Oncogene (2007) 26, 6750–6765

www.nature.com/onc

REVIEW

Differential contributions of haematopoietic stem cells to foetal and adult haematopoiesis: insights from functional analysis of transcriptional regulators

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An increasing number of molecules have been identified as candidate regulators of stem cell fates through their involvement in leukaemia or via post-genomic gene discovery approaches. A full understanding of the function of these molecules requires (1) detailed knowledge of the gene networks in which they participate and (2) an appreciation of how these networks vary as cells progress through the haematopoietic cell hierarchy. An additional layer of complexity is added by the occurrence of different haematopoietic cell hierarchies at different stages of ontogeny. Beyond these issues of cell context dependence, it is important from a mechanistic point of view to define the particular cell fate pathway impacted by any given regulator. Herein, we advance the notion that haematopoietic stem cells (HSC), which sustain haematopoiesis throughout adult life and are specified in foetal life, have a minimal or late contribution to foetal haematopoiesis but instead largely proliferate during the foetal period. In light of this notion, we revisit published data on mouse knockouts of haematopoietically-affiliated transcription factors highlighting novel insights that may be gained from taking such a view.
Oncogene (2007) **26,** 6750–6765; doi:10.1038/sj.onc.1210759

Keywords: cell fate; self-renewal; foetal liver; bone marrow

Haematopoiesis: the HSC and cellular hierarchies

Haematopoiesis is the process through which a restricted number of blood stem cells give rise to differentiated progeny of at least 10 distinct lineages, while maintaining a population of haematopoietic stem cells (HSC) capable of sustaining blood formation throughout the lifespan of the organism. It relies on the capacity of the HSC to regulate the balance between asymmetric and symmetric, self-renewing or differentiative divisions. It also requires adequate regulation of lineage commitment and appropriate differentiation down the various lineages.

In the adult organism, HSC give rise to differentiated progeny following a series of relatively well-defined steps during the course of which cells lose proliferative potential and multilineage differentiation capacity and progressively acquire characteristics of terminally differentiated mature cells (reviewed in Kondo *et al*., 2003). As depicted in Figure 1, the more primitive cells in the haematopoietic differentiation hierarchy are long-term repopulating HSC (LT-HSC), short-term repopulating HSC (ST-HSC) and multilineage progenitors. For the purposes of this review, we will refer to these three cell types as 'hierarchically primitive cells', so as to distinguish them from the primitive blood cell lineages first identified in the yolk sac (YS) during ontogeny.

True HSC (or LT-HSC) are defined by their capacity to long-term reconstitute the haematopoietic system of an irradiated adult organism (Morrison and Weissman, 1994). They possess multipotentiality, as well as the ability to produce faithful replicas upon cell division, that is to self-renew. ST-HSC, which are operationally defined by their ability to transiently contribute to the production of lymphoid and myelomonocytic cells in an irradiated recipient (Adolfsson *et al*., 2001; Christensen and Weissman, 2001), are often described as self-renewing cells. However, as ST-HSC are not able to contribute to haematopoiesis in a sustained manner, it is likely that they produce slightly more mature daughter cells upon each cell division, and therefore, although highly proliferative, they do not strictly self-renew. ST-HSC give rise to multilineage progenitors (Christensen and Weissman, 2001), which cannot contribute to haematopoiesis *in vivo* due to a more limited proliferative potential, but retain the capacity to differentiate into the various haematopoietic lineages when tested *in vitro*.

The mode of commitment of multilineage progenitors to bi- and unilineage progenitors is still a matter of debate (Adolfsson *et al*., 2005; Forsberg *et al*., 2006; Mansson *et al*., 2007) and will not be discussed here. Nevertheless, most of the existing evidence supports the notion of an earlier decision step driving commitment to the erythroid and megakaryocytic lineages, which occurs upstream of the separation between myelomonocytic and lymphoid lineage fates.

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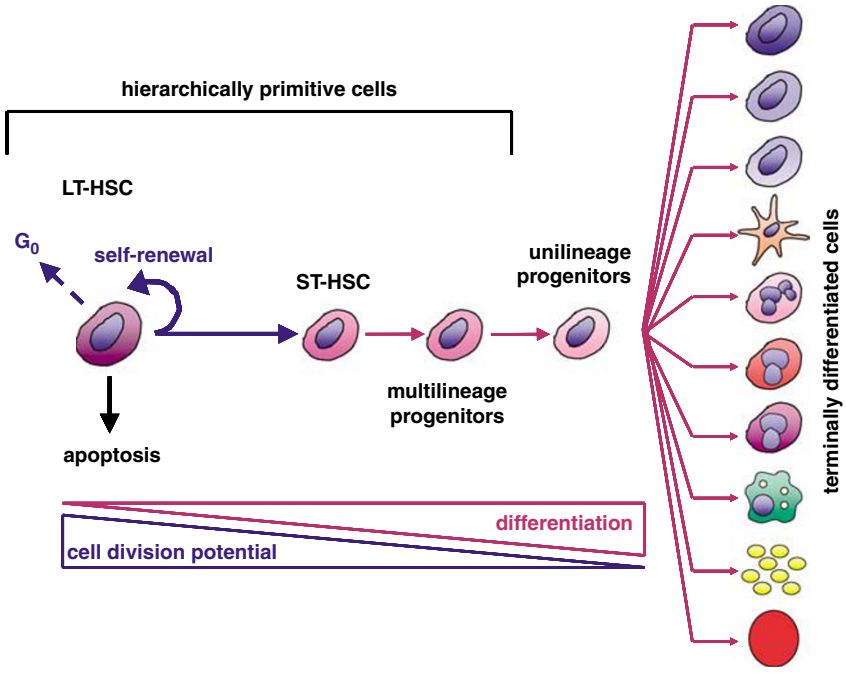

Figure 1 Haematopoietic stem cell (HSC) fates and cellular differentiation hierarchy in adult bone marrow. Long-term repopulating HSC (LT-HSC) are the only haematopoietic cells with self-renewing potential, which allows them to lifelong reconstitute the haematopoietic system of a lethally irradiated adult animal. LT-HSC maintain haematopoiesis by coordinating self-renewal with alternative fates of cell cycle exit, differentiation and cell death. A simplified diagram of haematopoietic differentiation is depicted. LT-HSC originate non-self-renewing cells with multilineage differentiation potential, which may be read transiently *in vivo* (short-term repopulating HSC, ST-HSC) or *in vitro* (multilineage progenitors). When analysed collectively, these three populations are referred to in the main text as 'hierarchically primitive cells'. Multilineage progenitors ultimately give rise to unilineage-committed progenitors (see text for a more detailed discussion), which lose proliferative potential and terminally differentiate into mature blood cells.

General concepts in regulation of haematopoiesis

Decision-making at each step in the haematopoietic hierarchy is presumed to be dependent on and regulated by a combination of intrinsic and extrinsic factors. In the past few years, a number of reports have started to elucidate the role of cellular and non-cellular components of the adult bone marrow (BM) microenvironment in regulating HSC numbers and fate decisions (reviewed in Wilson and Trumpp, 2006). HSC can be found in one of two niches—next to bone surfaces or in contact with the endothelium. Osteoblasts have been shown to contribute to HSC maintenance by expressing Jagged1 and signalling to the Notch pathway (Calvi *et al*., 2003); on the other hand, osteoblasts produce the matrix protein osteopontin (Stier *et al*., 2005), which negatively regulates HSC numbers. Furthermore, HSC seem to be able to sense the presence of calcium in the bone surfaces (Adams *et al*., 2006), which has implications for cellular homing during engraftment and migration of HSC to the BM during development. Other molecules, such as the chemokine receptor CXCR4 (Sugiyama *et al*., 2006), have also been shown to regulate HSC maintenance and homing at both the endosteal and the endothelial niches.

