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INVITED REVIEW

From hematopoietic stem cells to platelets

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Summary. Megakaryocytopoiesis is the process that leads to the production of platelets. This process involves the commitment of multipotent hematopoietic stem cells toward megakaryocyte (MK) progenitors, the proliferation and differentiation of MK progenitors, the polyploidization of MK precursors and the maturation of MK. Mature MK produce platelets by cytoplasmic fragmentation occurring through a dynamic and regulated process, called proplatelet formation, and consisting of long pseudopodial elongations that break in the blood flow. Recent insights have demonstrated that the MK and erythroid lineages are tightly associated at both the cellular and molecular levels, especially in the transcription factors that regulate their differentiation programs. Megakaryocytopoiesis is regulated by two types of transcription factors, those regulating the differentiation process, such as GATA-1, and those regulating proplatelet formation, such as NF-E2. The humoral factor thrombopoietin (TPO) is the primary regulator of MK differentiation and platelet production through the stimulation of its receptor MPL. Numerous acquired or congenital pathologies of the MK lineage are now explained by molecular abnormalities in the activity of the transcription factors involved in megakaryocytopoiesis, in the *Tpo* or *c-mpl* genes, as well as in signaling molecules associated with MPL. The recent development of MPL agonists may provide efficient agents for the treatment of some thrombocytopenias.

Keywords: endomitosis, megakaryocyte, platelets, proplatelet, transcription factors.

Introduction

Platelets are essential in hemostasis and thrombosis. Recent studies have shown that platelets also play an unexpected role in several other processes such as inflammation, innate immunity, neoangiogenesis and tumor metastasis. Each day in humans, approximately 1×10^{11} platelets are produced by the cytoplasmic fragmentation of megakaryocytes (MK), their marrow precursor cells. This enormous cell production continuing throughout the human lifespan is related to the organization of hematopoiesis that allows a regulated platelet production through the differentiation of hematopoietic stem cells (HSC) into MKs. This chapter will focus on the cellular and molecular aspects of MK differentiation from HSC with the exception of cytokine regulation.

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Cellular aspects

Platelet production derives from the commitment and differentiation of multipotent HSC. HSC are the only long-lived hematopoietic cells that are capable of regenerating all cellular components of the hematopoietic tissue by their self-renewal capacities [1,2]. During commitment decisions, HSC produce a progeny of cells, which first lose their ability to replenish themselves and then their multipotential properties. The loss of multipotency appears to occur through discrete steps leading to oligopotent progenitors and followed with monopotent progenitors committed into one cell lineage, such as the MK progenitors. This cellular hierarchy allows a huge cell amplification to happen mainly through proliferation of the hematopoietic progenitor cell compartment and thus permitting a low proliferation at the HSC compartment level.

Hematopoietic stem cells

In the mouse, HSC and multipotent progenitors (MPP) are highly enriched in a population of Lin^- c-kit^+ Sca-1^+ cells [3,4]. In this cell population, the HSC, capable of long-term hematopoietic reconstitution, are defined by markers such as Thy1.1 and the CD150 [5] and by the absence of CD34 and FLT3 [6,7]. The immediate HSC progeny, defined by a reduced self-renewal capacity, is capable only of short-term reconstitutions, and displays a similar phenotype except for the expression of both CD34 and FLT3, but not of CD150. A third step corresponds to the formation of the MPP defined by the loss of the Thy1.1 antigen expression. These past few years, the inability of stem cells to retain the Hoechst dye [8] has also been used for the characterization and purification of HSC. This cell population enriched in HSC, is called the side population (SP). However, recent evidence suggests that only a part of true HSC (about 50%) is present in SP [9].

It is generally believed that the MPP (without self-renewal capacity) give rise to a common lymphoid progenitor (CLP)

[10] and myeloid progenitor (CMP) [11] capable of yielding all myeloid lineages, including the MK lineage.

Compared with the mouse, this cell hierarchy is not as well defined in humans. HSC are defined by the expression of the CD34, CD133, Thy-1 and c-kit antigens and the absence of CD38 and of lineage markers [12]. It has been suggested that MPP were already $CD34^+CD38^+$ as other hematopoietic progenitors [13]. The recent assays using xenografts in immunodeficient mice depleted in NK cells may permit the definition of long-term and short-term reconstitutive cells for human hematopoietic tissues [14,15].

MK/Erythro progenitor (MEP)

The erythroid and megakaryocyte lineages are closely related. There is increasing evidence that in both mice and humans, they derive from a common bipotent progenitor, called either MEP or BFU-E/MK, that gives rise to colonies containing a large majority of erythroblasts and a minority of MKs [16]. The phenotype of this progenitor has been clearly assessed in the mouse (Lin^- c-kit^+ Sca-1^- $CD16/CD32^{low}CD34^{low}$) [2]. In humans, the MEP is defined as Lin^- $CD34^+CD38^+IL3Ra^-CD45RA^{-2}$, although some authors found this progenitor in the $CD34^+CD38^{low}$ Lin^- cell fraction [16]. A late bipotent E/MK progenitor that co-expresses erythroid (TER119, glycophorins) and MK (4A5, GPV) markers has been described in stress conditions in the mouse [17].

In the current scheme of hematopoietic development, the MEP derives from the CMP populations. Alternatively, a controversial model suggests that the MEP can derive directly from HSC [18,19]. Other models of stem cell commitment, not based on a linear hierarchy, have also been suggested.

MK progenitors

The MK lineage fundamentally begins at the developmental stage where the progenitor is restrictively committed towards the MK lineage. MK progenitor cells are capable of proliferating and give rise *in vitro* to MK colonies. Several clonal assays have been developed to establish a hierarchical classification of MK progenitors that is essentially based on cell proliferative capacities. BFU-MK are the most primitive MK-committed progenitors with the highest aptitude to proliferate. After 12 days for the mouse [20] and 21 days for the human [21], they give rise to colonies composed of more than 50 cells. CFU-MK have a lower proliferation capacity. They give rise to colonies composed of 3 to 50 cells in 5 days in the mouse [22] and 12 days in the human [23]. A third and more mature progenitor has been defined in the mouse and gives rise, in 2 to 3 days, to colonies composed of a few MK with a high ploidy level [24].

MK differentiation stage-specific markers

In humans, MK progenitors have been characterized by clusters of differentiation markers on their surface also present in other hematopoietic progenitors, such as the CD34, CD31 and the CD133 (Fig. 1). The expression of HLA-DR enables BFU-MK ($HLA\text{-}DR^{low}$) to be distinguished from CFU-MK ($HLA\text{-}DR^{high}$) [21].

Historically, one of the first platelet markers used to study MK differentiation in the mouse was the cytochemical marker acetylcholinesterase (AChE). AChE staining is easily detected using light microscopy and is very convenient for investigating murine megakaryocytopoiesis *in vitro*. Studies have shown the presence of a compartment of small AChE positive cells (SACHE) with a 2 N ploidy in the marrow [25]. These cells are the direct precursors of MK and differentiate into polyploid MK in a few days [26]. Most of these 2 N cells have no proliferative capacities and subsequently undergo endoreplication cycles.

