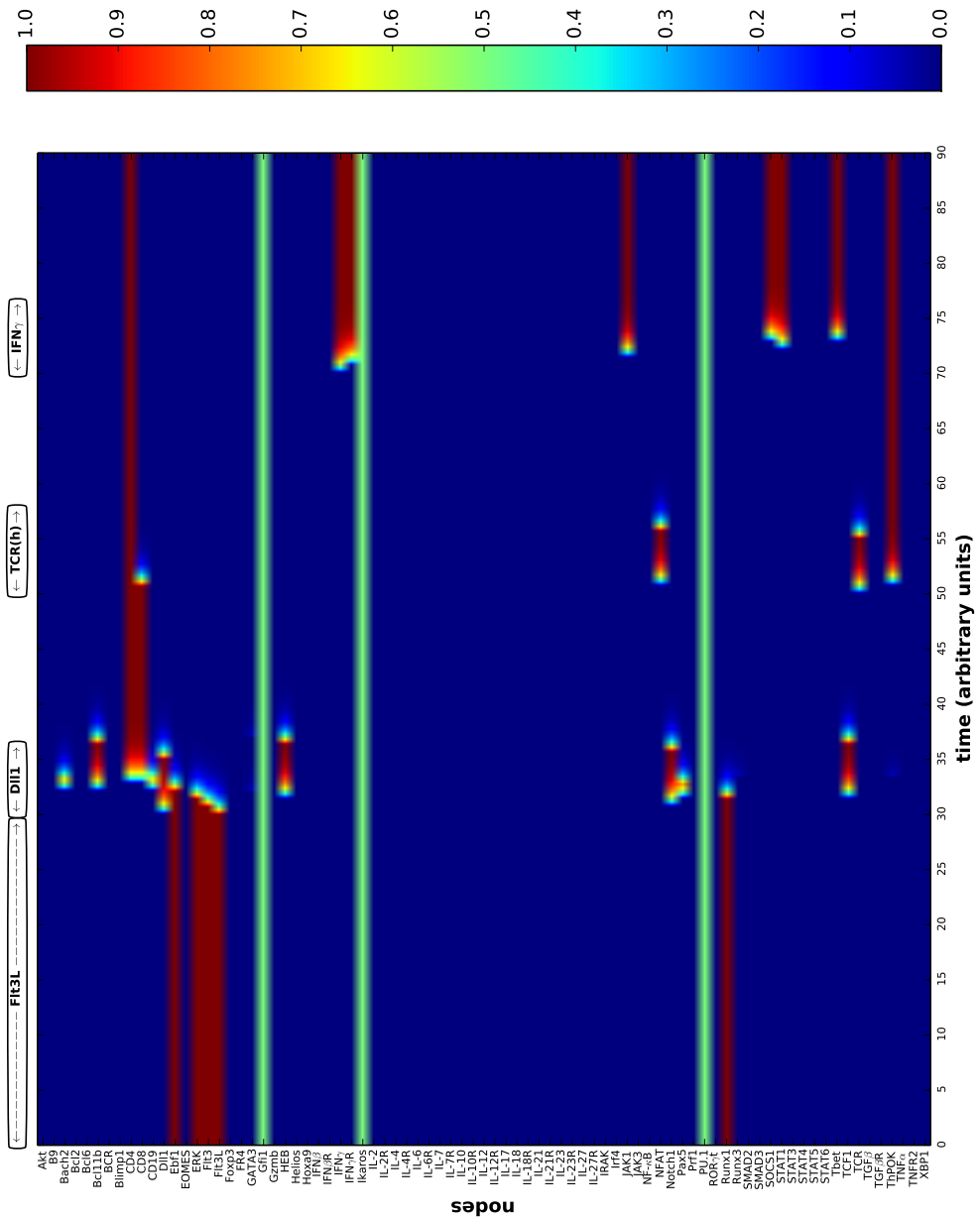

Figure A.4: Simulation of the differentiation process from CLP to Th1. The changes in the level of activation of the 81 nodes of the regulatory network are represented as a heatmap. Extracellular signals are simulated as temporal perturbations, indicated as rectangles atop of the heatmap. There are four stationary states, each corresponding to specific cell types: at time 0-30 (CLP), ∼40-50 (DP), ∼60-70 ($CD4^+$ naive), and ∼80 onward (Th1).

