The haematopoietic system is established during embryonic life through a series of developmental steps that culminates with the generation of haematopoietic stem cells. Characterisation of the transcriptional network that regulates blood cell emergence has led to the identification of transcription factors essential for this process. Among the many factors wired within this complex regulatory network, ETV2, SCL and RUNX1 are the central components. All three factors are absolutely required for blood cell generation, each one controlling a precise step of specification from the mesoderm germ layer to fully functional blood progenitors. Insight into the transcriptional control of blood cell emergence has been used for devising protocols to generate blood cells de novo, either through reprogramming of somatic cells or through forward programming of pluripotent stem cells. Interestingly, the physiological process of blood cell generation and its laboratory-engineered counterpart have very little in common.

Keywords: embryonic haematopoiesis; haemogenic endothelium; transcription network

In adult organisms, the haematopoietic system is sustained throughout life by a pool of haematopoietic stem cells (HSCs) that resides in the complex microenvironment of the bone marrow [1]. This pool of HSCs maintains itself through self-renewal; it is not clear, however, whether HSCs are slowly cycling, or mostly quiescent with only a subset of cycling HSCs [2]. Under homeostatic conditions, HSCs give rise to all blood cells of the erythroid, myeloid and lymphoid lineages through a discreet series of differentiation steps leading to fully mature blood cells. This equilibrium between self-renewal and differentiation is carefully controlled by the bone marrow microenvironment and is critical to the sustained production of all blood cells. Any alteration in this balancing act such as in certain haematological disorders leads to bone marrow failure, a condition in which HSCs become exhausted [3]. Left untreated, this is unfortunately a terminal illness as there is no de novo generation of HSCs in adult organisms. HSCs are only generated de novo during embryonic development when the haematopoietic system is first established.

Embryonic emergence of the haematopoietic system

During embryonic development, the haematopoietic system emerges in sequential waves, each characterised by its specific timing, location and type of progenitors generated. Soon after gastrulation, mesoderm
progenitors within the primitive streak migrate to the developing yolk sac to form mesodermal masses that, by E7.5 in the mouse embryo, form blood islands composed of primitive erythrocytes surrounded by endothelial cells [4]. This first wave of haematopoiesis also gives rise to megakaryocytes [5], macrophages and tissue-resident macrophages such as microglia of the brain [6]. This first wave is closely followed by a second wave of precursor emergence within the yolk sac vasculature of E8.5 mouse embryos. At this stage, erythro-myeloid progenitors are produced which, upon maturation, generate definitive erythrocytes, and all types of myeloid cells [7]. The generation of lymphoid progenitors shortly follows and occurs both within the yolk sac and the embryo proper by E9.0–9.5 [8,9]. The first HSCs, capable of adult engraftment, are only detected by E10.5, emerging from the major arteries of the developing embryo [10,11]. HSCs are found in the yolk sac and placenta later on, but it is still not clear whether they arise autonomously within those sites or if they are transported there from their site of emergence via the circulation [12,13]. Newly formed HSCs migrate to the liver where considerable expansion takes place [14]; from E14.5 onwards, HSCs start colonising the spleen, and ultimately the bone marrow, where they will reside thereafter [15].

**Endothelial origin of all blood cells**

Seminal observations dating back from the early 19th century suggested a very close lineage relationship between endothelium and blood cells during embryonic development, coining terms such as haematoblast [16], haemocytoblast [17] or haemangioblast [18]. The endothelial origin of blood cells was formally demonstrated decades later with the advance of experimental approaches allowing cellular marking [19] and lineage tracing [20]. All blood cells are derived from FLK1-expressing mesoderm [21] through endothelium intermediates; whether these FLK1 mesoderm progenitors can be termed haemangioblast remains a matter of debate discussed elsewhere [22]. Endothelium giving rise to blood cells are defined as haemogenic endothelium (HE) and are found at all sites of blood cell emergence. Through a process of endothelium-to-haematopoietic transition (EHT), HE subsets were shown to generate primitive erythrocytes [23], erythro-myeloid progenitors [24], B lymphocytes [9] and HSCs [25]. This EHT process is akin to the well-characterised epithelial to mesenchyme transition and entails a differentiation process involving dramatic morphological and transcriptional changes. In the literature, the definition of HE is often associated with the potential to generate both endothelial and haematopoietic cells. However, the current lack of specific markers hinders the distinction between HE and non-HE. Thus, at present, it is not possible to determine, and therefore to claim, that HE generates endothelium. Rather, HE can only be identified retrospectively, once it has produced blood cells.