In humans, the AChE staining is not specific for MK and several monoclonal antibodies directed against platelet proteins have been used. Most studies have focused on the main platelet glycoproteins, the GPIIb/IIIa ($\alpha IIb\beta 3$ integrin or CD41b) and the GPIb complex (GPIbα, GPIbβ, GPIX and GPV or CD42a, b, c and d) [27,28]. CD41a (GPIIb) or CD41b (GPIIb/IIIa complex) appear relatively specific for the MK lineage, especially in adults, although mast cells can express this integrin [29]. CD41 is present on a small fraction of marrow $CD34^+$ cells (about 3%). These $CD34^+CD41^+$ cells are enriched in MK progenitors, but they do not contain all the CFU-MK. CD41 expression precedes the detection of other major platelet proteins including CD42. However, CD42 is present on a fraction of $CD34^+CD41^+$ cells [30]. Thus, $CD34^+CD41^+CD42^-$ cells correspond to true CFU-MK whereas $CD34^+CD41^+CD42^+$ cells give rise to single MKs or clusters of less than 4 MKs. The surface expression of CD42 corresponds to a late differentiation step and is associated with a marked increase in the expression of Mpl, GPVI (collagen receptor), the $\alpha 2\beta 1$ integrin (collagen receptor), CD36 and in the detection of proteins contained in the α granules, such as PF4 or vWF. Thereafter, expression of these different platelet proteins markedly increases whereas the CD34 antigen disappears during the endomitotic process.

In the mouse, it is possible to purify CFU-MK nearly to homogeneity based on the expression of several antigens

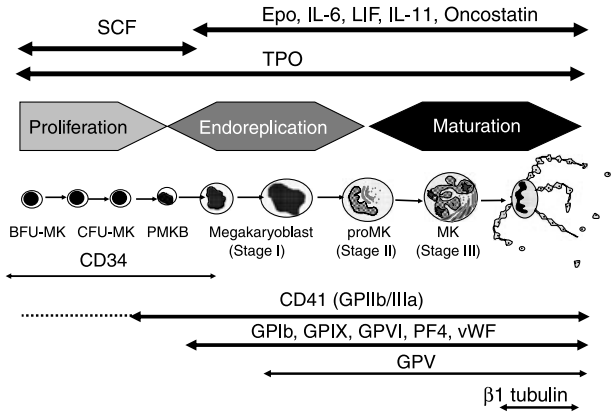

Fig. 1. Effects of cytokines and expression of differentiation markers along the human megakaryocytic differentiation.

Lin^- c-kit^+ $Sca\text{-}1^-$ $Fc\gamma R^{low}$ $IL7R\alpha^-$ $Thy1.1^-$ $CD34^{low}$ along with the presence of CD9 and CD41 [31].

Megakaryocytes and the endomitotic process

After initiating the synthesis of platelet proteins, the MK precursor begins to increase its ploidy by a process called endomitosis (Fig. 2). The megakaryocyte is one of the rare cells that are polyploid during normal differentiation, in contrast to other cells that become polyploid in response to stress. Polyploidy is a way of increasing platelet production, as the MK cytoplasm volume increases in parallel with ploidy. MK can stop DNA duplication at any stage between 2 N to 64 N, and possibly until 128 N. In humans, as well as in the majority of mammals, the modal ploidy is 16 N (about 50% of the MK). During polyploidization [32] and at the end of this process, the MKs increase the synthesis of platelet specific proteins and of important proteins for platelet formation and function [33]. The diameter of a 2 N MK is 21 ± 4 μm compared with 56 ± 8 μm for a 64 N MK [34]. This leads to an 81-fold increase in MK volume and, theoretically, in the number of platelets produced. It has been suggested that only MKs with a ploidy over 4 N are capable of forming proplatelets. However, low ploidy MKs (2 N and 4 N micro MKs) are able to shed platelets in *in vitro* cultures as well as in human malignant pathologies.

The term 'endomitosis' was originally given to the polyploidization mechanism because it was thought that mitosis was occurring without rupture of the nuclear envelope [35]. However, ultrastructural studies performed on both the cells taken from the marrow and the MKs grown in culture have allowed a partial description of the endomitotic process. Endomitosis corresponds to a mitosis that skipped anaphase B and cytokinesis [36,37]. An endomitosis, similarly to a mitosis, begins with the duplication of the centrosomes, the development of a mitotic spindle, the prophase with chromatin condensation, the rupture of the nuclear envelope, the alignment of the chromosomes on the equatorial plate during the metaphase and finally the separation of the sister chromatids at anaphase. However, the spindle of a polyploid MK is multipolar with the number of poles corresponding to the ploidy level. In MK polyploidy, the spindle remains short and does not elongate as in a normal mitosis. Chromatids move towards each pole and appear as a round mass circling each pole at anaphase, but each DNA mass remains tight as a consequence of the absence of spindle elongation. Nevertheless, a true midzone develops as during normal mitosis [38,39]. Time-lapse experiments have shown that endomitotic MKs begin the cytokinesis step (unpublished results). However, a late reversal of cytokinesis occurs, characterized by a backward movement in the daughter cells that leads to their fusion, including their nuclei. At the end of endomitosis, the MK contains a single nucleus with a single nuclear membrane. Each nuclear lobe corresponds to each pole of the multipolar spindle and their number is the direct reflection of the ploidy [38].

The cell cycle during MK polyploidization is clearly composed of a succession of G1, S, G2 and M phases, but the M phase is incomplete [35,37,40]. After M phase, MKs re-enter into G1 to initiate a subsequent cell cycle in order to duplicate their DNA. None of the molecular mechanisms suggested at the origin of the endomitotic process are involved in MK polyploidization. Polyploidization was first suggested to be due to a defect in cyclin B1 [40]. Yet, no marked defect in the expression of cyclin B1 has ever been demonstrated during endomitosis [37]. Subsequently, it was believed that Aurora B was not expressed in endomitotic MK [41–43]. However, a normally localized and functional Aurora B is present in

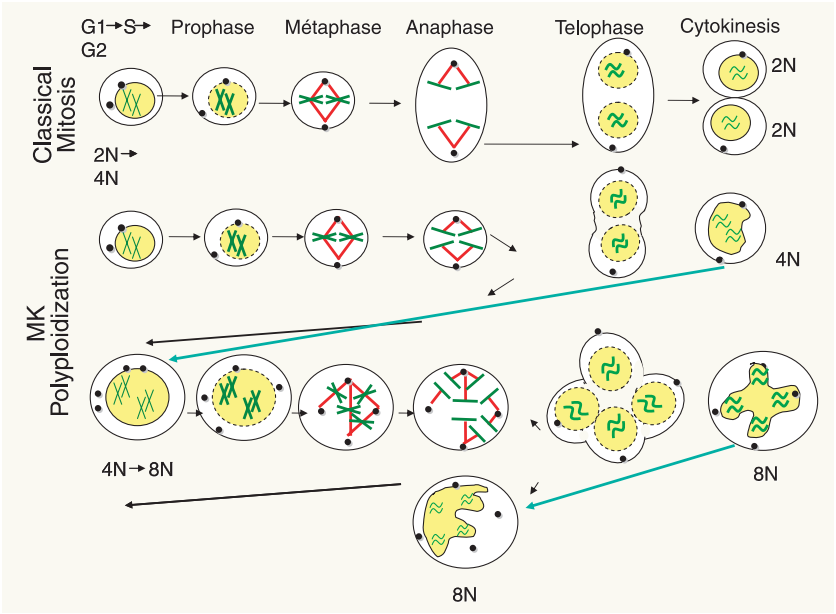

Fig. 2. Scheme of the endomitotic process.

endomitotic human MKs [44]. More recently, it has been suggested that endomitosis could be the consequence of a lack of certain chromosome passenger proteins, especially of survivin [41,43]. Although results are still controversial among laboratories, survivin seems to be normally expressed in human endomitotic MKs [44] (personal results). Endomitosis appears now to be related to a failure in cytokinesis and thus may be due rather to an abnormal depolymerization of microtubules and/or a defect in contractile forces related to the actin/myosin II complex.