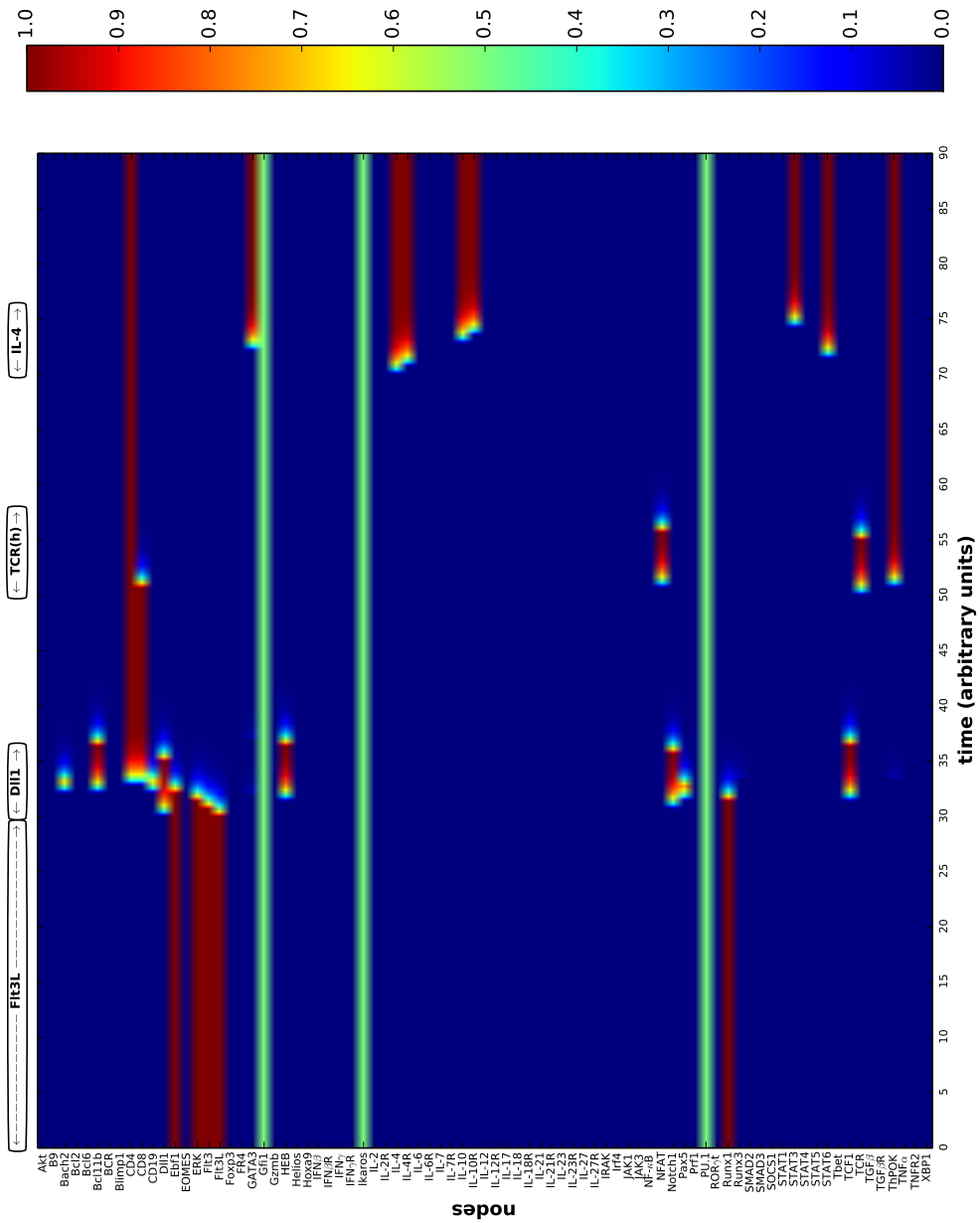

Figure A.5: Simulation of the differentiation process from CLP to Th2. The changes in the level of activation of the 81 nodes of the regulatory network are represented as a heatmap. Extracellular signals are simulated as temporal perturbations, indicated as rectangles atop of the heatmap. There are four stationary states, each corresponding to specific cell types: at time 0-30 (CLP), ∼40-50 (DP), ∼60-70 (CD4$^+$ naive), and ∼80 onward (Th2).