In the post-genomic era, global expression profiling studies have been fruitful in defining sets of regulators expressed by HSC and other cell types in the haematopoietic hierarchy (Ivanova *et al*., 2002; Park *et al*., 2002; Forsberg *et al*., 2005; Mansson *et al*., 2007). They have been successful at identifying intrinsic regulators such as transcription factors and associated molecules (Park *et al*., 2003; Shojaei *et al*., 2005) as well as in defining receptors and signalling cascades through which the niche may influence fate decisions (Reya *et al*., 2003). Gene expression profiling has also lent support (Akashi *et al*., 2003; Bruno *et al*., 2004) to previous observations (Hu *et al*., 1997; Delassus *et al*., 1999; Miyamoto *et al*., 2002) that several alternative molecular programmes co-exist as accessible in hierarchically primitive haematopoietic cells, including HSC—a phenomenon referred to as multilineage priming.

The fact that at least some programmes characteristic of alternative differentiation fates are accessible in a given cell prior to lineage commitment has several implications. First, it underlines the capacity of hierarchically primitive cells to rapidly integrate extrinsic and intrinsic cues necessary to decision-making. Second, it reveals a degree of flexibility in fate adoption that can be important in maintaining effective and adaptable blood production under conditions of stress. Third, it implies that differentiation or other fate decision involves activation as much as repression of alternative molecular programmes. Fourth, it means that regulators present at different steps in the haematopoietic hierarchy may use different partners and see different targets at different stages, implying that the understanding

of haematopoiesis and HSC regulation requires the identification of individual factors as much as the characterisation of their interactions and combinatorial outputs.

Nature of HSC regulators

Many regulators of HSC decisions were initially identified as targets of chromosomal rearrangements associated with leukaemia: transcription factors SCL/TAL1 (Begley *et al*., 1989), LMO2/RBTN2 (Boehm *et al*., 1988), RUNX1/AML1 (Miyoshi *et al*., 1991) and TEL/ETV6 (Golub *et al*., 1995), as well as the membrane receptor NOTCH1/TAN (Ellisen *et al*., 1991) are classical examples. In addition to the Notch pathway, signalling cascades of central importance in various developmental systems, such as the Wnt and the Hedgehog pathways, have been shown to play major roles in the regulation of the HSC compartment (Bhardwaj *et al*., 2001; Reya *et al*., 2003). Attention has also focused on chromatin modifiers: the trithorax orthologue MLL/ALL1 (Collins and Rabbitts, 2002) is another frequent target of chromosomal rearrangements in leukaemia; BMI1 (Lessard and Sauvageau, 2003; Park *et al*., 2003) and other members of the Polycomb family (Lessard *et al*., 1999; Ohta *et al*., 2002; Iwama *et al*., 2004; Arai and Miyazaki, 2005) were shown to play important roles in regulation of HSC and progenitors.

Despite the increasing number of known HSC regulators, we are still very far from understanding how they contribute to particular functions and phenotypes. Transcription factors, for example, act as on/off switches of transcription at different *loci*, and frequently function as part of multi-molecular complexes with consequences that depend on the nature of the partners (Rodriguez *et al*., 2005). Furthermore, the same transcription factor can be active at different steps in the haematopoietic hierarchy, in which not only can it associate with different partners, but its contributions to the regulation of fate decisions also depend on the accessibility of potential targets. To some extent, accessibility is determined by previous binding by other transcription factors, and the order in which transcriptional regulators interact with their respective targets can decide the cellular output (Iwasaki *et al*., 2006). It has also become increasingly evident that, at least under certain circumstances, it is the level rather than the presence of the transcription factors that determine the functional consequences (reviewed in Rosenbauer *et al*., 2005). In this framework, transcription factors should no longer be regarded as isolated critical determinants of cell fate decisions, but rather as modulatory elements in complex and dynamic networks whose output effects the fate choices (Soneji *et al*., 2007).

A similar discussion is pertinent to components of signalling cascades and chromatin modifiers. The first incorporate intra- and extracellular stimuli and often modulate gene expression through activation or inactivation of transcription factors. The activity of the second is in a certain way comparable to that of transcription factors as these molecules function to mark and modulate *loci* accessibility to the transcriptional machinery; however, they have the potential to exert their function in a more widespread manner across interrelated genes as well as to affect the status of the *loci* on a more permanent basis.

HSC fate decisions

In addition to understanding the molecular details and potential interconnections of the activities of regulatory factors in haematopoiesis, and indeed as a means of directing the search for pathways and partners, it is important to carefully position the role of each determinant in relation to alternative HSC fate decisions (Figure 1). HSC can either divide or reversibly exit the cell cycle into quiescence; upon cell division, they can either generate new multipotential HSC, or give rise to more mature progeny of restricted lineage potential, thus initiating differentiation. At the population level, this corresponds to asymmetric cell division; at a single-cell level, it may equate with symmetric self-renewing divisions that originate two HSC; symmetric differentiative divisions that only give rise to more mature cells; or true asymmetric divisions that maintain but do not expand the HSC pool (Figure 2; reviewed in Lessard *et al*., 2004). At each stage, HSC and their progeny can undergo apoptosis, which functions as an additional means of regulating cell number and allows for elimination of mutated clones.

When considering functional roles of individual regulatory factors, it is worth noting that similar global outputs can be obtained by different combinations of impacts on alternative cell fates. For example, an

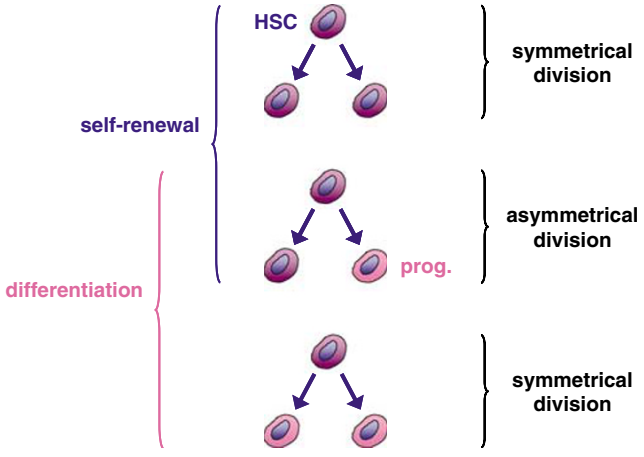

Figure 2 Alternative modes of haematopoietic stem cell (HSC) division. Symmetrical divisions may originate two HSC or two more differentiated daughter cells, thus expanding the HSC or the progenitor compartments, respectively. Asymmetrical divisions preserve the number of HSC and maintain haematopoietic production at steady-state.

expansion of HSC number can be obtained by promoting symmetrical self-renewing divisions, by blocking differentiation, by inhibiting apoptosis in the stem-cell compartment, as well as by different combinations of the above, which may or may not include recruitment of the quiescent HSC pool into cell cycle. The challenge is to appropriately dissect the final output so as to pinpoint the specific fate or fates impacted upon and ultimately to define the regulatory networks controlling each fate decision.

Developmental contexts in haematopoiesis

Most of the analyses on HSC regulators have been performed using mouse knockout models (non-conditional and conditional) and embryonic stem cell (ESC) chimaeras. As a consequence, most of the effects of the deletion of specific regulators have been read and interpreted during mouse development. It is therefore important to review and delineate key events in the establishment of haematopoiesis during foetal development and in the adult animal, and to define cell types and cell fates relevant to each stage.

Despite a recent resurgence of the debate over the exact topography of HSC emergence in the mammalian embryo (Samokhvalov *et al*., 2007), the prevalent view on blood formation is that it follows a two-wave model (reviewed in Godin and Cumano, 2002). The first wave of haematopoiesis—mouse embryonic days (E) 7–8.5—produces primitive erythrocytes (both nucleated and enucleated; Kingsley *et al*., 2004), megakaryocytes (Tober *et al*., 2007) and macrophages (Naito *et al*., 1996), that emerge as uni- and bilineage-committed progenitors (Tober *et al*., 2007); it does not produce HSC as defined by the capacity to reconstitute haematopoiesis in an adult organism (Cumano *et al*., 2001). In mammals, this inaugural blood production is extra-embryonic and locates to the blood islands in the YS.