In addition, the 'polyploidy' checkpoint which blocks a 4 N cell from re-entering the S phase does not operate in MK. It is noteworthy that MK express very high levels of cyclin D3 and that an ectopic increase in cyclin D1 raises the MK ploidy level [40,45]. In addition, cyclin $E^{-/-}$ mice have a marked defect in MK ploidization while the proliferation of the other hematopoietic cells is not impaired [46].

The advantages of polyploidization over a mitotic process for platelet formation are unclear at present. Polyploidization may be a way to increase protein synthesis and to modify gene expression. Indeed, all the alleles of a gene remain functional during polyploidization, thus leading to a true gene amplification [47]. Recent results suggest that polyploidization may not affect gene expression *per se*, as in the yeast, but may modify gene expression in a differentiation-related manner [32], and thus polyploidization may be directly integrated into the MK differentiation program.

Megakaryocyte and platelet shedding

At the end of their maturation, MKs form platelets by fragmentation. This process must absolutely occur in the blood circulation or otherwise the platelets would remain trapped in the marrow. The mechanism of platelet shedding is quite well understood and requires the formation of long cytoplasmic extensions (proplatelet formation) [48,49]. There are three main determinants in platelet shedding.

The demarcation membrane system (DMS)

Demarcation membranes arise from the invagination of the MK plasma membrane and constitute a membrane reservoir for proplatelet formation [50]. The DMS associates with both the microtubules and the actin filaments, prior to proplatelet formation [51], and is evaginated to form pseudopodal processes during proplatelet formation.

The microtubules

Microtubules are essential for proplatelet formation and defects in their assembly lead to an absence of proplatelet generation and thrombocytopenia. Indeed, microtubules constitute the protrusion forces that allow proplatelet formation [49,52,53]. They align into bundles to form a loop at the free end of the proplatelet [49]. It is believed that platelets only form at the free end of the proplatelet extension, but not at the swellings along the extensions. The driving force for proplatelet elongation is not microtubule polymerization but microtubule sliding [51]. Microtubules also play an important role in organelle transport in proplatelets [54].

Acto-myosin

Inhibition of actin polymerization has no effect on the extension of proplatelets, but impedes branching necessary for the amplification of platelet production. However, there is increasing evidence that the actin/myosin complex may play a role both in the early stages of proplatelet formation and in the forces allowing platelets to separate from proplatelets [55].

Platelet shedding must occur directly in the circulation. In the marrow, MKs are located in the sub-endothelium region in close contact with endothelial cells, which may be involved in MK terminal differentiation. Long cytoplasmic pseudopods may cross the endothelial barrier and enter into the marrow sinusoids. Alternatively, mature MKs also migrate through this blood barrier and are detected in the circulating blood [56]. Several authors have pointed out that MKs are present in significant numbers in the small vessels of the lungs and are trapped in the pulmonary capillary beds where they release platelets [57]. Increasing evidence indicates that proplatelet formation is tightly regulated by the stroma and the extracellular matrix (ECM). In the marrow, collagen inhibits proplatelet formation [58]. When an MK begins to migrate towards the endothelium and through the blood barrier it interacts with other components of the ECM such as fibrinogen, which may induce or increase proplatelet formation [59]. FGF4 and SDF-1 are thought to play important roles in MK marrow localization and migration, and thus proplatelet formation [60]. At the end of maturation, MKs have an altered response to SDF-1, leading to a decrease in the marrow retention force and thus facilitating their exit from the marrow [61]. This is related to a decrease in the function of the SDF-1 receptor, CXCR4, due to overexpression of RGS16, a negative regulator of G protein-coupled receptor signaling [62].

Megakaryocytes and ontogeny

Megakaryocytes can be detected very early during ontogeny. Indeed, MK progenitors together with primitive erythrocytes are present in the yolk sac (in the mouse, E7.5)[63–65]. At this early embryonic stage, the erythroid and MK lineages are tightly associated and derive from a common E/MK progenitor. This early hematopoiesis is a transient phenomenon but a similar link exists between the erythroid and MK lineages when definitive hematopoiesis emerges [65]. Platelets are observed in the fetal blood at E10.5. During human ES cell differentiation, preliminary results suggest that MK differentiation arises from a bipotent E/MK progenitor expressing erythro/MK markers [66,67]. In the mouse, it was demonstrated that CD41 is an ontogenic marker of hematopoiesis [68,69], which means it is the first hematopoietic differentiation marker for both the ES cells and embryonic hemopoiesis. Its expression seems to be

related to definitive hematopoiesis [68]. It is noteworthy that, in the embryos, CD41 is expressed by HSC emerging in sub-aortic patches [70]. Later during development, CD41 becomes restricted to the megakaryocytic lineage. In humans, CD41 is also an ontogenic marker [67,71].

During human ontogeny, MKs increase their ploidy, but in culture the majority of fetal MKs are 2 N or 4 N [72]. Preliminary evidence indicates that this may be due to a different response of fetal and adult MKs to thrombopoietin [73,74].

Molecular regulation of megakaryocytopoiesis

Numerous investigations on the molecular regulation of megakaryocytopoiesis have permitted significant progress in understanding normal MK differentiation as well as in the comprehension of congenital or acquired MK pathologies (Fig. 3). Various transcription factors regulating the expression of genes specific for the platelet/MK lineages, genes involved in the polyploidization process, and genes implicated in proplatelet formation, have been identified. In addition, major advances have been made in the characterization of the close relationship between the differentiation of the erythroid and the MK lineages.

The sequencing of MK-specific gene promoters, such as GPIIb, PF4, GPIbα, β-TG, GPIX or GPV, has revealed the presence of a consensus binding sequence (WGATAR) for the transcription factors of the GATA family, also found in erythroid-specific genes [75,76]. MK-specific gene promoters also possess a DNA consensus sequence (GGA/T) for transcription factors of the ETS family, some of them found in tandem with the GATA motifs [77]. GATA and ETS binding sites are equally important for the activity of MK gene promoters. ETS binding sites are absent from erythroid-specific genes, while other CGCC or CACC motifs are present associated with GATA binding sites.

The GATA-1 protein was first thought to be a specific regulator for erythroid-specific genes but has since been detected in MKs [78,79]. The GATA-1 knock-out leads to a lethal anemia in the embryo [80]. The directed knock-out of GATA-1 in the MK lineage has permitted the generation of viable mice presenting marked abnormalities in megakaryocytopoiesis characterized by a profound macro-thrombocytopenia with an excess of small immature MKs in the marrow and combined with an increase in cell proliferation [81]. In addition, these animals presented a significant decrease in the synthesis of all the main platelet glycoproteins.

The GATA-1 protein has to be associated with several other transcription factors in order to be functionally active. One of its binding partners, called FOG-1 (Friend of GATA-1), was identified by a yeast double-hybrid approach [82]. The knock-out of FOG-1 gives a phenotype similar to that of GATA-1 in the erythroid lineage and dramatically affects the megakaryocytic lineage (i.e. a total absence of MK progenitors), suggesting that FOG-1 associates with another partner during the early stage of the MK lineage. Several pieces of evidence suggest that this partner is GATA-2, another member of the GATA family [83]. GATA-1 forms distinct, activating or inhibitory complexes in erythroid and MK cells. In erythroid cells, the GATA-1, TAL-1, Ldb1, E2A, LMO2 complexes, which bind to bipartite E box-GATA motifs, seem to correlate with the activation of gene transcription whereas the GATA-1, GFI-1b, ETO complexes appear to mediate gene repression [84–86]. At present it is not known whether MK-specific genes are similarly regulated.

The importance of the association between GATA-1 and FOG-1 has been underscored by the study of human pathologies. Some chromosome X-linked congenital thrombocytopenia characterized by very large sized platelets (macro-platelets) and a moderate dyserythropoiesis are linked to mutations in the *GATA-1* gene at positions 205 and 218 in the interaction site of GATA-1 with FOG-1 [87]. More recently, megakaryoblastic leukemia (AML-M7) of Down's syndrome has been associated with mutations in the second exon of *GATA-1*, preventing the synthesis of a full-length GATA-1 protein, but allowing the synthesis of a shorter variant (GATA-1s) [88].

