Transcriptional Regulation of Haematopoietic Stem Cells

11

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Abstract

Haematopoietic stem cells (HSCs) are a rare cell population found in the bone marrow of adult mammals and are responsible for maintaining the entire haematopoietic system. Definitive HSCs are produced from mesoderm during embryonic development, from embryonic day 10 in the mouse. HSCs seed the foetal liver before migrating to the bone marrow around the time of birth. In the adult, HSCs are largely quiescent but have the ability to divide to self-renew and expand, or to proliferate and differentiate into any mature haematopoietic cell type. Both the specification of HSCs during development and their cellular choices once formed are tightly controlled at the level of transcription. Numerous transcriptional regulators of HSC specification, expansion, homeostasis and differentiation have been identified, primarily from analysis of mouse gene knockout experiments and transplantation assays. These include transcription factors, epigenetic modifiers and signalling pathway effectors. This chapter reviews the current knowledge of these HSC transcriptional regulators, predominantly focusing on the transcriptional regulation of mouse HSCs, although transcriptional regulation of human HSCs is also mentioned where relevant. Due to the breadth and maturity of this field, we have prioritised recently identified examples of HSC transcriptional regulators. We go on to highlight additional layers of control that regulate expression and activity of HSC transcriptional regulators and discuss how chromosomal translocations that result in fusion proteins of these HSC transcriptional regulators commonly drive leukaemias through transcriptional dysregulation.

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11.1 Introduction

The haematopoietic system performs a number of critical functions for mammalian physiology including transport of oxygen and nutrients, as well as immune protection. Blood cells have a rapid turnover and the entire haematopoietic system is maintained by haematopoietic stem cells (HSCs), a rare cell type normally found in the bone marrow of adult mammals.

11.1.1 Functional Definition of an HSC

The gold standard functional definition of an HSC comes from transplantation assays. In the mouse for example, a single HSC has the ability to reconstitute the entire haematopoietic system when injected intravenously into a sublethally irradiated recipient mouse (irradiation destroys the haematopoietic system), and stably maintain the haematopoietic system for the life of the recipient [1]. This so-called long-term reconstitution ability defines the key characteristics of HSCs: (1) the ability to home to and colonise the bone marrow in adult mammals, (2) the ability to expand and self-renew to form and maintain a stable population size for the lifetime of the organism, and (3) the capacity to differentiate into any mature haematopoietic cell type (multipotency). Long-term self-renewal and expansion can additionally be determined by serial or competitive transplantation assays, while multipotency can also be analysed using in vitro colony forming assays.

Large efforts have been made to prospectively isolate pure HSC populations using a range of cell surface marker combinations (both their presence and absence), cellular characteristics such as their ability to efflux certain dyes, and molecular signature such as gene expression patterns. Other combinations of markers have been identified that mark the various haematopoietic progenitor and mature cell populations, alongside functional colony forming assays and morphological identification.

11.1.2 Key Experimental Approaches

A key approach used to characterise transcriptional regulators of mouse HSCs has been gene targeting in embryonic stem (ES) cells followed by the generation of knock-out mice, which can then be used to assay the consequences of gene deletion on haematopoiesis during embryonic development, the adult HSC pool and differentiation potential. Conditional gene targeting protocols, such as those using the Cre-Lox system, allow genes to be deleted later during development or in an adult cell population. Dosage effects can be analysed using heterozygous (+/null) mice, retrovirally inserted shRNAs or overexpression vectors. Recently, ES cell differentiation to embryoid bodies (EBs) has been validated as an in vitro model of developmental haematopoiesis (reviewed elsewhere [2]), and has allowed analysis of some of these critical developmental transcriptional regulators in haematopoiesis.

Techniques such as phylogenetic footprinting, DNase I hypersensitive (DHS) assays, chromatin immunoprecipitation (ChIP) assays and mutagenesis have been used to identify *cis*-regulatory elements within critical gene loci and determine upstream transcriptional regulators. Importantly, the tissue and developmental time specific activity of regulatory regions identified using the above techniques can be validated using powerful in vivo assays including transient (F0) transgenic mouse embryo assays and comprehensive analysis of haematopoietic parameters in the bone marrow of established transgenic mouse lines. The advent

of next generation DNA sequencing coupled to ChIP (ChIP-seq) has allowed identification of genome-wide binding sites of specific transcription factor within a given cell population and identifies putative regulatory sites and downstream targets. A current limitation of this technique is the large number of cells required, typically several million.

11.2 Transcriptional Regulation of HSC Formation

11.2.1 Biology of Mammalian Developmental Haematopoiesis

The haematopoietic system is derived from the mesoderm lineage in the developing embryo in a process called developmental haematopoiesis. Developmental haematopoiesis occurs at several distinct spatiotemporal locations in the developing embryo and can be broadly divided into two stages: (1) embryonic haematopoiesis and (2) definitive haematopoiesis [3]. Embryonic haematopoiesis occurs from E7.5 in the yolk sac, initially producing primitive erythroid cells, and later multilineage progenitors [4–7]. However, these cell types do not fulfill the criteria of a true HSC as they are unable to reconstitute the entire haematopoietic system of an irradiated mouse.

True HSCs are only produced during the second wave, definitive haematopoiesis, which occurs from approximately E10 in the developing mouse embryo, when the first cells are generated that have the ability to both self-renew and reconstitute the entire haematopoietic system [6, 7]. Definitive HSCs are believed to bud off from the ventral wall of the dorsal aorta in a part of the embryo labelled the aorta-gonad-mesonephros (AGM) region [7]. Additional contribution to the pool of definitive HSCs from extraembryonic tissue is currently unresolved (reviewed in [3]).

11.2.2 Specification of HSCs

Two models of haematopoietic specification, the haemangioblast and haemogenic endothelium models (reviewed in [3]) have recently been reconciled by Lancrin et al. who proposed a linear pathway of haematopoietic specification from mesoderm, through a tri-potent haemangioblast cell type (with the capacity of forming haematopoietic, endothelial and smooth muscle cells) to a bi-potent haemogenic endothelium (HE) cell type (with the capacity to commit to either haematopoietic or endothelial cell types), from which definitive blood cells can be derived [8] (Fig. 11.1). A number of transcriptional regulators have been identified as playing a crucial role in the specification of HSCs during development, and can be fitted into the pathway described above.

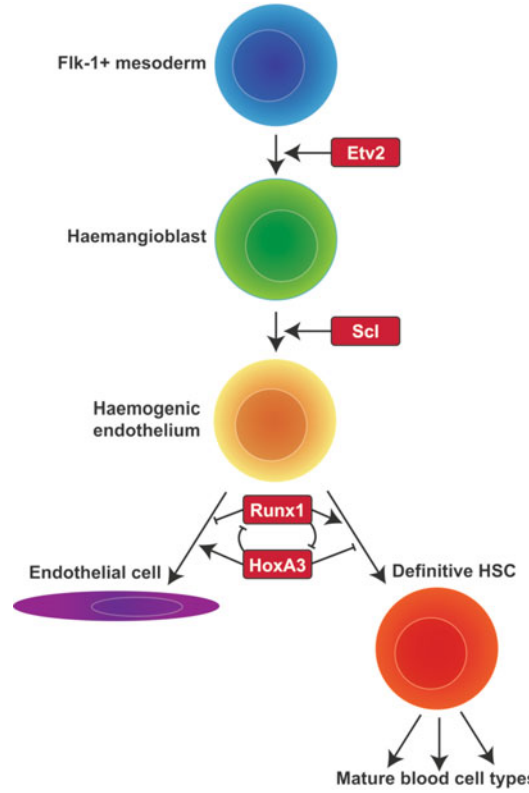

Fig. 11.1 Model of definitive HSC specification from mesoderm during embryonic development. Definitive HSCs are derived from Flk-1$^+$ mesoderm, which are specified through a tri-potent haemangioblast stage (Etv2-dependent), and bi-potent haemogenic endothelial stage (Scl-dependent). Haemogenic endothelium lineage specification to haematopoietic or endothelial cell types is dependent on the expression of antagonistic transcription factors Runx1 and HoxA3