The second wave—mouse E10.5–E12.5—produces HSC capable of long-term multilineage reconstitution of adult haematopoiesis (Medvinsky and Dzierzak, 1999); HSC proliferate during foetal life and sustain blood production throughout the lifespan of the organism. This later wave of definitive haematopoiesis starts in the embryo proper in the ventral wall of the major arterial vessels, namely the dorsal aorta (Medvinsky and Dzierzak, 1996; de Bruijn *et al*., 2002) in the aorta–gonad–mesonephros (AGM) region, and the umbilical and vitelline arteries (de Bruijn *et al*., 2000). From their sites of origin in the major blood vessels, and later in other tissues including the YS (Kumaravelu *et al*., 2002) and probably the placenta (Gekas *et al*., 2005; Ottersbach and Dzierzak, 2005), HSC migrate to the foetal liver (FL), which functions as the major haematopoietic organ for the remainder of foetal development and where HSC expand (Kumaravelu *et al*., 2002). The haematopoietic site shifts once more in the perinatal period, this time to the BM cavities of the axial skeleton, where adult haematopoiesis will become definitively established. There is evidence suggesting that the BM can be seeded by emergent HSC as part of the same migratory wave that colonises the FL (Delassus and Cumano, 1996; Kumaravelu *et al*., 2002). The contribution of these early BM HSC to foetal or adult haematopoiesis has not been elucidated.

In between these two waves of primitive and definitive blood formation, there is production of progressively more immature blood cells in extra- and intra-embryonic locations (reviewed in Medvinsky and Dzierzak, 1998), which we will refer to as intermediate-stage. Initial production of unilineage-committed progenitors in the YS is followed by the formation of multilineage myeloid progenitors (Palis *et al*., 2001); slightly later, the YS contains multipotent lympho-myeloid progenitors capable of reconstituting haematopoiesis in neonatal (Yoder and Hiatt, 1997), but not adult recipients (Cumano *et al*., 2001). The intra-embryonic para-aortic splanchnopleura (P-Sp) and its successor, the AGM region, are sites of emergence of progressively more immature myeloid (Medvinsky *et al*., 1993; Muller *et al*., 1994) and myelo-lymphoid progenitors (Godin *et al*., 1999), which can be identified prior to the emergence of the first LT-HSC.

Arguably, determinants of haematopoiesis regulate three distinct though probably interconnected developmental processes. The first key event is the formation of primitive and intermediate-stage blood. This involves the initial unilineage production of primitive erythroid, megakaryocytic and macrophage cells, followed by the emergence of progressively more immature multilineage progenitors whose progeny is similar to that found in definitive haematopoiesis. By definition, this pre-definitive haematopoiesis is not sustained in the long term, but the extent to which it contributes to foetal blood has not been elucidated.

The second key event is the intra-embryonic formation of true LT-HSC and the consequent establishment of multilineage definitive haematopoiesis. Since the progeny of definitive HSC is broadly similar to cells identified in primitive and intermediate-stage haematopoiesis, it is plausible that they share molecular regulators of lineage commitment and differentiation. Regulators of alternative HSC fates may or may not be different from regulators found during pre-definitive blood formation.

The actual contribution of HSC to the formation of foetal blood poses an interesting conundrum. It is commonly perceived that blood production in the FL predominantly reflects the progeny of recently emerged HSC; it is simultaneously accepted that HSC expand in the FL so as to make up the number of stem cells necessary to sustain haematopoiesis throughout adulthood (Figure 3—model I). While it is possible that the two processes co-exist and are powerful enough to produce and expand HSC and HSC-derived progenitors, it is difficult to envisage how newly-emerged HSC engaged in symmetric self-renewing divisions can generate enough progenitors to produce fully differentiated circulating progeny in a short period. Moreover,

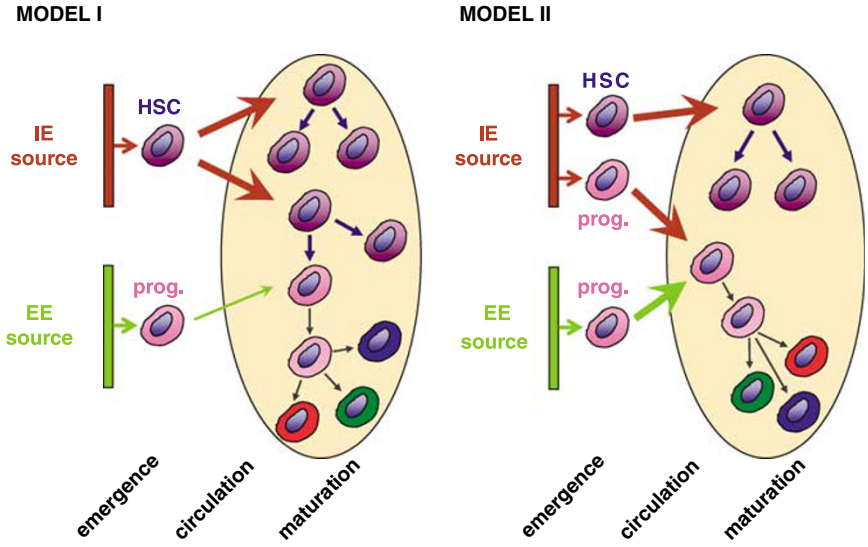

Figure 3 Alternative models of haematopoietic stem cell (HSC) and progenitor contribution to foetal blood production. Model I illustrates the more classical view of haematopoiesis in the foetal period. HSC are specified in the AGM region (IE, intra-embryonic source) and later migrate to the foetal liver; they expand and contribute to foetal blood production for the remainder of foetal development. HSC expansion is represented by a symmetric self-renewing division; HSC contribution to foetal haematopoiesis is represented by an asymmetric division and the subsequent multilineage differentiation of the daughter progenitor cell. At the early stages of foetal blood production, HSC progeny co-exist with yolk sac (EE, extra-embryonic source) progenitors, which make a relatively minor contribution to foetal blood. Model II illustrates the proposed notion that foetal blood production is mainly sustained by progenitors emerged from IE and EE sources, while IE-derived HSC predominantly engage in symmetrical self-renewing divisions, thereby expanding their number. According to this view, HSC only start to contribute to haematopoiesis at late stages in foetal development or in adult life.

detailed enumeration of LT-HSC in the AGM, YS, FL and circulation of mouse embryos between E11 and E13 (Kumaravelu *et al*., 2002) revealed that expansion of HSC numbers in the FL during that period cannot be accounted for solely by cell division and requires continued production at intra- and extra-embryonic sites. Therefore, at least until E13 it seems implausible that HSC engage in differentiation. On the other hand, in the AGM, emergence of HSC co-exists with production of progenitors (Taoudi *et al*., 2005). Moreover, it is plausible that the intermediate-stage multilineage progenitors generated extra- and intra-embryonically prior to HSC emergence contribute to foetal blood formation. In this setting, it is conceivable that definitive HSC make a minimal and late contribution to blood production in the foetus and mostly readout postnatally, while remaining engaged in cell division and expansion during foetal life (Figure 3—model II).