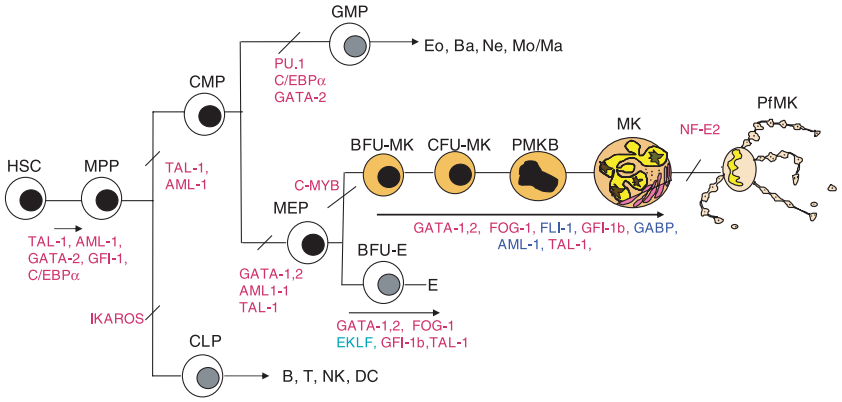

Fig. 3. Scheme of transcription factors involved in the development of megakaryocytic lineage. HSC, hematopoietic stem cell; MPP, multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte/monocyte progenitor; Eo, eosinophil; Ba, basophil; Ne, neutrophil; Mo/Ma, monocyte/macrophage; MEP, megakaryocyte/erythrocyte progenitor; BFU-MK, burst-forming unit megakaryocyte; CFU-MK, colony-forming unit megakaryocyte; PMKB, promegakaryoblast; MK, megakaryocyte; PfMK, proplatelet forming megakaryocyte.

GATA-1s, which lacks the N-terminal activation domain but can still bind to the DNA and interact with FOG-1 to the same extent as the full-length GATA-1, presents a reduced transactivation potential. These mutations are already present in the transient myeloproliferative syndrome of Down's syndrome, suggesting that (i) these mutations occur *in utero* and (ii) that a second genetic event is necessary for the development of an acute leukemia. These GATA-1 mutations were identified only in AML-M7 with trisomy 21, suggesting that GATA-1 may cooperate with a protein encoded by a gene located on chromosome 21 and thus over-expressed. It is noteworthy that GATA-1s increases the proliferation of MK progenitors but has no effect on MK maturation [89,90]. In contrast, GATA-1 lacking the binding domain of FOG has no effect on MK progenitor proliferation, but fails to induce terminal differentiation.

The bHLH transcription factor TAL-1 is also associated with GATA-1 in a large complex involved in the regulation of erythroid genes. TAL-1 is expressed in MKs [91]. As the *TAL-1* knock-out is embryonal lethal due to the absence of HSC and, in consequence, of hematopoiesis [92], it was necessary to develop an inducible knock-out model of *TAL-1* to assess its precise role in MK differentiation. Using such an approach, it was demonstrated that TAL-1 is not necessary in the adult to get a stem cell activity but in the embryo it is the main transcription factor necessary for the commitment of a mesenchymatous cell towards hematopoiesis. In addition, TAL-1 is absolutely required for the development of the erythroid and MK lineages [93]. Recent data indicate that TAL-1 plays a major role in platelet production during stress thrombopoiesis by regulating NF-E2 transcriptional activity [94].

The ETS family of transcription factors includes more than 30 different members. Four of them, ETS-1, PU.1/SPI-1, FLI-1 and GABPα, are expressed in the MK lineage and are able to transactivate different promoters of MK genes *in vitro*. Fli-1 knock-out is lethal in the embryo due to a defect in the vascular development and megakaryocytopoiesis characterized by a marked thrombocytopenia with an excess of small immature MKs undergoing apoptosis [95]. The same abnormalities were observed in the Paris Trousseau thrombocytopenia and the Jacobsen syndrome. In these syndromes, a deletion in chromosome 11q23 is always present and involves both ETS-1 and FLI-1. It has been shown that over-expression of FLI-1 can restore the dysmegakaryocytopoiesis linked to these syndromes [96]. FLI-1 cooperates with GATA-1 and FOG-1 to activate the transcription of late MK genes such as GPIX, GPIbα and PF4 [97]. In contrast to GATA-1 and the other transcription factors involved in the regulation of MK genes, FLI-1 is not significantly detected in the erythroid lineage. Furthermore, its over-expression in the murine erythroid lineage may lead to an erythroleukemia. More recently it has been shown that GABPα is also expressed in MKs and regulates essentially early MK genes such as GPIIb or c-mpl [98]. Another transcription factor of the ETS family cooperating with GATA-1 is PU-1. There is increasing evidence that GATA-1 and PU-1 have cross-antagonism at the level of an MPP and that an excess of GATA-1 compared with PU-1 may induce differentiation of a CMP towards a MEP [99,100]. Similarly, there are now data suggesting a functional cross-antagonism between FLI-1 and EKLF in the transcription of erythroid- and MK-specific genes. Preliminary evidence supports the involvement of FLI-1 in the commitment of a MEP towards the MK lineage [101].

Recently it has been shown that AML-1 (RUNX1), a DNA-binding subunit of the core binding factor (CBF) transcription complex, may cooperate with GATA-1 [102,103]. Germ-line heterozygous missense mutations, frameshifts, nonsense mutations, and the single nucleotide deletion in a single allele of the gene encoding for AML-1, all resulting in a lack of binding to the DNA, are detected in cases of familial thrombocytopenia with propensity to develop AML [104,105]. Thus, the thrombocytopenia may be related to this haplo-insufficiency. Point mutations of AML-1 have been also described in sporadic leukemia [106]. The constitutive knock-out of AML-1 in mice showed that it is essential for the establishment of a definitive hematopoiesis [107–109] and an inducible AML-1 knock-out revealed its important role in megakaryocytopoiesis [110,111]. Similarly, dysmegakaryocytopoiesis was also observed in mice deficient in CBFβ, a partner of AML-1.

$p45^{NF\text{-}E2}$ is a bZip (basic-leucine zipper protein) transcription factor. It forms a heterodimer with proteins of the MAF family to form an active NF-E2 transcription factor. $p45^{NF\text{-}E2}$ has been identified in erythroid cells, where it regulates genes involved in heme synthesis. Surprisingly, $p45^{NF\text{-}E2}$ knock-out mice die at birth from a profound thrombocytopenia with no defect in the erythroid lineage. In these $p45^{NF\text{-}E2-/-}$ mice, there is an increase in the number of MKs, presenting a marked defect in MK maturation, especially in the development of the demarcation membranes and in the distribution of α-granules [112]. More interestingly, $p45^{NF\text{-}E2-/-}$ MKs are unable to form proplatelets *in vitro*, correlating with the profound thrombocytopenia [112,113]. The partner of $p45^{NF\text{-}E2}$ in megakaryocytes is essentially MAFG and to a lesser extent MAFK [114]. The double knock-out mice $MafG^{-/-}$ $MafK^{-/-}$ have the same degree of thrombocytopenia as the $p45^{NF\text{-}E2-/-}$ mice [115]. Thus, the discovery of the target genes of $p45^{NF\text{-}E2}$ was an opportunity to characterize the genes involved in proplatelet formation. Three of the $p45^{NF\text{-}E2}$ targets are effectively involved in this process. β1-tubulin was the first to be identified and is restricted to the MK lineage and expressed at late stages of differentiation [116]. β1-tubulin knock-out mice have a thrombocytopenia with abnormal shaped platelets (absence of discoid shape) [117]. Nevertheless, complementation of β1-tubulin in $p45^{NF\text{-}E2-/-}$ MKs did not completely restore proplatelet formation. A polymorphism in the β1 tubulin gene leading to macrothrombocytopenia has been described in humans [118]. More recently, it has been shown that the 3β-HSD gene involved in estrogen synthesis is regulated by NF-E2 [119]. Normal murine MKs synthesize estradiol, which in turn regulates proplatelet formation in an autocrine or paracrine manner. Ectopic expression of the 3β-HSD in $p45^{NF\text{-}E2-/-}$

MKs partially restores their capacity to form proplatelets, while that of both β1-tubulin and 3β-HSD completely restores proplatelet formation from $p45^{NF\text{-}E2-/-}$ MKs. It has also been shown that Rab27b, a small G protein involved in granule trafficking, is a target gene of $p45^{NF\text{-}E2}$ and may also play a role in proplatelet formation [120].

