Transcriptional hierarchies regulating early blood cell development

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Abstract

Hematopoiesis represents one of the paradigmatic systems for studying stem cell biology, but our understanding of how the hematopoietic system develops during embryogenesis is still incomplete. While many lessons have been learned from studying the mouse embryo, embryonic stem cells have come to the fore as an alternative and more tractable model to recapitulate hematopoietic development. Here we review what is known about the embryonic origin of blood from these complementary systems and how transcription factor networks regulate the emergence of hematopoietic tissue from the mesoderm. Furthermore, we have performed an integrated analysis of genome-wide microarray and ChIP-seq data sets from mouse embryos and embryonic stem (ES) cell lines deficient in key regulators and demonstrate how this type of analysis can be used to reconstruct regulatory hierarchies that both confirm existing regulatory linkages and suggest additional interactions.

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Introduction

Hematopoietic stem cells (HSCs) are paradigmatic for studying stem cell biology as a result of their ability to maintain hematopoiesis throughout the life of an organism as well as the relative ease with which the hematopoietic system can be accessed for study [1]. However, while much is known about the function and regulation of HSCs in the adult, the mechanisms regulating their emergence during embryogenesis are less well understood.

The developing hematopoietic system has been the subject of over 100 years of study, since the appearance of hemoglobinised cells in the yolk sac was first noticed (reviewed by Refs. [2–4]). Hematopoietic cells develop from the embryonic mesoderm in two waves – the primitive wave that produces cells to sustain the developing embryo and the definitive wave that generates the HSCs that will populate the adult marrow – at multiple spatiotemporal sites within the embryo. As with adult hematopoiesis, transcription factors have emerged as key regulators of blood cell development. However, efforts to understand developmental hematopoiesis have been hampered by the difficulty in accessing the embryo during development. Here we will review what is known from our best-studied organism, the mouse, and discuss what has been learned from using the differentiation of embryonic stem cells (ES cells) as a model of development (see also [5]). We will focus particularly on the transcriptional hierarchies active during blood specification, including a meta-analysis of recently published gene expression profiles reporting loss of function analyses for the key transcriptional regulators, ETV2, SCL/TAL1 and RUNX1.

Overview of early blood cell development

In the mouse, hematopoiesis initiates at embryonic day 7.0–7.5 in the extra-embryonic yolk sac, where hematopoietic cells are first found neighboring endothelial cells within structures named blood islands [6,7]. The hemogenic potential of the yolk sac and its autonomous role in initiating the first stages of blood development was later demonstrated in vitro using explant cultures [8,9], confirming the yolk sac as the first site of hematopoiesis. Primitive erythrocytes are the first differentiated cells to be produced, with other cells of the myeloid lineages identified by E8.0 [10], indicating that multipotent progenitors are present at this stage. However, such progenitors do not yet have the ability to reconstitute the entire hematopoietic system of an irradiated mouse, the gold standard assay for hematopoietic stem cell activity, indicating that definitive HSCs are not produced during the earliest stages of developmental hematopoiesis [11–13].

The yolk sac remains the main site of hematopoiesis until around E10.0, when mammalian HSCs can first be detected within the embryo proper. It was originally thought that the mammalian hematopoietic system developed directly from yolk sac hematopoietic cells that migrated to the embryo after the initiation of the circulation at around E8.5. However, transplantation assays demonstrated that the yolk sac

Abbreviations: AGM, aorta-gonad-mesonephros; BL-CFC, blast colony-forming cells; E#, embryonic days post-fertilization; HSC, hematopoietic stem cell; VEGF, vascular endothelial growth factor.
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does not contain transplantable adult-type definitive HSCs until E11.5 [11,12,14], which is later than can be detected within the embryo proper. The intra-embryonic origin of HSCs was first demonstrated in the avian system using chimeras in which quail donor embryos were transplanted onto chicken recipient yolk sacs. In this system, definitive hematopoietic cells were shown to be embryonic in origin as they always derive from the donor [15], and were identified as originating from a mesodermal region containing the dorsal aorta. A similar region was later identified as having hematopoietic activity in the mouse, with E10.0 cells from the aorta–gonads–mesonephros (AGM) region able to form spleen colonies at a higher rate than yolk sac cells when transplanted into lethally irradiated mice [12], and able to reconstitute the entire hematopoietic system [13]. Within the AGM, the dorsal aorta was then shown to be the site of hematopoiesis [11], with clusters of cells expressing hematopoietic markers appearing to bud out from the ventral aortic endothelium and be released into the circulation [16–18].

