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Genetic regulatory networks programming hematopoietic stem cells and erythroid lineage specification

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Abstract

Erythroid cell production results from passage through cellular hierarchies dependent on differential gene expression under the control of transcription factors responsive to changing niches. We have constructed Genetic Regulatory Networks (GRNs) describing this process, based predominantly on mouse data. Regulatory network motifs identified in *E. coli* and yeast GRNs are found in combination in these GRNs. Feed-forward motifs with autoregulation generate forward momentum and also control its rate, which is at its lowest in hematopoietic stem cells (HSCs). The simultaneous requirement for multiple regulators in multi-input motifs (MIMs) provides tight control over expression of target genes. Combinations of MIMs, exemplified by the SCL/LMO2 complexes, which have variable content and binding sites, explain how individual regulators can have different targets in HSCs and erythroid cells and possibly also how HSCs maintain stem cell functions while expressing lineage-affiliated genes at low level, so-called multi-lineage priming. MIMs combined with cross-antagonism describe the relationship between PU.1 and GATA-1 and between two of their target genes, Fli-1 and EKLF, with victory for GATA-1 and EKLF leading to erythroid lineage specification. These GRNs are useful repositories for current regulatory information, are accessible in interactive form via the internet, enable the consequences of perturbation to be predicted, and can act as seed networks to organize the rapidly accumulating microarray data.

Keywords: Stem cell; Transcriptional regulatory network; Network motif; Blood; Hematopoiesis; Erythroid lineage

Introduction

Over recent years, the volume of data concerning the programming of blood lineages has expanded considerably. One way to collate these data is to construct genetic regulatory networks (GRNs) depicting the interactions between the individual genes. A number of developmental processes have now been represented as GRNs, providing insight into their underlying molecular mechanisms (Cripps and Olson, 2002; Davidson et al., 2002; Howard and Davidson, 2004; Loose and Patient, 2004).

A comprehensive analysis of regulatory interactions in yeast and *E. coli* GRNs has resulted in the distillation of distinct network motifs (Table 1) (Lee et al., 2002; Shen-Orr et al., 2002). The individual network motifs can be considered as small networks with distinct properties, which in combination define the genetic control of the cells' transcription programmes (Howard and Davidson, 2004). For example, autoregulation and multi-component loops, where transcription factors (TFs) stimulate their own expression either directly or through an intermediate, function to maintain gene expression programmes (Table 1A–B). In the single-input motif (SIM), one TF activates multiple targets (Table 1C), while multi-input motifs (MIMs) require several TFs to activate a group of targets (Table 1D–E). Regulatory chains, a number of TFs acting in a series, and the more complex feed-forward loop, provide temporal control within networks (Table 1F–G).

Here, we have constructed and analyzed GRNs underlying the specification of the hematopoietic stem cell (HSC) and its subsequent differentiation to the erythroid lineage, mainly focusing on the mouse. We have analyzed the use of different network motifs within the GRNs and relate this to the biology of hematopoietic and erythroid development. Interactions are

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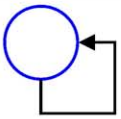
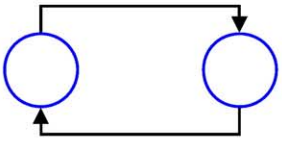
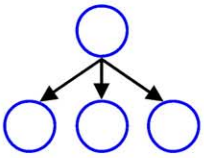
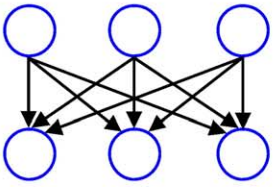
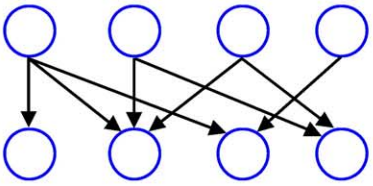
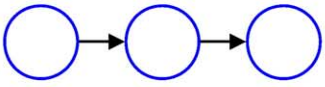
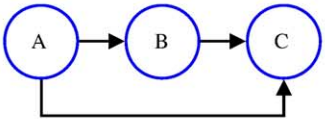
Table 1
Network motifs

Network motif	Description
a 	Autoregulation: A factor binds and regulates its own expression. There are many examples in the blood network including Fli-1, GATA-2, c-myb, GATA-1 and PU.1.
b 	Multicomponent Loop: Two (or more) transcription factors regulate one another in a loop. No examples of this network motif have yet been identified in this network.
c 	Single-Input Motif (SIM): A single transcription factor regulates multiple target genes. No examples of this network motif have yet been identified in this network.
d 	Multi-Input Motif (MIM): A set of transcription factors all regulate a set of targets. This network motif is often a subset of the more frequently observed 'Dense Overlapping Region' (DOR). See Fig. 4A for examples including Fli-1, Elf-1, GATA-2, Hex and SCL.
e 	Dense Overlapping Region (DOR): A set of TFs that overlap to regulate a set of targets, each target being regulated by different combinations of regulators. See Fig. 4B for examples involving the SCL complex.
f 	Regulatory Chain: An ordered chain of three or more transcription factors in series. A chain ends if a gene has no target, or autoregulates. Examples include beta-catenin regulating HoxB4, which in turn regulates c-myc.
g 	Feed-Forward Motif: The first transcription factor (A) regulates an intermediate (B), with both required for target gene (C) regulation. There are 8 subtypes of feed-forward network motif dependent on the sign of the regulation (positive or negative) (Ma et al., 2004). See Fig. 5 for examples including GATA-1, EKLF, Fog-1, β-globin, GATA-2 and c-myb.

derived from published expression profiles, perturbation experiments and *cis*-regulatory elements identified in promoters and enhancers of target genes, as previously described in our GRN for mesendoderm formation in *Xenopus* (Loose and Patient, 2004). Note that only those genes and interactions specifying the erythroid lineage via the HSC are depicted. Some significant TFs, for example Runx1, currently play minor roles in the GRNs, reflecting a lack of information rather than importance (see Otto et al. (2003) for a review of Runx1). A summary of the evidence and data for each link is available via the accompanying website and Table S1, along with interactive versions of these networks, which allow the upstream regulators and downstream targets of individual TFs to be highlighted (http://www.nottingham.ac.uk/genetics/networks/mouse and Table S1). We acknowledge that there are many links and genes missing from the network in its current form and that the reliability of individual links within the network depends on the evaluation of the source data. As a consequence, we will continue to update the network and welcome submission of new links via our website.