The third and last key event is the establishment of adult haematopoiesis. Regardless of their effective contribution to foetal haematopoiesis, HSC expand in the FL and eventually home pre-natally to the BM. HSC then undergo a dramatic change in their proliferative and differentiative properties (Figure 4), in what seems to constitute an intrinsically regulated phenomenon (Bowie *et al*., 2006, 2007). They switch from a proliferative HSC compartment that, in the mouse, sustains an interleukin (IL)7-independent B-lymphopoiesis (Kikuchi and Kondo, 2006) and gives rise to myeloid-skewed grafts in experimental recipients (Bowie *et al*., 2007), to a relatively quiescent adult HSC population that generates B lymphocytes in a strictly IL7-dependent manner and that produces lymphoid-skewed grafts in reconstitution experiments. In the mouse, the switch occurs in the first few weeks after birth: the proliferative switch was observed between the third and fourth weeks (Bowie *et al*., 2006); the lymphoid switch may occur 2 weeks earlier. Although it has not been formally demonstrated in the human system, the cell cycle status and engraftment characteristics of hierarchically primitive haematopoietic populations from FL (Wilpshaar *et al*., 2002), cord blood (Wilpshaar *et al*., 2000) and BM (Gothot *et al*., 1997) suggest that a similar developmental switch may take place in the perinatal period.

HSC fate regulators and their contributions

In this scenario of alternative HSC fate decisions with presumed distinct relative contributions to haematopoiesis at different developmental stages, it is important to address which phenotypes to expect from gain or loss-of-function models of key regulators.

Haematopoietic defects (and therefore functions) have broadly been classified into three categories: specification or development of primitive blood; specification or development of definitive haematopoiesis, which should imply strict dependence on HSC and HSC maintenance in the adult. The first has been equated

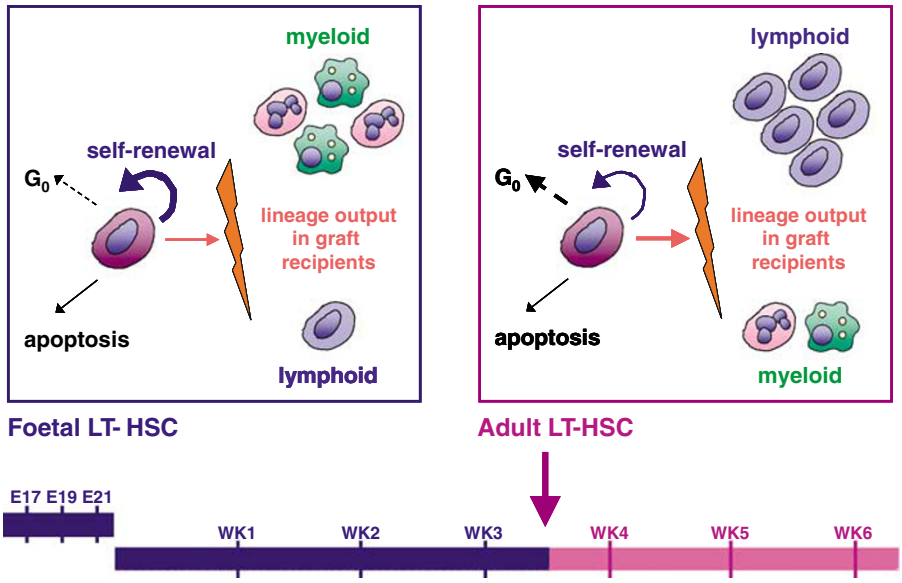

Figure 4 Intrinsic foetal-to-adult haematopoietic stem cells (HSC) switch in proliferation, self-renewal and lineage output properties. Foetal and neonatal mouse HSC are proliferative cells commonly engaged in symmetrical self-renewing divisions, and originate myeloid lineage-skewed grafts when transplanted into irradiated animals. Between the third and the fourth weeks after birth, HSC undergo an intrinsically-regulated switch (Bowie *et al*., 2006, 2007) that changes the balance between alternative cell fates to generate an adult HSC phenotype. Adult HSC are largely quiescent or slowly proliferating, engage in asymmetrical rather than symmetrical self-renewing divisions and generate lymphoid lineage-skewed grafts in lethally irradiated recipients.

with defects in YS blood formation; the second with the establishment of FL haematopoiesis; the third with haematopoietic function in the adult, often read as defective self-renewal and tested by transplantation of BM cells. Regulation of haematopoiesis has been more extensively analysed in the mouse system. YS regulatory functions have been assessed and inferred in non-conditional knockout animals; contributions to HSC emergence and FL haematopoiesis in non-conditional knockouts and ESC chimaeras; contribution to HSC maintenance in the adult BM has been studied in conditional knockout models and by competitive or serial transplantation.

Arguably, relatively standardised conclusions drawn from these experimental approaches may overlook explanations that, when considered, suggest a subtly different view of the establishment of haematopoiesis and the relative contributions of different cells during ontogeny. Paradigmatic classical and novel transcriptional regulators can be used to illustrate this point. Broadly, factors fall into two groups. Group I: factors required for the establishment of haematopoiesis in the embryo, but not for HSC function in the adult; and Group II: factors dispensable during foetal development that play roles in maintenance of the HSC compartment in the adult. The fact that later factors such as BMI1 or TEL (see below) are not strictly required during embryonic development may point to the existence of a distinct set of regulators of cell fate decisions in the adult, but may also support the notion of a relatively minor or late contribution of HSC to blood production in the embryo. The loss-of-function phenotype of factors in the first category is equally compatible with both hypotheses. However, the fact that FL and BM HSC null for BMI1 produce similar haematopoiesis reconstitution defects upon transplantation, while in the knockout animal FL blood production is broadly unaffected (see below), may suggest a significant contribution of progenitors to the latter, and bring into question the true definitive nature of at least some of the blood production in the FL. The correct answer possibly lies between the two extremes. Intra-embryonic haematopoiesis may have mixed contributions of progenitor and HSC populations, the latter becoming progressively more important in later stages in development and corresponding to *bona fide* definitive blood formation.

One of the features of the recently described switch between foetal and adult HSC probably reflects the change from a highly proliferative stage whereby HSC have to accommodate symmetric and asymmetric self-renewing divisions, so as to expand and produce differentiated progeny, to a less proliferative phase that couples asymmetric self-renewing divisions with exit of cell cycle into dormancy. Thus, key molecular mechanisms emerging as distinct between the two populations should be regulators of quiescence and cell division or HSC commitment to differentiate.

We will review the data on the function of exemplary transcriptional regulators and discuss it in terms of the proposed late contribution of HSC to haematopoiesis in the embryo. Regulators have been classified as 'early' or 'late' depending on the developmental stage at which the haematopoietic defect becomes manifest; we have also grouped them in terms of their putative contribution to specification, proliferation, lineage commitment or

survival of HSC and/or progenitors as understood at present. We will limit our discussion to data obtained in mammalian models, namely in mouse, as these are the only systems where the function of HSC (as defined by the ability to long-term reconstitute the haematopoietic system of an adult animal) can be specifically assessed.

Group I: 'early' regulators

Specification of multipotent progenitors and/or HSC

SCL/TAL1. *SCL* was initially identified as a target of chromosomal rearrangements leading to gene overexpression in childhood T-cell acute lymphoblastic leukaemia (T-ALL). Knockout mice are embryonic lethal (Robb *et al*., 1995; Shivdasani *et al*., 1995): embryos die between E8.5 and E10.5 due to severe anaemia, which results from the inability to form blood at the YS stage. $SCL^{-/-}$ ESC chimaeras do not contribute to any definitive myeloid or lymphoid lineage (Porcher *et al*., 1996; Robb *et al*., 1996), a phenotype that can be rescued upon SCL expression (Porcher *et al*., 1996). However, continuous expression of *SCL* was shown to be dispensable for HSC maintenance and normal multilineage haematopoiesis in adult mice (Mikkola *et al*., 2003), while it persisted as a requirement in terminal erythroid differentiation. These data were interpreted as reflecting the absolute requirement for SCL during the establishment of primitive and definitive haematopoiesis, but not for HSC maintenance in the adult BM.