After the emergence of HSCs in the AGM, HSCs migrate to the fetal liver and undergo a period of expansion, finally colonizing the bone marrow shortly before birth where they remain throughout life (reviewed by Refs. [1–4]). Other auxiliary sites have been proposed for hematopoiesis, including the placenta, but they have been reviewed elsewhere and will not be discussed here [2–4]. Moreover, the yolk sac has long been suggested to contain precursor cells that can mature into fully competent and transplantable HSCs [19,20] and a very recent paper reported independent emergence of HSCs in the embryo head [21]. Taken together, therefore, a rather complex picture of developmental hematopoiesis is emerging.

In addition to the ongoing uncertainties regarding the anatomical sites of hematopoiesis, the cellular origin of hematopoietic cells has also been the source of some dispute. In the yolk sac, the observation that blood islands comprise inner hematopoietic cells surrounded by endothelium [22] lead to the hypothesis that these cell types emerge from a common precursor, the hemangioblast [6,7]. Furthermore, single-gene mutations have been shown to severely affect both endothelial and hematopoietic lineages, most notably those involving Flk1 and Ev12, suggesting that they at least share common regulators, if not a common progenitor. The observation of hematopoietic clusters in the dorsal aorta of many species lead to an opposing theory by which HSCs develop from an endothelium with hematopoietic potential, the hemogenic endothelium. While it has been difficult to test these theories in the embryo, the use of ES cells as a model for development has provided evidence for both [2,3,5], and has recently allowed the two to be reconciled [23].

ES cells can be induced to differentiate, either in adherent culture on extracellular matrix or stromal cells, or through embryoid bodies – three-dimensional cystic structures reminiscent of the developing embryo - and can generate hematopoietic tissue with similar characteristics to the yolk sac including the development of hemoglobinised blood islands [5,24]. Detailed analyses have suggested that the kinetics of hematopoietic development, including expression of cell surface markers and hematopoietic genes, closely resemble that of the yolk sac (reviewed in [5]), indicating that ES cells provide an excellent model in which to study the developing hematopoietic system. When ES cells were plated in methylcellulose with vascular endothelial growth factor (VEGF) and Kit ligand, two cytokines important for hematopoietic development, they produced cells with blast morphology, called blast colony-forming cells (BL-CFCs). BL-CFCs could generate cells of multiple hematopoietic lineages and expressed the hematopoietic markers Flk1, CD34, Scl and Gata1, but not the pan-hematopoietic marker Brachyury [25]. The same group later showed that in liquid culture these cells could form both adherent cells with endothelial characteristics, including expression of CD31, Flk1 and the ability to uptake low-density lipoproteins (LDLs), as well as non-adherent hemoglobinised cells that could produce multiple hematopoietic lineages in colony assays [26]. They were then able to use the same culture conditions to identify analogous cells from the posterior mesoderm of gastrulating embryos that could form blast colonies with both hematopoietic and vascular potential, the characteristics of the hemangioblast, and also the potential to give rise to vascular smooth muscle [27].