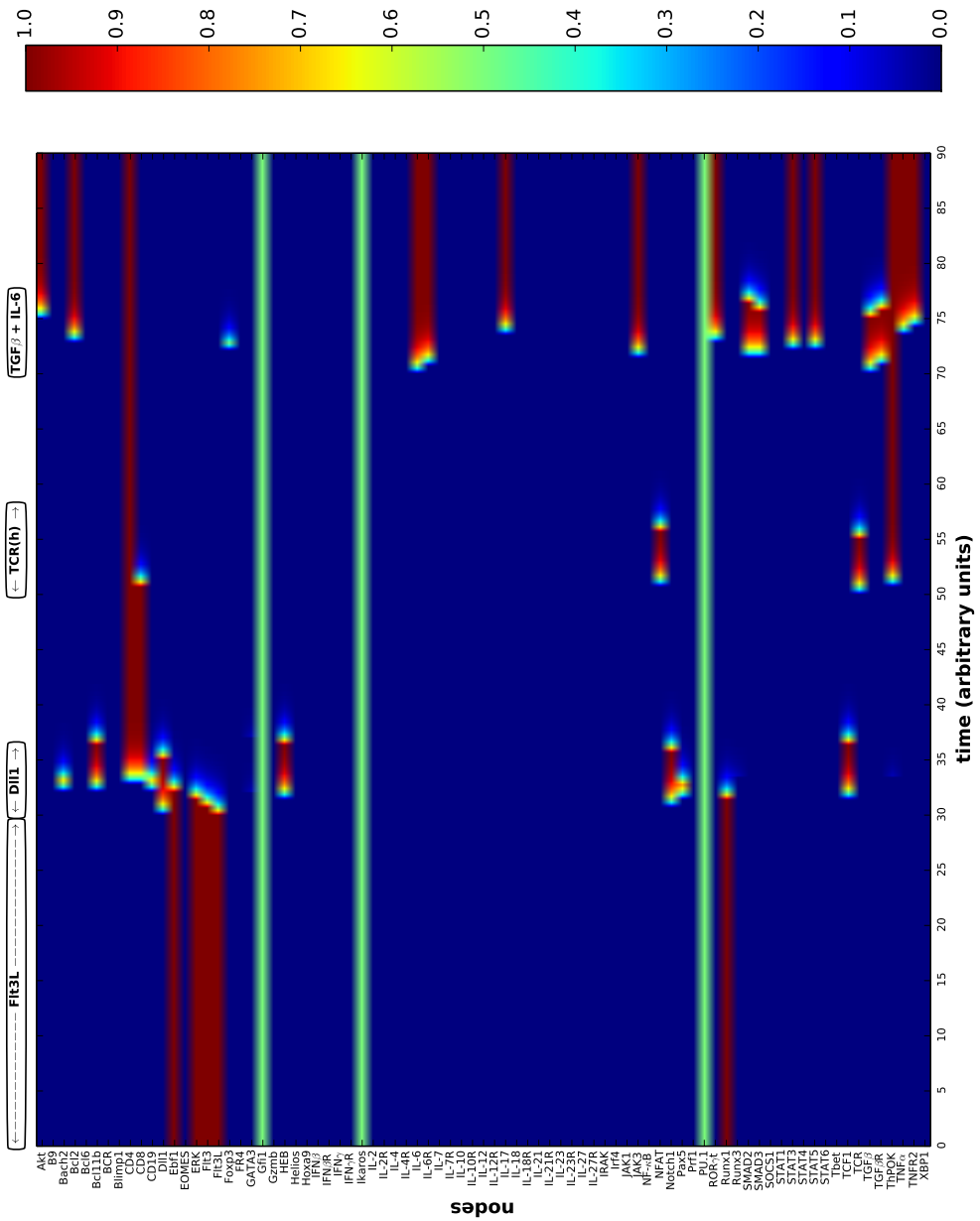

Figure A.6: Simulation of the differentiation process from CLP to Th17. The changes in the level of activation of the 81 nodes of the regulatory network are represented as a heatmap. Extracellular signals are simulated as temporal perturbations, indicated as rectangles atop of the heatmap. There are four stationary states, each corresponding to specific cell types: at time 0-30 (CLP), ∼40-50 (DP), ∼60-70 ($CD4^+$ naive), and ∼80 onward (Th17).