**Transcriptional control of mesoderm specification to endothelium and haemogenic endothelium**

**ETV2**

Once mesoderm is formed, the first known transcription factor regulating further specification towards haematopoiesis is the ETS family member ETV2. This ETS transcription factor is expressed between embryonic day E6.5 and E9.5 in the mouse embryo, with an expression pattern primarily restricted to the yolk sac, where its expression marks all nascent endothelium [26]. Remarkably, ETV2 deficiency leads to a complete absence of all blood cells and organised vasculature [27]. However, the conditional deletion of ETV2 in FLK1-expressing cells [28] or TIE2-expressing cells [29] does not affect blood cell emergence or vasculature organisation. This suggests that ETV2 acts as a temporal switch for these lineages, during early embryonic development, at the onset of FLK1 expression. Analysis of the downstream targets of ETV2 implicated in these developmental processes established this transcription factor as a master regulator of both blood and endothelium programs (Fig. 1), regulating the expression of genes such as Sox7, Sel or Gata2 [30–32]. Using embryonic stem cells (ESCs) in vitro differentiation to study haematopoietic specification, Wareing et al. observed that the expression of SCL was sufficient to fully restore blood cell emergence in Etv2<sup>−/−</sup> cells [28], demonstrating the unique role of ETV2 in switching on the haematopoietic program via SCL; similar observations were made using the Zebrafish model system [33].

It is interesting to note that the function of ETV2 is not fully conserved across evolution: the Etsrp/ER71 Zebrafish homolog is required for vascular development and myeloid lineages but is dispensable for erythroid lineages [34] while the ER71 Xenopus homolog is only required for vascular development and does not control blood cell emergence [35]. The work of Liu and colleagues suggests that FLI1, another member of the ETS family, orchestrates the earliest stage of mesoderm specification to the cardiovascular system in Xenopus and Zebrafish and that ETV2 acts...
downstream of FLI1 in these model organisms [36]. In an in vitro mouse ESC model of blood cell emergence, the expression of FLI1 in ETV2−/− FLK1 mesoderm was, however, unable to restore haematopoiesis [28], demonstrating that in the mouse system the function of these two ETS factors is not interchangeable. The reason behind the progressive changes in ETV2 and FLI1 functions during evolution remains to be determined.

**SCL**

As discussed above, directly downstream of ETV2, the basic Helix-loop-helix transcription factor SCL (also known as TAL1) controls blood cell emergence (Fig. 1). SCL was originally identified at breakpoint of chromosomal translocations associated with the occurrence of leukaemias [37,38] and was later shown, through knockout approaches, to be indispensable for the generation of all blood cells in vivo [39,40] and in vitro [41,42]. In Scl−/− embryos, vascular development is not as dramatically affected as observed in Ev2−/− embryos, but SCL deficiency does lead to remodelling defects in the yolk sac vascular network [43,44]. Similar to ETV2, the expression of SCL is only temporally required for blood cell emergence as its conditional deletion in TIE2-expressing cells does not affect blood cell emergence [45]. In line with this restricted temporal requirement, in vitro studies have demonstrated the critical role of SCL for the formation of haemangioblast [46] and HE [47]. As discussed elsewhere, SCL is also an important player in adult haematopoiesis and erythropoiesis [48].

SCL functions within multi-factor complexes containing LMO2, E2A, LDB1, FLI1 and GATA2 [49,50]. The genome-wide analysis of SCL targets via chromatin immunoprecipitation has revealed a large number of downstream transcriptional targets, including many genes with known implication in both endothelial and haematopoietic cell fate, including many transcription factors such as Sox7, Sox17, Gata1, Gata2, Erg, Flil or Myb [51–53]. Among those, Runx1 has been recurrently identified as a critical downstream transcriptional target of SCL at the onset of blood cell emergence [54–56].