The first demonstration of the hemogenic endothelium came from studying the chicken where a subset of endothelial cells in the dorsal aorta was found to express the hematopoietic marker CD45. A lineage-tracing study demonstrated that Dil-LDL-labeled endothelium gives rise to hematopoietic cells bearing the same marker [17]. Hemogenic endothelium has been observed in both the dorsal aorta [18] and yolk sac [28–30], challenging the hemangioblast theory, and the first HSCs capable of repopulating the adult hematopoietic system in the mouse express endothelial cell surface markers [31,32] which are lost during fetal liver hematopoiesis [33]. The ES cell model has also been useful in demonstrating the hematopoietic potential of endothelium in the mouse. Isolation and culture of VE-cadherin+CD45– endothelial cells and VE-cadherin–CD45+ hematopoietic cells showed that both cell types could produce myeloid and lymphoid cells, but that the potential was higher in endothelial cells [30,34]. Live-imaging studies have attempted to visualize the so-called endothelial-to-hematopoietic transition where hematopoietic cells form clusters in the lumen of the dorsal aorta and bud off into the circulation. FLK1+-VE-cadherin+ mesodermal precursors have been isolated from ES cells and the development of these cells on an OP-9 stromal layer observed by time-lapse imaging [35]. Endothelial colonies were able to generate non-adherent proliferative hematopoietic cells expressing CD45 through a semi-adherent stage analogous to intra-aortic clusters in which cells expressed both CD41 and VE-cadherin. The adherent hemogenic endothelial cells were able to produce endothelium but not cardiac or smooth muscle, negating the possibility that they are an earlier mesodermal progenitor. Furthermore, the authors isolated equivalent cells from the E7.5 embryo and demonstrated similar hemogenic endothelial potential. Additionally, lineage tracing and live imaging of sections of Ly6a(Sca-1)-GFP mice at E10.5 indicated that rare GFP+ cells budded directly from CD31+GFP+ endothelial cells and emerged into the aortic lumen [36].

Recently, the two theories have been reconciled by showing that the development of hematopoietic cells proceeds sequentially from mesoderm through the hemangioblast to the hemogenic endothelium and hematopoietic progenitors [23] (Fig. 1). Hemangioblasts derived from ES cells gave rise to blast colonies that contained both endothelial and hematopoietic cells, and visualized the transition from adherent endothelial cells to free hematopoietic cells with concurrent up and down-regulation of markers for hematopoietic and endothelial cells, respectively. Again, analogous cells were identified within the embryo, indicating that the ES cell system faithfully recapitulates hematopoietic development in the embryo.

Efforts have also been made to identify key regulators of the above developmental stages and transitions, and the ability to use ES cells to introduce gene knockouts into embryos has been invaluable. These studies have opened the door to understanding the transcriptional regulatory hierarchies controlling development and have implicated three factors in particular in the regulation of key stages of early hematopoiesis: ETV2, SCL and RUNX1.

Transcriptional control of hemangioblast formation

While no single absolute marker has yet been identified for the hemangioblast, both blood and endothelium arise from cells expressing the VEGF receptor, FLK1 [37–39]. Flk1−/− embryos die between E8.5 and 9.5 due to defects in blood and endothelial development, including a lack of yolk sac blood islands, and Flk1+/− ES cells do not contribute to the hematopoietic system in chimeras [40], indicating a very early defect in hematopoiesis.

In zebrafish, the cloche mutant similarly affects the earliest stage of development of blood and endothelial cells, with a resultant loss of
expression of many known blood- and endothelial-specific genes. Study of this mutant identified a number of candidate regulators of hemangioblast formation including Etsrp, an Ets-family transcription factor expressed specifically in vascular endothelial cells [41]. The Ets family of transcription factors includes key hematopoietic regulators such as ETS1, FLI1 and PU.1 ([53,54]). Knockdown of Etsrp resulted in the complete absence of circulation of zebrafish morphants, while over-expression could induce vascular endothelial markers in multiple cell types [42]. Etsrp was also a strong inducer of FLK1+ cells. The same group later studied the related mammalian Ets factor Etv2/Ert2, which was able to expand the hemangioblast lineage when expressed ectopically in zebrafish [43]. Etv2−/− mouse embryos die during gestation with severe blood vessel defects similar to the Fli1 phenotype, and Flk1 expression is reduced. Over-expression leads to the induction of FLK1+ mesoderm and can rescue its formation when normal induction is blocked by the inhibition of bone morphogenetic protein, Notch and Wnt signaling [44].

Attempts have been made to define the temporal window of Etv2 requirement during development. Etv2 is expressed in early mesoderm in a subset of the FLK1+ population with enhanced with endothelial and endothelial potential, but is down-regulated by E9.5 [45,46]. In a subset of the FLK1+ population with enhanced hematopoietic and requirement during development.