However, it is also possible for the results to reflect a role for SCL in specification and/or incipient differentiation decisions of an early multipotent progenitor but not of HSC. This would be consistent with an absolute requirement for the formation of erythroid and myeloid progenitors in the YS, as well as for multilineage haematopoiesis in the FL; it would also prevent HSC readout in the adult BM. Recent studies suggest that SCL may play a comparable role in adult life. Using a conditional knockout approach, Begley and collaborators (Curtis *et al*., 2004) have shown that SCL is required for short-term, but not long-term repopulation of primary transplant recipients, a pattern that is preserved in (and therefore transplantable to) secondary recipients. Once again, this is compatible with a role in survival, proliferation or differentiation of an early progenitor. Moreover, recent analyses in human cells using forced expression (Reynaud *et al*., 2005) and knockdown (Brunet de la Grange *et al*., 2006) approaches have indicated that SCL plays a role in erythromyeloid lineage commitment both *in vitro* and *in vivo*. These papers also indicate that SCL enhances long-term engraftment of human cells in the non-obese diabetic/severe combined immunodeficiency (NOD/SCID) xenotransplantation model, an assay of human HSC function. Therefore, it is possible that SCL may contribute to HSC proliferation or survival, although the data are equally compatible with increased proliferation or survival of an early multilineage progenitor, the readout of which impacts upon the levels of engraftment.

SCL plays a central regulatory role in haematopoiesis throughout development. Arguably, the data obtained to present does not precisely identify the target cells in the haematopoietic hierarchy, nor does it permit to clearly discriminate between effects on cell specification, survival, proliferation or differentiation in different ontogenic moments. While not disputing the current understanding of the functions of SCL, we argue that the results are open to alternative interpretations, and more detailed analysis is required to define SCL functions in blood formation.

LMO2/RBTN2. LMO2 is a known partner of SCL in a multiprotein complex, which also includes GATA1 or GATA2, LDB1 and E47 (Osada *et al*., 1997; Wadman *et al*., 1997; Anguita *et al*., 2004). Like *SCL*, it is a target of chromosomal rearrangements in childhood T-ALL, being expressed at high levels in the leukaemic cells. The *LMO2* knockout mouse (Warren *et al*., 1994) is embryonic lethal around E10.5, due to lack of production of primitive erythrocytes in the YS. Unlike the *SCL* knockout, YS macrophage development is apparently unimpaired in the absence of LMO2. $LMO2^{-/-}$ ESC chimaeras (Yamada *et al*., 1998), on the other hand, do not contribute to any definitive-type myeloid or lymphoid lineages. LMO2 has thus been considered as required for the establishment of definitive haematopoiesis, as well as primitive erythropoiesis. However, no phenotypical or functional analyses of the HSC or progenitor compartment have been performed, and there are no reports of conditional knockout models where the role of LMO2 in adult haematopoiesis has been assessed. By analogy with its binding partner SCL, it is possible that LMO2 may prove to be dispensable for HSC maintenance in the adult BM and that the entire phenotype may be attributable to a defect in specification, survival or early differentiation of a multipotent progenitor. However, to date there is no data to prove or disprove the hypothesis.

RUNX1/AML1. The theme of transcription factor requirement for early haematopoiesis (or HSC) decisions in the embryo, but not in adult BM is once again repeated in the case of RUNX1 (reviewed in de Bruijn and Speck, 2004). However, there are also important differences in comparison with SCL.

RUNX1 was the first transcription factor described to be exclusively required for the formation of definitive haematopoiesis in the mouse embryo. It is the most commonly mutated gene in human acute leukaemia and a target of leukaemia-associated translocations, the most common ones being *TEL/AML1* in childhood pre B-ALL and *AML1/ETO* in adult acute myeloid leukaemia (AML) (reviewed in Speck and Gilliland, 2002).

Knockout mice (Okuda *et al*., 1996; Wang *et al*., 1996) are embryonic lethal. Embryos die between E12.5 and E13.5 as a consequence of massive bleeding into the brain ventricular spaces and mesothelium-lined cavities. There are no observable haematopoietic cells in the FL,

and that is the consequence of a complete abrogation of blood cell emergence in the dorsal aorta, the umbilical and vitelline arteries and in the YS vasculature (North *et al*., 1999). At this stage, HSC and progenitor compartments are equally affected; however, primitive erythropoiesis was unperturbed. Unlike the *SCL* knockout phenotype, loss of *RUNX1* establishes a clear distinction between the first and second waves of blood cell formation in the embryo in terms of fundamental regulatory mechanisms. However, it does not discriminate between relative contributions to HSC and progenitor compartments in intra-embryonic blood, as no emergence of haematopoietic cells is observed.

As is the case for SCL, analysis of conditional knockout mice (Ichikawa *et al*., 2004) revealed that RUNX1 is dispensable for maintenance of a functional HSC compartment in adult BM. It is specifically required for megakaryocyte maturation as well as for B- and T-cell development downstream of the common lymphoid progenitor (CLP) stage. Interestingly, *RUNX1* is expressed at relatively constant levels in nearly all the haematopoietic lineages in the BM (North *et al*., 2004; de Bruijn and Speck, 2004). The erythroid series is the exception, as levels of RUNX1 become undetectable downstream of the erythroblast stage; levels of RUNX1 also decrease moderately upon terminal granulocytic and monocytic differentiation.

Interestingly, RUNX1 effects on developmental haematopoiesis are dosage-dependent, underlining the functional relevance of the levels of at least some transcription factors. *RUNX1*$^{lz/+}$ mice have detectable adult long-term repopulating activity in the AGM region one day earlier than wild-type animals, and LT-HSC are heterogeneously distributed among CD45^{+}, endothelial and mesenchymal cells (North *et al*., 2002). Furthermore, analysis of progenitor activity in E9.5 P-Sp and E11.5 AGM reveals a global reduction in colony formation in *RUNX1*$^{+/-}$ mice when compared to controls (Mukouyama *et al*., 2000). Dosage-dependent effects of RUNX1 are also observed in adult human haematopoiesis: haploinsufficiency of RUNX1 associates with an autosomal dominant form of familial thrombocytopenia with increased predisposition to development of AML (Song *et al*., 1999).

Overall, the combination of embryonic and adult data indicates an absolute requirement for RUNX1 in the specification and emergence of intra-embryonic haematopoiesis. Persistent expression of RUNX1 in most blood series may highlight a more general programme in haematopoiesis, the regulation of which may depend on RUNX1 levels. There is no evidence to support a sustained and necessary role in HSC after intra-embryonic specification.

CBFβ. CBFβ is the dimerisation partner of RUNX1 (as well as RUNX2 and RUNX3). It was also initially identified as a target of chromosomal rearrangement in AML, being juxtaposed to the smooth muscle myosin heavy chain gene by inv(16), which associates with AML-M4 with eosinophilia. Similarly to *RUNX1*, *CBFβ* knockout mice (Niki *et al*., 1997) die *in utero* between E12.5 and E13.5 due to massive intracranial haemorrhage and absent FL haematopoiesis. YS primitive erythropoiesis is unaffected. Restoration of CBFβ expression under the control of *TIE2* regulatory regions (Miller *et al*., 2002) rescues the establishment of FL haematopoiesis, although with diminished formation or maintenance of the hierarchically more primitive multilineage progenitors (colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM)). There has been no detailed characterisation of the HSC compartment. Thus, similarly to RUNX1, CBFβ expression appears to be necessary for the emergence, survival or early differentiation of intra-embryonic haematopoietic cells, while being dispensable for primitive YS haematopoiesis.

CBFβ knockout mice rescued with a *CBFβ* transgene present later defects in myelomonocytic and lymphoid, but not erythroid differentiation at E17.5; this is compatible with the lineage-associated expression pattern described for CBFβ and RUNX1 in BM cells (North *et al*., 2004; de Bruijn and Speck, 2004). There is currently no data on the role of CBFβ in HSC maintenance in the BM, though one may speculate that it may prove to be dispensable, as seen for RUNX1.

Maintenance of multipotent progenitors and/or HSC

MLL. MLL is the orthologue of *Drosophila* Trithorax, the founding component of a multiprotein complex that functions to regulate appropriate expression of *HOX* genes during development. The Trithorax complex is a chromatin-modifier complex responsible for methylation of lysine 4 of histone 3 (H3K4), a mark associated with active accessible chromatin (see Ringrose and Paro, 2004 for a review).