11.2.3 Formation of the Haemangioblast from Mesoderm

The E-twenty-six specific (Ets) factor Etv2 (ER71) has recently been identified as a key transcriptional regulator of the mesoderm to haemangioblast transition [9]. Etv2 is essential for development of both endothelial and haematopoietic lineages at an early stage; mesodermal precursors of haemangioblasts are generated in Etv2 null embryos and during ES cell differentiation, but further specification is blocked. Etv2 is expressed early in developing mesoderm, and marks a subset of the Flk-1$^+$ mesodermal population with enhanced haematopoietic and endothelial potential [9]. Etv2 expression is downregulated by E9.5 and silenced by E10.5 in endothelial cells, suggesting it only plays a role in the early steps of mesoderm specification towards endothelial and haematopoietic cell fates [9]. Lee et al. have previously identified a potential regulatory cascade acting upstream of Etv2 including Notch, BMP and Wnt signalling [10]. Liu et al. recently suggested that Etv2 plays a role in specifying a haematopoietic rather than cardiogenic fate of Flk-1$^+$ mesoderm through regulating Wnt signalling [11].

11.2.4 Commitment of the Haemangioblast to Haemogenic Endothelium

Lancrin et al. demonstrated that the transition between haemangioblast and haemogenic endothelium was dependent on expression of the basic helix-loop-helix (bHLH) transcription factor Scl (Tal-1), when analysed using ES cell in vitro differentiation assays [8]. Scl is first expressed at the haemangioblast stage, and its expression is maintained through haemogenic endothelial and HSC stages [8, 12]. Expression of *Scl* is regulated by several developmental tissue-specific enhancers, including three important for haematopoiesis. The -4 *Scl* enhancer was found to drive expression to endothelium and foetal haematopoetic progenitors, mediated by Ets factor binding (including Fli-1 and Elf-1) [13]. The +19 enhancer is active in endothelial and haematopoietic cells, and critically depends on an Ets/Ets/Gata motif that binds Ets factors Fli-1 and Elf-1, and Gata2 [14]. These two enhancers appear to have overlapping roles in HSC specification, with the +19 enhancer being sufficient to drive *Scl* expression and blood formation in *Scl*$^{-/-}$ embryos, but not necessary as its deletion does not result in loss of haematopoiesis [13]. The third enhancer is the +40 region, which drives *Scl* expression in embryonic and definitive haematopoietic cells. The +40 enhancer may be particularly important to sustain rather than initiate *Scl* expression as its activity is regulated by Scl protein, thereby forming an autoregulatory loop [15, 16].

11.2.5 Specification of HSCs from Haemogenic Endothelium

Several critical factors have been identified as transcriptional regulators of definitive HSC specification including Runx1, Mll1, TFIIS, Gata2, Notch1, Meis1, Erg, c-Myb and c-Myc (see references below). The role of c-Myb and c-Myc in transcriptional regulation of stem cells is reviewed in Chaps. 15 and 19.

The core binding factor Runx1 (AML1) and its binding partner, CBFβ, are both required for definitive haematopoiesis [17–19]. Using conditional knockout mice models, Chen et al. recently identified the HE to definitive HSC transition as dependent on Runx1 [20]. Nottingham et al. identified an important Runx1 enhancer, the +23, which regulates Runx1 expression during HSC emergence, through binding of Gata, Ets and Scl factors [21].

The Trithrorax-related Mll1, a histone H3 lysine 3 (H3K4) methyltransferase, is required for definitive haematopoiesis from analysis of *Mll1* null mouse embryos and chimera contribution [22]. However, a second *Mll1* knockout mouse model created by McMahon et al. survived up to E16.5 and contained a limited number of foetal HSCs [23]. The reason for this discrepancy is unclear. Mll1 forms a large multi-protein complex with many proteins, which is thought to activate and maintain transcription

and epigenetic memory (reviewed in [24]). Although Mll1 contains a CXXC-type zinc finger DNA binding domain, its recruitment to DNA is not fully understood and the recent identification of the possible involvement of non-coding RNAs (ncRNAs) suggesting at least in part non-classical modes of recruitment to target regions [25]. The transcription elongation factor S-II (TFIIS), which is known to interact and synergistically function with the Mll1-interacting PAF1 complex [26], is also required for definitive haematopoiesis [27]. Recently, a physical interaction between the C-terminal SET domain of Mll1 and the Runt domain of Runx1 has been identified, responsible for recruitment of Mll1 to, and the regulation of, the Runx1 target gene *Spi-1/PU.1* [28]. Recruitment of Mll1 by Runx1 may in part explain the apparent functional overlap of these two transcriptional regulators in haematopoiesis.

The zinc finger transcription factor Gata2 is essential for definitive haematopoiesis. Gata2 is expressed prior to HSC emergence and thought to mark haematopoietic-specified cells [29]. However, a reduction of Gata2 expression or activity appears necessary for haematopoietic commitment [30]. Once again, the Ets/Ets/Gata motif and E-box motifs were found in a *Gata2* enhancer region (the -3 enhancer) [31, 32]. Gata2 appears to have an overlapping role with Runx1 in definitive haematopoiesis, as $Gata2^{+/-}Runx1^{+/-}$ mice are not viable and display haematopoietic defects at midgestation, while single heterozygous mice are viable with only a minor haematopoietic phenotype [33].

The Ets transcription factor Erg was recently shown to be critical for early maintenance, but not specification, of definitive HSCs as deletion results in rapid loss of HSCs [34]. Erg is thought to achieve this by acting as an upstream regulator of *Scl*, *Gata2* and *Runx1* [14, 21, 31].

Notch proteins are major constituents of a highly conserved signalling pathway. Notch proteins are membrane bound receptors, which when bound by their ligand Jagged, proteolytically cleave their intracellular domain, the so-called Notch-IC domain, which translocates to the nucleus where it participates in the formation of multiprotein DNA-binding complexes to regulate transcription [35]. Notch1 (but not Notch2-4) is required for generating definitive HSCs [36]. Further analysis using ES cell differentiation models and the generation of chimeric mouse embryos demonstrated that *Notch1*-deficient ES cells are capable of producing definitive haematopoietic progenitors, but fail to establish long-term definitive HSCs [37]. *Runx1* appears to be a key target of Notch signalling during definitive haematopoiesis [38, 39].

Meis1, a member of TALE subfamily of homeobox proteins, is a Hox protein cofactor that modulates their DNA binding affinity and specificity. Several *Meis1*-deficient mouse models have been created and show similar phenotypes; mouse embryos die by E14.5 with haemorrhaging and liver hypoplasia due to defective developmental haematopoiesis [40, 41]. Definitive haematopoiesis is compromised but is not completely ablated, and *Meis1*-deficient foetal livers at E12.5 have reduced HSC populations, which lack reconstitution ability [41]. *Meis1* is expressed in definitive haematopoietic clusters in the AGM, which are reduced in number and size, and have reduced *Runx1* expression in *Meis1*-deficient embryos [40].

