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REVIEW ARTICLE

Megakaryocytes and beyond: the birth of platelets

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Summary. Megakaryocytes are highly specialized precursor cells that differentiate to produce blood platelets via intermediate cytoplasmic extensions known as proplatelets. Recent advances in the understanding of megakaryocyte differentiation and platelet formation rely on a combination of genetic and cell biological studies with detailed structural analysis of cultured cells. Visualization of sequential steps in endomitosis has expanded our views on how megakaryocytes acquire polyploid DNA content, whereas studies in mouse models of platelet disorders provide clues into transcriptional pathways and those leading to the assembly of platelet-specific secretory granules. The experimental findings forge stronger links between cellular processes and molecular mechanisms, while observation of the underlying morphologic events in beginning to yield insights into the cytoskeletal mechanics of proplatelet formation. Here we review salient aspects of the emerging appreciation of the cellular and molecular basis of thrombopoiesis.

Keywords: megakaryocyte, platelet assembly, platelet biogenesis, proplatelet, thrombopoiesis.

Although the origin of blood platelets from bone marrow megakaryocytes (MKs) was recognized early in the 20th century, the mechanisms by which terminally differentiated MKs release their platelet load into the circulation have been more elusive. Wright commented on 'the detachment of plate-like fragments or segments from pseudopods' of MKs in 1906 [1], but the MK pseudopodia known as proplatelets [2] were not characterized in any detail until much later [3–6]. Early observations, although heavily reliant on MK ultrastructure alone, gradually led to the idea that platelet release entails reorganization of the MK cytoplasm into an extensive pseudopodial network that harbors nascent platelets and releases them into bone marrow sinusoids. Alternative models that invoked preformation of 'platelet territories' within the maturing MK cytoplasm retained credibility for some time, but the weight of experimental evidence now supports proplatelet-based mechanisms for platelet biogenesis and release. The discovery of thrombopoietin (Tpo) in 1994 permitted expansion of MKs in culture, enhanced the ability to combine structural studies with biochemical analysis, and renewed interest in the cellular and molecular basis of proplatelet formation. A number of mouse models of platelet disorders have since yielded cohesive genetic insights into thrombopoiesis. Here we attempt to review historic and recent studies that inform current concepts in platelet biogenesis. Although the field is still young, with many more questions than answers, the present synthesis suggests that deeper insights into the processes of MK maturation and platelet release will be forthcoming. This review focuses on the cell biological pathways in late MK differentiation.

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Regulation of unique aspects of megakaryocyte differentiation

MK development and platelet formation are regulated by a multitude of cytokines, principally Tpo, which is produced in the liver and marrow stroma [7]. Interleukin (IL)-3, IL-6, IL-11, and the c-kit ligand may also regulate MK ontogeny but probably function mainly in concert with Tpo [8]; knockout mice have not demonstrated essential roles in thrombopoiesis for cytokines other than Tpo. In contrast, mice lacking Tpo or its surface receptor c-Mpl have about 15% of the normal platelet count but show no abnormalities in platelet or MK structure [9,10]. These observations reinforce the notion that Tpo serves predominantly to expand cell numbers and may be dispensable for terminal MK differentiation or proplatelet morphogenesis [11]; there is even a suggestion that Tpo inhibits proplatelet formation *in vitro* [12,13].

Early in MK ontogeny, Tpo drives mononuclear MK precursors to undergo endomitosis and become polyploid; human and rodent MKs typically attain a DNA content of at least 16N [14–16]. This presumably facilitates the increase in cell mass required to assemble hundreds of individual platelets, and a recent study that used fluorescent probes to visualize newly synthesized nuclear RNAs indeed demonstrates that all the alleles of MK-expressed genes present in polyploid cells are transcriptionally active [17]. Endomitosis results from prematurely terminated mitoses in which the nuclear envelope breaks down with each cycle and cells complete anaphase A [18,19].

