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CELL SCIENCE AT A GLANCE

Blood platelet formation at a glance

Julie Boscher*, Ines Guinard*, Anita Eckly, François Lanza and Catherine Léon‡

ABSTRACT

The main function of blood platelets is to ensure hemostasis and prevent hemorrhages. The 10^{11} platelets needed daily are produced in a well-orchestrated process. However, this process is not yet fully understood and *in vitro* platelet production is still inefficient. Platelets are produced in the bone marrow by megakaryocytes, highly specialized precursor cells that extend cytoplasmic projections called proplatelets (PPTs) through the endothelial barrier of sinusoid vessels. In this Cell Science at a Glance article and the accompanying poster we discuss the mechanisms and pathways involved in megakaryopoiesis and platelet formation processes. We especially address the – still underestimated – role of the microenvironment of the bone marrow, and present recent findings on how PPT extension *in vivo* differs from that *in vitro* and entails different mechanisms. Finally, we recapitulate old but recently revisited evidence that – although bone marrow does produce megakaryocytes and PPTs – remodeling and the release of bona fide platelets, mainly occur in the downstream microcirculation.

KEY WORDS: Megakaryocyte, Platelet, Cytoskeleton, Mechanobiology

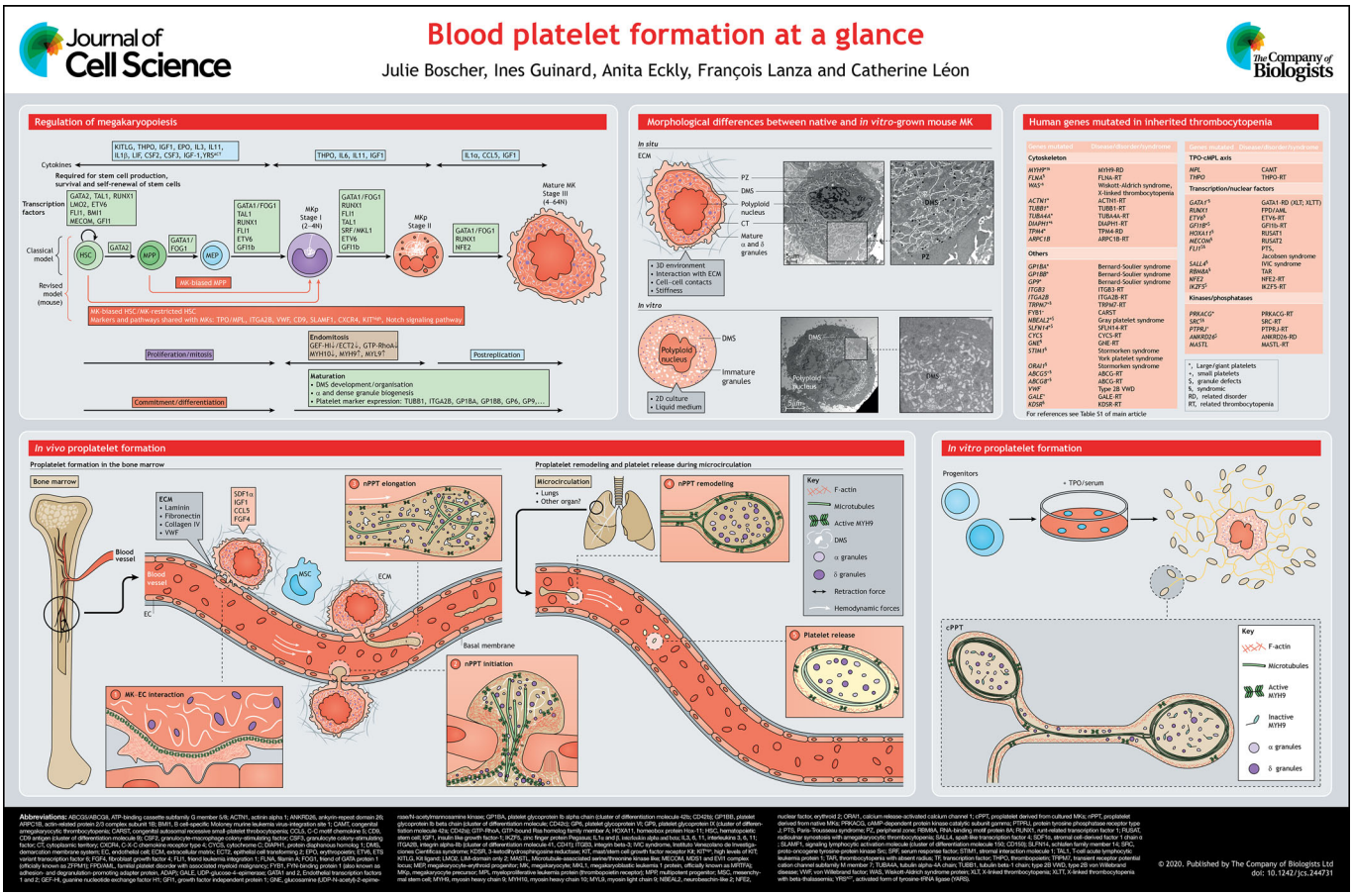
Université de Strasbourg, INSERM, EFS Grand Est, BPPS UMR-S 1255, F-67000 Strasbourg, France.
*These authors contributed equally to this work