### Cardiac versus haematopoietic fate

Both ETV2 and SCL are critical transcriptional activators of the haematopoietic and endothelial programs, and while they are actively promoting these cell fates, experimental evidence suggests that they are also actively repressing the cardiomyocyte fate (Fig. 1). In an ESCs differentiation system promoting cardiac, endothelial and haematopoietic lineage specification, the enforced expression of ETV2 was shown to inhibit the specification of cardiac mesoderm, which gives rise to cardiomyocytes and smooth muscle cells [57]. This was shown to occur via inhibition of the Wnt signalling pathway. Similar findings were also observed in Zebrafish embryos, in which ETV2 deficiency led to increase formation of cardiomyocytes [58]. Additional studies further established that the transcriptional inhibition of ETV2 expression was a necessary step for cardiac specification [59]. In a similar line of investigation, several studies demonstrated the repressive role of SCL on cardiomyocyte specification through enhancer competitive occupancy [53,60] and recruitment of Polycomb repressive complexes [52] to cardiomyocyte specific genes. It is not clear if both mechanisms of
cardiomyocyte lineage repression function in concert or whether they are each active at different stages of the process. Single-cell transcriptomic analysis of Scf−/− early embryos suggest that the primary role of SCL is blood specification and that cardiomyocyte specification might be a later event in cells unable to give rise to blood due to SCL deficiency [61]. Together, these findings suggest complex and stepwise mechanisms in which cell-fate specification to endothelium, blood and cardiomyocytes are controlled by positive and negative transcriptional inputs. How ETV2 and SCL each contribute to the repression of cardiomyocyte fate and whether they act in a cascade of events or merely in reinforcing each other activity still remains to be established.

Transcriptional control of endothelium-to-haematopoietic transition

While the generation of HE depends on the program initiated by the ETV2/SCL transcriptional cascade, blood cell emergence from HE is controlled by RUNX1 [47,62]. Similar to Scl, Runx1 was initially identified at breakpoints of chromosomal translocations in haematopoietic malignancies [63]. Through knockout approaches, RUNX1 was shown to control definitive haematopoiesis emergence (Fig. 1), its deletion only sparing primitive erythrocytes [64–66]. To date, it is not known whether tissue-resident macrophages of the brain, emerging alongside primitive erythrocytes, depend or not on RUNX1 for their formation. The conditional deletion of Runx1 at specific stages of development further refined our understanding of its requirement for blood cell emergence. RUNX1 function was shown to be essential in VE-cadherin or TIE2-expressing endothelium but no longer required in Vav-expressing newly formed blood cells [62,67], pinpointing RUNX1 specific and critical role at the HE stage. Similar to SCL, RUNX1 is also required at later stages of haematopoietic commitment to specific lineages, such as megakaryocytes or T and B cells [68].

The Runx1 locus encodes several isoforms (Runx1a, b and c) that are differentially expressed from alternative promoters during embryonic development and in adult haematopoiesis (Fig. 2) [69–72]. The proximal promoter P2 controls the transcription of Runx1a and Runx1b while the distal promoter P1 drives Runx1c expression. At the protein level, RUNX1b and RUNX1c only differ by a few amino acids in the N-terminal region; the functionality of this difference remains unclear. Runx1a encodes a truncated version of RUNX1b and may act as an inhibitor of the two other isoforms [73], but little is known about the role of Runx1a. Upon enforced expression, this isoform was shown to enhance haematopoiesis [74–76]; additionally, Runx1a was shown to be overexpressed in haematological malignancies [77,78]. In the mouse embryo, Runx1b is the first isoform expressed in HE prior to the endothelium-to-haematopoietic transition; Runx1c becomes expressed in newly formed blood progenitors [79]. As mentioned above, Runx1 complete knockout blocks the emergence of all definitive blood cells; in contrast, the specific deletion of the Runx1c isoform only marginally affects haematopoiesis [69,72,79]. Due to the locus structure (Fig. 2), it is not feasible to delete only the Runx1b isoform to test its specific requirement in blood cell generation; however, both Runx1b and Runx1c isoforms are largely functionally interchangeable, as they both equally restore the defects observed in Runx1 complete knockout embryos [80,81]. It is thought that the timing and expression level of Runx1b driven by the proximal promoter are the most critical parameters determining its essential role during endothelial-to-haematopoietic transition [71,82].