$p45^{NF\text{-}E2}$ and GFI-1b, a transcription factor with a SNAG domain considered as a transcriptional repressor, are transcriptionally regulated by GATA-1[121]. GFI-1b is mainly expressed during erythroid and MK differentiation. Its knockout is lethal in the embryo due to profound anemia and thrombocytopenia, the results of marked abnormalities in erythroid and MK differentiation [122]. GF-I1b is not only regulated by, but also associated with, GATA-1 in transcription complexes [86,123].

Recently, c-MYB was shown to play an important role in megakaryocytopoiesis. A decrease in c-MYB activity augments the commitment of MPP towards the MK lineage and also enhances the cytokine response independently of MPL [124,125].

In conclusion, a lot of progress has been made over the past few years in understanding megakaryopoiesis and the mechanisms responsible for platelet production. Several fundamental questions and hypotheses on the molecular mechanisms at the basis of MK commitment, the precise role of some transcription factors and the endomitotic process still need to be addressed. Determination of the detailed transition from MK to platelets and, more precisely, characterization of the molecules regulating proplatelet formation and platelet release will be also extremely important. However, current knowledge has already permitted the characterization of the mechanisms of numerous congenital or acquired disorders of platelet production including megakaryoblastic leukemia. The recent development of novel thrombopoietic agents, such as the agonist of the TPO receptor (MPL), offers promising strategies for the treatment of thrombocytopenia.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interests.

References

1 Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiar Res* 1961; **14**: 213–22.

2 Bryder D, Rossi DJ, Weissman IL. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am J Pathol* 2006; **169**: 338–46.

3 Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science* 1988; **241**: 58–62.

4 Osawa M, Nakamura K, Nishi N, Takahasi N, Tokuomoto Y, Inoue H, Nakauchi H. *In vivo* self-renewal of c-Kit + Sca-1 + Lin(low/-) hemopoietic stem cells. *J Immunol* 1996; **156**: 3207–14.

5 Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 2005; **121**: 1109–21.

6 Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 1996; **273**: 242–5.

7 Adolfsson J, Borge OJ, Bryder D, Theilgaard-Monch K, Astrand-Grundstrom I, Silnicka E, Saraki Y, Jacobsen SH. Upregulation of Flt3 expression within the bone marrow Lin(-)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity* 2001; **15**: 659–69.

8 Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J Exp Med* 1996; **183**: 1797–806.

9 Morita Y, Ema H, Yamazaki S, Nakauchi H. Non-side-population hematopoietic stem cells in mouse bone marrow. *Blood* 2006; **108**: 2850–6.

10 Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 1997; **91**: 661–72.

11 Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 2000; **404**: 193–7.

12 Terstappen LW, Huang S, Safford M, Lansdorp PM, Loken MR. Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34 + CD38- progenitor cells. *Blood* 1991; **77**: 1218–27.

13 Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. *Proc Natl Acad Sci U S A* 2002; **99**: 11872–7.

14 Dick JE, Bhatia M, Gan O, Kapp U, Wang JC. Assay of human stem cells by repopulation of NOD/SCID mice. *Stem Cells* 1997; **15**(Suppl. 1): 199–203; discussion 204–7.

15 McKenzie JL, Gan OI, Doedens M, Dick JE. Human short-term repopulating stem cells are efficiently detected following intrafemoral transplantation into NOD/SCID recipients depleted of CD122+ cells. *Blood* 2005; **106**: 1259–61.

16 Debili N, Coulombel L, Croisille L, Katz A, Breton Gorius J, Vainchenker W. Characterization of a bipotent erythro-megakaryocytic progenitor in human bone marrow. *Blood* 1996; **88**: 1284–96.

17 Vannucchi AM, Paoletti F, Linari S, Cellai C, Caporale R, Ferrini RR, Sanchez M, Migliaccio G, Migliaccio AR. Identification and characterization of a bipotent (erythroid and megakaryocytic) cell precursor from the spleen of phenylhydrazine-treated mice. *Blood* 2000; **95**: 2559–68.

18 Adolfsson J, Mansson R, Buza-Vidas N, *et al*. Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* 2005; **121**: 295–306.

19 Forsberg EC, Serwold T, Kogan S, Weissman IL, Passegue E. New evidence supporting megakaryocyte-erythrocyte potential of flk2/flt3 + multipotent hematopoietic progenitors. *Cell* 2006; **126**: 415–26.

20 Long MW, Gragowski LL, Heffner CH, Boxer LA. Phorbol diesters stimulate the development of an early murine progenitor cell: the burst forming unit-megakaryocyte. *J Clin Invest* 1985; **76**: 431–8.

21 Briddell RA, Brandt JE, Straneva JE, Srour EF, Hoffman R. Characterization of the human burst-forming unit-megakaryocyte. *Blood* 1989; **74**: 145–51.