‡Author for correspondence (Catherine.Leon@efs.sante.fr)

A.E., 0000-0001-9620-4961; F.L., 0000-0002-5802-4748; C.L., 0000-0002-8597-9929

Introduction

Blood platelets are crucial during hemostasis to prevent hemorrhages. At sites of vascular injury, circulating platelets immediately adhere and aggregate to form a platelet plug to stop bleeding (Nurden et al., 2008), and concomitantly release the contents of their granules to repair the lesion. Defective platelet production or function is life threatening due to the risk of hemorrhaging and may necessitate platelet transfusion. In humans, the reference range for a normal platelet count is 150–400×10^9/l blood. With a platelet lifetime of approximately eight to ten days, a



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production of 10^{11} platelets per day is needed to maintain a constant level (Daly, 2011).

Platelets are cytoplasmic fragments derived from megakaryocytes (MKs), giant cells (50–100 µm) that present at a low frequency within the bone marrow (BM) extravascular compartment (<1% of total marrow cells; Wright, 1910). Although it has been agreed that MKs are the precursor cells to platelets, the mechanisms and the site of platelet release *in vivo* has long remained a matter of debate. Fragmentation theory, whereby platelets are pre-formed within MKs (Kosaki, 2005), contradicted other hypotheses, i.e. that MKs extend cytoplasmic protrusions through the vessel wall (Radley and Scurfield, 1980) or directly enter the blood circulation to release platelets into the lungs (Zucker-Franklin and Philipp, 2000; Stenberg and Levin, 1989). With the development of intravital microscopy and the possibility to observe BM cells in real time, it is now firmly accepted that mature MKs extend cytoplasmic processes, the so-called proplatelets (PPTs), through the sinusoid vessel barrier (Bornert et al., 2020; Junt et al., 2007; Kowata et al., 2014). Once released into circulation, these extensions need to remodel in the downstream microcirculation in order to form bona fide platelets, and a recently revisited – but old – concept proposed the lungs as the main site for platelet release (Lefrançais et al., 2017). This Cell Science at a Glance article and accompanying poster highlight our latest knowledge of the molecular factors that regulate platelet formation. We also focus on recent considerations on how the native microenvironment uniquely contributes to full MK maturation and PPT extension (see poster).

Differentiation and maturation of megakaryocytes

MKs differentiate from hematopoietic stem cells (HSCs), located in the hematopoietic BM, towards the MK lineage, and develop the characteristic features of mature MKs (Deutsch and Tomer, 2013).

Megakaryopoiesis

In the classic stepwise hematopoietic differentiation model, asymmetric division of HSCs gives rise to multipotent progenitor (MPP) cells that gradually lose their self-renewal capacity and their multipotency, leading to the bipotent megakaryocytic/erythroid progenitors (MEPs) that finally differentiate into the unipotent MK progenitor and, then, MK precursor (MKp) cells (Akashi et al., 2000; Woolthuis and Park, 2016; see poster). This well-defined hierarchical model with homogeneous HSCs at the apex has been challenged in the past decade. HSCs and MPPs with a differentiation bias towards the MK lineage have been identified, including a subset of HSCs that can even give rise directly to unipotent MK progenitors (MK-restricted HSCs) (Carrelha et al., 2018; Psaila and Mead, 2019; Sanjuan-Pla et al., 2013; Yamamoto et al., 2013). The earlier observation that a HSC subpopulation also shares similar characteristics with MKs further supports this idea (Huang and Cantor, 2009; see poster). Such MK lineage bias may play a crucial role in the rapid replenishment of platelets in response to pathological conditions of acute platelet demand (Haas et al., 2015; Rodriguez-Fraticelli et al., 2018). However, although strong evidence exists in mice, MK-biased HSCs and MMPs have still not been convincingly demonstrated in human (Psaila and Mead, 2019).