However, spindle poles fail to move outward during anaphase B, telophase and cytokinesis are aborted, and the nuclear envelope reassembles around the entire set of sister chromatids, forming a single enlarged, multilobed nucleus. Cytoplasmic maturation is initiated during endomitosis and accelerates significantly after all DNA synthesis has ceased [14,15,20].

The signals that allow MKs to circumvent the typically tight coupling of DNA replication and cell division remain unknown. Studies in cell lines [21,22] support the idea that endomitosis results from reduced activity of mitosis promoting factor (MPF), a multiprotein complex between cdc2 and cyclin B1, whereas other studies point to a driving role for cyclin D3 [23]. Although yeast strains with inhibited cyclin B or cdc2 undergo an additional round of DNA replication without cytokinesis [24,25], cultured primary MKs reveal normal cyclin B1 and cdc2 levels and functional mitotic kinase activity in cells undergoing endomitosis [26]. Perhaps the most significant observations concern AIM-1, a serine/threonine kinase of the Aurora family that is implicated in aspects of mitosis and shows significantly reduced levels as MKs begin endomitosis [27,28]. Artificial suppression of AIM-1 induces polyploidy in MK cell lines, whereas its activation suppresses the process [28].

Functionally, the regulators of endomitosis must target relevant effector pathways, including microtubule-based forces during anaphase B. This process normally involves sliding of antiparallel interdigitating non-kinetochore (polar) microtubules past each other [29]. The mitotic kinesin-like protein 1 localizes at regions of overlapping microtubules during anaphase B and can slide microtubules past one another *in vitro* [30]. Thus, the lack of spindle pole separation during endomitosis may result from a failure to align overlapping microtubules correctly and/or from absence of signals that localize or activate the kinesin motor molecule that provides the requisite force. It will be interesting to investigate the relation, if any, between AIM-1 kinase and these cellular events.

Further MK maturation results in the accumulation of distinctive cytoplasmic components: platelet-specific proteins, organelles, and the demarcation membrane system (DMS), an elaborate network of membrane channels composed of flattened cisternae and tubules [31]. The DMS can be labeled with cell-impermeable tracers such as ruthenium red and tannic acid [32–34], which implies that it is derived from the MK plasma membrane and retains contact with the cell exterior. Although the DMS was once thought to define preformed 'platelet territories' within the MK cytoplasm [31,35], the evidence is now more consistent with its primary function as a membrane reserve for proplatelet formation. Thus, 'invagination membrane system' may reflect a more accurate designation than DMS [36] and the open canalicular system (OCS) of mature platelets, which functions as a channel for granule secretion, may represent a remnant of this unique membranous network. MKs also contain a dense tubular system (DTS) [37] that corresponds to the site of platelet prostaglandin synthesis [38] and fails to stain with membrane tracers, indicating absence of direct contact with the external milieu.