RUNX1 transcriptional activity is mediated by a close interaction with its co-factor CBFB, which confers increased DNA binding affinity and enhances RUNX1 stability by preventing degradation [83–85]. Transcriptional targets of RUNX1 at the onset of blood specification have been determined using genome-wide approaches both in vitro [51,86,87] and in vivo [88]. At the HE stage, RUNX1 was shown to regulate the expression of genes involved in adhesion and migration, suggesting a unique role of RUNX1 in positioning of the HE population within the vascular compartment [87]. Upon endothelial-to-haematopoietic transition, RUNX1 was shown to activate the blood program, in collaboration with other transcriptional players such as SCL, GATA2 or FLI1. Genome-wide analysis of the dynamics of transcription factor binding on target genes revealed that an important function of RUNX1 is to reshape the epigenetic landscape through the assembly of haematopoiesis-specific binding patterns [89].

Among the many transcriptional targets of RUNX1 identified at this stage of blood cell development, the transcriptional repressors GFI1 and GFI1B hold a special function [86,90]. Through gain-and-loss of function approaches both in vitro and in vivo, it was shown that GFI1 and GFI1B actively downregulate the endothelial program in HE (Fig. 1). Through the recruitment of the chromatin-modifying protein LSD1, a member of the CoREST repressive complex, GFI1/
GF1B epigenetically silences the endothelial program, allowing the emergence of blood cells. In effect, RUNX1 has a dual activity in repressing the endothelial program while promoting the haematopoietic program. This dual function is highly reminiscent of the functions of ETV2 and SCL in the repression of cardiac specification and concomitant promotion of endothelial and HE programs.

**Endothelial and haematopoietic programs: a balancing act**

Specification of the vascular system which entails vasculogenesis and angiogenesis is controlled by key transcription factors of the SOXF, ETS and FOX families [91,92]. Among the SOXF factors, both SOX7 and SOX17 have been implicated in the regulation of HE and early haematopoietic specification. SOX17 was shown to mark HE [93] as well as newly formed HSCs [94] and, upon enforced expression, to maintain HSCs undifferentiated over several passages in vitro [95]. Similarly, SOX7 was shown to mark HE and upon enforced expression to block the transition from endothelium-to-haematopoiesis and to promote HE proliferation [96,97]. Further insight into the molecular mechanism underlying this block revealed that SOX7 physically interacts with RUNX1 in HE and prevents RUNX1 from switching on the blood program and switching off the endothelium program [98]. This is thought to create a meta-stable HE state, poised to give rise to haematopoietic cells upon disruption of the SOX7/RUNX1 interaction. Which signalling pathways or factors disrupt this equilibrium remains to be investigated. Interestingly, SOX17 was also shown to impact RUNX1 activity by actively repressing Runx1 transcription [99].

From all the studies of blood cell emergence from HE, it is clear that the tight control of RUNX1 activity is one of the most critical parameters. Two recent studies have explored the plasticity of endothelium and its capacity at generating blood cells [100,101]. In both studies, it was shown that during embryonic development, there is a short window of time in which ectopic RUNX1 expression will promote blood cell emergence from non-HE. Together, these studies suggest that haemogenic competency in endothelial progenitors must be restrained through the active silencing of Runx1 expression.