Recently, a negative regulator of HE specification to HSC has been identified, the homeobox transcription factor HoxA3 [42]. HoxA3 is a positive regulator of HE specification to endothelial lineage, and with Runx1 plays a key role in this lineage decision process. Runx1 acts to induce a haematopoietic transcription factor cascade to promote HSC formation, while inhibiting essential endothelial lineage genes. HoxA3 acts to maintain these endothelial lineage genes within the HE, and represses the haematopoietic cascade, which appears to at least in part be achieved through direct repression of *Runx1* [42].

11.2.6 Migration, Expansion and Maintenance of Foetal HSCs

From approximately E12.5 of mouse embryonic development, definitive HSCs generated in the AGM region migrate to and colonise the foetal

liver, the site of foetal haematopoiesis [43]. Around the time of birth, HSCs move to the bone marrow niche for the rest of the life of the mammal [43]. It is estimated that at E11.5 there is one HSC produced in the AGM [44]. Expansion of these early HSCs is therefore critical to form a large enough population to maintain haematopoiesis for the life of the organism. This propensity to expand the HSC population, rather than maintain pool size is a key difference between foetal and adult HSCs, although adult HSCs retain this potential as demonstrated by transplantation assays.

Sox17 is a Sry-related high mobility group box transcription factor and within the haematopoietic system, is expressed in foetal and neonatal, but not adult HSCs [45]. *Sox17* deficiency severely impairs foetal haematopoiesis, and *Sox17*-null foetal and neonatal HSCs lose all reconstitution ability implicating Sox17 in generation or maintenance of definitive HSCs [45]. Loss of Sox17 expression correlates with acquisition of adult HSC characteristics; slow cell cycling and adult surface marker phenotype [45]. A number of other transcriptional regulators of both foetal and adult HSCs have been identified, but are discussed in Sect. 11.4.

11.3 Transcriptional Regulation of HSC Homeostasis

HSCs have the capacity to proliferate and self-renew to maintain their population for the lifetime of the organism, and balance this with differentiation into the committed haematopoietic cell types to replenish physiological turnover or injury. Additionally, to prevent population expansion to a physiologically dangerous size, programmed cell death (apoptosis) must also be regulated. In the adult, HSCs constitute an exceedingly rare cell population estimated at 1 in 10^4 to 1 in 10^5 bone marrow cells. Adult HSCs are believed to be predominantly quiescent, with recent estimates in the mouse suggesting one cell division every 145 days and may reversibly switch between this state and self-renewal during homeostasis and repair [46]. Further modelling suggested the existence of two kinetically distinct subpopulations of HSCs, one cycling every 149–193 days, and the other cycling every 28–32 days [47].

11.3.1 Concepts of HSC Fate Decisions

It is generally assumed that HSC fate choices are associated with cell division, as HSC differentiation without division would likely lead to HSC exhaustion [48]. These fate decisions would therefore be a result of the type of cell division; symmetrical division to produce either two HSCs or two progenitor cells, or asymmetric cell division into one HSC and one progenitor (Fig. 11.2a). These cell division options would allow HSC pool size to be regulated (e.g. expansion after transplantation) and respond to environmental stress [48].

Cell intrinsic (e.g. transcription factor protein concentrations and distribution in daughter cells) and extrinsic (e.g. cytokines and cell-cell signalling) cues determine lineage restriction. Two types of models have been proposed to explain HSC lineage commitment ([48, 49] summarised in Fig. 11.2b): (1) Instructive or deterministic models predict HSCs to respond to external stimuli, which directly guide lineage decisions during differentiation. (2) Selective or permissive models predict lineage choice is predominately random, which may be due to stochastic gene expression, and that external stimuli act to regulate survival and proliferation of these randomly produced progenitors and mature cells. Evidence for both models has been reported (see [48, 49] and references within, and [50, 51]). It is important to mention that these two models are not mutually exclusive, and it seems likely extrinsic events can be both instructive and selective [48, 49].

Cell intrinsic processes, in particular transcription factor networks, are central to defining the developmental stage and lineage potential, and determine the response of an external signal. External signals must act within these intrinsic parameters to instruct and/or select cell fate. Indeed, simply the regulation of cell surface receptor expression immediately determines the ability of a cell to respond to a particular extracellular ligand. Numerous intrinsic positive and negative transcriptional regulators of HSC

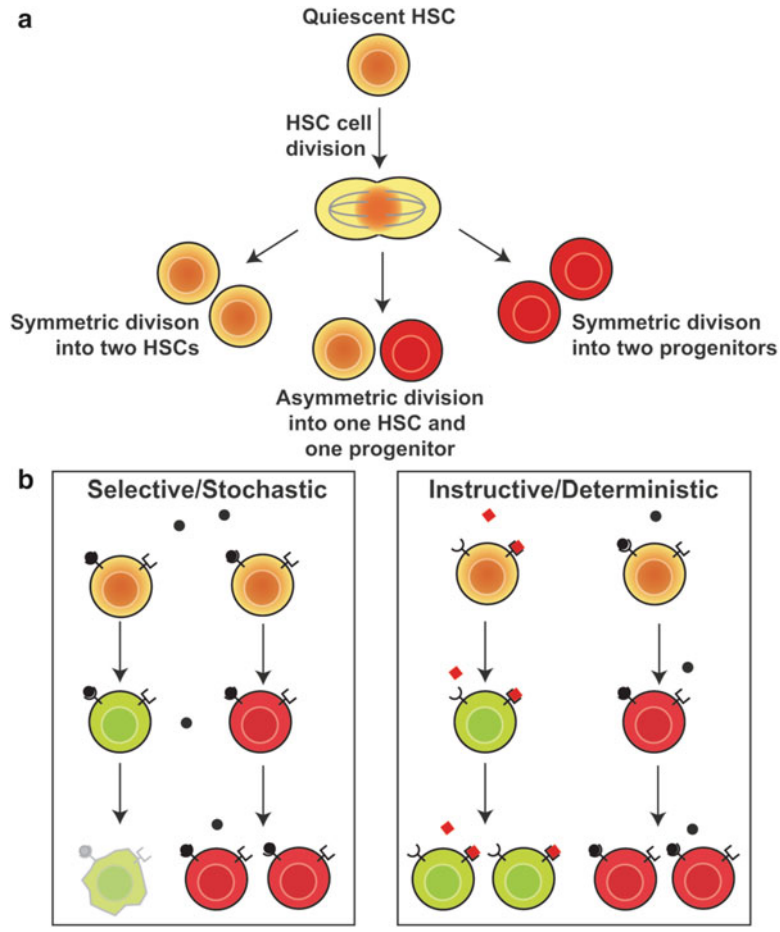

Fig. 11.2 Models of HSC fate choices. (**a**) HSCs may divide symmetrically into two HSCs or two progenitors, or asymmetrically into one HSC and one progenitor. (**b**) The two types of model of HSC fate determination. Selective/stochastic models predict HSC fate choice is random and signalling molecules (e.g. cytokines) act to promote survival and proliferation or apoptosis of the fated progenitors. Instructive/deterministic models predict signalling molecules directly determine HSC fate decisions

homeostasis have been identified, which control self-renewal, proliferation, quiescence and apoptosis, and include transcription factors, chromatin and DNA modifying enzymes, and signalling pathways, and are described below.