MLL is a frequent target of chromosomal translocations that fuse it with a growing number of partners, some of which may be functionally associated (Erfurth *et al*., 2004). The leukaemias generated by the fusions can be either AML or childhood B-ALL, probably depending on the fusion partner; some of the fusions have been shown to induce a molecular programme with both myeloid and lymphoid gene activation (So *et al*., 2003).

The *MLL* knockout mouse (Yu *et al*., 1998) is embryonic lethal around E10.5 and the mice die with hypoplasia of the branchial arches and involution of the maxillary process. At this stage, multilineage progenitor content of the YS, as assessed by *in vitro* colony formation, is greatly reduced, particularly the hierarchically more primitive CFU-GEMM and burst-forming unit-erythroid (BFU-E), which are nearly absent, and colony-forming unit-granulocyte, macrophage (CFU-GM), which are affected to a lesser extent; furthermore, colonies are small and morphologically atypical (Hess *et al*., 1997). Similar observations were made in E12.5 FL from mice homozygous for an *MLL*-null mutation (Yagi *et al*., 1998), which also showed delayed colony formation. This could suggest a role in proliferation of hierarchically primitive haematopoietic progenitors.

Study of ESC chimaeras (Ernst *et al*., 2004a) revealed the absence of $MLL^{-/-}$ lympho-myeloid cells in the FL. $MLL^{-/-}$ AGM produced a few macrophage-like cells, but no other lineages, in short-tem explant cultures; no HSC activity was detected in $MLL^{-/-}$ AGM upon transplantation. Although it is not possible to exclude that MLL may play a role in HSC specification, the joint interpretation of CFU and transplantation data more strongly suggests that MLL is required for proliferation or survival of HSC and/or hierarchically primitive haematopoietic progenitors.

Analysis of $MLL^{-/-}$ ESC-derived haematopoietic progenitors (Ernst *et al*., 2004b) showed a similar defect in colony formation. This could be rescued by expression of *CDX4*, a general activator of *HOX* gene expression, but not by *BCL2*, the latter favouring regulation of proliferation over a role in survival. Given the dependence of different clusters of *HOX* genes on MLL for proper expression, and the different roles of these genes in proliferation, commitment and differentiation of haematopoietic cells throughout adult life (reviewed elsewhere in this issue of *Oncogene*), it is reasonable to speculate that loss of *MLL* in adult HSC may lead to defective or delayed contribution to different blood lineages upon transplantation. In leukaemia, expression of MLL fusions results in abnormal high expression of certain clusters of MLL-target *HOX* genes (Hess, 2004), which have been putatively implicated in the developmental block and proliferation of the leukaemic clone. To date, there are no reports of specific ablation of MLL function in adult haematopoiesis in a conditional knockout setting.

GATA2. *GATA2* knockout mice (Tsai *et al*., 1994) die *in utero* between E10 and E11 with severe anaemia and pericardial enlargement. There is an almost complete abrogation of production or survival of mixed and erythroid progenitors in the YS; macrophage progenitors are also affected, but to a lesser extent. $GATA2^{-/-}$ ESC chimaeras (Tsai *et al*., 1994) do not show contribution of GATA2-null cells to myeloid or lymphoid lineages in FL or in the adult animal.

GATA2 haploinsufficient animals, unlike knockouts, have a normal lifespan and no gross blood phenotype. However, detailed characterisation of the stem cell and progenitor compartments in these animals revealed a defect in granulocyte-monocytic progenitors, as well as lower numbers of phenotypically defined HSC in adult BM (Rodrigues *et al*., 2005). HSC function, as tested by cobblestone area-forming cell (CAFC) assays and competitive transplantation, was also shown to be impaired in heterozygous animals, most probably due to increased apoptosis; there was no detectable self-renewal defect upon serial transplantation (Rodrigues *et al*., 2005).

Analysis of HSC during development of *GATA2* heterozygous mice (Ling *et al*., 2004) revealed a selective reduction in HSC numbers in the AGM region and a more general qualitative defect upon serial and competitive transplantation. Adult BM further showed delayed recovery following cytotoxic treatment (Ling *et al*., 2004). This is compatible with a proliferative defect, although the suggestion contradicts forced expression experiments in multipotent progenitors (using an oestrogen receptor fusion; Heyworth *et al*., 1999) and in mouse BM HSC and hierarchically primitive progenitors (Persons *et al*., 1999), which describe a proliferative defect in response to higher GATA2 levels.

To date, no conditional knockout model of *GATA2* was published and consequently a specific requirement for this transcription factor in the maintenance of adult HSC has not been addressed. Interestingly, GATA2 expression is clearly enhanced after the postnatal switch from foetal to adult haematopoiesis (Bowie *et al*., 2007). Whether this is a mere correlate with the proliferative status of the two populations or whether GATA2 regulates molecular programmes that play a mechanistic role in the transition remains to be elucidated and will undoubtedly shed light on the regulation of HSC maintenance.

Group II: 'late' regulators

Regulation of HSC commitment and differentiation

BMI1. *BMI1* is the mammalian orthologue of *posterior sex combs*, a component of the Polycomb group 1. Polycomb group proteins are involved in maintenance of gene silencing through chromatin modifications (reviewed in Sparmann and van Lohuizen, 2006). During development, opposite effects of Polycomb and Trithorax complexes serve to maintain appropriate temporal and spatial regulation of *HOX* gene expression and thus guarantee correct embryo segmentation and organ specification (reviewed in Ringrose and Paro, 2004).

BMI1 was initially identified as a common proviral integration site in murine B-cell lymphomas (van Lohuizen *et al*., 1991). Non-conditional knockout mice (van der Lugt *et al*., 1994) are born alive but typically die between 3 and 20 weeks after birth as a consequence of infection; the animals present multisystemic abnormalities, which include an ataxia of progressive severity and multiple posterior transformation defects of the axial skeleton. FL haematopoiesis is broadly normal *in vivo* although defects in *in vitro* colony formation in response to IL-7 and macrophage colony-stimulating factor (but not IL-3) suggested specific defects of lymphoid and myelomonocytic differentiation. Strikingly, a progressive hypoplasia and adipose replacement of the BM was observed after birth, which correlated with a progressive decline in mature cell numbers across lineages in the peripheral blood and in the haematopoietic organs. Selective defects in thymic T-cell differentiation and in B-cell proliferative responses were also reported.

Overall, the phenotype suggested a defect in BM HSC maintenance. Detailed analysis of the HSC compartment of $BMI1^{-/-}$ mice revealed normal numbers of phenotypically defined HSC in the FL and a progressive quantitative decline postnatally in the BM (Park *et al*.,

2003). Functionally, however, both FL and BM HSC were shown to be defective in terms of repopulating ability when analysed in competitive and serial transplantation settings (Lessard and Sauvageau, 2003; Park *et al*., 2003; Iwama *et al*., 2004). *In vitro* analysis of proliferation and self-renewal properties of *BMI1*$^{-/-}$ hierarchically primitive progenitor cells indicate rapid loss of proliferative capacity and accelerated differentiation with few self-renewal divisions (Iwama *et al*., 2004). Conversely, forced expression of *BMI1* (Iwama *et al*., 2004) sustains HSC self-renewal and/or survival as, unlike controls, transduced HSC cultured *ex vivo* for more than 1 week maintain their repopulating capacity.