In the ES cell system, several studies have demonstrated the requirement for Scl in the formation of the hemogenic endothelium from the hemangioblast [23,66]. FLK1+ cells from Scl−/− ESC lines cannot produce hematopoietic clusters in the aorta, blast colonies or CD41+ hematopoietic cells [23]. However, Scl, like Etv2, is required within a specific temporal window during development. When Scl was placed under the control of a tamoxifen-inducible Cre, it could rescue the generation of VE-cadherin+ hematogenic endothelial cells during a defined temporal window of differentiation 2–4 days after the initiation of differentiation of ES cells on OP9 stromal cells, but was ineffective at later stages [66]. When Scl was deleted using Tie2-Cre, which is expressed shortly after Scl in hemogenic and vasculogenic sites, fetal liver hematopoietic cells were still detected, indicating that the

Transcriptional control of hemogenic endothelium

Like ETV2, the transcription factor SCL (also known as TAL1) is also vital for the development of the hematopoietic system. Identified in the human through chromosomal translocations in acute T-cell lymphoblastic leukemia [49–51]. SCL is a basic helix–loop–helix transcription factor expressed from E7.5 in both embryonic and extra-embryonic mesoderm. It localizes to the blood islands of the yolk sac and later to the fetal liver and spleen, at the sites of hematopoiesis [52], as well as the dorsal aorta and endocardium between E10.5 and 13.5 and in multiple cell types of the adult hematopoietic system, including HSCs. Scl expression is highly conserved across vertebrates [53,54], and the Scl gene locus has become a paradigm for studying the conservation as well as evolution of transcriptional control mechanisms [53,55–60]. Scl−/− embryos die at around E9.5 with an absence of hematopoietic cells, while other mesodermal lineages are unaffected [61,62]. Complementary gain of function studies in zebrafish confirmed SCL’s key role in early hematopoietic specification [63].

Morpholino-based down-regulation of Scl in the zebrafish had no significant effect on the expression of Etsrp in angioblasts [42]. Scl is not expressed in Etv2−/− or Fli1−/− embryos [45,47,64], but Scl expression in Etv2−/− mouse cells can restore hematopoiesis [45,47]. Furthermore ETV2 binds directly to the Scl locus [45], implicating Scl as a direct target downstream of Etv2 in the developing hematopoietic system. When Scl−/− ES cells were induced to form hematopoietic tissues, BL-CFCs, the in vitro counterpart of hemangioblasts, were detected but were unable to generate endothelial and hematopoietic cells, instead forming colonies of vascular smooth muscle cells [65]. This places the function of SCL downstream of the specification of the hemangioblast.

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time Scl is ablated it is no longer required for hematopoietic develop-
ment, at least not in a non-redundant fashion [67–69].

As well as driving hematopoietic development directly by promot-
ing the formation of the hemogenic endothelium, Scl may also have a role in inhibiting the potential of the hemangioblast to form vascular smooth muscle. Use of a doxycycline-inducible Scl transgene in ES cells showed that an early pulse of Scl expression expanded the number of hematopoietic colony forming cells while repressing the expression of cardiac markers and the formation of beating cardiomyocytes [70]. Similar results were found in vivo where Scl−/− embryos initiated a cardiac transcriptional program in the yolk sac, with CD31+PDGFRα+ cardiac progenitors emerging and generating beating cardiomyocytes [71].

These studies indicate that Scl is essential for the specification of the hemogenic endothelium from the hemangioblast (Fig. 1), but not for the subsequent specification of hematopoietic cells from the endothelium.

Transcriptional control of endothelial-to-hematopoietic transition

As outlined above, hematopoietic cells bud off from the hemogenic endothelium into the aortic lumen in a process termed endothelial-to-

hematopoietic transition. The expression of the transcription factor Runx1 in hematopoietic cells as well as their neighboring endothelial cells [72], both in the yolk sac and the embryo proper, suggested that this protein is involved in this transition. Runx1−/− embryos die at around E12.5 with severe hemorrhaging [73–75] and hematopoietic clusters fail to form in the dorsal aorta [72]. Conversely, over-expression of Runx1 in a Runx1−/− ES cell line resulted in an increased number of CD41+ hematopoietic cells [23]. The level of Runx1 expres-
sion is also important, as heterozygotes have reduced, but not lost, production of definitive HSCs in the AGM [76]. Interestingly, Runx1 deficiency can be partially rescued by localized Runx1 expression in explanted para-aortic splanchopleura, the mesodermal precursor of the AGM, adding weight to the argument that this region is capable of definitive hematopoiesis independently of the yolk sac [77].