11.3.2 Transcription Factor Networks Active in Haematopoietic Cells

ChIP-seq experiments to define genome-wide occupancy of key transcription factors in HSCs have been limited by the relatively large cell numbers required for this technique, and the scarcity of HSCs. However, using cell line models, such as the mouse haematopoietic stem/progenitor cell line HPC7, has allowed analysis of transcription factor binding in early haematopoietic cells. So far, ChIP-seq of ten haematopoietic transcription factors has been published using this cell line, identifying combinatorial transcriptional regulation of key genes and putative *cis*-regulatory sites [33]. Combining such ChIP experiments to define transcription factor occupancy with knowledge of *cis*-regulatory elements within gene loci has allowed modelling of the

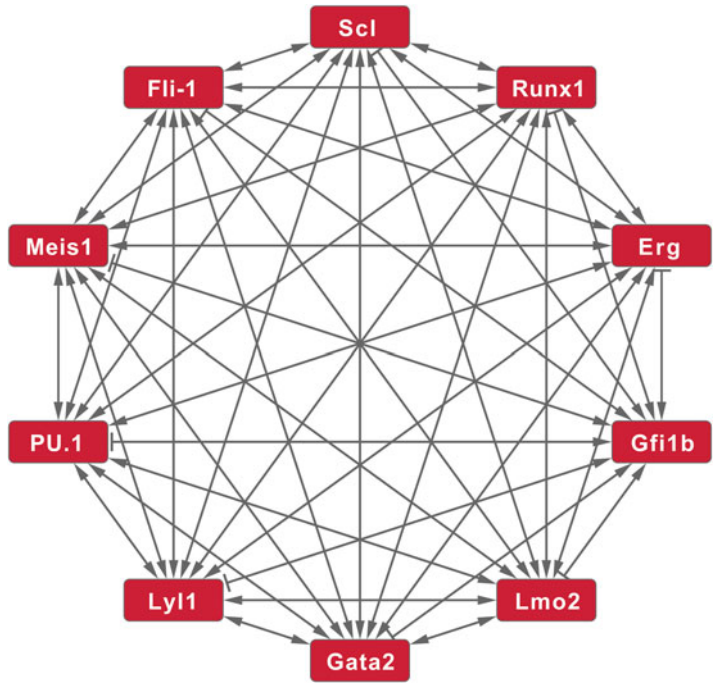

Fig. 11.3 A model of a core transcriptional regulatory network active in haematopoietic stem/progenitor cells consisting of ten transcription factors predicted from Wilson et al. [33]. Interactions identified from analysis of transcription factor enrichment from ChIP-seq experiments within gene loci at *cis*-regulatory elements. The transcription factor regulatory network is highly interconnected, rather than hierarchical in structure

interconnections within active transcription factor networks in haematopoietic cells (Fig. 11.3; reviewed in [52]). Due to the availability of mature haematopoietic cell types for ChIP-seq experiments, the regulatory networks in the later stages of haematopoiesis are more advanced. An alternate method has been used by Novershtern et al., who used gene expression analysis (which require lower cell numbers) of pure haematopoietic populations combined with known *cis*-regulatory interactions to identify tightly interconnecting networks that control HSC and mature haematopoietic cell state [53].

11.3.3 Basic Helix-Loop-Helix Transcription Factors

Scl is highly expressed in HSCs and regulates quiescence by inhibiting the G0 to G1 transition [54]. The same study also identified a dosage-dependent role for Scl in long-term HSC reconstitution potential. A paralogue of Scl, Lyl1, also regulates foetal and adult HSC reconstitution potential and lymphoid differentiation [55]. Lyl1 appears to have a partially overlapping role with Scl, as *Lyl1*/*Scl* conditional double knockout HSCs have complete loss of reconstitution ability, and increased HSC and progenitor apoptosis, a more severe phenotype than loss of Scl or Lyl1 alone [56]. Scl forms a multifactor complex with transcription factors Gata2 and E2A proteins, along with bridging molecules Lmo2 and Ldb1 in foetal and adult HSCs and differentiating haematopoietic cells. Formation of this complex is essential for regulation of HSC function, as loss of any component impairs HSC function (see below and [57, 58]). Depending on the context, the Scl complex may also include Lyl1, Gata1, Lmo4, HEB, Eto2 and Sp1 [59–62].

The E2A locus expresses two bHLH E-proteins; E47 and E12, which regulate HSC

cycling and promote early progenitor maturation [63]. Genetic deletion of E2A increases HSC cycling while reducing pool size, and HSCs lose long term reconstitution ability [63]. Recent analysis of pure HSC populations has identified a role for the E47 isoform in regulating HSC proliferation and energetics [64]; *E47*$^{-/-}$ HSCs progressively lose self-renewal potential with concomitant hyperproliferation of progenitor populations. E2A protein activity is regulated through interactions with inhibitors Id1-3; Id1 also regulates HSC homeostasis [65, 66], while Id2 and Id3 regulate haematopoietic lineage commitment [67–69].

Besides its role in definitive haematopoiesis, c-Myc also regulates HSC homeostasis, playing a crucial role in balancing HSC self-renewal versus differentiation decisions [70], as well as HSC survival and HSC lineage commitment [71, 72]. HSCs also express a second Myc protein, N-myc, which with c-Myc regulates HSC function and survival [71]. The role of Myc proteins in stem cells is considered in more detail in Chap. 19.

11.3.4 Homeobox Transcription Factors

Hox genes encode homeodomain transcription factors and are crucial for developmental patterning [73]. In mammals, 39 *Hox* genes are co-ordinately expressed from four loci. DNA binding of Hox transcription factors is modulated by interaction with DNA binding cofactors; one of three Pbx family members and/or one of four Meis family members (both families are also homeobox proteins) [74]. Several *Hox* genes have been implicated in HSC homeostasis, although deletion of a single *Hox* gene does not usually severely affect HSC homeostasis, possibly due to their functional redundancy. Within the haematopoietic system, *Hox* gene expression is largely confined to the HSC and progenitor compartment [75].

Ectopic expression of *HoxA9*, *HoxA10*, *HoxA6 HoxB4* and *HoxC5* expands HSCs in vitro [76–80]. Additionally, genetic deletion of *HoxA9* or *HoxB3* and *HoxB4* mildly impairs HSC proliferation [81, 82]. *HoxA9* null HSCs also have impaired differentiation and reduced reconstitution ability [82]. Compound deletion of *HoxA9*, *HoxB3* and *HoxB4* caused an increase in bone marrow HSCs, but did not affect in vitro colony forming ability [83]. Interestingly, the defect in reconstitution ability of compound null HSCs was no worse than single *HoxA9* deficiency [83].

Pbx1 can dimerise with a subset of Hox proteins, and can also trimerise with Hox and Meis proteins [84, 85]. Pbx1 is required to maintain definitive HSCs in the foetal liver; *Pbx1* null mice are embryonic lethal at E15-16, and have severe anaemia due to defective foetal liver haematopoiesis [86]. Conditional deletion of *Pbx1* from adult HSCs results in the upregulation of several cell cycle regulators with increased HSC cycling and progressive loss of HSC reconstitution ability [87].

11.3.5 Ets Transcription Factors

Several Ets transcription factors are known to regulate HSC homeostasis and differentiation including Erg, Fli-1, Tel/Etv6, GABPα, PU.1/Spi-1 and Elf4 [88–95]. Two of the most recently reported Ets factors are described below.

A role for Erg in adult HSC function was identified using a sensitised genetic screen in mice [88]. Erg is required to maintain HSC pool size and reconstitution ability, and differentiation to committed progenitors [88, 89]. Furthermore, additional mutation of *Fli-1* in *Erg*-deficient HSCs identified a partial functional redundancy of these two Ets factors, with the double deficiency causing a more severe phenotype [90]. Yu et al. recently identified GABPα to be a critical regulator of HSC homeostasis and differentiation [91]. GABPα heterodimerises with GABPβ to form the GABP complex and is essential for early embryogenesis [96]. Conditional deletion of GABPα in adult HSCs lead to a rapid loss of HSC self-renewal, increased apoptosis of HSCs and progenitors, and impaired differentiation [91].