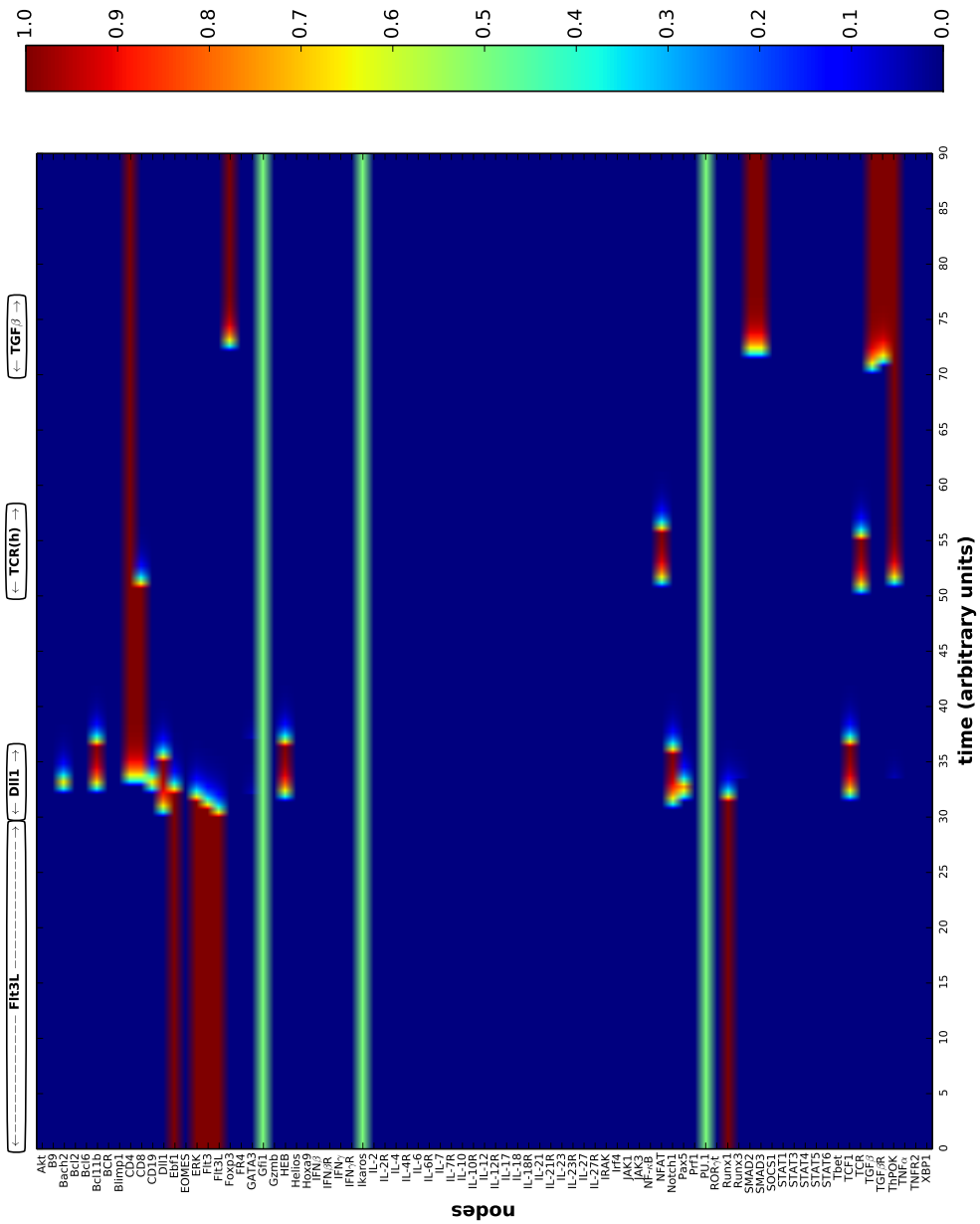

Figure A.7: Simulation of the differentiation process from CLP to Treg. The changes in the level of activation of the 81 nodes of the regulatory network are represented as a heatmap. Extracellular signals are simulated as temporal perturbations, indicated as rectangles atop of the heatmap. There are four stationary states, each corresponding to specific cell types: at time 0-30 (CLP), ∼40-50 (DP), ∼60-70 ($CD4^+$ naive), and ∼80 onward (Treg).

[1] Roffe S, Hagai Y, Pines M, Halevy O. Halofuginone inhibits Smad3 phosphorylation via the PI3K/Akt and MAPK/ERK pathways in muscle cells: Effect on myotube fusion. Exp Cell Res. 2010;316(6):1061–1069.

[2] Zhang Q, Cui F, Fang L, Hong J, Zheng B, Zhang JZ. TNF-α impairs differentiation and function of TGF-β-induced Treg cells in autoimmune diseases through Akt and Smad3 signaling pathway. J Mol Cell Biol. 2013;5(2):85–98.

[3] Nutt SL, Taubenheim N, Hasbold J, Corcoran LM, Hodgkin PD. The genetic network controlling plasma cell differentiation. Semin Immunol. 2011;23(5):341–349.

[4] Ochiai K, Katoh Y, Ikura T, Hoshikawa Y, Noda T, Karasuyama H, et al. Plasmacytic transcription factor Blimp-1 is repressed by Bach2 in B cells. J Biol Chem. 2006;281(50):38226–38234.

[5] Alinikula J, Nera KP, Junttila S, Lassila O. Alternate pathways for Bcl6-mediated regulation of B cell to plasma cell differentiation. Eur J Immunol. 2011;41(8):2404–2413.

[6] Kusam S, Vasanwala FH, Dent AL. Transcriptional repressor BCL-6 immortalizes germinal center-like B cells in the absence of p53 function. Oncogene. 2004;23(3):839–844.

[7] Tunyaplin C, Shaffer AL, Angelin-Duclos CD, Yu X, Staudt LM, Calame KL. Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. J Immunol. 2004;173(2):1158–1165.

[8] Teodorovic LS, Babolin C, Rowland SL, Greaves SA, Baldwin DP, Torres RM, et al. Activation of Ras overcomes B-cell tolerance to promote differentiation of autoreactive B cells and production of autoantibodies. Proc Natl Acad Sci USA. 2014;111(27):2797–27806.

[9] Yasuda T, Hayakawa F, Kurahashi S, Sugimoto K, Minami Y, Tomita A, et al. B cell receptor-ERK1/2 signal cancels PAX5-dependent repression of BLIMP1 through PAX5 phosphorylation: a mechanism of antigen-triggering plasma cell differentiation. J Immunol. 2012;188(12):6127–6134.

[10] De Silva NS, Simonetti G, Heise N, Klein U. The diverse roles of IRF4 in late germinal center B-cell differentiation. Immunol Rev. 2012;247(1):73–92.

[11] Cimmino L, Martins GA, Liao J, Magnusdottir E, Grunig G, Perez RK, et al. Blimp-1 attenuates Th1 differentiation by repression of ifng, tbx21, and bcl6 gene expression. J Immunol. 2008;181(4):2338–2347.

[12] Klein B, Tarte K, Jourdan M, Mathouk K, Moreaux J, Jourdan E, et al. Survival and proliferation factors of normal and malignant plasma cells. Int J Hematol. 2003;78(2):106–113.

[13] Kallies A, Hasbold J, Tarlinton DM, Dietrich W, Corcoran LM, Hodgkin PD, et al. Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. J Exp Med. 2004;200(8):967–977.

[14] Kikuchi H, Nakayama M, Takami Y, Kuribayashi F, Nakayama T. EBF1 acts as a powerful repressor of Blimp-1 gene expression in immature B cells. Biochem Biophys Res Commun. 2012;422(4):780–785.

[15] Lin KI, Angelin-Duclos C, Kuo TC, Calame K. Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. Mol Cell Biol. 2002;22(13):4771–4780.

[16] Kunisawa J, Hashimoto E, Ishikawa I, Kiyono H. A pivotal role of vitamin B9 in the maintenance of regulatory T cells in vitro and in vivo. PLoS One. 2012;7(2):e32094.

[17] Yamaguchi T, Hirota K, Nagahama K, Ohkawa K, Takahashi T, Nomura T, et al. Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. Immunity. 2007;27(1):145–159.

