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Transcriptional regulatory networks in haematopoiesis

Diego Miranda-Saavedra and Berthold Göttgens

The coordinated expression of genes lies at the heart of developmental programmes, with complex regulatory networks controlling the spatial and temporal aspects of gene expression. Haematopoiesis (blood formation) has long served as a model process for studying the specification and subsequent differentiation of stem cells and represents the best characterised adult stem cell system. In this review, we outline how the integration of experimental and computational approaches as applied to haematopoiesis has resulted in some of the most advanced models of transcriptional regulatory networks in mammals.

Addresses
Department of Haematology, Cambridge Institute for Medical Research, University of Cambridge, Hills Road, Cambridge CB2 0XY, UK

Corresponding author: Göttgens, Berthold (bg200@cam.ac.uk)

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Introduction

The specification of haematopoietic stem cells (HSCs) and their subsequent differentiation into at least 14 types of mature blood cells, all of which perform different essential functions, is one of the best-characterised vertebrate developmental systems. Markers and biological assays are available for mature progeny of all haematopoietic lineages as well as for a plethora of progenitors with varying potentials including the pluripotent HSC. A major strength of the mouse blood system is the ability to transplant distinct subpopulations or indeed single cells and to assess their functionality *in vivo*. *In vivo* functionality is based on the ability of transplanted cells to re-establish a complete haematopoietic system in recipients that had their blood system destroyed either by exposure to gamma radiation or by cytotoxic drugs. The availability of this very powerful *in vivo* assay has resulted in HSCs becoming a paradigm for adult stem cells. Haematopoiesis involves a progressive restriction of differentiation potential and the establishment of lineage-specific expression profiles, which rely on lineage-specific transcription factors (TFs). Several TFs such as *Scl/Tal1*, *Gata2*, *Tel*, *Fli1* or *Runx1* are known to be critical for the specification and/or biological function of HSCs. Moreover, many of these essential haematopoietic genes not only play a role in HSCs but may also be required for the development of specific subsets of mature blood lineages (Figure 1).

The output of transcriptional programmes is controlled by the interplay of cellular context and extracellular inputs. Typically, an external stimulus activates a signal transduction pathway which leads to the modification of the activities of several TFs which in turn target enhancer/regulatory regions. However, the precise phenotypic response triggered by a given signalling molecule critically depends on cellular context, which at the transcriptional level is largely determined by cell type specific TF networks. Current strategies for characterising such transcriptional networks fall into two major classes: 'bottom-up' and 'top-down'.

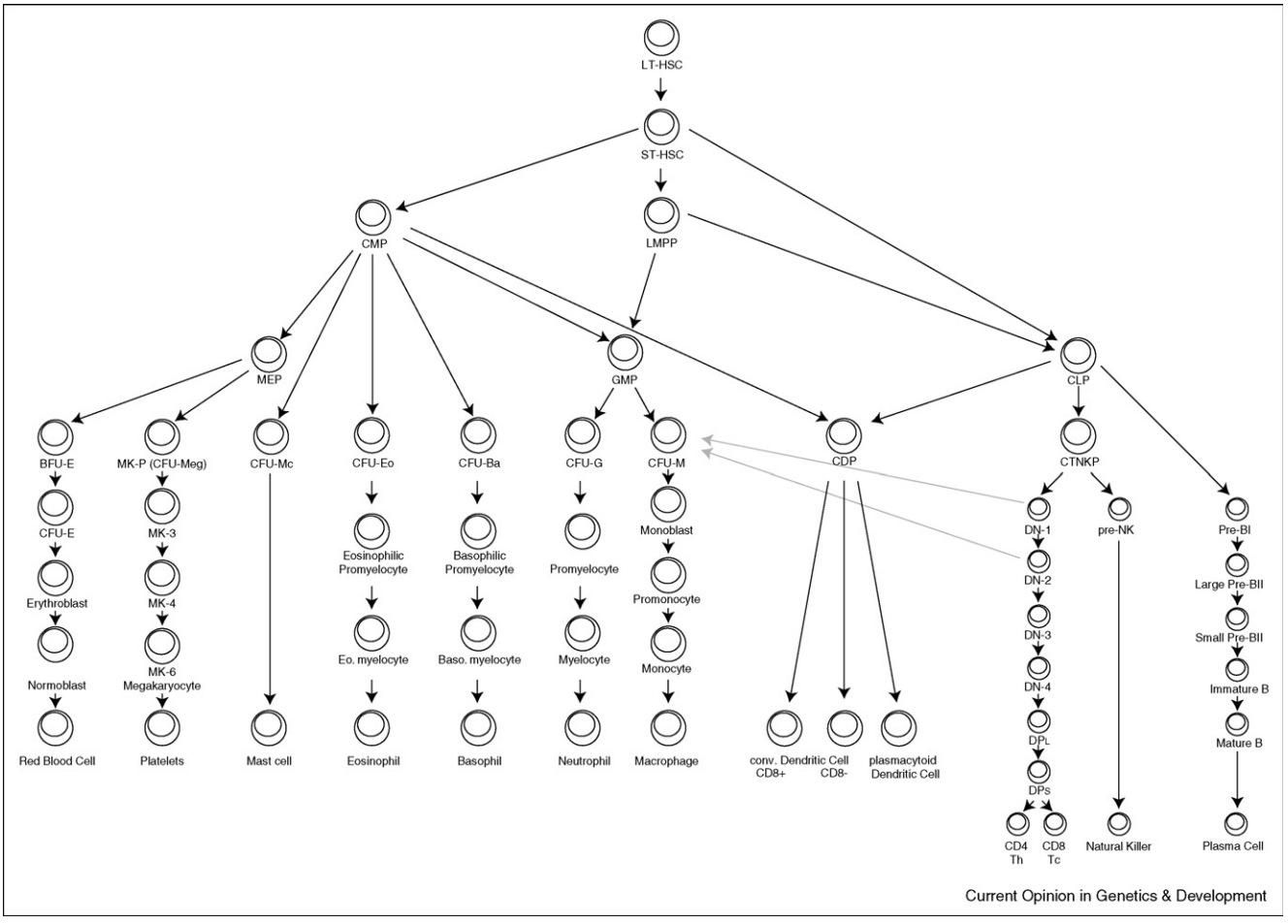
Reconstruction of gene regulatory networks: bottom-up approaches