**Reprogramming and forward programming for blood cell generation**

Understanding the transcriptional network that leads to the formation of haematopoietic stem and progenitor cells (HSPCs) should provide the critical knowledge required for generating these cells via reprogramming or forward programming. It is thought that mastering the de novo generation of HSPCs from unlimited cell sources (somatic or pluripotent) will provide tailor-made cell populations usable in the clinic to cure a large range of haematological and autoimmune diseases [102,103]. Many studies have explored the reprogramming of somatic cells to blood cells using a variety of transcription factors (Table 1) [104–112]. Similarly, forcing pluripotent stem cells to adopt a HSC or in vivo engrafting blood progenitor identity upon differentiation has been achieved via forward programming mediated by transcription factors (Table 2) [108,113–116]. In most studies, the experimental approaches were very similar with lentiviral or retroviral vectors used for expressing potential reprogramming factors in the cell populations of choice. The factors conferring reprogramming were identified from an initial pool of selected transcription factors typically involved in HSC specification or self-renewal. When large panels of factors were tested, factors
effective at reprogramming were identified via selective functional assays in vivo or in vitro. In most studies, the requirement of each identified factor was further determined by individual removal from the reprogramming pool. While all studies reported the generation of blood progenitors as measured by clonogenic replating assays, the generation of cells with long-term in vivo engraftment was by far not as successful. While all the studies reported in Tables 1 and 2 describe the conversion of somatic cells into blood progenitors, the further characterisation of the reprogrammed blood progenitors and their biological output varied quite widely. The study by Pulecio et al. [107] described the reprogramming of fibroblasts to unipotent monocyte-like progenitors. The study performed by Pereira et al. [105] showed the generation of blood progenitors with myeloid-restricted potential. In their study, Szabo et al. [104] reprogrammed fibroblasts to progenitors endowed with myeloid and erythroid potential in vitro but in vivo engraftment was mostly limited to myeloid and no

| Reprogrammed population | Reprogramming factors | Expression system | Species | In vitro clonogenicity | Long-term engraftment | References |
|-------------------------|-----------------------|-------------------|---------|------------------------|-----------------------|-----------|
| Fibroblasts             | OCT4                  | Lentivirus        | Human   | Yes                    | Limitedd              | Szabo et al., 2010 [104] |
| Fibroblasts             | Gata2, Gh1b, cFos, Etv6| LentivirusRetrovirus | Mouse   | Limitedb               | No                    | Pereira et al., 2013 [105] |
| Fibroblasts             | Erg, Gata2, Lmo2, Runx1c, Scl | Lentivirus | Mouse   | Yes                    | No                    | Batta et al., 2014 [106] |
| Fibroblasts             | SOX2, miR125b         | LentivirusRetrovirus | Human   | Limitedc               | Not tested           | Pulecio et al., 2014 [107] |
| Fibroblasts             | Scl, Lmo2,Gata2, Ptx2, Sox7,MyCN | TransposonPiggy Bac | Mouse   | Not tested             | No                    | Vereide et al., 2014 [108] |
| Committed blood progenitors | Run111, Hlf, Lmo2, Prdm6, Pbx1, Zfp37, Mycn, Meis1 | Lentivirus | Mouse   | Yes                    | Yes                   | Riddel et al., 2014 [109] |
| Endothelial cells       | FOSB, GFI1, RUNX1, SPI1 | Lentivirus | Human   | Yes                    | Yes                   | Sandler et al., 2014 [110] |
| Fibroblasts             | Scl, Lmo2, Runx1, Bmi1 | LentivirusRetrovirus | Mouse   | Yes                    | No                    | Cheng et al., 2016 [111] |
| Endothelial cells       | Fosb, Gfi1, Runx1, Spi1 | Lentivirus | Mouse   | Yes                    | Yes                   | Lis et al., 2017 [112] |

| Reference | |
|-----------|-----------|
| Szabo et al., 2010 [104] | |
| Pereira et al., 2013 [105] | |
| Batta et al., 2014 [106] | |
| Pulecio et al., 2014 [107] | |
| Vereide et al., 2014 [108] | |
| Riddel et al., 2014 [109] | |
| Sandler et al., 2014 [110] | |
| Cheng et al., 2016 [111] | |
| Lis et al., 2017 [112] | |

* Long-term in vivo engraftment means at least 6 months engraftment of all haematopoietic lineages with secondary engraftment. Reprogrammed cells only gave rise to very few myeloid colonies in CFU assays. Reprogrammed cells only gave rise to monocyte-like cells. Reprogrammed cells gave rise mostly to myeloid with low-CD45 expression level in primary engraftment and very limited secondary engraftment.