[18] Martínez-Sosa P, Mendoza L. The regulatory network that controls the differentiation of T lymphocytes. BioSystems. 2013;113(2):96–103.

[19] Ciofani M, Knowles GC, Wiest DL, von Boehmer H, Zúñiga-Pflücker JC. Stage-specific and differential notch dependency at the $\alpha\beta$ and $\gamma\delta$ T lineage bifurcation. Immunity. 2006;25(1):105–116.

[20] Bouamar H, Abbas S, Lin AP, Wang L, Jiang D, Holder KN, et al. A capture-sequencing strategy identifies IRF8, EBF1, and APRIL as novel IGH fusion partners in B-cell lymphoma. Blood. 2013;122(5):726–733.

[21] Hirokawa S, Sato H, Kato I, Kudo A. EBF-regulating Pax5 transcription is enhanced by STAT5 in the early stage of B cells. Eur J Immunol. 2003;33(7):1824–182.

[22] O'Riordan M, Grosschedl R. Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. Immunity. 1999;11(1):21–31.

[23] Intlekofer AM, Takemoto N, Wherry EJ, Longworth SA, Northrup JT, Palanivel VR, et al. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. Nat Immunol. 2005;6(12):1236–1244.

[24] Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, et al. Control of Effector CD8+ T Cell Function by the Transcription Factor Eomesodermin. Science. 2003;302(5647):1041–1043.

[25] Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, Beavo JA, et al. Foxp3-dependent programme of regulatory T-cell differentiation. Nature. 2007;445(7129):771–775.

[26] Wan YY, Flavell RA. How Diverse—CD4 Effector T Cells and their Functions. J Mol Cell Biol. 2009;1(1):20–36.

[27] Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat Immunol. 2007;8(3):277–284.

[28] Zhou L, Lopes JE, Chong MMW, Ivanov II, Min R, Victora GD, et al. TGF-β-induced Foxp3 inhibits Th17 cell differentiation by antagonizing RORγt function. Nature. 2008;453(7192):236–240.

[29] Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFβ in the Context of an Inflammatory Cytokine Milieu Supports De Novo Differentiation of IL-17-Producing T Cells. Immunity. 2006;24(2):179–189.

[30] Zhong X, Gao W, Degauque N, Bai C, Lu Y, Kenny J, et al. Reciprocal generation of Th1/Th17 and T(reg) cells by B1 and B2 B cells. Eur J Immunol. 2007;37(9):2400–2404.

[31] Åhsberg J, Tsapogas P, Qian H, Zetterblad J, Zandi S, Månsson R, et al. Interleukin-7-induced Stat-5 acts in synergy with Flt-3 signaling to stimulate expansion of hematopoietic progenitor cells. J Biol Chem. 2010;285(47):36275–36284.

[32] Gwin KA, Shapiro MB, Dolence JJ, Huang ZL, Medina1 KL. Hoxa9 and Flt3 signaling synergistically regulate an early checkpoint in lymphopoiesis. J Immunol. 2013;191(2):745–754.

[33] Wan YY, Flavell RA. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature. 2007;445(7129):766–770.

[34] Ouyang W, Löhning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, et al. Stat6-Independent GATA-3 Autoactivation Directs IL-4-Independent Th2 Development and Commitment. Immunity. 2000;12(1):27–37.

[35] Ranganath S, Murphy KM. Structure and Specificity of GATA Proteins in Th2 Development. Mol Cell Biol. 2001;21(8):2716–2725.

[36] Schmidt-Weber CB, Alexander SI, Henault LE, James L, Lichtman AH. IL-4 Enhances IL-10 Gene Expression in Murine Th2 Cells in the Absence of TCR Engagement. J Immunol. 1999;162(1):238–244.

[37] Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6(11):1133–1141.

[38] Usui T, Nishikomori R, Kitani A, Strober W. GATA-3 Suppresses Th1 Development by Downregulation of Stat4 and Not through Effects on IL-12Rβ2 Chain or T-bet. Immunity. 2003;18(3):415–428.

[39] Wang L, Wildt KF, Zhu J, Zhang X, Feigenbaum L, Tessarollo L, et al. Distinct functions for the transcription factors GATA-3 and ThPOK during intrathymic differentiation of CD4(+) T cells. Nat Immunol. 2008;9(10):1122–1130.

[40] Wang L, Bosselut R. CD4-CD8 Lineage Differentiation: Thpok-ing into the Nucleus. J Immunol. 2009;183(5):2903–2910.

[41] Möröy T, Khandanpour C. Growth factor independence 1 (Gfi1) as a regulator of lymphocyte development and activation. Semin Immunol. 2011;23(5):368–378.

[42] Spooner CJ, Cheng JX, Pujadas E, Laslo P, Singh H. A Recurrent Network Involving the Transcription Factors PU.1 and Gfi1 Orchestrates Innate and Adaptive Immune Cell Fates. Immunity. 2009;31(4):576–586.

[43] Braunstein M, Anderson MK. HEB-deficient T-cell precursors lose T-cell potential and adopt an alternative pathway of differentiation. Mol Cell Biol. 2011;31(5):971–982.

[44] Gwin K, Frank E, Bossou A, Medina KL. Hoxa9 regulates Flt3 in lymphohematopoietic progenitors. J Immunol. 2010;185(11):6572–6583.