Extracellular and intracellular signals that mediate cell differentiation

Megakaryopoiesis is coordinated through the concerted action of cytokines and transcription factors (TFs), with thrombopoietin (THPO) as its main physiological regulator (Hitchcock and Kaushansky, 2014). This glycoprotein is mainly synthesized in the liver and, to a lesser extent, in the kidney and some marrow stromal cells. The binding of THPO to its specific receptor myeloproliferative leukemia protein (MPL) promotes its dimerization, leading to activation of janus kinase 2 (JAK2). In turn, JAK2 phosphorylates MPL, resulting in the recruitment of multiple effectors, such as signal transducers and activators of transcription (STATs), phosphoinositide-3-kinase (PI3K) and mitogen-activated protein kinases (MAPKs) (Behrens and Alexander, 2018; Hitchcock and Kaushansky, 2014). *Thpo*- and *Mpl*-deficient mice show an ∼85% decrease in their platelet and MK levels, confirming THPO as the main megakaryopoiesis cytokine, but also pointing to the existence of additional regulating factors. Other cytokines play an important role in synergy with THPO (see poster; Behrens and Alexander, 2018), but THPO-independent pathways have also been identified, including a role of insulin-like growth factor 1 (IGF1) and the activated form of tyrosine-tRNA ligase (YARS) YRS^{ACT} (Kanaji et al., 2018), C-C motif chemokine 5 (CCL5) and interleukin 1α (IL1α) (Noetzli et al., 2019). However, the respective roles of these extracellular factors in normal versus inflammatory and/or emergency megakaryopoiesis remain to be clarified.

At the intracellular level, several TFs, together with cofactors and chromatin modifiers, govern the lineage-specific transcription program required for normal commitment and maturation of MKs by activating or repressing gene expression. Mutations of these TFs or their target proteins often lead to congenital thrombocytopenia, a condition associated with low platelet count (Box 1; see also poster and Table S1; Almazni et al., 2019; Balduini et al., 2017; Léon et al., 2016). Among the crucial TFs, interaction between GATA1 and its cofactor friend of GATA1 (FOG1, officially known as ZFPM1) is essential for erythroid-megakaryocytic differentiation (Mancini

Box 1. Defective platelet production of hereditary origin

A decrease in the level of circulating platelets (thrombocytopenia) can have different origins and can be classified into two main categories: (i) peripheral thrombocytopenia, due to an increase in platelet destruction, consumption or sequestration in the spleen and, (ii) central thrombocytopenia, due to impaired platelet production. Abnormalities of platelet production can be either acquired, i.e. resulting from a pathological condition or medical therapy, or of hereditary origin (Smock and Perkins, 2014). These genetic forms display various clinical manifestations and platelet defects, including the degree of thrombocytopenia and abnormal platelet morphology, such as larger, smaller or spherical cells, with defective granule contents or an abnormal microtubule organization (Almazni et al., 2019; see poster and Table S1). Some are associated with other clinical symptoms (syndromic thrombocytopenia), either congenital or of later onset, which can be far more serious than the bleeding phenotype itself as, for instance, in the case of immunodeficiency, lung or kidney failure (Melazzini et al., 2017). Identification of mutated genes responsible for thrombocytopenia has helped to reveal new pathways involved in platelet biogenesis. Among the various mutated genes, a number of them affect early megakaryopoiesis and MK differentiation due to defects in the THPO–MPL axis or in transcription factors (Almazni et al., 2019; see poster and Table S1). Others are associated with the target genes of these TFs, especially those encoding cytoskeletal proteins, and result mainly in late MK maturation defects and abnormal platelets. So far, mutations have been identified in ∼40 genes (Almazni et al., 2019; see poster and Table S1). Long misdiagnosed and poorly studied, our knowledge of these rare hereditary diseases has increased considerably over the last twenty years with the availability of high-throughput and whole-exome sequencing techniques, and by studying these mutations in animal models (Léon et al., 2016). Nevertheless, ∼40% of diagnosed cases of constitutional thrombocytopenia remain of unknown origin.