Gene expression profiling of *BMI1*$^{-/-}$ HSC (Park *et al*., 2003) suggested a role for the *INK4a locus* products p16 and p19 in the BMI-null phenotype. Overexpression of p16 induces a G_1-to-S phase progression block, while high levels of p19 cause p53-dependent growth arrest and apoptosis; both factors are implicated in cell senescence (Jacobs *et al*., 1999). However, several lines of evidence suggest that repression of *p19* may not be a major effector mechanism of BMI1 in HSC (Iwama *et al*., 2004; Oguro *et al*., 2006). On the other hand, while deletion of the *INK4a locus* was capable of restoring multilineage engraftment of *BMI1*$^{-/-}$ HSC, it could only partially rescue proliferation of these cells *in vitro* (Oguro *et al*., 2006); the same study suggested the existence of p16/p19-independent roles of BMI1 in the BM microenvironment. This suggests that BMI1 has HSC intrinsic and extrinsic roles and that its relevant transcriptional repressor properties extend beyond the *INK4a locus*. For instance, it would be very interesting to assess the relevance of BMI1 regulation of *HOX* gene expression in the haematopoietic context and how the balance of Polycomb and Trithorax factors help to regulate HSC decisions.

Interestingly, expression levels of BMI1 are not increased as HSC switch from a foetal proliferative to an adult slowly-cycling phenotype (Bowie *et al*., 2007). Although one cannot exclude a discrepancy between mRNA and protein levels of BMI1, the results are not necessarily surprising since FL and BM HSC function was shown to be equally affected upon transplantation. This is consistent with the hypothesis we formulated that HSC make a small and late contribution to blood formation during the foetal period. The putative dependence of foetal blood formation on progenitors rather than HSC would explain the lack of a haematopoietic defect in the FL stage in the absence of BMI1; the defect is only apparent as haematopoiesis becomes dependent on HSC contribution postnatally; it is also evident in a transplantation setting when HSC, regardless of their FL or BM origin, have to actively contribute to repopulating an irradiated host.

The similar BMI1 expression levels observed before and after the foetal-to-adult switch also suggest that BMI1 may exert its effects on HSC maintenance through inhibition of differentiation rather than through control of the cell cycle, or at least in addition to it. This would explain the normal numbers of HSC in the FL (Park *et al*., 2003), where HSC are preferentially engaged in symmetric rather than asymmetric self-renewing divisions. Suggested roles for BMI1 in osteoblasts (Iwama *et al*., 2004) may be relevant in this context.

Analysis of the effects of *p16* deletion in *in vitro* differentiation of HSC, both in isolation and in the *BMI1*$^{-/-}$ background (Iwama *et al*., 2004), suggest that repression of *p16* may contribute to generation or maintenance of hierarchically primitive multilineage progenitors, as opposed to uni- or bilineage-committed CFU. As reviewed below, *CEBPA*$^{-/-}$ HSC express increased levels of BMI1: this may be relevant both in terms of the selective advantage of the cells in competitive transplantation, but also to the early differentiation block of *CEBPA*$^{-/-}$ cells and subsequent myeloproliferative phenotype.

CEBPA. CEBPA is a regulator of myelomonocytic lineage commitment that acts at the level of transition from the common myeloid protein (CMP) to the granulocyte/macrophage progenitor (GMP) stage (Zhang *et al*., 2004); it participates in a cross-antagonistic regulatory network with PU.1. (Laslo *et al*., 2006) that defines the balance between neutrophilic versus monocytic output downstream of the GMP. CEBPA further interacts with cell cycle regulators, namely E2F and regulates cell cycle arrest associated with terminal differentiation (Porse *et al*., 2001).

CEBPA knockout mice (Zhang *et al*., 1997) die antenatally of severe hypoglycaemia; FL haematopoiesis presents a selective block in granulocytic differentiation without changes in other lineages. In conditional knockout models, *CEBPA*$^{-/-}$ FL and BM-derived HSC have selective advantage in competitive transplantation settings (Zhang *et al*., 2004), suggesting a role for CEBPA as a negative regulator of HSC self-renewal and/or maintenance. Notably, conditional *CEBPA*$^{-/-}$ mice, as well as mice expressing either a shorter 30 kDa dominant-negative isoform of CEBPA (Mueller and Pabst, 2006) or mutant forms that affect protein–protein interaction with the cell cycle machinery (Porse *et al*., 2005), develop a transplantable myeloproliferative disorder similar to AML. CEBPA-null cells express higher levels of BMI1 than wild-type cells (Zhang *et al*., 2004), and it is possible that BMI1 contributes to the increased self-renewal and/or blocked differentiation of CEBPA-null cells.

The similar repopulating advantage shown by FL and BM-derived CEBPA-null HSC upon competitive transplantation argues in favour of the equivalent functionality of foetal and adult HSC when asked to participate in downstream haematopoiesis. Furthermore, in the adult, as HSC contribute to blood formation, the combination of a block in early myelomonocytic lineage decisions and a putative proliferative advantage of *CEBPA*$^{-/-}$ cells may result in myeloproliferation.

The myeloproliferative phenotype associated with reduced or ablated expression of CEBPA is relevant to human leukaemia, as *CEBPA* is commonly mutated in AML (usually subtypes M1 or M2) (reviewed in Leroy

et al., 2005), or expressed at reduced levels in the context of AML1-ETO-associated leukaemias. *CEBPA* mutations in AML associate with a favourable prognosis, but the pathophysiological mechanism behind this observation is unknown.

Regulation of HSC quiescence and cell cycle entry

GFI1. *GFI1* is, similarly to *BMI1*, a common site of retroviral integration associated with murine lymphomas (van Lohuizen *et al*., 1991). It is a known target of CEBPA at the level of the GMP that promotes differentiation down the neutrophilic lineage by cross-antagonism with the PU.1 target EGR2 (Laslo *et al*., 2006). Furthermore, it plays roles at several levels in lymphoid differentiation (reviewed in Hock and Orkin, 2006): it promotes the commitment to the lymphoid-primed multipotent progenitor (LMPP) and the CLP compartments, as well as differentiation and/or proliferation of $CD4^+$ and Th2 cells.

GFI1 knockout mice (Hock *et al*., 2004a; Zeng *et al*., 2004) have a relatively normal HSC compartment during foetal development and in adult mice and GFI1-null HSC are able to reconstitute haematopoiesis in lethally irradiated hosts in a non-competitive transplantation setting. However, GFI1-null cells are at severe disadvantage upon competitive transplantation (Hock *et al*., 2004a; Zeng *et al*., 2004) and in secondary transplants (Hock *et al*., 2004a) and have a progressively reduced contribution to BM haematopoiesis in ESC chimaeras (Hock *et al*., 2004a). The defect was pinpointed to regulation of cell cycle exit into quiescence (Hock *et al*., 2004a; Zeng *et al*., 2004). In contrast with the pro-proliferative role of GFI1 in T cells, the HSC compartment of $GFI1^{-/-}$ BM presents a greater proportion of cells in S-phase with concomitant increased BrdU incorporation, and a significant reduction of cells in G_0. This effect may be mediated through p21 (Hock *et al*., 2004a; Zeng *et al*., 2004) and E2F5 and 6 (Zeng *et al*., 2004).

Given the putative role of GFI1 in maintaining the HSC compartment through promotion of cell cycle exit into quiescence, one would expect to observe increased expression of GFI1 upon switch from foetal to adult haematopoiesis. However, transcript levels of GFI1 remain unchanged (Bowie *et al*., 2007). It is possible that there is a discrepancy between mRNA and protein levels or that downstream effectors of GFI1 only become accessible or functional in adult BM. Nevertheless, given the relatively normal function of the HSC compartment of $GFI1^{-/-}$ mice under physiological conditions, one cannot exclude that it plays a relatively minor or redundant role in steady-state BM becoming relevant under haematopoietic stress.

MEF/ELF4. MEF, on the other hand, has a clear change in expression levels during the transition from foetal to adult haematopoiesis, with a marked reduction in 4-week and adult BM (Bowie *et al*., 2007). This correlates with its recently described role as a negative regulator of HSC quiescence (Lacorazza *et al*., 2006).