[45] Novelli F, D'Elios MM, Bernabei P, Ozmen L, Rigamonti L, Almerigogna F, et al. Expression and role in apoptosis of the alpha- and beta-chains of the IFN-gamma receptor on human Th1 and Th2 clones. J Immunol. 1997;159(1):206–213.

[46] Rigamonti L, Ariotti S, Losana G, Gradini R, Russo MA, Jouanguy E, et al. Surface Expression of the IFN-γR2 Chain Is Regulated by Intracellular Trafficking in Human T Lymphocytes. J Immunol. 2000;164(1):201–207.

[47] Kerra IM, Costa-Pereira AP, Lillemeier BF, Strobl B. Of JAKs, STATs, blind watchmakers, jeeps and trains. FEBS Lett. 2003;546(1):1–5.

[48] Kotenko SV, Pestka S. Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. Oncogene. 2000;19(21):2557–2565.

[49] Goodbourn S, Didcock L, Randall RE. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J Gen Virol. 2000;81(10):2341–2364.

[50] Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 Signaling via STAT5 Constrains T Helper 17 Cell Generation. Immunity. 2007;26(3):371–381.

[51] Shevach EM, DiPaolo RA, Andersson J, Zhao DM, Stephens GL, Thornton AM. The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells. Immunol Rev. 2006;212:60–73.

[52] Hamalainen H, Zhou H, Chou W, Hashizume H, Heller R, Lahesmaa R. Distinct gene expression profiles of human type 1 and type 2 T helper cells. Genome Biol. 2001;2(7):RESEARCH0022.

[53] Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol. 2002;2(12):933–944.

[54] Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006;126(6):1121–1133.

[55] Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med. 2007;13(2):139–145.

[56] Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J. 1998;334(2):297–314.

[57] Jiang Q, Li WQ, Aiello FB, Mazzucchelli R, Asefa B, Khaled AR, et al. Cell biology of IL-7, a key lymphotrophin. Cytokine Growth Factor Rev. 2005;16(4-5):513–533.

[58] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the Interleukin-10 Receptor. Ann Rev Immunol. 2001;19:683–765.

[59] Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature. 1996;382(6587):171–174.

[60] Chang JT, Segal BM, Nakanishi K, Okamura H, Shevach EM. The costimulatory effect of IL-18 on the induction of antigen-specific IFN-γ production by resting T cells is IL-12 dependent and is mediated by up-regulation of the IL-12 receptor β2 subunit. Eur J Immunol. 2000;30(4):1113–1119.

[61] Yi JS, Cox MA, Zajac AJ. Interleukin-21: a multifunctional regulator of immunity to infections. Microbes Infect. 2010;12(14-15):1111–1119.

[62] Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, et al. A Receptor for the Heterodimeric Cytokine IL-23 Is Composed of IL-12Rβ1 and a Novel Cytokine Receptor Subunit, IL-23R1. J Immunol. 2002;168(11):5699–5708.

[63] Hunter CA, Kastelein R. Interleukin-27: balancing protective and pathological immunity. Immunity. 2012;37(6):960–969.

[64] Hirahara K, Ghoreschi K, Laurence A, ping Yang X, Kanno Y, O'Shea JJ. Signal transduction pathways and transcriptional regulation in Th17 cell differentiation. Cytokine Growth Factor Rev. 2010;21(6):425–434.

[65] Yoshida H, Nakaya M, Miyazaki Y. Interleukin 27: a double-edged sword for offense and defense. J Leukoc Biol. 2009;86(6):1295–1303.

[66] Saito M, Gao J, Basso K, Kitagawa Y, Smith PM, Bhagat G, et al. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. Cancer Cell. 2007;12(3):280–292.

[67] Sciammas R, Shaffer AL, Schatz JH, Zhao H, Staudt LM, Singh1 H. Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. Immunity. 2006;25(2):225–236.

[68] Decker T, di Magliano MP, McManus S, Sun Q, Bonifer C, Tagoh H, et al. Stepwise activation of enhancer and promoter regions of the B cell commitment gene Pax5 in early lymphopoiesis. Immunity. 2009;30(4):508–520.

[69] Kanakaraj P, Ngo K, Wu Y, Angulo A, Ghazal P, Harris CA, et al. Defective Interleukin (IL)-18–mediated Natural Killer and T Helper Cell Type 1 Responses in IL-1 Receptor–associated Kinase (IRAK)-deficient Mice. J Exp Med. 1999;189(7):1129–1138.

[70] Krebs DL, Hilton DJ. SOCS proteins: negative regulators of cytokine signaling. Stem Cells. 2001;19(5):378–387.

[71] Sommer VH, Clemmensen OJ, Nielsen O, Wasik M, Lovato P, Brender C, et al. In vivo activation of STAT3 in cutaneous T-cell lymphoma. Evidence for an antiapoptotic function of STAT3. Leukemia. 2004;18(7):1288–1295.

[72] Qin JZ, Zhang CL, Kamarashev J, Dummer R, Burg G, Döbbeling U. Interleukin-7 and interleukin-15 regulate the expression of the bcl-2 and c-myb genes in cutaneous T-cell lymphoma cells. Blood. 2001;98(9):2778–2783.

[73] Serre K, Bénézech C, Desanti G, Bobat S, Toellner KM, Bird R, et al. Helios is associated with CD4 T cells differentiating to T helper 2 and follicular helper T cells in vivo independently of Foxp3 expression. PLoS One. 2011;6(6):e20731.