11.3.6 Zinc Finger Transcription Factors

Zinc fingers are a protein domain that commonly interacts with DNA and are found in a wide range

of transcription factors, several of which are known to regulate HSC homeostasis including Gata2, Gata3, Gfi1, Gfi1b, Klf4, Ikaros, Evi-1, Sall4, Zfx and Prdm16 [97–108]. Interestingly, several of these zinc finger transcription factors also regulate ES cell self-renewal and pluripotency (Klf4, Sall4 and Zfx [100, 109, 110]).

Analysis of *Gata2* heterozygous mouse embryos identified a role for Gata2 in expansion of definitive HSCs in the AGM and their proliferation after foetal colonisation [111]. However, Gata2 is generally thought to restrict cell cycle entry in adult HSCs (reviewed elsewhere [102]). Additionally, GATA2 also regulates human HSC quiescence, with enforced expression increasing G0 residency [112]. More recently, a second Gata factor, Gata3, has also been identified as a regulator of HSC maintenance, with *Gata3* null mice having a smaller HSC population [101].

Gfi1 is a transcriptional repressor that promotes HSC quiescence, and maintains HSC self-renewal and reconstitution potential [103, 104]. Additionally, Gfi1 appears to be a direct target of p53 in HSCs, a key cell cycle regulator. The paralogue Gfi1b is also responsible for maintaining HSC quiescence, although *Gfi1b*$^{-/-}$ HSCs retain self-renewal capacity [105]. Gfi1 and Gfi1b appear to have partially overlapping functions as *Gfi1*/*Gfi1b* double deletion result in complete loss of HSCs [105].

Ikaros and related family of transcription factors were initially identified as regulators of lymphoid lineages (reviewed in [113]). However, Ikaros is also expressed in HSCs, although different isoforms to those expressed in lymphoid progenitors [114], and plays a role in HSC activity. Ikaros mutant mice have reduced numbers of HSCs and progenitors, and have reduced reconstitution ability [115]. More recently, a role for Ikaros in lymphoid lineage priming of HSCs has been identified [106].

Evi-1 contains a SET/PR-domain with a total of ten zinc fingers [116]. Deletions of *Evi-1* results in embryonic lethality at E10-16.5 (depending on the mouse model), and the development and expansion of definitive HSCs is severely impaired [107, 108], and reviewed in [116]. Conditional deletion of *Evi-1* from adult HSCs causes a shift from quiescence to cell cycling, reduction of the HSC pool size and loss of reconstitution ability [108]. Evi-1 expression has also been used as a marker of long term haematopoietic reconstitution potential [117]. Interestingly, dosage of Evi-1 appears important as *Evi-1* heterozygosity causes partial loss of HSC self-renewal while overexpression enhances HSC self-renewal at the expense of differentiation [107]. However, *Evi-1* is dispensable for lineage commitment [107].

A second SET/PR-domain protein, Prdm16, is also important for HSC homeostasis, and specifically expressed in HSCs and early progenitors in the adult haematopoietic system [118]. Overexpression of *Prdm16* has previously been found to expand HSCs in vitro, and also causes myeloproliferative disease in vivo after transplantation [119]. A transposon mutagenesis screen identified a role for *Prdm16* in regulation of adult stem cell reactive oxygen species (ROS) levels, apoptosis and cell cycle, and its loss lead to HSC depletion [120]. Aguilo et al. identified a role of Prdm16 in HSCs using *Prdm16* null mice embryos [118]; foetal HSC and progenitors were reduced in number, had mild defects in vitro colony forming ability, severely impaired reconstitution ability, and increased apoptosis.

11.3.7 Myb Proteins

C-Myb plays an important role in HSC self-renewal and adult haematopoiesis; its conditional deletion causing a defect in HSC proliferation, increased differentiation, and loss of reconstitution ability [121]. A genome-wide mutagenesis screen identified the ability of p300 to interact with the transactivation domain of c-Myb to be necessary for proper HSC proliferation and differentiation [122]. The role of c-Myb in stem cells is discussed in further detail in Chap. 15.

The cyclin-D binding myb-like transcription factor 1 (Dmtf1) has been implicated in regulating HSC quiescence [123]. *Dmtf1* null mice are viable, but have increased blood counts, and *Dmtf1* null HSCs have increased proliferation, self renewal and long term reconstitution ability [123].

11.3.8 Core Binding Factors

A number of conflicting papers have been published about the role of Runx1 in HSCs [124–128]. The most recent from Cai et al., has sought to resolve the experimental discrepancies by highlighting that Runx1 regulates the expression of several key markers commonly used to identify HSCs by flow cytometry, and report that conditional deletion of *Runx1* only moderately decreases the number of HSCs, while increasing those of early progenitors [129]. Loss of *Runx1* also causes slight increases in HSC quiescence and reduces apoptosis, and combined suggest Runx1 promotes HSC proliferation and differentiation. However, the three major Runx1 isoforms (Runx1a, b and c) appear to have at least partially distinct functions in the haematopoietic system [130–132]. Ectopic expression of a short isoform of *Runx1*, *Runx1a*, expands HSCs in vitro (which retain in vivo reconstitution ability), while ectopic expression of *Runx1b* promotes differentiation [130, 132]. No functional difference between the two long isoforms, *Runx1b* and *Runx1c*, has been identified [131]. Regulation of Runx1 in haematopoietic cells is considered in more detail in Sect. 11.6.

By comparison, HSCs appear to be more sensitive to CBFβ from hypomorph experiments; 15–30 % of WT CBFβ levels promote HSC and progenitor expansion as well as mature cell differentiation [133], and suggest a role for CBFβ in HSC quiescence. Interestingly, Miller et al. suggest a Runx1-independent role for CBFβ in foetal haematopoiesis in differentiation of haematopoietic progenitors, which is not due to a defect in bone marrow niche [134]. CBFβ can also heterodimerise with the two paralogues of Runx1, Runx2 and Runx3, to form protein complexes that can bind to the same consensus DNA sequence (reviewed in [135]). Partial overlap in function of Runx1– 3 in regulating HSCs would help explain the difference in Runx1 and CBFβ phenotypes, although are yet to be identified. Defective bone marrow haematopoiesis in *Runx2* null mice has been identified, but is thought to be a result of altered HSC niche due to defective osteoblast differentiation [136, 137].

11.3.9 Cell Cycle Regulators

Unsurprisingly, cell cycle regulators have also been identified as regulating HSC homeostasis. Two of these involved in transcriptional regulation are retinoblastoma (pRB) and p53 families [138–141]. pRb, with family members p107 and p130, inhibit cell cycle entry by repressing E2F target gene expression, and have an overlapping function in regulating HSC quiescence and self-renewal [138]. Conditional deletion of all three pRB proteins causes increased HSC proliferation, expansion of HSC numbers, loss of reconstitution activity and a lethal myloproliferative phenotype [138]. However, an extrinsic role for pRB in regulating HSC has also been identified [142]. The functionally similar Necdin, also regulates HSC quiescence state, and interactions with p53 [141, 143].

A third cell cycle regulator has also been found to regulate HSCs; NF-Y, a trimeric transcription factor complex composed of NF-Ya, NF-Yb and NF-Yc [144]. NF-Y is an important developmental regulator, with genetic deletion of *NF-Ya* causing embryonic lethality around E8.5 [145]. NF-Ya overexpression promotes HSC self-renewal [146] while conditional deletion of *NF-Ya* causes defective cell cycle G2/M progression and increased apoptosis, resulting in death [147].