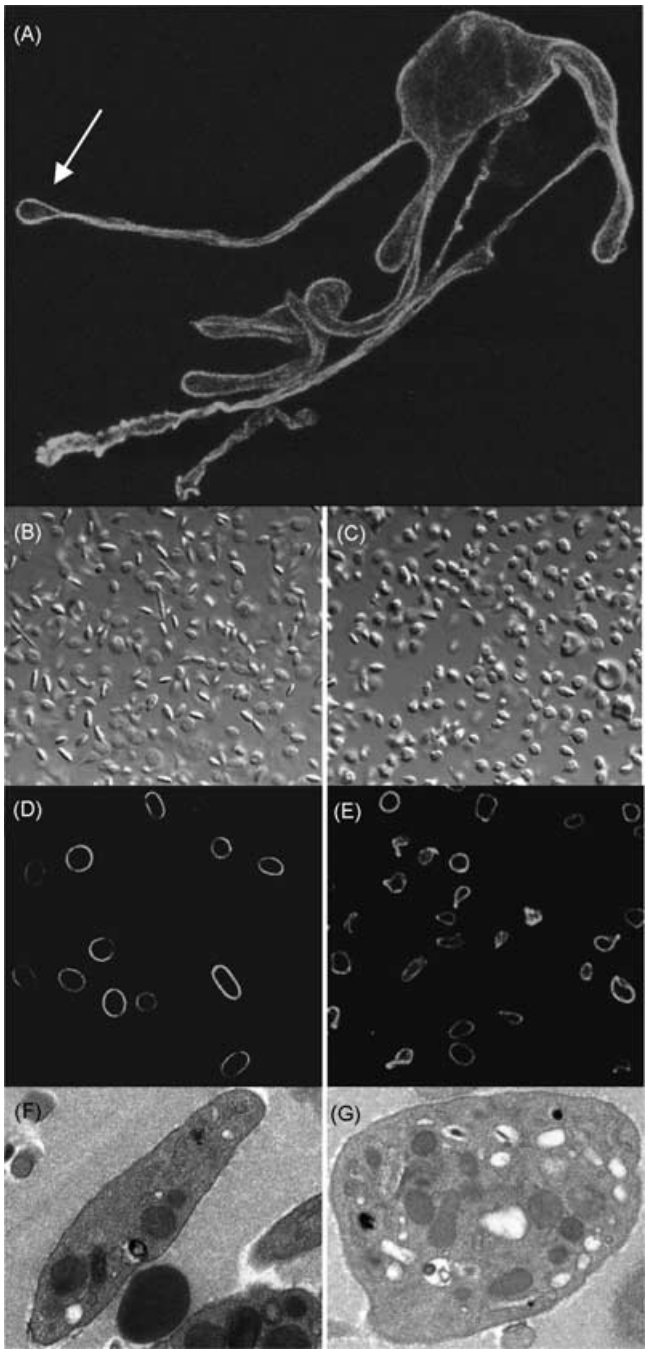
Steps that precede platelet release

The biogenesis of secretory granules is a cardinal feature of thrombopoiesis. The most abundant are α-granules, 200–500-nm spheres that have a dense central nucleus within a finely granular matrix and contain proteins essential for platelet adhesion. Dense granules are 200–300 nm in diameter, characterized by a clear halo encircling an electron opaque core, and contain small molecules such as serotonin, epinephrine and ADP. Both types of granules originate from the Golgi complex, are present in maturing MKs from early stages [39,40], and acquire their contents through a combination of endogenous synthesis and uptake of plasma components by receptor-mediated endocytosis and pinocytosis [41,42]. Endogenous proteins such as platelet factor 4 (PF4), β-thromboglobulin, and von Willebrand factor are detected in MKs earlier than endocytosed proteins such as fibrinogen. Several proteins critical to platelet function are incorporated in the limiting membranes of both α- and dense granules, including P-selectin and the $\alpha_{IIb}\beta_3$ integrin, whereas dense granule membranes uniquely express the marker granulophysin [40]. Ultrastructural studies reveal the presence of multivesicular bodies (MVBs) that probably reflect intermediate structures in the biogenesis of mature α-granules [43]. There is also some evidence that MVB morphology evolves over time [43] and that they transiently house components of α- and dense granules, so they may serve both to assemble and sort these two granule classes [40]. The 30–70-nm vesicles present in MVBs contain endogenous and endocytosed material, as is the case in other cell types, and they are sometimes seen in the periphery of platelet α-granules. The topic of secretory granule biogenesis was recently reviewed thoroughly and succinctly [44].