To identify the temporal window of Runx1 activity, Chen and colleagues used VE-cadherin− or Vav1-mediated Cre to ablate Runx1 at different stages of hematopoietic development [78]. At E8.5, VE-cadherin is restricted to endothelial cells of the yolk sac, dorsal aorta and heart, and Runx1 ablation at this stage caused severe defects in hematopoiesis, in line with the Runx1 knockout [72,78]. However, when Cre was under the control of Vav1, which is restricted to hematopoietic cells and implicated as a RUNX1 target, fetal and adult viability were unaffected, demonstrating that Runx1 expression is no longer re-
quired after the endothelial-to-hematopoietic transition [78]. This was further confirmed in the ES cell system where Runx1 expression is crit-
ical for the formation of hematopoietic precursors from the hemogenic endothelium [23]. Time-lapse imaging identified the formation of clusters of tightly associated adherent cells in Runx1−/− ES cells. However, these cells expressed the endothelial marker CD31 but not CD41, and very few generated blast colonies.

The temporal window of RUNX1 activity focused attention on the identification of pathways downstream of RUNX1 that regulate the maturation of definitive HSCs from CD41+ cells. ChIP-seq for RUNX1 in early hematopoietic stages in the ES cell system identified multiple direct RUNX1 targets with expression correlated to Runx1 and which were enriched for biological functions related to hematopoietic develop-
ment [79]. Direct targets with negatively correlated expression were enriched for genes associated with non-hematopoietic tissue differ-
etiation, indicating that RUNX1 may directly repress alternative fates in the hemogenic endothelium as well as promoting hematopoietic develop-
ment. Runx1 alone can completely reverse the endothelial program initiated by HoxA3 in HE cells, while HoxA3 can restrain or even reverse the differentiation of the earliest endothelial progenitors into CD41+ hematopoietic cells in the ES cell system, suggesting how the HE may balance the production of blood and endothelium [80].

In the hematopoietic lineage, Gfi1 and Gfi1b are both able to partially rescue Runx1 defects, triggering the down-regulation of endothelial markers and the formation of hematopoietic cells budding from the HE [81]. Expression of both factors increases when the hemogenic endo-
thelium forms and is reduced in the absence of Runx1, but deletion of either alone does not phenocopy Runx1−/− embryos. However, in double knockout embryos, the cells of the hemogenic endothelium are unable to bud off into the yolk sac and embryo, highlighting the key regulatory role of these factors downstream of Runx1.

Revealing regulatory cascades from loss of function analyses

As outlined in the previous sections, a number of key regulatory transcription factors have been identified to function during the develop-
mental progression from mesodermal cells to blood progenitors. In order to investigate the relationship between the three key develop-
mental transcription factors, ETv2, RUNX1 and SCL, and the genes that they regulate, we performed an integrated analysis of five microarray knockout comparison studies and two ChIP-sequencing studies from ES cell-derived cultures. These data were analysed in the context of existing literature on the regulatory network of early hematopoiesis. FLK1+ cells from Etv2−/− and wild-type cells were compared in two studies, revealing 170 [45] and 143 [47] differentially expressed genes, respectively. Together, these studies suggest that SCL, FLJ1 and GATA2, which form a recursively wired gene-regulatory circuit in the hemangioblast [82], are key downstream targets of ETv2. Also, wild type CD31+ cells from the yolk sac were compared to those cells from Scl−/− embryos. In the latter circumstance, developmental hematopoiesis is arrested before the hemogenic endothelium stage and 2260 differentially expressed genes were identified. No equivalent study in the ES cell differentiation system has yet been described. Two studies have been described that compare Runx1−/− and Runx1+/+ embryonic stem cell lines [79,83]. The genome-wide binding pattern of Runx1 was also reported for these cells [79], which was combined with the gene expression data in an integrated analysis. A second RUNX1 ChIP-seq analysis study has also been reported [84].