11.3.10 Immediate Early Response Transcription Factors

Two immediate early response transcription factors, JunB and Egr1, have been implicated in regulating HSCs. Inactivation of JunB in HSCs results in increased proliferation and differentiation [148], but does not affect reconstitution ability. Additionally, JunB inactivation decreases HSC response to Notch and TGFβ signalling through loss of *Hes1* expression [148]. *JunB* is also a target of TGFβ signalling [149]. Egr1 regulates HSC quiescence as well as retention within the HSC niche [150]. Interestingly, *Egr1* knockout HSCs expand and mobilise without losing reconstitution ability [150].

11.3.11 Epigenetic Regulation

A key mechanism by which transcription factors are thought to regulate eukaryotic gene expression is through their recruitment of epigenetic modifying enzymes, which catalyse histone or DNA modifications. Epigenetic modifications affect chromatin structure, recruit secondary factors and regulate transcription. Several epigenetic modifiers have been identified to play an important role in HSC homeostasis, summarised below.

Three histone lysine acetyltransferases and transcriptional co-activators are essential for HSC self-renewal; CBP, p300 and MOZ [151–153]. The H3K79 methyltransferase Dot1l is also crucial for maintaining HSC function [154, 155].

Both Mll1, and its cofactor Menin, are required for HSC self-renewal [156]. As described earlier, two mouse Mll1 knockout models display differing severity in phenotype, although both agree that Mll1 is necessary to maintain adult HSC self-renewal [23, 157]. Using a conditional gene knockout model, Gan et al. identified a role for Mll1 in post-natal but not foetal HSC maintenance [158]. The distantly related Mll family member, Mll5, is also involved in regulating HSC self-renewal and haematopoietic differentiation [159–161].

Multiple polycomb group (PcG) proteins, which epigenetically regulate transcriptional repression, have been found to regulate HSCs (review in [162]). PcG proteins form two discrete complexes, polycomb repressive complex 1 and 2 (PRC1 and PRC2), which have distinct enzymatic activity (H2AK119 monoubiquitination and H3K27 trimethylation activities respectively) and discrete functions in HSCs [163]. Various gene knockout models suggest PRC2 limits HSC self-renewal [162–167]. Ezh2, a core component of PRC2 is also required for maintenance of foetal HSCs [168].

Genetic deletion of PRC1 core components generally results in the loss of HSC self-renewal [169–173]. Bmi-1 is a particularly important core component, with overexpression promoting mouse and human HSC self-renewal and ex vivo expansion [170, 174]. A paralogue of Bmi-1, Mel-18, which replaces Bmi-1 to form a PRC1-like complex, promotes HSC proliferation and differentiation [175, 176]. The balance between Bmi-1 and Mel-18 expression may regulate HSC fate. A functional crosstalk between Bmi-1 and Mll1/HoxA9 has also been identified in establishing HSCs [177].

DNA methylation, generally 5′-cytosine methylation (mC) in a CpG dinucleotide context, is a key epigenetic mark and is thought to inhibit transcription. DNA methyltransferases Dmnt3a and Dmnt3b (involved in de novo DNA methylation), and Dnmt1 (involved in maintaining DNA methylation patterns) have been implicated in regulating HSC self-renewal [178–180]. Additionally, Tet2, a methylcytosine dioxygenase that converts mC to 5-hydroxymethyl-cytosine (hmC), is required for HSC homeostasis [181–183]. Chromatin remodelling complexes such as the Mi-2β containing NuRD complex are also important for maintaining HSC quiescence and self-renewal [184].

11.3.12 Signalling Pathways

Several signalling pathways have been identified to regulate HSC self-renewal and differentiation through regulating transcription. These appear to play important, although not usually essential roles in HSC homeostasis. Functional redundancy of signalling molecules within these pathways, as well as overlap and integration of different signalling pathways help explain the often conflicting phenotypes after in vitro activation, in vivo genetic deletion, depletion, inhibition, constitutive activation or overexpression of the mediators of these pathways.

The downstream signalling transcriptional regulators Notch-IC (Notch signalling) and β-catenin (Wnt signalling) have a fairly established role in HSC self-renewal and expansion (reviewed in [35, 185–187]). Signalling through Smad transcription factors (TGFβ and BMP signalling) and Gli1-3 (Hedgehog signalling) are also thought to regulate HSC

self-renewal (reviewed elsewhere [187, 188]). In contrast, retinoic acid receptor γ (retinoic acid signalling) stimulates HSC and progenitor cell differentiation [189].

Activation of the receptor tyrosine kinases c-kit (SCF receptor), c-mpl (thrombpoietin receptor) and Tie-2 (angiopoietin receptor) also regulate HSC maintenance, although through multiple signalling pathways including JAK-STAT, phosphoinositide-3 kinase (PI3K), and MAPK. JAK-STAT signalling activation but PI3K signalling inhibition appears important to maintain HSC self-renewal (reviewed in [187, 190–192]).

11.3.13 Oxidative Stress

Regulation of oxidative stress is critical for HSC homeostasis. FoxO transcription factors are regulated by PI3K signalling, and also play a critical role in HSC resistance to oxidative stress [193, 194]. *FoxO1/3/4* null HSCs have increased ROS levels, increased cell cycling and apoptosis, are reduced in number and defective in their reconstitution activity. Interestingly, anti-oxidative treatment alleviates the *FoxO*-deficient phenotype [194]. Even single deletion of *FoxO3a* results in elevated ROS in HSCs, which impairs HSC function [195]. As mentioned above, Prdm16 is also involving in regulation of adult stem cell ROS levels [120]. Additionally, proper regulation of the hypoxia-inducible factor 1 alpha (HIF-1α) is essential for HSC quiescence and reconstitution ability [196].

11.4 Transcriptional Regulation of HSC Differentiation

11.4.1 Cellular Hierarchy of Mammalian Adult Haematopoiesis

HSCs have the ability to differentiate into at least ten different specialised mature cell types with a diverse range of functions, morphologies, lifetimes and proliferative abilities, and their relative proportions are dependent on extracellular and external influences. Adult HSCs differentiate through progressively more lineage-committed stages (progenitor cells) to form mature, terminally differentiated haematopoietic cells. This lineage commitment is represented as a haematopoietic hierarchy or tree (Fig. 11.4). Numerous cell surface markers and functional assays have been used to identify and define these intermediate progenitors and mature cell types, although the complete definition of in vivo potential of the many different progenitor populations is still ongoing. This detailed understanding of HSC differentiation pathways has greatly facilitated the identification and dissection of the role of transcriptional regulators of this process. Almost all transcriptional regulators of HSC homeostasis also regulate later lineage commitment decisions. Numerous other transcriptional regulators of this process have also been identified, predominantly controlling relatively late lineage commitment decisions. We refer to a number of recent reviews of the transcriptional regulation of these later lineage commitment decisions for further detail [198–203].

11.4.2 HSC Differentiation and Lineage Specification

HSC differentiation is closely linked to proliferation, and many of the transcriptional regulators of HSC homeostasis also play a role in differentiation, and have been mentioned above. Few transcriptional regulators of the initial steps of HSC differentiation and commitment have so far been identified that do not also regulate HSC homeostasis. Here we briefly describe two example transcriptional regulators of HSC differentiation: C/EBPα and Hmgb3.