'Bottom-up' approaches use small sets of key network components to experimentally define new interactions and thus incrementally generate regulatory network models. Initial network components or building blocks can be TFs, regulatory elements or a combination of both.

An example of a bottom-up approach starting with a regulatory element took an enhancer of the *Scl/Tal1* gene as a starting point. SCL/TAL1 is a basic helix–loop–helix TF essential for the development of blood and blood vessels [1]. *Scl/Tal1* is expressed in HSCs and its expression is maintained during differentiation along the erythroid, mast and megakaryocytic lineages but is repressed after commitment to all other lineages. *Scl/Tal1* was the first HSC TF for which enhancer-specific regulatory activity was validated *in vivo* [2,3,4•,5,6]. A 600-bp enhancer +19 kb downstream of the transcription start site was found to be sufficient for expression in HSCs and endothelium in transgenic mice. *In vivo* activity of the +19 enhancer was dependent upon two conserved ETS-binding sites and one conserved GATA-binding site which were bound by the Ets factors FLI1, ELF1, ERG and the Gata factor GATA2, respectively [7,8].

A subsequent computational screen identified 67 ETS–ETS–GATA TF-binding site clusters that were conserved between the human, mouse and dog genomes with the same orientation and spacing constraints as the *Scl/Tal1* +19 element [9••,10]. Four of these clusters were situated in gene loci of TF genes expressed in

Figure 1



Roadmap of haematopoiesis in the mouse. The multipotent long-term haematopoietic stem cell (LT-HSC) is the source of all blood cell types in this hierarchy with progressively restricted developmental potentials. LT-HSCs give rise to progenitor populations all of which can as well be isolated by using antibodies against cell-surface proteins, followed by fluorescence-activated cell sorting (FACS). These include short-term HSCs (ST-HSCs), lymphoid primed multipotent progenitors (LMPP) and the common lymphoid and myeloid progenitors (CLP and CMP). The CMP is the source of all myeloid lineages plus the common dendritic progenitor (CDP). Myeloid cells develop from CMPs via a number of intermediate progenitors (see below). The CLP develops into B cells and T and NK cells via the CTNKP (common T and natural killer progenitor), and also gives rise to the CDP. T cell development is especially complex and occurs in a series of stages of their 'double-negative' (DN) cells followed by the double-positive large (DP_L) and double-positive small (DP_S) cells to ultimately give rise to T helper (Th) and T cytotoxic (Tc) cells. MEP, megakaryocyte and erythrocyte progenitor; BFU-E, blast-forming unit erythrocyte; CFU-E, colony-forming unit erythrocyte; MK-P, megakaryocyte progenitor; CFU-Mc, colony-forming unit mast cell; CFU-Eo, colony-forming unit eosinophil; CFU-Ba, colony-forming unit basophil; GMP, granulocyte and macrophage progenitor; CFU-G, colony-forming unit granulocyte; CFU-M, colony-forming unit macrophage. The previously recognised distinction between clear-cut lymphoid and myeloid developmental pathways has recently been challenged by the finding that double-negative 1 and double-negative 2 (DN-1 and DN-2) cells of the T cell differentiation pathway retain their potential to generate myeloid cells, predominantly macrophages [36,37]. Therefore, our knowledge of the well-studied haematopoietic system is continually evolving.

embryonic HSCs and three of these clusters (controlling *Fli1*, *Prh* and *Smad6* genes) were found to possess an *in vivo* activity similar to that of the *Scl/Tal1* +19 element when assayed in transgenic mice [9••,10]. Using further bioinformatic and transgenic analyses, additional key regulators of blood development have progressively been integrated into this nascent gene regulatory network, such as the BMP4/*Smad* pathway and *Runx* TFs [10–15] (Figure 2). Interestingly, concerted biochemical and cell biological analyses revealed functional cross-talk between the BMP/*Smad* axis and *Runx1*. Previously unsuspected cross-talk between Smads and other TF families has recently also emerged as an important control mechanism of skin stem cell ageing [16] and may therefore represent a recurring theme in stem cell regulatory networks. Finally, the emerging HSC network laid the foundation for the first topological description of a regulatory network motif in blood stem cells, namely the fully connected triad composed of the SCL/TAL1, GATA2 and FLI1 TFs [17•] (Figure 2). In analogy to the *Oct4/Sox2/Nanog*