[74] Li P, Burke S, Wang J, Chen X, Ortiz M, Lee SC, et al.. Reprogramming of T cells to natural killer-like cells upon Bcl11b deletion; 2010.

[75] Li L, Leid M, Rothenberg EV. An early T cell lineage commitment checkpoint dependent on the transcription factor Bcl11b. Science. 2010;329(5987):89–93.

[76] Michie AM, Chan AC, Ciofani M, Carleton M, Lefebvre JM, He Y, et al. Constitutive Notch signalling promotes CD4 CD8 thymocyte differentiation in the absence of the pre-TCR complex, by mimicking pre-TCR signals. Int Immunol. 2007;19(12):1421–1430.

[77] Rothenberg EV, Taghon T. Molecular genetics of T cell development. Annu Rev Immunol. 2005;23:601–649.

[78] Nie L, Perry SS, Zhao Y, Huang J, Kincade PW, Farrar MA, et al. Regulation of lymphocyte development by cell-type-specific interpretation of Notch signals. Mol Cell Biol. 2008;28(6):2078–2090.

[79] Braunstein M, Anderson MK. HEB in the spotlight: Transcriptional regulation of T-cell specification, commitment, and developmental plasticity. Clin Dev Immunol. 2012;2012:678705.

[80] Weber BN, Chi AWS, Chavez A, Yashiro-Ohtani Y, Yang Q, Shestova O, et al. A critical role for TCF-1 in T-lineage specification and differentiation. Nature. 2011;476(7358):63–68.

[81] Kallies A, Nutt SL. Bach2: plasma-cell differentiation takes a break. EMBO J. 2010;29(23):3896–3897.

[82] Nutt SL, Kee BL. The transcriptional regulation of B cell lineage commitment. Immunity. 2007;26(6):715–725.

[83] Holmes ML, Carotta S, Corcoran LM, Nutt SL. Repression of Flt3 by Pax5 is crucial for B-cell lineage commitment. Genes Dev. 2006;20(8):933–938.

[84] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441:235–238.

[85] Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, et al. Surface phenotype and antigenic specificity of human interleukin 17–producing T helper memory cells. Nat Immunol. 2007;8:639–646.

[86] Seo W, Ikawa T, Kawamoto H, Taniuchi I. Runx1-Cbfβ facilitates early B lymphocyte development by regulating expression of Ebf1. J Exp Med. 2012;209(7):1255–1262.

[87] Wong WF, Kurokawa M, Satake M, Kohu K. Down-regulation of Runx1 expression by TCR signal involves an autoregulatory mechanism and contributes to IL-2 production. J Biol Chem. 2011;286(13):11110–11118.

[88] Woolf E, Xiao C, Fainaru O, Lotem J, Rosen D, Negreanu V, et al. Runx3 and Runx1 are required for CD8 T cell development during thymopoiesis. Proc Natl Acad Sci USA. 2003;100(13):7731–7736.

[89] Xiong Y, Castro E, Yagi R, Zhu J, Lesourne R, Love PE, et al. Thpok-independent repression of Runx3 by Gata3 during CD4+ T-cell differentiation in the thymus. Eur J Immunol. 2013;43(4):918–928.

[90] Kappes DJ. Expanding roles for ThPOK in thymic development. Immunol Rev. 2010;238(1):182–194.

[91] Cruz-Guilloty F, Pipkin ME, Djuretic IM, Levanon D, Lotem J, Lichtenheld MG, et al. Runx3 and T-box proteins cooperate to establish the transcriptional program of effector CTLs. J Exp Med. 2009;206(1):51–59.

[92] Giambra V, Jenkins CR, Wang H, Lam SH, Shevchuk OO, Nemirovsky O, et al. NOTCH1 promotes T cell leukemia-initiating activity by RUNX-mediated regulation of PKC-θ and reactive oxygen species. Nat Med. 2012;18(11):1693–1698.

[93] Naito T, Taniuchi I. The network of transcription factors that underlie the CD4 versus CD8 lineage decision. Int Immunol. 2010;22(10):791–796.

[94] Gu AD, Wang Y, Lin L, Zhang SS, Wana YY. Requirements of transcription factor Smad-dependent and -independent TGF-β signaling to control discrete T-cell functions. Proc Natl Acad Sci USA. 2012;109(3):905–910.

[95] Malhotra N, Robertson E, Kang J. SMAD2 is essential for TGF beta-mediated Th17 cell generation. J Biol Chem. 2010;285(38):29044–29048.

[96] Martinez GJ, Zhang Z, Reynolds JM, Tanaka S, Chung Y, Liu T, et al. Smad2 positively regulates the generation of Th17 cells. J Biol Chem. 2010;285(38):29039–29043.

[97] Losman JA, Chen XP, Hilton D, Rothman P. Cutting Edge: SOCS-1 Is a Potent Inhibitor of IL-4 Signal Transduction. J Immunol. 1999;162(7):3770–3774.

[98] Gascoigne NRJ. CD8+ thymocyte differentiation: T cell two-step. Nat Immunol. 2010;11(3):189–190.

[99] Knosp CA, Johnston JA. Regulation of CD4+ T-cell polarization by suppressor of cytokine signalling proteins. Immunology. 2012;135(2):101–111.