The signals that promote dense-granule assembly are largely understood through genetic analysis of platelet storage-pool diseases in mice and the results highlight the regulation of vesicular traffic (reviewed in [45]). Investigation into the molecular basis of the Hermansky–Pudlak syndrome has been especially revealing, and to date 11 mouse and six human genes involved in this disease have been identified at the molecular level [46]. The *mocha* and *pearl* mutants harbor defects in the δ and β3a subunits, respectively, of the adaptor protein (AP)-3, which participates in clathrin-mediated budding of vesicles from the *trans*-Golgi network. Several newly identified gene products appear to interact with the same machinery and with each other, but as yet their biochemical characterization is limited. *pallid* mice carry mutations in a novel gene whose product interacts physically with syntaxin-13, a t-SNARE protein that localizes to endosomal membranes and mediates vesicle fusion [47]. The products of the mouse *ruby-eye* and *ruby-eye2* loci interact with each other in a novel complex that governs biogenesis of at least two lysosome-related organelles, platelet dense bodies and melanosomes [46]. Thrombocytopenia in the *gunmetal* mouse results from reduced activity of the enzyme (geranylgeranyl transferase) that attaches lipid groups to the Rab family of small Ras-like GTPases [48]. One or more Rab proteins are therefore required to avoid the mutant

phenotype, which includes disorganized MK internal membranes, reduced granule numbers, and impaired cellular retention of α-granule proteins [49].

The sum of these genetic studies reveals a close relationship between mechanisms that generate lineage-specific organelles in MKs and melanocytes, while mutations in some critical genes also result in leukocyte or neuronal anomalies. Thus, the relatively recent evolution of platelets in mammals [50] appears to have exploited cellular mechanisms that may originally have served essential roles in other cell lineages. Indeed, many of the genes affected by mutations in humans and mice with variants of the Hermansky–Pudlak syndrome are expressed widely, yet the phenotype is restricted to a few cell types, including platelets and pigmented cells of the skin and retina [46]. Along these lines, it is interesting to consider the extent to which proplatelet morphogenesis resembles neuronal outgrowth and to speculate on the likely overlap between the underlying cell biological mechanisms.



Proplatelets and the site of platelet release *in vivo*

Pseudopodial proplatelet extensions (Fig. 1A), which constitute the essential intermediate structures in platelet release, have been observed extending into bone marrow sinusoids [51–53] and in culture they generate particles with many or all of the properties of blood platelets [11,54,55]. Proplatelets are recognized in many mammalian species [5,13,56,57] and their absence correlates with thrombocytopenia in genetic mouse models [58–60]. Although proplatelets were first recognized in scanning EM images of bone marrow sinuses [2,3,61,62], recent understanding of their morphogenesis derives largely from studies on cultured primary MKs. Transformation of the MK cytoplasm initiates with erosion at one pole of the cell and subsequently concentrates virtually all of the cellular contents into proplatelet extensions [55]. Initially thick pseudopodia elongate into thin tubules of 2–4 μm diameter that develop periodic densities along their length and undergo repeated, dynamic cycles of extension, retraction, bending, and branching. The nucleus is eventually surrounded by an arborized network of proplatelet processes, and rapid retraction separates individual or short chains of proplatelet fragments from the cell body.

A gradient of MK maturity is observed to extend toward bone marrow sinusoids [51,53]. Accordingly, mature MKs probably migrate into the close proximity of sinus endothelial cells and thus enable projecting proplatelets to enter the vascular space. Although young MKs contain the chemokine receptor CXCR4

Fig. 1. Proplatelet formation and the defects resulting from absence of β1 tubulin. (A) Proplatelet formation observed in a cultured murine megakaryocyte, with the location and roles of the microtubule cytoskeleton highlighted through immunofluorescent staining of β tubulin. Note the early network of thick pseudopodia that precedes elaboration of thinner proplatelets, one of which is evident in the top left of this micrograph. Microtubules with a morphology characteristic of the platelet marginal band (white arrow) are only observed at the ends of mature proplatelet extensions. (B,C) Differential interference contrast micrographs of wild-type (B) and β1 tubulin-null (C) platelets, demonstrating the contrast between normal discoid or elliptical shape (B) and the spherocytosis (C) that characterizes β1 tubulin-null platelets as a result of harboring a thin and defective marginal microtubule band. (D,E) Confocal α-tubulin immunofluorescence micrographs of wild-type (D) and β1 tubulin-null (E) platelet cytospin preparations, illustrating the contrast between the smooth contours seen in normal platelets and the misshapen, kinked marginal bands formed in the absence of β1 tubulin. The exposure time is longer for panel E than for panel D to account for the substantially reduced microtubule polymer content of β1 tubulin-null platelets. (F,G) Thin-section transmission electron micrographs of wild-type (F) and β1 tubulin-null (G) platelets, showing that besides a change in cell shape, absence of β1 tubulin does not result in abnormal platelet contents.

