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Mathematical model of a gene regulatory network reconciles effects of genetic perturbations on hematopoietic stem cell emergence

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ABSTRACT

Interlinked gene regulatory networks (GRNs) are vital for the spatial and temporal control of gene expression during development. The hematopoietic transcription factors (TFs) Scl, Gata2 and Fli1 form one such densely connected GRN which acts as a master regulator of embryonic hematopoiesis. This triad has been shown to direct the specification of the hemogenic endothelium and emergence of hematopoietic stem cells (HSCs) in response to Notch1 and Bmp4–Smad signaling. Here we employ previously published data to construct a mathematical model of this GRN network and use this model to systematically investigate the network dynamical properties. Our model uses a statistical-thermodynamic framework to describe the combinatorial regulation of gene expression and reconciles, mechanistically, several previously published but unexplained results from different genetic perturbation experiments. In particular, our results demonstrate how the interactions of Runx1, an essential hematopoietic TF, with components of the Bmp4 signaling pathway allow it to affect triad activation and acts as a key regulator of HSC emergence. We also explain why heterozygous deletion of this essential TF, Runx1, speeds up the network dynamics leading to accelerated HSC emergence. Taken together our results demonstrate that the triad, a master-level controller of definitive hematopoiesis, is an irreversible bistable switch whose dynamical properties are modulated by Runx1 and components of the Bmp4 signaling pathway.

Introduction

Hematopoietic stem cells (HSCs) are a rare population of cells with self-renewal potential to divide and contribute cells to all blood lineages throughout the life of an organism. The ontogeny of HSCs has been carefully studied in terms of anatomical locations and stages of cellular progression (Medvinsky et al., 2011; Orkin and Zon, 2008). Studies using mouse, zebrafish and embryonic stem cells have demonstrated that blood progenitor cells (with limited self-renewal ability) are formed early during embryogenesis, initially in the yolk sac and then in the embryo (Medvinsky et al., 2011). This is followed by the emergence of definitive HSCs (with long-term self-renewal potential) initially in the aorta-gonads-mesonephros region of both mice and humans (Medvinsky et al., 2011). Furthermore, it has been shown that a specialized part of the blood vessel network termed the 'hemogenic endothelium' undergoes an endothelial-to-hematopoietic transition (EHT) to form blood stem/progenitor cells (Bertrand et al., 2010; Boisset et al., 2010; Eilken et al., 2009; Lancrin et al., 2009; Zovein et al., 2008). Understanding the molecular mechanisms that drive HSC and blood formation in the developing embryo will be crucial in designing novel regenerative medicine protocols.

Tight spatial and temporal control of gene expression is vital for the proper development of an organism (Davidson, 2006). Gene expression programs are coordinately regulated by the combinatorial binding of tissue-specific transcription factors (TFs) and external cues that are communicated to cells via signaling pathways. Several TFs regulating key stages of blood cell development have been identified (Marks-Bluth and Pimanda, 2012). Scl, Gata2 and Fli1 act early during development to specify the hemogenic endothelium and are necessary for HSC emergence (Hart et al., 2000; Ling et al., 2004; Schlaeger et al., 2005; Shivdasani et al., 1995; Tsai et al., 1994). On the other hand, Runx1 is required in the hemogenic endothelium for the EHT but not subsequently (Chen et al., 2009; Li et al., 2006; Liakhovitskaia et al., 2009). TF activities and signaling pathways are integrated by *cis*-regulatory modules such as promoters and enhancers which have been characterized for numerous TFs involved in HSC emergence (Pimanda and Gottgens, 2010). Enhancers for *Gata2, Fli1* and *Scl* are bound by

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themselves and each other to form a fully connected triad (Pimanda et al., 2007b), and the HSC enhancer for *Runx1* is bound by all three triad proteins (Nottingham et al., 2007).

Two signaling pathways, Bmp and Notch, are required for HSC and progenitor development (Durand et al., 2007; Kaimakis et al., 2013; Kumano et al., 2003; Marks-Bluth and Pimanda, 2012). The Notch1 intracellular mediator binds at the *Gata2* locus, whereas Bmp-induced signaling mediator, Smad1, binds at the *Runx1* promoter and at the *Gata2* and *Fli1* enhancers (Fig. 1A; (Oren et al., 2005; Pimanda et al., 2007a; Robert-Moreno et al., 2005)). Smad6, an inhibitory Smad, participates in the Bmp4-signaling pathway by hindering Smad1 activation and targeting it for proteolytic degradation (Knezevic et al., 2011). The *Smad6* enhancer is bound by the triad proteins, Smad1 and Runx1, and a negative feedback loop from Smad6 regulates Runx1 by promoting its proteosomal degradation (Knezevic et al., 2011). Runx1 binding at the *Smad6* enhancer is mediated by triad TFs, thus triad activation temporally balances Runx1 activity by up-regulating both Runx1 and its negative regulator Smad6 (Knezevic et al., 2011). Altogether these interactions form a gene regulatory network (GRN) that controls hematopoietic stem and progenitor cell emergence in the developing embryo (Fig. 1B). Multiple feed-forward and feedback loops present in the GRN (Fig. 1A) lead to complex dynamical properties that allow tight control over the network's response to external and internal cues. Understanding these complex emergent properties with purely experimental approaches is challenging; mathematical modeling of networks can serve as an important complementary approach. Models can combine qualitative and quantitative information about network architecture and parameters, and thereby serve as an integrative platform for understanding the results of various genetic perturbations and for making novel predictions.