[100] Elser B, Lohoff M, Kock S, Giaisi M, Kirchhoff S, Krammer PH, et al. IFN-γ Represses IL-4 Expression via IRF-1 and IRF-2. Immunity. 2002;17(6):703–712.

[101] Chen XP, Losman JA, Rothman P. SOCS Proteins, Regulators of Intracellular Signaling. Immunity. 2000;13(3):287–290.

[102] Saito H, Morita Y, Fujimoto M, Narazaki M, Naka T, Kishimoto T. IFN Regulatory Factor-1-Mediated Transcriptional Activation of Mouse STAT-Induced STAT Inhibitor-1 Gene Promoter by IFN-γ1. J Immunol. 2000;164(11):5833–5843.

[103] Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, et al. T-bet is rapidly induced by interferon-γ in lymphoid and myeloid cells. Proc Natl Acad Sci USA. 2001;98(26):15137–15142.

[104] Fujino M, Li XK. Role of STAT3 in regulatory T lymphocyte plasticity during acute graft-vs.-host-disease. JAKSTAT. 2013;2(4):24529.

[105] Laurence A, Amarnath S, Mariotti J, Kim YC, Foley J, Eckhaus M, et al. STAT3 transcription factor promotes instability of nTreg cells and limits generation of iTreg cells during acute murine graft-versus-host disease. Immunity. 2012;37(2):209–222.

[106] Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, et al. STAT3 Regulates Cytokine-mediated Generation of Inflammatory Helper T Cells. J Biol Chem. 2007;282(13):9358–9363.

[107] Mullen AC, High FA, Hutchins AS, Lee HW, Villarino AV, Livingston DM, et al. Role of T-bet in Commitment of TH1 Cells Before IL-12-Dependent Selection. Science. 2001;292(5523):1907–1910.

[108] Malin S, McManus S, Cobaleda C, Novatchkova M, Delogu A, Bouillet P, et al. Role of STAT5 in controlling cell survival and immunoglobulin gene recombination during pro-B cell development. Nat Immunol. 2010;11(2):171–179.

[109] Heltemes-Harris LM, Willette MJL, Vang KB, Farrar MA. The role of STAT5 in the development, function, and transformation of B and T lymphocytes. Ann NY Acad Sci. 2011;1217:18–31.

[110] Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the Interleukin (IL)-12R β2 Subunit Expression in Developing T Helper 1 (Th1) and Th2 Cells. J Exp Med. 1997;185(5):817–824.

[111] Smeltz RB, Chen J, Hu-Li J, Shevach EM. Regulation of Interleukin (Il)-18 Receptor α Chain Expression on Cd4+ T Cells during T Helper (Th)1/Th2 Differentiation. J Exp Med. 2001;194(2):143–154.

[112] Morishima N, Owaki T, Asakawa M, Kamiya S, Mizuguchi J, Yoshimoto T. Augmentation of Effector CD8+ T Cell Generation with Enhanced Granzyme B Expression by IL-27. J Immunol. 2005;175(3):1686–1693.

[113] Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A Novel Transcription Factor, T-bet, Directs Th1 Lineage Commitment. Cell. 2000;100(6):655–669.

[114] Rothenberg EV. Transcriptional drivers of the T-cell lineage program. Curr Opin Immunol. 2012;24(2):132–138.

[115] Brugnera E, Bhandoola A, Cibotti R, Yu Q, Guinter TI, Yamashita Y, et al. Coreceptor reversal in the thymus: signaled CD4+8+ thymocytes initially terminate CD8 transcription even when differentiating into CD8+ T cells. Immunity. 2000;13(1):59–71.

[116] Singer A, Adoro S, Park JH. Lineage fate and intense debate: myths, models and mechanisms of CD4- versus CD8-lineage choice. Nat Rev Immunol. 2008;8(10):788–801.

[117] Park JH, Adoro S, Lucas PJ, Sarafova SD, Alag AS, Doan LL, et al. 'Coreceptor tuning': cytokine signals transcriptionally tailor CD8 coreceptor expression to the self-specificity of the TCR. Nat Immunol. 2007;8(10):1049–1059.

[118] Huang Y, Wange RL. T Cell Receptor Signaling: Beyond Complex Complexes. J Biol Chem. 2004;279(28):28827–28830.

[119] Shi Y, Massagué J. Mechanisms of TGF-β Signaling from Cell Membrane to the Nucleus. cell. 2003;113(6):685–700.

[120] Massagué J, Xi Q. TGF-β control of stem cell differentiation genes. FEBS Lett. 2012;586(14):1953–1958.

[121] So T, Croft M. Regulation of PI-3-kinase and Akt signaling in T lymphocytes and other cells by TNFR family molecules. Front Immunol. 2013;4.

[122] Wildt KF, Sun G, Grueter B, Fischer M, Zamisch M, Ehlers M, et al. The Transcription Factor Zbtb7b Promotes CD4 Expression by Antagonizing Runx-Mediated Activation of the CD4 Silencer. J Immunol. 2007;179(7):4405–4414.

[123] Egawa T. Runx and ThPOK: A balancing act to regulate thymocyte lineage commitment. J Cell Biochem. 2009;107(6):1037–1045.

[124] Naito T, Tanaka H, Naoe Y, Taniuchi I. Transcriptional control of T-cell development. Int Immunol. 2011;23(11):661–668.