and respond to its ligand stromal cell-derived factor 1 (SDF-1) in chemotaxis assays [63], neither mature MKs nor platelets respond to SDF-1, suggesting attenuation of CXCR4 signaling pathways late in MK development. This could provide one mechanism for retaining immature MKs in the marrow and later permitting mature MKs to migrate toward endothelial cells and liberate platelets in the circulation [64,65].

It is unclear whether MKs release fully formed individual platelets or proplatelet chains within the bloodstream. Whole or fragmented MKs are also known to circulate [66–70] and it is conceivable that platelet maturation terminates in blood vessels [71], specifically in the first capillary beds encountered in transit, i.e. in the lung. MKs have been seen in pulmonary vessels [67,72–74], and platelet counts are reportedly higher in pulmonary artery blood than in the aorta [75]. However, the fraction of platelets first released in the lung is unlikely to be significant because MKs and their naked nuclei, which are readily detected in the marrow [4,20], are rarely observed in the mouse lung [76] after stimulation of thrombopoiesis. Moreover, two independent findings suggest that neither the marrow environment nor the pulmonary circulation is essential for platelet release *per se*. First, cultured MKs can engender platelets *in vitro* [11,58,77]. Second, the spleen can be a significant site of platelet production in bone marrow failure states, at least in rodents [78] and probably in other species, including man. However, these observations do not preclude an important role for stromal or other cell-autonomous elements in accelerating or otherwise influencing thrombopoiesis, as suggested in some studies [13].

Role of the cytoskeleton in proplatelet morphogenesis

Drugs that depolymerize microtubules, such as nocodazole or vincristine, block proplatelet formation [6,36,56], and close inspection of proplatelet-producing MKs provides clues as to how microtubules mediate platelet development [55]. In the blunt pseudopodia formed in initial stages of proplatelet formation, microtubules consolidate into thick cortical bundles situated just beneath the plasma membrane. As the pseudopodia elongate and become thinner, linear microtubule arrays form the core of the cytoplasmic extensions. The distal end of each proplatelet stalk contains a microtubule bundle that forms several peripheral loops before reentering the shaft to form a teardrop-shaped structure; the mechanism of microtubule coiling remains unclear. Thus, a simple model where *de novo* microtubule assembly at the tip drives proplatelet elongation is unlikely because microtubules in the bulbous swellings appear to lack the free ends required for tubulin polymerization. Indeed, proplatelet shafts can even elongate while the tip is tethered, which suggests a role for microtubule sliding in this process (J.E. Italiano, unpublished data). Besides their role in proplatelet elongation, microtubules probably provide the tracks to transport mitochondria, granules and other vesicular organelles from the cell soma into nascent platelets. However, the relative importance of the actin and microtubule cytoskeletons in this function is unresolved.