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et al., 2012). Together, the proto-oncogene friend leukemia integration 1 (FLI1) and GATA1 activate specific genes responsible for the regulation of late MK progenitor cells (Huang et al., 2009). Runt-related transcription factor 1 (RUNX1) favors progenitor proliferation and modulates the late stages of MK maturation. Other TFs include MDS1 and EVI1 complex locus protein (MECOM) and the nuclear factor, erythroid 2 (NFE2), which is involved in late MK maturation (Mazzi et al., 2018; Tijssen and Ghevaert, 2013). Another degree of complexity is brought about by a number of microRNAs (miRNAs), whose expression is up- or downregulated upon megakaryopoiesis (reviewed by Li et al., 2011; Raghuwanshi et al., 2019). Notably, this is the case for miR-22, which inhibits expression of growth factor interdependent 1 (*GFI1*) to promote normal MK differentiation (Weiss and Ito, 2019). By controlling the expression of their target genes, miRNAs appear to be important regulators of MK differentiation and represent an emerging field with novel therapeutic perspectives.

Megakaryocyte maturation

Once differentiated, MKp cells undergo a process of maturation, during which they acquire the components of the future platelets and the competence for their production. In contrast to fish, reptiles and birds – all of which have diploid (2N) thrombocytes – mammals evolved towards anucleate platelets, i.e. platelets without a nucleus, which present with improved shear-resistant thrombi (Schmaier et al., 2011) that might have hemostatic advantages for reproduction (Martin and Wagner, 2019). Anucleated platelets are produced by polyploid MKs that contain 8–64 sets of chromosomes, the result of endomitosis, i.e. chromosomal replication without division of the cell or nucleus. Although not mandatory *per se* for the formation of platelets (Mattia et al., 2002; Potts et al., 2014), polyploidization might be a more efficient and energy-saving process as compared to that yielding diploid cells, allowing the body to rapidly adapt to acute platelet demands (Vainchenker and Raslova, 2019).

Endomitosis results from defective cytokinesis and karyokinesis (Ravid et al., 2002; Vitrat et al., 1998; Geddis et al., 2007; Lordier et al., 2010). Cytokinesis in normal mitosis requires RhoA-regulated actomyosin contraction of the cleavage furrow for abscission (Basant and Glotzer, 2018). Upon differentiation, RUNX1 downregulates myosin-10 (MYH10) expression. Concomitantly, expression of the guanine exchange factors GEF-H1 and ECT2 is downregulated, preventing RhoA activation and accumulation at the midzone, hence preventing myosin-9 (MYH9) activation, and precluding abscission (Gao et al., 2012; Mazzi et al., 2018; Shin et al., 2011).

Concomitantly, cytoplasmic maturation takes place; this is characterized by a large increase in MK volume, and associated with substantially increased protein and lipid synthesis (Odell and Jackson, 1968; Behnke, 1968). During these steps, MKp cells begin to express platelet-specific receptors, such as integrins ITGA2B and ITGB3 required for platelet aggregation, the glycoprotein receptor GPVI (GP6) required for adhesion onto subendothelial collagen I and the glycoprotein receptor complex GPIb–IX–V – comprising four subunits, i.e. GPIbA (GP1BA), GPIbB (GP1BB), GPIX (GP9) and GPV (GP5) – required for adhesion onto von Willebrand factor (VWF) (Nurden, 2014). In addition, the machinery for the biogenesis of the future typical platelet granules becomes fully functional (Sharda and Flaumenhaft, 2018), so that granules are almost mature at the time of PPT formation. Granule content is crucial for hemostasis, allowing amplification of platelet responses (McNicol and Israels, 1999). Granules are also important for the contribution of platelets to non-hemostatic functions, such as angiogenesis, wound healing, inflammation, innate immunity and cancer metastasis (reviewed by Margraf and Zarbock, 2019; Palacios-Acedo et al., 2019).