In this study, we build a mathematical model of the GRN shown in Fig. 1B based on previously published details of *cis*-regulatory modules, TF-binding and protein–protein interactions. The model integrates Runx1 regulation as well as Bmp4 and Notch1 signaling with the Scl–Gata2–Fli1 triad module. Using this model we elucidate the role of Runx1 in the network. Dynamical properties of the network predicted by the model are in good agreement with in vitro and in vivo experimental observations. Moreover, *in silico* perturbations of Runx1, Notch1 and Bmp4 in the simulations closely match the observations in knockout and over-expression phenotypes. Importantly, our model provides mechanistic insight into the early emergence of blood progenitors observed in *Runx1* haploid embryos. Taken together these results suggest that the GRN analyzed here can act as a master-level switch in the signal pathway controlling definitive hematopoiesis.

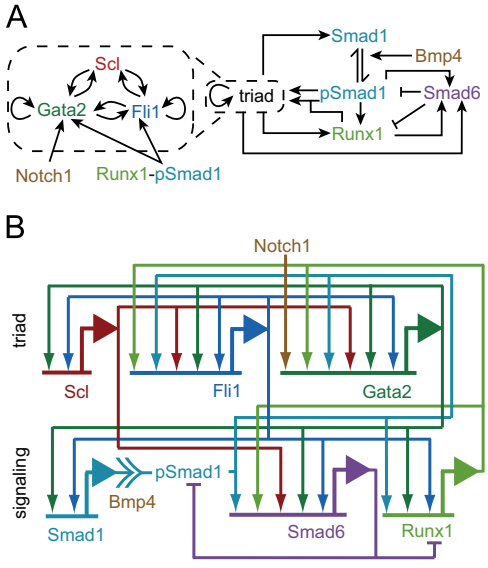

Fig. 1. GRN responsible for regulating HSC specification. (A) The GRN responsible for regulating HSC cell specification contains TFs Scl, Gata2, and Fli1 that are connected via multiple positive feedback loops (dashed box). This triad is regulated directly via Notch1 and indirectly via Bmp4 through a peripheral circuit containing Smad1, Smad6, and Runx1. Bmp4 affects the triad indirectly by regulating the Smad1 phosphorylation rate. Smad6 negatively regulates pSmad1 and Runx1 (blunted arrows) by targeting them for proteasomal degradation. Arrows represent positive transcriptional regulation. (B) Detailed representation of the regulatory connections in the GRN that explicitly shows the various promoters and binding sites (using the notation from (8)). The top half of the diagram shows the triad module, whereas the bottom half shows the signaling module.

Results

Notch1 is necessary for irreversible activation of the triad

Definitive hematopoiesis is the production of blood progenitor cells with the potential to form mature erythroid and myeloid cells, and occurs in multiple sites of the developing embryo including the yolk sac, placenta, AGM and head regions (Li et al., 2012; Lux et al., 2008; Medvinsky and Dzierzak, 1996; Rhodes et al., 2008). The Scl–Gata2–Fli1 triad (Fig. 1A, dashed box) is at the core of the GRN analyzed here; its activation with Notch1 and Bmp4 signals is known to play an important role in definitive hematopoiesis (Durand et al., 2007; Kataoka et al., 2011; Pimanda et al., 2007b; Wareing et al., 2012). Previously, we used a mathematical model to show that Notch1 and Bmp4 cause an irreversible switch to high levels of triad gene expression and thereby explained their role in the activation of these master regulatory genes of definitive hematopoiesis (Narula et al., 2010). Here we extend this model to incorporate recently uncovered interactions between components of the Bmp4 signaling pathway and Runx1, another key regulator of definitive hematopoiesis (Knezevic et al., 2011; Pimanda et al., 2007a).

In this extended model we explicitly include the components involved in Bmp4 signaling—Smad1, Smad6 and Runx1. We briefly outline the major interactions and assumptions of the model (see the "Methods" section and SI for details). Bmp4 promotes the phosphorylation of Smad1, following which pSmad1 translocates to the nucleus and upregulates the transcription of the triad genes as well as of Runx1 and Smad6 (Attisano and Wrana, 2002; Bee et al., 2009a; Ishida et al., 2000). Runx1 forms a complex with pSmad1 in the nucleus (Zaidi et al., 2002). We assume that the formation of this complex enhances the effect of pSmad1 on triad gene expression although it is not essential for triad upregulation. As a result, in our model, Runx1 participates in triad regulation but is not essential for triad gene expression. Smad6 post-translationally modulates Bmp4 signaling by forming complexes with Runx1 and pSmad1, and thereby targeting them for proteolytic degradation (Knezevic et al., 2011; Murakami et al., 2003). In addition the triad feeds back to the signaling module by transcriptionally upregulating Runx1, Smad6 and Smad1 (see the "Methods" and Fig. S1; (Bee et al., 2009a, 2009b; Knezevic et al., 2011; Nottingham et al., 2007; Pimanda et al., 2007b)). It should be noted that our model focuses specifically on the emergence of HSCs from the hemogenic endothelium and as such cannot be used to infer the effects of either Bmp4 and Notch1 signals or triad gene expression levels on the eventual fate (i.e. differentiation and/or proliferation potential) of these cells.

To understand the role of the Smad1–Smad6–Runx1 signaling module we first examine the steady-state response of the network to Notch1 and Bmp4 signals. To this end we compute how the steady-state concentrations of the triad proteins depend on the