Platelet assembly occurs continuously as an intrinsic feature of proplatelet morphogenesis and is completed at the ends of proplatelets, the only sites that house a microtubule coil similar in structure and dimensions to the platelet marginal band [55]. Efficient thrombopoiesis therefore requires a large number of free proplatelet ends, which are amplified in number through a dynamic mechanical process that bends the shaft of an elongating proplatelet and allows a new branch to emerge. This process probably depends on the actin cytoskeleton, as actin filament assemblies are concentrated at branch points and cytochalasins, which disrupt actin assembly, abolish proplatelet branching [6,55,56]. Proplatelet bending and branching may be powered by the actin-based molecular motor myosin. Each myosin molecule has two heads and a long, rod-like tail that functions to assemble myosin monomers into bipolar filaments. Interestingly, a mutation in the tail domain of the human *non-muscle myosin heavy chain 9* (*MYH9*) gene results in the May–Hegglin anomaly [79–81], a rare inherited human disorder characterized by thrombocytopenia, giant platelets with dispersed microtubules, and leukocyte inclusions [82,83]. MYH9 makes up 2–5% of total platelet protein and, as a class II myosin, is an ATPase motor that binds to actin filaments and generates force for contraction. Although the precise role of MYH9 and other myosins in proplatelet morphogenesis is unknown, the May–Hegglin macrothrombocytopenia suggests a crucial requirement for filamentous myosin II forms in efficient thrombopoiesis and regulation of platelet size.

Another essential cytoskeletal protein is β1 tubulin, a divergent and exquisitely lineage-specific mammalian β-tubulin isoform. β1-tubulin is a major component of the proplatelet cytoskeleton and of the platelet marginal band [58,84], which contains a single microtubule coiled 8–12 times in the cytoplasmic periphery just beneath the plasma membrane [62,85]. β1 tubulin knockout mice are thrombocytopenic as a result of the greatly reduced ability of their MKs to generate proplatelets. Moreover, β1 tubulin-null platelets (Fig. 1) are uniformly spherical, probably as a direct consequence of possessing structurally defective marginal bands that contain only two to three coils instead of the usual 8–12 coils [60]. The marginal microtubule band is fragile and frequently kinked or misshapen (Fig. 1D,E). Platelet spherocytosis is also a feature of human giant platelet disorders, including the May–Hegglin and gray platelet syndromes, where disorganized microtubule bundles often substitute for the planar marginal band [86]. Whereas these observations emphasize the role of the microtubule cytoskeleton in maintaining discoid platelet shape, the properties that endow β1 tubulin with an ability to generate tight coils or MYH9 and other molecules to determine platelet size remain unknown.

Besides the crucial role of the glycoprotein (GP)Ib-IX-V surface protein complex in mediating platelet attachment to damaged vascular walls, this complex is also an essential structural component of the resting platelet cytoskeleton. Filamin molecules extend through pores in the spectrin network, connecting underlying actin filaments to the cytoplasmic tail of the GPIbα chain on the cell surface [87–89]. The importance of this linkage is revealed by the abnormal morphology and

extreme fragility of platelets observed in the Bernard–Soulier syndrome (BSS) [90], which results from dysfunction or lack of the GPIb-IX-V complex, usually due to a mutation in the GPIb α chain [91]. BSS is characterized by severe bleeding, thrombocytopenia, and giant spherical platelets; MKs show abnormal distribution of the DMS, inhomogeneous granule distribution, and randomly distributed microtubules [92,93]. This phenotype is recapitulated in GPIbα-deficient mice [94] and depends on the C-terminal cytoplasmic half of the protein [95]. These findings point to an important but mechanistically unclear role for the GPIb-IX-V complex in MK differentiation and, probably, in proplatelet morphogenesis.

Is platelet release a form of apoptosis?

Mature MKs derived from cultured CD34+ cells display apoptotic features [96,97], perhaps as a reflection of cellular senescence. However, because proplatelet formation represents the terminal phase in the MK life cycle, parallels with programmed cell death have been suggested at many levels [98]. Indeed, transgenic mice overexpressing the anti-apoptotic factor Bcl-2 in MKs show reduced platelet counts [99], as do knockout mice lacking the proapoptotic gene Bim [100]. Similarly, overexpression of Bcl-xL (another anti-apoptotic protein) in MKs delays platelet recovery after experimentally induced thrombocytopenia [101]. Although the reasons for reduced platelet numbers in these diverse mouse models are poorly understood, it is certainly reasonable to speculate that some components of the machinery that normally regulates or drives apoptosis also participate in MK fragmentation.