| Reprogrammed population | Reprogramming factors | Expression system | Species | In vitro clonogenicity | Long-term engraftment | References |
|-------------------------|-----------------------|-------------------|---------|------------------------|-----------------------|-----------|
| ESC-derived cells       | Hoxb4                 | IntegratedInducible | Mouse   | Yes                    | Yes                   | Kyba et al., 2004 [113] |
| ESC-derived cells       | Cdx4                  | IntegratedInducible | Mouse   | Yes                    | Yes                   | Wang et al., 2005 [114] |
| ESC-derived cells       | ERG, HOXA9, RORA, SOX4, MYB | Lentivirus | Human   | Yes                    | Short-termb           | Doulatov et al., 2013 [115] |
| ESC-derived cells       | Scl, Lmo2,Gata2, Ptx2, Sox7, MyCN | TransposonPiggy Bac | Mouse   | Not tested             | Vereide et al., 2014 [108] |
| ESC-derived cells       | ERG, HOXA5, HOXA9, HOXA10, LCOR, RUNX1, SPI1 | Lentivirus | Human   | Yes                    | Sugimura et al., 2017 [116] |

| Reference | |
|-----------|-----------|
| Kyba et al., 2004 [113] | |
| Wang et al., 2005 [114] | |
| Doulatov et al., 2013 [115] | |
| Vereide et al., 2014 [108] | |
| Sugimura et al., 2017 [116] | |
lymphoid potential was observed either in vitro or in vivo [104]. In the study by Batta et al. [106], fibroblasts were reprogrammed to blood progenitors with erythroid, myeloid, and lymphoid potential that conferred short-term in vivo engraftment. The only successful reprogramming approaches towards the generation of blood progenitors with long-term in vivo engraftment used starting cell populations with a close relationship to HSC. Riddel et al. [109] reprogrammed committed lymphoid and myeloid progenitors to HSCs. Sandler and Lis studies demonstrated long-term multilineage repopulation (with the exception of T cells for Sandler) using human and murine endothelial cells, respectively, as starting material for reprogramming [110,112]. Sugiura and collaborators obtained long-term multilineage repopulation by expressing a set of seven transcription factors in haematopoietic cells emerging from HE during the in vitro differentiation of human pluripotent stem cells [116]. A possible explanation for the successful reprogramming of those cells to HSCs is that endothelium and committed blood progenitors are more amenable to reprogramming towards HSC due to their specific chromatin landscape, likely quite divergent from fibroblast chromatin configuration. To date, it is unknown if the combination of factors used in those studies could reprogram somatic cells more distantly related to the blood lineage.

One striking observation, when surveying these studies, is the wide range and variety of transcription factors used for programming (Tables 1 and 2). There is clearly no consensus towards a subset of defined factors promoting blood specification. A few of the listed studies used RUNX1 (5 out of 14 studies) or SCL (4 out of 14 studies), but these two master regulators of blood cell emergence seem dispensable for reprogramming or forward programming. It can be argued, however, that either the reprogrammed population already expressed these factors, as for example in the case of the Riddell study [109], or that Scl and Runx1 are switched on as downstream transcriptional targets of the factors used for reprogramming. An interesting observation is the frequent use of homeotic/homeobox genes as reprogramming factors (5 out of 14 studies), suggesting the important role for re-patterning, re-specification or enhanced self-renewal.

Overall, it seems that the reprogramming of somatic cells to HSPCs does not follow the orderly transcriptional path of developmental haematopoiesis and that multiple combination of factors can promote blood specification in somatic cells. However, one needs to consider that different somatic cell landscapes may require different driving forces to push them towards HSPCs. Additionally, the HSPCs derived from these reprogramming experiments might be qualitatively very different from each other. To date, side-by-side comparisons of reprogramming protocols have not been published. Forward programming of pluripotent stem cells represent a different challenge as this consists in pushing undifferentiated cells towards a lineage of choice. It is noteworthy that most of these studies employ homeotic genes to drive forward programming. While great progress has been made towards the de novo generation of HSPCs via reprogramming, it remains uncertain whether these protocols can be translated to the clinic, given the expression of potential harmful factors (some of them known to confer leukaemias) or the random genomic insertion of exogenous factors that may activate harmful genes.

**Conclusion and perspective**

We have come a long way in understanding how blood cell emergence is controlled at the molecular level and what are the main players in this developmental process. However, there are still many unanswered questions: what differentiates HE from non-HE at the molecular level? How is the level of RUNX1 regulated during the transition from HE to haematopoietic cells? Are there different types of HE, is the microenvironment critical in conferring specificity to HE or is it a combination of both? Will we be able to use all this basic knowledge towards the clinic for the benefit of patients?

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