Another hallmark of mature MKs is the presence of a large and complex intracellular membrane network called the demarcation membrane system (DMS) that supplies the membrane of future platelets. DMS biogenesis starts with invaginations of the plasma membrane (Behnke, 1968) located in GPIb-positive clusters, further fueled by fusion with Golgi vesicles and the endoplasmic reticulum (Eckly et al., 2014). Ultimately, the membranes become highly organized so that, in mature MKs, the DMS demarcates characteristic cytoplasmic territories (Aguilar et al., 2016; see poster). PACSIN2 and CIP4 (officially known as TRIP10) are F-BAR domain-containing proteins that regulate DMS invaginations (Begonja et al., 2015; Chen et al., 2013). Filamin A (FLNA) interacts with PACSIN2 to ensure its correct localization at the sites of membrane tubulation, as well as with GPIb (Meyer et al., 1997). Several cytoskeletal components have been found to play a key role in establishing the DMS. DMS membranes are enriched in PtdIns(4,5)P_2 that facilitates the recruitment and activation of the WASP-Arp2/3 complex to nucleate short F-actin (Schulze et al., 2006). Mutations in human genes or deletions in mouse genes, such as *Gp1ba*, *Gp1bb*, *Gp9* and *Flna*, or in proteins that regulate F-actin dynamics – such as small Rho GTPases – result in an abnormal DMS that is usually accompanied by a defect in the capacity of MKs to extend PPTs and, ultimately, in thrombocytopenia (Ghalloussi et al., 2019).

Importance of the bone marrow microenvironment for full MK maturation

When differentiated in culture, MKs do not exhibit the degree of maturation observed *in vivo*. They have a decreased ploidy and poorly organized DMS with few cytoplasmic territories, and lack the typical organelle-free peripheral zone and immature granules, implying that the physiological process is not fully reproduced (Aguilar et al., 2016; see poster). This may explain why the *in vitro* production of platelets is still very inefficient (Box 2). *In vivo*, in addition to cytokines (see poster), MKs also interact with other cells and the extracellular matrix (ECM), and are exposed to mechanical stress, all of which contributes to their physiological maturation.

The role of endothelial cells is probably key in the late steps of MK maturation, as all mature MKs reside in proximity to sinusoid vessels (Stegner et al., 2017). Stromal cell-derived factor-1 (CXCL12, also known as SDF1) promotes migration of progenitors and their interaction with endothelial cells, which contributes to their maturation (Avraham et al., 1993; Hamada et al., 1998), notably through release of fibroblast growth factor 4 (FGF4) (Avecilla et al., 2004) and VWF (Ouzegdouh et al., 2018). Stromal cells might also contribute, either through direct cell–cell interaction, or the secretion of ECM proteins or chemokines (Brouard et al., 2017; Tamura et al., 2016).

The role of the mechanical properties of the BM has only recently been explored. The marrow is the softest tissue of the body, with a stiffness of only 300 Pa as measured by atomic force microscopy (AFM) (Shin et al., 2014). It is, however, confined to the inside of the bone – the most rigid tissue with a stiffness of >1 GPa – which confers strong constraints to the marrow. Currently, only a few studies reported an effect of substrate stiffness on MK differentiation and PPT formation. Studies indicated that type-I collagen fibrils, which inhibit PPT formation, are stiffer than collagen IV, which promotes PPT formation (Balduini et al., 2008; Malara et al., 2011). Further work showed that MKs extended

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Box 2. *In vitro* platelet production