22 McLeod DL, Shreeve MM, Axelrad AA. Induction of megakaryocyte colonies with platelet formation in vitro. *Nature* 1976; **261**: 492–4.
23 Vainchenker W, Bouget J, Guichard J, Breton-Gorius J. Megakaryocyte colony formation from human bone marrow precursors. *Blood* 1979; **54**: 940–7.
24 Chatelain C, Debast M, Symann M. Identification of a light density murine megakaryocyte progenitor (LD-CFU-M). *Blood* 1988; **72**: 1187–92.
25 Jackson CN. Cholinesterase as a possible marker for early cells of the megakaryocytic series. *Blood* 1973; **42**: 413–21.
26 Long MW, Williams N, P MT. Immature megakaryocytes in the mouse: in vitro relationship to megakaryocyte progenitor cells and mature megakaryocytes. *J Cell Physiol* 1982; **112**: 339–44.
27 Mazur EM, Hoffman R, Chasis J, Marchesi S, Bruno E. Immunofluorescent identification of human megakaryocyte colonies using an antiplatelet glycoprotein antiserum. *Blood* 1981; **57**: 277–86.
28 Vinci G, Tabilio A, Deschamps J-F, Van Haeke O, Henri A, Guichard J, Tetteroo P, Lansdorp PM, Hercend T, Vainchenker W, Breton-Gorius J. Immunological study of in vitro maturation of human megakaryocytes. *Br J Haematol* 1984; **56**: 589–605.
29 Kirshenbaum AS, Akin C, Goff JP, Metcalfe DD. Thrombopoietin alone or in the presence of stem cell factor supports the growth of KIT(CD117)low/ MPL(CD110)+ human mast cells from hematopoietic progenitor cells. *Exp Hematol* 2005; **33**: 413–21.
30 Debili N, Issaad C, Masse JM, Guichard J, Katz A, Breton-Gorius J, Vainchenker W. Expression of CD34 and platelet glycoproteins during human megakaryocytic differentiation. *Blood* 1992; **80**: 3022–35.
31 Nakorn TN, Miyamoto T, Weissman IL. Characterization of mouse clonogenic megakaryocyte progenitors. *Proc Natl Acad Sci U S A* 2003; **100**: 205–10.
32 Raslova H, Kauffmann A, Sekkai D, Ripoche H, Larbert F, Robert T, Tronik Leroux D, Kroemer G, Debili N, Dessen P, Lazer V, Vainchenker W. Interrelation between polyploidization and megakaryocyte differentiation: a gene profiling approach. *Blood* 2006.
33 Paulus J-M. DNA metabolism and development of organelles in guinea-pig megakaryocytes: a combined ultrastructural, autoradiographic and cytophotometric study. *Blood* 1970; **35**: 298–311.
34 Deutsch VR, Tomer A. Megakaryocyte development and platelet production. *Br J Haematol* 2006; **134**: 453–66.
35 Ravid K, Lu J, Zimmet JM, Jones MR. Road to polyploidy: the megakaryocyte example. *J Cell Physiol* 2002; **190**: 7–20.
36 Nagata Y, Muro Y, Todokoro K. Thrombopoietin-induced polyploidization of bone marrow megakaryocytes is due to a unique regulatory mechanism in late mitosis. *J Cell Biol* 1997; **139**: 449–57.
37 Vitrat N, Cohen-Solal K, Pique C, *et al*. Endomitosis of human megakaryocytes are due to abortive mitosis. *Blood* 1998; **91**: 3711–23.
38 Roy L, Coullin P, Vitrat N, Hellio R, Debili N, Weinstein J, Bernheim A, Vainchenker W. Asymmetrical segregation of chromosomes with a normal metaphase:anaphase checkpoint in polyploid megacaryocytes. *Blood* 2001; **97**: 2238–47.
39 Geddis AE, Kaushansky K. Endomitotic megakaryocytes form a midzone in anaphase but have a deficiency in cleavage furrow formation. *Cell Cycle* 2006; **5**: 538–45.
40 Wang Z, Zhang Y, Kamen D, Lees E, Ravid K. Cyclin D3 is essential for megakaryocytopoiesis. *Blood* 1995; **86**: 3783–8.
41 Kawasaki A, Matsumura I, Miyagawa J, Ezoe S, Tanaka H, Terada Y, Tatsuka M, Machii T, Miyazaki H, Furukawa Y, Kanakura Y. Downregulation of an AIM-1 kinase couples with megakaryocytic polyploidization of human hematopoietic cells. *J Cell Biol* 2001; **152**: 275–88.
42 Zhang Y, Sun S, Chen WC, Kaluzhny Y, Chinnappan D, Yu G, Ravid K. Repression of AIM-1 kinase mRNA as part of a program of genes regulated by Mpl ligand. *Biochem Biophys Res Commun* 2001; **282**: 844–9.
43 Zhang Y, Nagata Y, Yu G, Nguyen HG, Jones MR, Toselli P, Jackson CW, Tatsuka M, Todokoro K, Ravid K. Aberrant quantity and localization of Aurora-B/AIM-1 and survivin during megakaryocyte polyploidization and the consequences of Aurora-B/AIM-1-deregulated expression. *Blood* 2004; **103**: 3717–26.
44 Geddis AE, Kaushansky K. Megakaryocytes express functional Aurora-B kinase in endomitosis. *Blood* 2004; **104**: 1017–24.
45 Sun S, Zimmet JM, Toselli P, Thompson A, Jackson CW, Ravid K. Overexpression of cyclin D1 moderately increases ploidy in megakaryocytes. *Haematologica* 2001; **86**: 17–23.
46 Geng Y, Yu Q, Sicinska E, Das M, Schneider JE, Bhattacharya S, Rideout WM, Bronson RT, Gardner H, Sicinski P. Cyclin E ablation in the mouse. *Cell* 2003; **114**: 431–43.
47 Raslova H, Roy L, Vourc'h C, Le Couedic JP, Brison O, Metivier D, Feunteun J, Kroemer G, Debili N, Vainchenker W. Megakaryocyte polyploidization is associated with a functional gene amplification. *Blood* 2003; **101**: 541–4.
48 Radley JM, Scurfield G. The mechanism of platelet release. *Blood* 1980; **56**: 996–9.
49 Italiano JE, Lecine P, Shivdasani RA, Hartwig JH. Blood platelets are assembled proncipally at the ends of proplatelet process produced by differentiated megakaryocytes. *J Cell Biol* 1999; **147**: 1299–342.
50 Radley JM, Haller CJ. The demarcation membrane system of the megakaryocyte: a misnomer. *Blood* 1982; **60**: 213–9.
51 Patel SR, Richardson JL, Schulze H, Kahle E, Galjart N, Drabek K, Shivdasani RA, Hartwig JH, Italiano JE Jr. Differential roles of microtubule assembly and sliding in proplatelet formation by megakaryocytes. *Blood* 2005; **106**: 4076–85.
52 Cramer EM, Norol F, Guichard J, Breton-Gorius J, Vainchenker W, Masse JM, Debili N. Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand. *Blood* 1997; **89**: 2336–46.
53 Italiano JE Jr, Shivdasani RA. Megakaryocytes and beyond: the birth of platelets. *J Thromb Haemost* 2003; **1**: 1174–82.
54 Richardson JL, Shivdasani RA, Boers C, Hartwig JH, Italiano JE Jr. Mechanisms of organelle transport and capture along proplatelets during platelet production. *Blood* 2005; **106**: 4066–75.
55 Schulze H, Korpal M, Hurov J, Kim SW, Zhang J, Cantley LC, Graf T, Shivdasani RA. Characterization of the megakaryocyte demarcation membrane system and its role in thrombopoiesis. *Blood* 2006; **107**: 3868–75.
56 Tavassoli M, Aoki M. Migration of entire megakaryocyte through the marrow-barrier. *Br J Hematol* 1981; **48**: 25–9.
57 Trowbridge EA, Martin JF, Slater DN. Evidence for a theory of physical fragmentation of megakaryocytes implying that all platelets are produced in the pulmonary circulation. *Thromb Res* 1982; **28**: 461–75.
58 Sabri S, Jandrot-Perrus M, Bertoglio J, Farndale RW, Mas VM, Debili N, Vainchenker W. Differential regulation of actin stress fiber assembly and proplatelet formation by alpha2beta1 integrin and GPVI in human megakaryocytes. *Blood* 2004; **104**: 3117–25.
59 Larson MK, Watson SP. A product of their environment: do megakaryocytes rely on extracellular cues for proplatelet formation? *Platelets* 2006; **17**: 435–40.
60 Avecilla ST, Hattori K, Heissig B, Tejada R, Liao F, Shido K, Jin DK, Dias S, Zhang F, Hartman TE, Hackett NR, Crystal RG, Witte L, Hicklin DJ, Bohlen P, Eaton D, Lyden D, de Sauvage FSR. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. *Nat Med* 2004; **10**: 64–71.
61 Riviere C, Subra F, Cohen-Solal K, Cordette-Lagarde V, Letestu R, Auclair C, Vainchenker W, Louache F. Phenotypic and functional evidence for the expression of CXCR4 receptor during megakaryocytopoiesis. *Blood* 1999; **93**: 1511–23.
62 Berthebaud M, Riviere C, Jarrier P, Foudi A, Zhang Y, Compagno D, Galy A, Vainchenker W, Louache F. RGS16 is a negative regulator of SDF-1-CXCR4 signaling in megakaryocytes. *Blood* 2005; **106**: 2962–8.