The CCAAT-enhancer binding protein alpha (C/EBPα) is required for development of granulocytes [204], but also functions in HSCs to promote differentiation. *C/EBP*α null HSCs have increased repopulating ability and self-renewal, and also display a block of early myeloid differentiation [205]. Additionally, C/EBPα determines

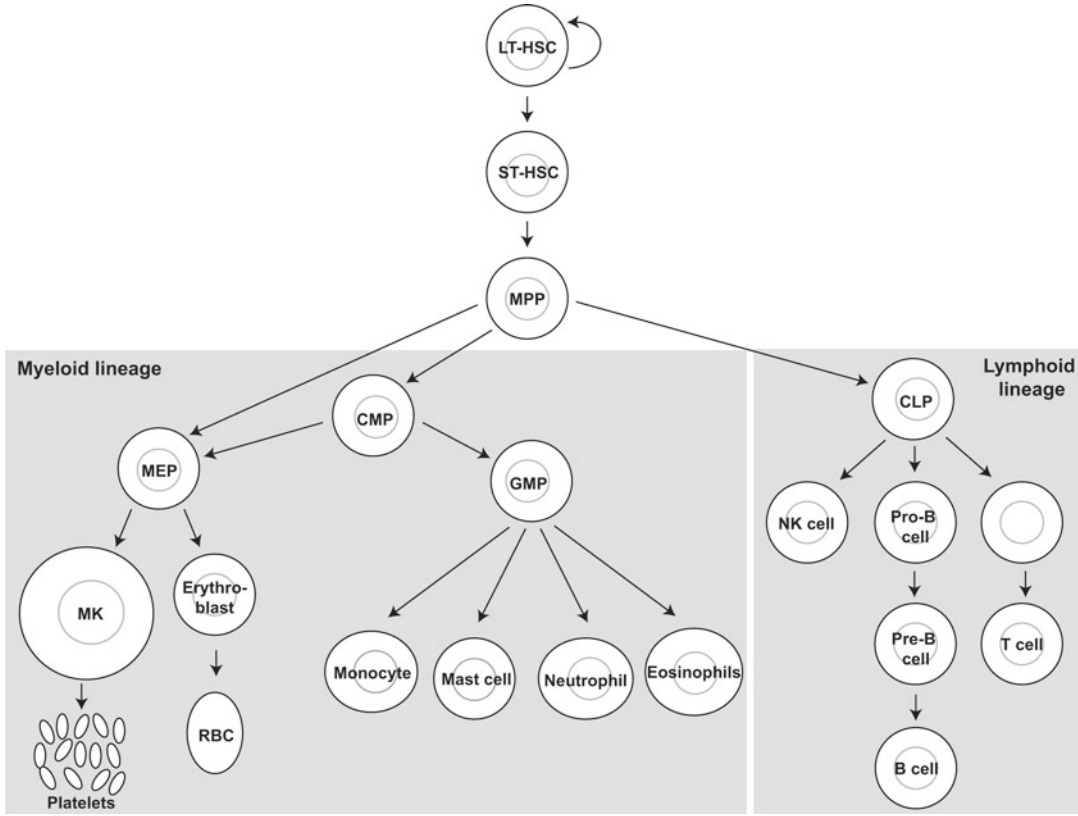

Fig. 11.4 The haematopoietic lineage tree illustrates HSC differentiation potential. HSCs differentiate through progressively more committed progenitors into at least ten mature blood cell types with diverse functions (which can be divided into myeloid and lymphoid cell types). The haematopoietic tree shows stable cell populations, which have been defined by surface marker expression, although the exact branching points and potential of progenitors are still a matter of debate (see for example [197] for further details). *LT-HSC* long-term haematopoietic stem cell, *ST-HSC* short-term haematopoietic stem cell, *MPP* multipotent progenitor, *CLP* common lymphoid progenitor, *CMP* common myeloid progenitor, *GMP* granulocyte monocyte progenitor, *MEP* myeloerythroid progenitor, *MK* megakaryocyte, *RBC* red blood cell

cell fate of multipotent progenitors, inducing myeloid differentiation while inhibiting erythroid differentiation [206].

The high mobility group binding protein B3 (Hmgb3) is a sequence-independent chromatin binding protein. Loss of *Hmgb3* does not affect HSC numbers, self-renewal or reconstitution ability, but does result in reduced CLP and CMP numbers [207, 208]. Of note, even though in vitro differentiation of *Hmgb3*-deficient CLP and CMP are unaffected, loss of *Hmgb3* appears to bias HSCs to self-renewal rather than differentiation into progenitors [207].

11.5 Regulation of HSC Transcriptional Regulator Expression

Much of our understanding of the regulation of HSCs is at the transcriptional level, and transcriptional regulation of haematopoietic transcription factors by *cis*-regulatory elements has allowed modelling of transcription factor networks. However, additional regulatory mechanisms overlay and interconnect with these networks, including alternative promoter usage and splicing, post-transcriptional and translational control

mechanisms, and post-translational regulation of protein activity and degradation. To highlight this additional complexity, we discuss the regulatory mechanisms known to control expression and activity of a single transcription factor, Runx1, within the haematopoietic system.

11.5.1 Transcriptional and Co-transcriptional Regulation

Runx1 is expressed from two promoters, a distal P1 and proximal P2, which play nonredudant roles in definitive haematopoiesis, with the P2 being critically required [209]. Different transcription factor binding at the *Runx1* promoters confers specificity of promoter activity, and explain differential promoter activity during developmental haematopoiesis [131]. Haematopoietic expression is also regulated by the activity of the *Runx1* +23 enhancer [21]. As mentioned above, three major isoforms of Runx1 appear to have partially distinct functions [130]. However, over 12 differentially spliced *Runx1* cDNAs have so far been identified, which may play additional roles in the haematopoietic system [209, 210].

11.5.2 Post-transcriptional and Translational Regulation

MicroRNAs (miRNAs) are a class of small ncRNA that play a critical role in regulating gene expression (see Chap. 18 for further details). Ben-Ami et al. identified five miRNAs with the ability to bind the *Runx1* 3′UTR and inhibit expression [211]. Alternative splicing determines the length of the 3′UTR, and therefore the ability of these miRNAs to bind and interfere with translation of Runx1. Ben-Ami et al. went onto describe a feedback loop active during megakaryocytic differentiation (of a myeloid cell line) between Runx1 and miR-27a [211]. MiR-27 has also been identified as inhibiting *Runx1* expression during granulocyte development [212].

Runx1 promoter activity determines the 5′UTR transcribed and site of translational initiation. Transcripts from the distal P1 promoter are translated by a Cap-dependent mechanism, while transcripts from the proximal P2 promoter are translated from an internal ribosome entry site (IRES) [213]. Regulation of these two translational start sites by different mechanisms adds an additional level of control to Runx1 expression. Interestingly, several studies suggest miRNAs do not inhibit translation from IRES [214, 215], and could represent a further mechanism by which expression of alternative isoforms is differentially regulated.

11.5.3 Post-translational Modification by Phosphorylation, Acetylation and Methylation

Post-translational modification of proteins by phosphorylation, acetylation and methylation are common mechanisms to regulate protein activity through modulating tertiary structure and protein-protein or protein-DNA interactions. Runx1 is phosphorylated by cyclin-dependent kinases (CDKs) in a cell cycle-specific manner, which regulates Runx1 activity, protein-protein interactions, stability and degradation [216–218]. Runx1-DNA binding stability is also regulated by transcriptional co-activator p300- and MOZ-mediated lysine acetylation [219, 220]. Runx1 methylation has also been reported to alter its activity and transcriptional co-activator interactions [221, 222]. Post-translational modification also appears important for the ability of transcriptional regulator fusion proteins to drive leukaemias; lysine acetylation of RUNX1-ETO is necessary for its ability to mediate leukaemogenesis [223].