For each of the five microarray experiments the list of differentially expressed genes was downloaded and filtered for hematopoietic transcription factors, based upon annotation in the Mouse Genome Database [85] for the hematopoietic phenotype, and the RIKEN Transcription Factor Database [86] and GO annotation [87] for transcription factors. Hierarchical clustering was performed on the resulting merged expression is no longer re-
ed for hematopoietic transcription factors, based upon annotation in the Mouse Genome Database [85] for the hematopoietic phenotype, and the RIKEN Transcription Factor Database [86] and GO annotation [87] for transcription factors. Hierarchical clustering was performed on the resulting merged matrix of discrete expression values (Fig. 2A). We next constructed putative network diagrams by considering the clustered expression data in combination with the two RUNX1 ChIP-seq experiments in hemogenic endothelium, and in light of what is already established in the literature. Binding by a transcription factor was taken as evidence of direct regulation.

Our analysis supports a model in which key hematopoietic trans-
cription factors are activated sequentially and then later collaborate to control gene expression (Fig. 2B) [79,84]. This process begins with activation of Env2 by PAK/CREB signaling at an early differentiation stage [48]. ETV2 subsequently activates the core circuit comprised of Scl, Gata2 and FLJ1 [82]. The small number of genes directly regulated...
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by ETV2 suggests that its primary role is to activate this self-regulating triad, which controls further specification of differentiation. This is in agreement with the finding that expression of Scf alone is sufficient to restore hematopoietic and endothelial potential in Etv2−/− cells [45,47]. However, direct regulation of Lmo2 by ETV2 has also recently been described [88].

At the hemangioblast stage, the SCL-GATA2-FLI triad forms a positive feedback loop that is independent of further activation by ETV2. The three transcription factors cooperate to activate Ly61, Hhox, Erg and Runx1. In the hemogenic endothelium, we see a more densely connected network in which RUNX1 cooperates with SCL, FLI1 and GATA2 to activate some factors while independently regulating others. Among these factors are the transcriptional repressors GFI1 and GFI1B, which are key mediators of RUNX1’s role in extinguishing the endothelial program during the transition to definitive hematopoietic progenitor cells [80,81].

It is illustrative to compare our predicted network links to well-established regulatory interactions active in HSCs. Notably, our analysis predicts the activation of Gata1 by Runx1, while existing models do not account for this activation. GATA1 can disable GATA2 auto-regulation by binding to the Gata2 3 kb regulatory region and mediating domain-wide chromatin remodeling. This mechanism may be involved in deactivating the autoregulatory loop of SCL, FLI1 and GATA2 in preparation for differentiation [82,89]. It is possible that RUNX1 activation of Gata1 could be involved in priming early hematopoietic progenitors for an erythroid fate. Another noteworthy observation is the characterisation of Sox17 as a direct target repressed by Runx1. Since Sox17 is required for endodermal differentiation [90,91], this is consistent with RUNX1’s role in extinguishing non-hematopoietic transcriptional programs [80,90].

Modeling hematopoietic regulatory networks

The previous section outlined how integrated analysis of a number of published datasets can be utilized to reconstruct likely regulatory hierarchies operating during the development of blood progenitors from mesoderm. Regulatory cascades are best understood in lower model organisms, where comprehensive functional studies in sea urchin for example have revealed in-built forward momentum, so that once a cell has embarked on a given developmental trajectory, this will be executed more or less automatically, and is largely dictated by the regulatory network wiring of feed forward loops between key regulators [92]. This forward momentum, however, needs to be halted at some stage, because at midgestation hematopoietic development entails the specification of blood stem cells, most of which do not automatically proceed to differentiate into mature blood cells because they are responsible for long-term maintenance of the blood system, both under homeostatic and stress conditions. When compared with intermediate developmental stages such as the hemangioblast or hemogenic endothelium, HSC regulatory network states have to show increased stability because HSCs not only need to have the ability to “move forward” and produce mature cell types, but also to “remain static” and contribute to stem cell self-renewal.