de Botton and colleagues recently demonstrated that prior to extending proplatelets, MKs undergo proteolytic maturation of procaspases 3 and 9, cleave the caspase substrate gelsolin, and release mitochondrial cytochome c, a hallmark feature of apoptosis [102]. Although these observations suggest mechanistic parallels between programmed cell death and thrombopoiesis, it is important to distinguish clearly between platelet release and cell death. Terminal as the fragmentation process may be, its end-products are live platelets that require intact mitochondria and preservation of some amount of proteins such as gelsolin, so proplatelet formation cannot represent indiscriminate apoptosis. Indeed, the de Botton study suggests that caspase activation may be compartmentalized within the MK cytoplasm and that proplatelet membranes in particular fail to express surface phosphatidylserine [102]. Thus, if portions of the apoptotic program are recruited to facilitate thrombopoiesis, some mitochondria and other cellular elements must be protected to a considerable degree to permit platelet viability and function. Further elucidation of how effectors of apoptosis signal to the proplatelet apparatus will be of obvious interest.

Transcriptional control of platelet formation and release

Foremost among transcription factors that regulate platelet release is the basic-leucine zipper heterodimer NF-E2. Mice lacking either the hematopoietic-specific p45 subunit or two of the 18–20 kDa small-Maf subunits show a MK maturation arrest, absence of proplatelet formation, and lethal thrombocytopenia; early MK differentiation is unaffected [59,103]. There is a considerable increase in expression of the p45 subunit as MKs approach terminal maturity and it is likely that NF-E2 coordinates a broad transcriptional program to drive platelet biogenesis. Candidate transcriptional target genes include β1 tubulin, which is absent from NF-E2-deficient MKs [104], regulators of inside-out signaling through the $\alpha_{IIb}\beta_3$ integrin [105], thromboxane synthase [106,107], and selected Rab GTPases implicated in intracellular organelle traffic (S. Tiwari and R.A.Shivdasani, unpublished data). Understanding how these diverse components orchestrate platelet formation and release constitutes a central challenge in the field.

NF-E2-null MKs complete endomitosis and develop an abundant albeit defective cytoplasm, but fail to elaborate proplatelets. In contrast to the almost exclusively late role for NF-E2 in the MK life cycle, the transcription factor GATA1 has essential functions at multiple distinct stages. GATA1 and its obligate cofactor FOG-1 are required to generate MKs from a common bipotential progenitor [108,109], but more germane to this discussion is their apparent role in terminal cell differentiation. Mice and humans with absent or mutant GATA1 protein are thrombocytopenic and carry abnormal MKs (Fig. 2) with a small, immature cytoplasm and reduced platelet granules [110–113]. Although the tissue-specific promoters of most megakaryocytic genes show a functional dependence on GATA1 *in vitro*, the physiological targets of GATA1 that are important for platelet biogenesis remain unknown. One attractive target is p45 NF-E2, whose promoter depends on GATA/FOG function *in vitro* and whose mRNA is substantially reduced in GATA1-deficient MKs [108,111]. Thus, GATA1 and NF-E2 may function within a linear transcriptional cascade in thrombopoiesis. A recent study [114] shows that GATA1–FOG complexes interact further with Ets-family proteins and may provide a basis for the common proximity of GATA and Ets consensus sequences in the promoters of MK genes [115]. A small group of lineage-restricted transcription factors may thus control expression of a large fraction of the genes that help define the MK–platelet axis.