11.5.4 Regulation of Runx1 Activity by Smad6

Besides protein-protein interactions with transcriptional co-activators and co-repressors that regulate Runx1 activity, Runx1 is also regulated by interaction with Smad6, a downstream regulator of the BMP and TGFβ signalling pathways. Smad6 regulates Runx1 (as well as Runx2) activity by acting as an adaptor, mediating ubiquitination of Runx1 by Smurf2 (an E3 ubiquitin ligase),

which results in proteosomal degradation [224, 225]. A novel self-regulatory mechanism has recently been identified by Knezevic et al., whereby Runx1 controls its own expression during definitive haematopoiesis through regulation of *Smad6* expression, an inhibitor of Runx1 activity [226]. Three key *Runx1* expression regulators Scl, Gata2 and Fli-1 also regulate *Smad6* expression, in combination with Runx1 [226]. Runx1 activity therefore determines *Smad6* expression, which in turn regulates Runx1 activity, and acts to maintain steady Runx1 activity during this process [226].

11.6 Transcriptional Regulation in Leukaemogenesis

11.6.1 Mutation, Translocation and Aberrant Expression of Haematopoietic Transcriptional Regulators

Haematological malignancies are a heterogenous group of diseases, genotypically and phenotypically, and include leukaemias and lymphomas. Chromosomal translocations that produce gene fusions are particularly common in haematological malignancies, with over 264 different gene fusions identified so far [227]. Mutation, translocation, or aberrant expression of many transcriptional regulators discussed above is associated with haematological malignancies, in particular leukaemias (reviewed elsewhere [197, 227, 228]). A large number were in fact originally identified from cytogenetic analysis of chromosomal abnormalities in leukaemias. The molecular pathogenesis of translocations of the haematopoietic transcriptional regulator, MLL1, one the best understood examples, is discussed below.

11.6.2 *MLL1* Translocations and Fusion Proteins

Chromosomal translocations involving *MLL1* account for approximately 10 % of all leukaemias and cause a variety of phenotypes (from which MLL1 gets its name; Mixed Lineage Leukaemia 1). Over 60 different in-frame gene fusion partners of *MLL1* have been identified as well as *MLL1* partial duplication events [229, 230]. However, over 90 % of cases are accounted for by gene fusion with *AF4*, *AF9*, *ELL*, *ENL*, *AF6* or *AF10* [231]. Expression from the *MLL1* promoter after translocation produces an MLL fusion protein consisting of the N-terminus of MLL1 and the C-terminus of the fusion partner, which does not contain H3K4 methyltransferase activity [230]. An increasing understanding of the molecular mechanisms by which MLL fusion proteins initiate and maintain leukaemias has helped developed targeted therapies.

MLL fusion proteins appear to "hijack" normal transcriptional regulators to mediate leukaemogenesis. Continued expression of the MLL fusion protein is required to maintain leukaemic growth [232], and MLL-AF9 also requires expression of wild-type MLL1 to initiate and maintain leukaemia [233]. The MLL1 cofactor Menin is also required for maintenance of *MLL1* leukaemias [234], and Menin-MLL inhibitors have recently been found to ablate leukaemogenic activity of MLL fusion proteins [235]. Additionally, a PcG protein Cbx8 (and PRC1 component) has recently been found to be necessary for initiation and maintenance of MLL-AF9 leukaemias, suggesting cooperation between PcG and MLL fusion proteins in leukaemogenesis [236].

Four of the most common fusion partners of MLL1 (AF4, AF9, ENL and ELL), along with the transcriptional coactivator pTEFb, the polymerase associated factor 1 complex, the H3K79 methyltransferase DOT1L, and the BET family protein BRD4 are thought to form large molecular complexes with MLL fusion proteins at target genes [237–241]. These data, combined with reports that H3K79 methylation profiles define multiple MLL fusion protein leukaemias [242], led to the design of a DOT1L inhibitor, which was recently reported to selectively kill MLL1 leukaemias [243]. BET inhibitors prevent BET proteins (including BRD4) from binding to acetylated histones. BET inhibitors are thought to destabilise MLL fusion protein complexes at

target genes, and have also recently been reported to be an effective treatment of *MLL1* leukaemias, inducing downregulation of *MYC*, cell cycle arrest and apoptosis [244].

The reports summarised above highlight the notion of how a molecular understanding of the transcriptional dysregulation that occurs in leukaemias can facilitate the design of effective targeted therapies. Furthermore, they suggest MLL fusion proteins may mediate leukaemogenesis through a common molecular mechanism involving inappropriate recruitment of transcriptional elongation promoting factors to MLL target genes. MLL fusion proteins are thought to target a subset of wild-type MLL1 targets, their aberrant expression promoting cellular proliferation and survival [245]. Perhaps unsurprisingly, several key MLL fusion protein targets are transcriptional regulators of HSC self-renewal: Hox genes (in particular *HOXA9*, *HOXA10*), *MEIS1*, *EVI-1*, *MYC* and *MYB*, which contribute to *MLL1* leukaemogenesis [244, 246–249].

However, a comparison of two ChIP-seq data sets of genome-wide MLL fusion protein occupancy (MLL-AF4 and MLL-AF9) identified few common gene targets [250]. This suggests that although MLL fusion proteins may act by a common mechanism to dysregulate transcription of target genes, many of these target genes are likely to be unique to the particular *MLL1* leukaemia, and may depend on cell of origin, MLL fusion partner, and/or additional mutations present. This may help to explain the heterogeneity in cellular phenotype and pathology of *MLL1* leukaemias.

11.7 Conclusions

Over the last 30 years, transcription factors have been identified as key regulators of every stage of normal and malignant haematopoiesis. However, most work to date has involved focusing on the role of single transcription factors within this system. However, it is becoming increasingly clear that transcription factors act within large regulatory networks, often functionally and physically interacting. Further work is needed to synthesise all this information, as well as integrating the additional layers of regulation acting on these transcription factors, into a wider, coherent network model.

Besides serving as a model of mammalian development, the overall aim of such research is its application to clinical problems, such as production or expansion of HSCs for bone marrow transplantation and mature blood cell types for transfusion medicine, as well as rational design of treatments of HSC-associated diseases, such as leukaemias. As mentioned in Sect. 11.7, several small molecule inhibitors have recently been identified as potential revolutionary treatments of *MLL1* leukaemias. However, our understanding of the leukaemogenic mechanisms of many other fusion proteins is less well advanced. Recent cancer genome sequencing projects are discovering ever more transcriptional regulators as candidate leukaemic oncogenes and/or tumour suppressors [251, 252]. However, further work is required to confirm their role and determine their function in driving leukaemia, as well as in normal haematopoiesis.

Mouse models have provided powerful tools to investigate the transcriptional regulation of HSCs, and in many ways account for our greater understanding of mouse HSCs over human HSCs. However, an over-reliance on mouse experiments must be avoided if research is to be successfully translated into clinical application. Although the roles of many transcriptional regulators of HSCs are likely conserved, differences in the basic biology of mice and humans (such as life expectancy) as well as those specific to HSCs will limit translation of knowledge. For example, HoxB4 is a potent regulator of mouse HSC expansion [78], but has very limited ability to expand human HSCs [253]. Additionally, current isolation protocols for human HSCs provide less pure cell populations than mouse HSCs. Further characterisation and dissection of human HSCs will therefore be important in the future.

In summary, transcriptional regulation of HSCs is a mature area of research that is continuing at an exciting pace, and one which holds real promise for further clinical application in the near future.

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