In the absence of comprehensive experimental approaches, several abstract models of hematopoietic stem and progenitor cell maintenance have been developed [93-96]. Models more explicitly anchored in molecular experimental data originally focussed on simple 2-factor or 3-factor models [97-103], which may capture some essential properties of core regulatory circuits but are clearly too simplistic to model complex network behavior. More recently, a qualitative model of the core transcription factor network active in common myeloid progenitors was constructed following a comprehensive literature survey [104] (Fig. 3). In their Boolean model, each transcription factor is represented by a variable which can take one of two values; 1, corresponding to an “on” or expressed state; and 0, corresponding to an “off” or unexpressed state. The complex combinatorial logic governing the interactions between transcription factors is encoded as Boolean update rules using the logical functions AND, OR and NOT. For example, GATA-2 positively regulates its own expression, and is inhibited by GATA-1 and FOG-1. As both GATA-1 and FOG-1 are required to fully repress GATA-2 expression, they are combined using AND in the Boolean update rule for GATA-2.

Computational analysis of this model, beginning from an initial transcription factor expression state representing the common myeloid progenitor, revealed an acyclic, 232-element hierarchical state space which recapitulated the steps of myeloid differentiation. This state space contained four terminal steady states, which were found to be in good agreement with microarray expression profiles of megakaryocytes, erythrocytes, granulocytes and monocytes. Once it had been established that this model recapitulated myeloid differentiation, further analyses based upon perturbations to the network were conducted. These simulations demonstrated that in silico knockouts were able to reproduce known experimental lineage depletion results and that in silico overexpression reproduced known experimental reprogramming results.

However, the transcription factor network model constructed did not identify the multipotential progenitor cell as a stable state, and

Fig. 3. A qualitative Boolean model of the core transcription factor network active in common myeloid progenitors. A. Visual representation of the common myeloid progenitor regulatory network model as encoded by the Boolean update rules from Krumsieck et al. [104]. Blue edges represent activation and red edges repression. Square boxes connecting edges represent AND operations. B. Schematic of the adult hematopoietic hierarchy in bone marrow. The CMP is regulated by the network in A to produce multiple outputs: the granulocyte–monocyte progenitor which gives rise to granulocytes, monocytes and other myeloid cells, and the megakaryocyte–erythroid progenitor, which produces erythrocytes and megakaryocytes. HSC, hematopoietic stem cell; MPP, multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte–erythroid progenitor; GMP, granulocyte–monocyte progenitor.
therefore clearly did not capture the relative stability that one would expect to characterize blood stem cell network states [104]. Boolean modeling based on experimental knowledge of the connectivity between eleven HSC transcription factors by contrast did produce a stable attractor state reminiscent of the HSC [109], which indeed required simulation of external triggers to allow exit from this state. HSCs balance self-renewal and differentiation, which suggests that more sophisticated modeling approaches will be required to combine the faithful representation of differentiation reported [104] with an ability to simultaneously endow the multipotent stem cell with a degree of inherent stability. Given the types of experimental data that will be available, it is unlikely that the field will be able to employ, at least not in the short term, large-scale stochastic process modeling [105], which aims to capture the kinetics of molecules inside the cell but requires accurate quantitative measurements of many parameters. Qualitative modeling on the other hand has demonstrated its utility to drive the design of future experimentation in many other contexts [106–108], even though it relies only on the network topology, and therefore only makes qualitative statements about the network. It is likely therefore that close cooperation of experimental and theoretical biologists could result in an iterative workflow of model predictions followed by experimental validation, which in turn would advance our understanding of the underlying biological processes.

Concluding remarks

The field of developmental hematopoiesis is rapidly entering a new era where genome-scale experiments combined with downstream computational analysis are likely to provide unparalleled insights into early regulatory hierarchies. The speed of future progress will depend on both conceptual and technological parameters, such as (1) miniaturization of genome-wide profiling techniques to accommodate the small cell numbers present in early embryos, (2) continued improvement of our understanding of early developmental lineage relationships to ensure for example that snap-shot gene expression measurements can be viewed within an appropriate “developmental” sequence, (3) an enhanced emphasis on multi-disciplinary approaches where classical developmental and cell biologists will work in close collaboration with bioinformaticians, imaging experts and mathematical biologists. Finally, analysis of the more tractable ES cell differentiation systems alongside developing embryos will not only aid our understanding of developmental hematopoiesis but will also be essential for the production of true HSCs in vitro from ES cells or induced pluripotent stem cells, and therefore has not only fundamental but also translational relevance.

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