The phenotypes of knockout mice reveal the unanticipated importance of two additional transcription factors in platelet release. MKs cultured from the fetal livers of mice lacking the atypical Ets-family transcription factor Fli-1 show a poorly developed cytoplasm with disorganized internal membranes and a paucity of α-granules [116,117]. In the absence of another zinc-finger protein, Hzf, mouse blood platelets show reduced α-granule contents and hence superficially resemble those seen in human gray platelet syndromes [118]. The role of Hzf in binding DNA or regulating transcription remains to be established, but if this is indeed the case, it is likely that genes under Hzf control function within the intracellular organelle traffic pathways discussed above. Finally, it is worth recording the possible role in late MK maturation of the basic helix-loop-helix protein tal-1/SCL (for stem-cell leukemia), which is required for the earliest development of the vertebrate hematopoietic and vascular systems. Conditional targeting of the

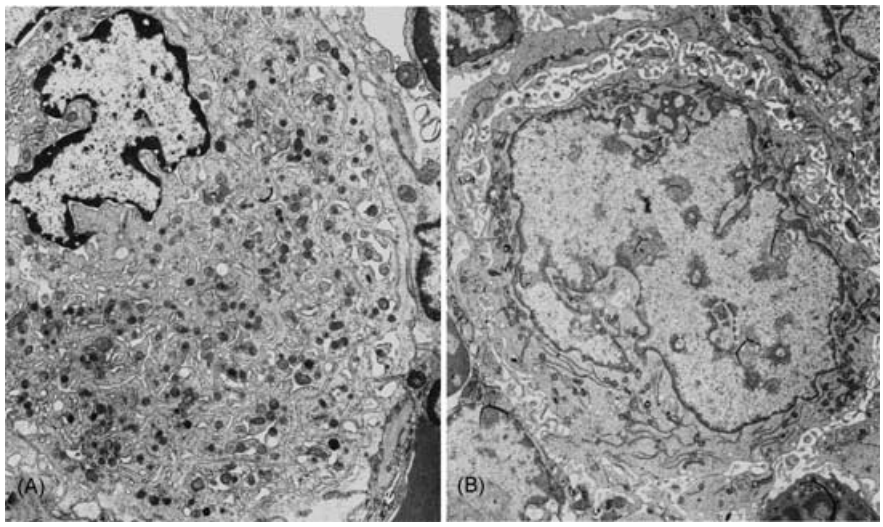


Fig. 2. Defective megakaryocyte ultrastructure in the absence of transcription factor GATA1. (A) Representative section of a normal mature megakaryocyte (MK) (original magnification × 6000), illustrating the dimensions of the cytoplasm and the relationship between developing organelles: internal membranes and platelet-specific granules. (B) In contrast, the typical MK that develops in the absence of GATA1 (original magnification ×4000) shows abnormally high nuclear : cytoplasmic ratio and extremely limited development of membrane or granular organelles. Instead, the cell reveals excess rough endoplasmic reticulum. Collectively, these markers of cellular immaturity correlate well with impaired proplatelet formation and thrombocytopenia. Similar defects are observed in patients with congenital thrombocytopenia resulting from GATA1 mutations.

mouse SCl gene further implicates it in differentiation of erythroid cells and MKs [119,120], but the precise stage at which MK maturation is arrested in the absence of SCL remains unclear. Both reports that address this question describe poorly differentiated MKs, with a subpopulation of cells that may resemble those produced in the absence of NF-E2 function, but more detailed characterization will be required before Fli-1, Hzf and tal-1/SCL are placed reliably within a transcriptional regulatory pathway or network.

Conclusions

Megakaryocyte maturation and platelet release entail a number of processes that are virtually unique to this cell lineage, including endomitosis, assembly and translocation of specific organelles, and proplatelet morphogenesis. Recent genetic and cell biological studies have built on a strong foundation of seminal observations to extend the mechanistic and molecular understanding of each of these processes. Although the limited scope of this review precluded us from considering a broader spectrum of ongoing investigations, we have touched on several areas of particular activity or controversy. The emerging concepts might some day permit novel approaches toward disorders of platelet numbers and function.

Acknowledegments

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