MEF knockout mice have no gross blood defect as seen by full blood counts of adult animals. However, detailed phenotypical analysis of hierarchically primitive BM compartments revealed significant increases in LT and ST-HSC and a concomitant decrease in multipotent progenitors suggestive of a delayed commitment to differentiation. Functionally, this translates in increased frequencies of CAFC and LTC-IC, but not CFU. $MEF^{-/-}$ HSC engraft primary recipients with similar efficiency to competitor cells, but show an advantage in secondary transplants; moreover, MEF-null cells are relatively resistant to chemotherapeutic agents and radiation injury, which is consistent with an expanded compartment of quiescent hierarchically primitive cells. Indeed, $MEF^{-/-}$ HSC have a greater proportion of cells in G_0 and reduced BrdU incorporation, and show a deficient cytokine-driven cell cycle entry *in vitro*.

As discussed for GFI1, the lack of an obvious haematopoietic phenotype in the absence of MEF may bring into question its role as a major regulator of HSC. However, higher expression levels in foetal HSC are compatible with a role in preventing HSC from exiting cell cycle at a stage where they are engaged in expansion.

Analysis of both factors further suggests that cell cycle regulation in the HSC compartment is probably dependent on several redundant pathways that contribute to its fine-tuning when in steady-state, and allow for an appropriate response to unexpected situations of increased haematopoietic demand.

Regulation of HSC survival

TEL/ETV6. *TEL* is the target of several chromosomal rearrangements that determine loss of function. The most common one is *TEL/AML1* that associates with pre B-ALL and constitutes the most common type of childhood cancer (reviewed in Zelent *et al*., 2004).

Knockout mice die *in utero* between E10.5 and E11.5 with a defective YS angiogenesis but no apparent defect in primitive blood cell production (Wang *et al*., 1997). Analysis of ESC chimaeras (Wang *et al*., 1998) revealed that TEL is specifically required for multilineage haematopoiesis in the BM, while being dispensable in the FL. There is a minimal contribution of $TEL^{-/-}$ ESC-derived cells to postnatal erythro-myeloid colony formation and splenic B cells, but progenitor formation in the BM is not sustained after the first week.

Analysis of selective TEL deficiency in committed progenitors using conditional knockout models showed that it is dispensable for most lineages, with the notable exception of megakaryocytes (Hock *et al*., 2004b). Ablation of *TEL* in the BM HSC compartment (Hock *et al*., 2004b) using the *Mx-Cre* transgene and interferon-α activation induces a transient decline in granulocyte and platelet peripheral blood counts, with no major changes in haemoglobin or peripheral blood lymphocytes, consistent with loss of the short-lived and frequently replenished fractions of mature cells. However, 4 weeks post-induction, peripheral blood counts were normal and all the haematopoietic cells expressed TEL, while the excised allele was still observable in non-haematopoietic tissues. This was accompanied by a

rapid decline in BM HSC numbers and consequent loss of progenitors; both compartments were repopulated to normal levels by TEL-expressing cells in 4 weeks. $TEL^{-/-}$ BM cells did not contribute to long-term repopulation of lethally irradiated mice in either non-competitive or competitive transplantation settings (Hock *et al*., 2004b).

These changes are compatible with a rapid loss of HSC upon *TEL* excision. Although alternative mechanisms have not been specifically assessed, the combined loss of phenotypically defined HSC and committed progenitors argues against accelerated differentiation and exhaustion due to increased proliferation. It was thus proposed that TEL is necessary for HSC survival. While not disputing this as a probable explanation, it is noteworthy that the data do not formally exclude a role for TEL in HSC (but not progenitor) specification.

In either scenario and in accordance with our hypothesis of minimal and late contribution of HSC to FL haematopoiesis, the haematopoietic effects of *TEL* loss would only be observable in the BM. Analysis of the AGM region and of the FL HSC compartment is still lacking, but should distinguish between alternative roles of TEL in HSC specification or survival. If the latter and most commonly accepted hypothesis proves correct, it is predictable that FL HSC numbers may only be mildly affected due to a positive balance of proliferation over apoptosis; $TEL^{-/-}$ FL HSC should be functionally deficient and not contribute to long-term repopulation of lethally irradiated mice.

ZFX. ZFX is the most recently characterised HSC transcriptional regulator (Galan-Caridad *et al*., 2007). Moreover, it was the first factor shown to be relevant for maintenance of ESC as well as adult stem cells, namely HSC. It is specifically required for stem cell survival. As discussed for TEL, $ZFX^{-/-}$ HSC (and ESC) show increased apoptosis, without evidence of accelerated or perturbed differentiation, or differences in proliferation. $ZFX^{-/-}$ haematopoietic progenitors did not show increased cell death.

Selective knockout of *ZFX* in the haematopoietic system revealed broadly normal YS and FL haematopoiesis. HSC numbers in the FL were mildly reduced with no quantitative change in the progenitor compartment. However, contribution of ZFX-null cells to BM haematopoiesis was severely affected with negative selection against $ZFX^{-/-}$ cells and emergence of dominant ZFX-expressing clones. This correlated with selective loss of LT and ST-HSC, but no significant change in the frequency or CFU activity of committed erythro-myeloid progenitors; the frequency of early lymphoid progenitors was reduced. ZFX-null cells made a minimal contribution to haematopoiesis upon competitive transplantation. Similar results were observed in analysing the BM of adult conditional knockouts, as well as in the BM of recipient animals where Cre recombinase was activated following transplantation, thus confirming an intrinsic regulatory mechanism.

The transcriptional programme activated in $ZFX^{-/-}$ ESC and HSC includes stress-induced and immediate-early response genes, which correlate with the increased susceptibility to apoptotic stimuli. It also includes previously uncharacterised genes, some of which may underline common and specific molecular signatures in stem cell survival and maintenance.

Concluding remarks

The functional categories discussed to contribute to HSC maintenance, namely cell survival, lineage commitment and differentiation and cell cycle entry are normally loosely referred to as self-renewal. Strictly speaking, self-renewal corresponds to the perpetuation of cellular characteristics in daughter cells after cell division and is no more synonymous with stem cells than it is with other dividing cells.

What is unique about stem cells is their capacity to generate both differentiated and stem cell progeny and to regulate both outputs so as to produce enough differentiated progeny and self-sustain a stem cell pool. This may be achieved through asymmetric cell division, but once again, asymmetrical partitioning of cellular components or fate determinants is not an exclusive of stem cells (Knoblich, 1997; Betschinger and Knoblich, 2004). Stem cell function therefore depends on adequate coordination of regulatory programmes balancing commitment and differentiation with survival or apoptosis in the context of cell division.

Once it becomes accepted that the mode of HSC division is not an alternative HSC fate *per se* but a function of extrinsic or intrinsic contexts, it easily follows that regulatory mechanisms of HSC function can be present and accessible throughout ontogeny and may act in similar ways if and when allowed to act. Returning to our original discussion on the late contribution of HSC to haematopoiesis in the foetus, it explains why loss of factors such as BMI1 elicits the same functional consequences in FL and BM HSC when tested out of developmental context, but only or more overtly manifest themselves in adult BM. Nevertheless, the divisional properties of HSC or the regulatory programmes that elicit them undoubtedly modulate HSC output and their contribution to blood formation (Lessard *et al*., 2004; Bowie *et al*., 2006, 2007).

The major challenge of stem cell biology is to define effector programmes downstream of each individual regulator and to understand how these programmes interact to promote or inhibit alternative outputs.

Acknowledgements

We thank Marella de Bruijn, Aldo Ciau-Uitz and Catherine Porcher for their critical reading of this manuscript and helpful comments and suggestions; we also thank Alexander Medvinsky for helpful discussions. Cristina Pina is a student of the Gulbenkian PhD programme in Biomedicine and is funded by Fundação para a Ciência e Tecnologia, Portugal (SFRH/BD/11812/2003). Work in Tariq Enver's laboratory is funded by the Leukaemia Research Fund and the Medical Research Council, United Kingdom.

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