63 Xu MJ, Matsuoka S, Yang FC, Ebihara Y, Manabe A, Tanaka R, Eguchi M, Asano S, Nakahata T, Tsuki K. Evidence for the presence of murine primitive megakaryocytopoiesis in the early yolk sac. *Blood* 2001; **97**: 2016–22.

64 McGrath KE, Palis J. Hematopoiesis in the yolk sac: more than meets the eye. *Exp Hematol* 2005; **33**: 1021–8.

65 Tober JM, Koniski A, McGrath KE, Vermishetti R, Emerson R, de Mery-Bentley KK, Waugh R, Palis J. The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. *Blood* 2006.

66 Vodyanik MA, Bork JA, Thomson JA, Slukvin, II. Human embryonic stem cell-derived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. *Blood* 2005; **105**: 617–26.

67 Vodyanik MA, Thomson JA, Slukvin, II. Leukosialin (CD43) defines hematopoietic progenitors in human embryonic stem cell differentiation cultures. *Blood* 2006; **108**: 2095–105.

68 Mikkola HK, Fujiwara Y, Schlaeger TM, Traver D, Orkin SH. Expression of CD41 marks the initiation of definitive hematopoiesis in the mouse embryo. *Blood* 2003; **101**: 508–16.

69 Mitjavila-Garcia MT, Cailleret M, Godin I, Nogueisa MM, Coher-Solal K, Schiavon V, Lecluse Y, Pesteur F, Lagrue AH, Vainchenker W. Expression of CD41 on hematopoietic progenitors derived from embryonic hematopoietic cells. *Development* 2002; **129**: 2003–13.

70 Bertrand JY, Giroux S, Golub R, Klaine M, Jalil A, Boucontet L, Godin I, Cumano A. Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. *Proc Natl Acad Sci U S A* 2005; **102**: 134–9.

71 Debili N, Robin C, Schiavon V, Letestu R, Pflumio F, Mitjavila-Garcia MT, Coulombel L, Vainchenker W. Different expression of CD41 on human lymphoid and myeloid progenitors from adults and neonates. *Blood* 2001; **97**: 2023–30.

72 Hegyi E, Nakazawa M, Debili N, Navarro S, Katz A, Breton-Gorius J, Vainchenker W. Developmental changes in human megakaryocyte ploidy. *Exp Hematol* 1991; **19**: 87–94.

73 Slayton WB, Wainman DA, Li XM, Hu Z, Jolwani A, Cogle CR, Walker D, Fisher RC, Wingard JR, Scott EW, Sola MC. Developmental differences in megakaryocyte maturation are determined by the microenvironment. *Stem Cells* 2005; **23**: 1400–8.

74 Pastos KM, Slayton WB, Rimsza LM, Young L, Sola-Visner MC. Differential effects of recombinant thrombopoietin and bone marrow stromal-conditioned media on neonatal vs adult megakaryocytes. *Blood* 2006; **108**: 3360–2.

75 Ravid K, Doi T, Beeler L, Kuter DJ, Rosenberg RD. Transcriptional regulation of the rat platelet factor 4 gene : interaction between an enhancer/silencer domain and the GATA-1 site. *Mol Cell Biol* 1991; **11**: 6116–27.

76 Martin F, Prandini M-H, Thevenon D, Marguerie G, Uzan G. The transcription factor GATA-1 regulates the promoter activity of the platelet glycoprotein IIb gene. *J Biol Chem* 1993; **268**: 21606–12.

77 Lemarchandel V, Ghysdael J, Mignotte V, Rahuel C, Roméo PH. Gata and Ets *cis*-acting sequence mediate megakaryocyte-specific expression. *Mol Cell Biol* 1993; **13**: 668–76.

78 Martin DIK, Zon LI, Mutter G, Orkin SH. Expression of an erythroid transcription factor in megakaryocytic and mast cell lineages. *Nature* 1990; **344**: 444–7.

79 Roméo P-H, Prandini MH, Joulin V, Mignotte V, Prenant M, Vainchenker W, Marguerie G, Uzan G. Megakaryocytic and erythrocytic lineages share specific transcription factors. *Nature* 1990; **344**: 447–9.

80 Pevny L, Simon MC, Roberston E, Klein WH, Tsai S-F, D'Agati V, Orkin SH, Constantini F. Erythroid differentiation in chimeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. *Nature* 1991; **349**: 257–61.

81 Shivdasani RA, Fujiwara Y, McDevitt MA, Orkin SH. A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. *EMBO J* 1997; **16**: 3965–73.

82 Tsang AP, Visvader JE, Turner CA, Fujiwara Y, Yu C, Weiss MJ, Crossley M, Orkin SH. FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell* 1997; **90**: 109–19.

83 Chang AN, Cantor AB, Fujiwara Y, Lodish MB, Droho S, Crispino JD, Orkin SH. GATA-factor dependence of the multitype zinc-finger protein FOG-1 for its essential role in megakaryopoiesis. *Proc Natl Acad Sci U S A* 2002; **99**: 9237–42.

84 Rodriguez P, Bonte E, Krijgsveld J, Kolodziej KH, Guyot B, Heck AJ, Vyas P, deBoer E, Grosveld F, Strouboulis J. GATA-1 forms distinct activating and repressive complexes in erythroid cells. *EMBO J* 2005; **24**: 2354–66.

85 Schuh AH, Tipping AJ, Clark AJ, Hamlett I, Guyot B, Iborra FJ, Rodriguez P, Strouboulis J, Enver T, Vyas P, Porcher C. ETO-2 associates with SCL in erythroid cells and megakaryocytes and provides repressor functions in erythropoiesis. *Mol Cell Biol* 2005; **25**: 10235–50.

86 Goardon N, Lambert JA, Rodriguez P, Nissaire P, Herblot S, Thibault P, Dumenil D, Strouboulis J, Romeo PH, Hoang T. ETO2 coordinates cellular proliferation and differentiation during erythropoiesis. *EMBO J* 2006; **25**: 357–66.

87 Nichols KE, Crispino JD, Poncz M, White TG, Orkins SH, Maris JM, Weiss MJ. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in *GATA1*. *Nat Genet* 2000; **24**: 266–70.

88 Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, Le Beau MM, Crispino JD. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. *Nat Genet* 2002; **32**: 148–52.

89 Li Z, Godinho FJ, Klusmann JH, Garriga-Canut M, Yu C, Orkin SH. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. *Nat Genet* 2005; **37**: 613–9.

90 Kuhl C, Atzberger A, Iborra F, Nieswandt B, Porcher C, Vyas P. GATA1-mediated megakaryocyte differentiation and growth control can be uncoupled and mapped to different domains in GATA1. *Mol Cell Biol* 2005; **25**: 8592–606.

91 Mouthon M-A, Bernard O, Mitjavila M-T, Romeo P-H, Vainchenker W, Mathieu-Mahul D. Expression of tal-1 and GATA-binding proteins during human hematopoiesis. *Blood* 1993; **81**: 647–55.

92 Shivdasani RA, Mayer E, Orkin SH. Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature* 1995; **373**: 432–4.

93 Hall MA, Curtis DJ, Metcalf D, Hefanty AG, Sourris K, Robb L, Gothert JR, Jane SM, Begley CG. The critical regulator of embryonic hematopoiesis, SCL, is vital in the adult for megakaryopoiesis, erythropoiesis, and lineage choice in CFU-S12. *Proc Natl Acad Sci U S A* 2003; **100**: 992–7.

94 McCormack MP, Hall MA, Schoenwaelder SM, Zhao Q, Ellis S, Prentice JA, Clarke AJ, Slater NJ, Salmon JM, Jackson SP, Jane SM, Curtis DJ. A critical role for the transcription factor Scl in platelet production during stress thrombopoiesis. *Blood* 2006; **108**: 2248–56.