At present, donor-derived platelets are the only source for clinical platelet transfusion. In recent years, the fear of shortages with regard to ageing societies, combined with the residual risk of disease transmission – notably for emergent pathogens – or the development of alloantibodies, has prompted laboratories to develop *in vitro* platelet production. However, to obtain a sufficient number platelets for transfusion ($\sim 5\times10^{11}$ platelets/unit) remains a technological challenge. Substantial progress has, however, been made, concentrating especially on the expansion of progenitors and using two main approaches. The first relies on human induced pluripotent stem cells (hiPSCs) and the generation of expandable MK lines. Here, one strategy used ectopical expression of the TFs myelocytomatosis proto-oncogene (MYC), B-cell-specific Moloney murine leukemia virus integration site 1 proto-oncogene (BMI1) and B-cell lymphoma-extra large (BCL-xL); also known as B-cell lymphoma 2-like 1, BCL2L1), controlled by a doxycycline-inducible promoter (Nakamura et al., 2014). Another strategy involves overexpression of GATA1, FLI1 and T-cell acute lymphocytic leukemia protein 1 (TAL1) (Moreau et al., 2016). As long-term self-renewable progenitors, hiPSCs are also amenable to genetic manipulations of human leukocyte antigen (HLA) or human platelet antigen (HPA) to prevent immune responses (Sugimoto and Eto, 2017). The second approach employs native $CD34^+$ hematopoietic progenitors derived from either embryonic cord blood, adult BM or peripheral blood (Lee et al., 2014; Strassel et al., 2016). These progenitors have the advantage of their ease of differentiation into mature MKs and their relatively high capacity to produce platelets. However, as $CD34^+$ cells do not indefinitely self-replicate, there is a need for a continuous supply from donors with related issues of apheresis costs and alloreactivity (Strassel et al., 2018). Optimization of the platelet fragmentation process, which is quite inefficient in static cultures, has also considerably increased platelet yields. Several laboratories have made use of various flow conditions to improve platelet release, for instance through repeated pipetting (Do Sacramento et al., 2020) or in flow perfusion bioreactors (Di Buduo et al., 2015; Dunois-Lardé et al., 2009; Thon et al., 2017). Finally, the use of a turbulence-controllable bioreactor in a scaled-up system succeeded in efficiently producing functional platelets in clinically relevant numbers (Ito et al., 2018).

more PPTs on collagen I or IV coated on soft silk film ($E \leq 10$ MPa) compared to stiffer films ($E \geq 90$ MPa), indicating an impact of mechanical factors (Abbonante et al., 2017). Matrix stiffness depends on ECM crosslinking, notably through lysyl-oxydase (LOX). Inhibition of LOX-mediated collagen crosslinking in mouse marrow lead to increased platelet count, further indicating that soft matrix promotes platelet formation (Abbonante et al., 2017; Leiva et al., 2018). Another study showed that soft 2D collagenous matrices maximize PPT extension compared to 3D or stiffer matrices (Shin et al., 2011). Independently of matrix–protein interactions, 3D culture using methylcellulose hydrogels has provided evidence that confinement and stiffness ($E \leq 0.1$ kPa) – which mimic BM mechanical cues – is preferable to liquid culture and promotes a MK maturation more similar to that in BM (Aguilar et al., 2016). Of note, such 3D culture has – by creating physical constraints – allowed to reveal defects in MYH9-deficient MKs that are present inside the marrow but went unnoticed in liquid culture (Aguilar et al., 2016).

How MKs sense and react to mechanical cues is currently still unclear. MKs express several integrins, i.e. receptors for ECM proteins known to sense matrix stiffness in many cells, and their mechanosensitive properties in MKs are still under evaluation (Ward and Ravid, 2020). One study proposed a role of the TRPV4 cation channel in sensing stiffness, leading to β1 integrin activation and internalization, thereby promoting PPT formation (Abbonante et al., 2017). At the intracellular level, MKs – like all cells – adapt to stiffness by modulating their actomyosin cytoskeleton. Confinement in soft hydrogels has been shown to modify MYH9 distribution and to favor the activation of the mechanosensitive myocardin-related transcription factor A (MRTFA; also known as MKL1) (Aguilar et al., 2016). Thus, extracellular constraints are thought to affect MK differentiation. Understanding how MKs sense and react to mechanical cues is of particular interest when considering pathologies that are linked to a modification of marrow stiffness, such as myelofibrosis, a severe condition associated with abnormal megakaryopoiesis for which still no effective treatment is currently available (Leiva et al., 2018).

Proplatelet extension

Once mature, MKs are able to enter the process of platelet release *per se*, i.e. thrombopoiesis, whose first step is the extension of PPTs. The structure of PPTs and the mechanisms for their extension appear to differ between *in vitro* PPTs derived from cultured MKs (hereafter referred to as cPPTs) and *in vivo* PPTs derived from native MKs (hereafter referred to as nPPTs) (Bornert et al., 2020).