95 Hart A, Melet F, Grossfeld P, Chien K, Jones C, Tunnacliffe A, Favier R, Bernstein A. Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. *Immunity* 2000; **13**: 167–77.

96 Raslova H, Komura E, Le Couedic JP, Larbret F, Debili N, Feunteun J, Danos O, Albagli O, Vainchenker W, Favier R. FLI1 monoallelic expression combined with its hemizygous loss underlies Paris-Trousseau/Jacobsen thrombopenia. *J Clin Invest* 2004; **114**: 77–84.

97 Wang X, Crispino JD, Letting DL, Nakazawa M, Poncz M, Blobel GA. Control of megakaryocyte-specific gene expression by GATA-1

and FOG-1: role of Ets transcription factors. *EMBO J* 2002; **21**: 5225–34.
98 Pang L, Xue HH, Szalai G, Wang X, Wang Y, Watson DK, Leonard WJ, Blobel GA, Ponez M. Maturation stage-specific regulation of megakaryopoiesis by pointed-domain Ets proteins. *Blood* 2006; **108**: 2198–206.
99 Nerlov C, Querfurth E, Kulessa H, Graf T. GATA-1 interacts with the myeloid PU.1 transcription factor and represses PU.1-dependent transcription. *Blood* 2000; **95**: 2543–51.
100 Zhang P, Zhang X, Iwama A, Smith KA, Mueller BU, Narravula S, Torbett BE, Orkin SH, Tenen DG. PU.1 inhibits GATA-1 function and erythroid differentiation by blocking GATA-1 DNA binding. *Blood* 2000; **96**: 2641–8.
101 Starck J, Cohet N, Gonnet C, Sarrazin S, Doubeikovskaia Z, Doubeikovski A, Verger A, Duterque-Coquillaud M, Morle F. Functional cross-antagonism between transcription factors FLI-1 and EKLF. *Mol Cell Biol* 2003; **23**: 1390–402.
102 Waltzer L, Ferjoux G, Bataille L, Haenlin M. Cooperation between the GATA and RUNX factors Serpent and Lozenge during Drosophila hematopoiesis. *EMBO J* 2003; **22**: 6516–25.
103 Elagib KE, Racke FK, Mogass M, Khetawat R, Delehanty LL, Goldfarb AN. RUNX1 and GATA-1 coexpression and cooperation in megakaryocytic differentiation. *Blood* 2003; **101**: 4333–41.
104 Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, Ratajezak J, Resende IC, Haworth C, Hock R, Loh M, Felix C, Roy DC, Busque L, Kurnit D, Willman C, Gewirtz AM, Speck NA, Bushweller JH, Li FP, *et al*. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet* 1999; **23**: 166–75.
105 Heller PG, Glembotsky AC, Gandhi MJ, Cummings CL, Pirola CJ, Marta RF, Kornblihu LI, Drachman JG, Molinas FC. Low Mpl receptor expression in a pedigree with familial platelet disorder with predisposition to acute myelogenous leukemia and a novel AML1 mutation. *Blood* 2005; **105**: 4664–70.
106 Harada H, Harada Y, Niimi H, Kyo T, Kimura A, Inaba T. High incidence of somatic mutations in the AML1/RUNX1 gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. *Blood* 2004; **103**: 2316–24.
107 Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 1996; **84**: 321–30.
108 Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. *Proc Natl Acad Sci U S A* 1996; **93**: 3444–9.
109 North T, Gu TL, Stacy T, Wang Q, Howard L, Binder M, Marin-Padilla M, Speck NA. Cbfa2 is required for the formation of intra-aortic hematopoietic clusters. *Development* 1999; **126**: 2563–75.
110 Ichikawa M, Asai T, Saito T, Seo S, Yamazaki I, Yamagata T, Mitani K, Chiba S, Ogawa S, Kurokawa M, Hirai H. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. *Nat Med* 2004; **10**: 299–304.
111 Sun W, Downing JR. Haploinsufficiency of AML1 results in a decrease in the number of LTR-HSCs while simultaneously inducing an increase in more mature progenitors. *Blood* 2004; **104**: 3565–72.
112 Shivdasani RA, Rosenblatt MF, Zucker-Franklin D, Jackson CW, Hunt P, Saris JM, Orkin SH. Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. *Cell* 1995; **81**: 695–704.
113 Lecine P, Villeval JL, Vyas P, Swencki B, Xu Y, Shivdasani R. Mice lacking transcription factor NF-E2 provide *in vivo* validation of the proplatelet model of thrombocytopoiesis and show a platelet production defect that is intrinsic to megakaryocytes. *Blood* 1998; **92**: 1608–16.
114 Shavit JA, Motohashi H, Onodera K, Akasaka J, Yamamoto M, Engel JD. Impaired megakaryopoiesis and behavioral defects in mafG-null mutant mice. *Genes Dev* 1998; **12**: 2164–74.
115 Onodera K, Shavit JA, Motohashi H, Yamamoto M, Engel JD. Perinatal synthetic lethality and hematopoietic defects in compound mafG::mafK mutant mice. *EMBO J* 2000; **19**: 1335–45.
116 Lecine P, Italiano JEJ, Kim SW, Villeval JL, Shivdasani RA. Hematopoietic-specific beta 1 tubulin participates in a pathway of platelet biogenesis dependent on the transcription factor NF-E2. *Blood* 2000; **96**: 1366–73.
117 Schwer HD, Lecine P, Tiwari S, Italiano JEJ, Hartwig JH, Shivdasani RA. A lineage-restricted and divergent beta-tubulin isoform is essential for the biogenesis, structure and function of blood platelets. *Curr Biol* 2001; **11**: 579–86.
118 Freson K, De Vos R, Wittevrongel C, Thys C, Defoor J, Vanhees L, Vermylen J, Peerlinck K, Van Geet C. The TUBB1 Q43P functional polymorphism reduces the risk of cardiovascular disease in men by modulating platelet function and structure. *Blood* 2005; **106**: 2356–62.
119 Nagata Y, Yoshikawa J, Hashimoto A, Yamamoto M, Payne AH, Todokoro K. Proplatelet formation of megakaryocytes is triggered by autocrine-synthesized estradiol. *Genes Dev* 2003; **17**: 2864–9.
120 Tiwari S, Italiano JE Jr, Barral DC, Mules EH, Novak EK, Swank RT, Seabra MC, Shivdasani RA. A role for Rab27b in NF-E2-dependent pathways of platelet formation. *Blood* 2003; **102**: 3970–9.
121 Huang DY, Kuo YY, Chang ZF. GATA-1 mediates auto-regulation of Gfi-1B transcription in K562 cells. *Nucleic Acids Res* 2005; **33**: 5331–42.
122 Saleque S, Cameron S, Orkin SH. The zinc-finger proto-oncogene Gfi-1b is essential for development of the erythroid and megakaryocytic lineages. *Genes Dev* 2002; **16**: 301–6.
123 Schipper LF, Brand A, Reniers N, Melief CJ, Willemze R, Fibbe WE. Differential maturation of megakaryocyte progenitor cells from cord blood and mobilized peripheral blood. *Exp Hematol* 2003; **31**: 324–30.
124 Emambokus N, Vegiopoulos A, Harman B, Jenkinson E, Anderson G, Frampton J. Progression through key stages of haemopoiesis is dependent on distinct threshold levels of c-Myb. *Embo J* 2003; **22**: 4478–88.
125 Metcalf D, Carpinelli MR, Hyland C, Mifsud S, Dirago L, Nicola NA, Hilton DJ, Alexander WS. Anomalous megakaryocytopoiesis in mice with mutations in the c-Myb gene. *Blood* 2005; **105**: 3480–7.