Proplatelet formation *in vitro*

In vitro, MKs are not highly polarized as protrusions may be initiated at several points around the cell membrane (Radley and Haller, 1982; see poster). These protrusions are rapidly converted into long, thin projections (see poster). cPPTs are characterized by a thin shaft, numerous branching and terminal buds. Microtubules play a crucial role in the extension of cPPTs. They are organized in bundles that line the cPPT shaft and coil inside the bud, prefiguring the microtubule coils known as the marginal band in platelets (see poster; Patel-Hett et al., 2008). Microtubules have been clearly shown to be the main drivers for cPPT extension, as incubation of MKs in the presence of microtubule-depolymerizing drugs prevents cPPT formation (Italiano et al., 1999; Tablin et al., 1990). Furthermore, low temperature or addition of microtubule-depolymerizing agents after cPPTs have extended causes cPPT to retract (Italiano et al., 1999; Tablin et al., 1990). Microtubule sliding controlled by dynein motors rather than *de novo* polymerization, seems to be important for cPPT extension (Bender et al., 2015). Actomyosin is also involved because F-actin promotes the branching process and, therefore, controls the extent of cPPTs per MK (Italiano et al., 1999). By contrast, the RhoA/Rho-kinase/MYH9 pathway restrains cPPT formation (Chang et al., 2007; Chen et al., 2007; Eckly et al., 2010).

Proplatelet formation *in vivo*

In vivo, and in contrast to *in vitro* formation, native MKs differentiate in a multifactorial complex environment, in which nPPTs must traverse the endothelial physical barrier to reach the circulating blood (see poster). The signals by which MKs become polarized to extend nPPTs in the correct direction are still unclear but regulation of F-actin dynamics might contribute to the polarization. Inactivation of genes that encode proteins directly or indirectly involved in the dynamics of F-actin, such as proteins of the WASP/WAVE family, Arp2/3, profilin or FYB1 (also known as ADAP), leads to an abnormal release of MK fragments inside the extravascular BM compartment (Ghalloussi et al., 2019; Spindler et al., 2018). Furthermore, an increase in Cdc42 activity results in MK hyperpolarization and increased transendothelial migration of whole MKs (Dütting et al., 2017).

Interaction with endothelial cells might be important for initial protrusions. It has been proposed that MKs extend podosomes to sense their surroundings (Schachtner et al., 2013). Such actin-rich

podosome-like protrusions can be visualized *in situ*, i.e. within the BM, extending from the MK plasma membrane and deforming endothelial cells located beneath (Eckly et al., 2020; Tavassoli and Aoki, 1981). These podosome-like extensions might provide the force required for fusion of the apical and basal endothelial membranes, creating transendothelial pores through which MKs extend their initial nPPT protrusions (Becker and De Bruyn, 1976; Eckly et al., 2020). Once inside the sinusoid, these protrusions form actin-rich shoulders that are anchored to the luminal face of the endothelial lining and might help the nPPT to extend into the circulation (Behnke and Forer, 1998; Bornert et al., 2020; Brown et al., 2018). nPPTs elongate by several hundred micrometers, fueled by membrane replenishment through fusion of the DMS with the plasma membrane (Brown et al., 2018). Microtubule organization within nascent nPPTs is rather individual with microtubules being heterogeneously distributed (Bornert et al., 2020; Brown et al., 2018), which is in contrast to the microtubule bundles found in cPPT. In fact, the role of microtubules appears to be less crucial *in vivo*, where hemodynamic forces are strong enough to substantially contribute to nPPT elongation (Bornert et al., 2020). Accordingly, inactivation or mutation of β1-tubulin or α4a-tubulin result in almost total absence of cPPTs *in vitro*, compared with only ∼50% decrease in nPPTs and circulating platelets observed *in vivo* (Bornert et al., 2020; Strassel et al., 2019).

Proplatelet remodeling and release in the microcirculation

The final step in platelet biogenesis requires the detachment, remodeling and fragmentation of nPPT into individual platelets by mechanisms that are still unknown. nPPTs usually detach from the remaining cell body as elongated fragments, having a volume encompassing that of several hundred platelets (Becker and De Bruyn, 1976). The released nPPTs are rapidly swept away and need further remodeling in the downstream circulation to form bona fide platelets. It has long been proposed that the pulmonary microcirculation might be the site of platelet release (Levine et al., 1993; Zucker-Franklin and Philipp, 2000). MK fragments, including whole MKs, have been first described in pulmonary circulation by Ludwig Aschoff in 1893 (Aschoff, 1893). Later on, several authors published observations of MKs within the blood circulation (Levine et al., 1993; Pedersen, 1978; Tavassoli and Aoki, 1981) and MKs lodged within the pulmonary vascular bed, capable to extend nPPTs (Howell and Donahue, 1937; Zucker-Franklin and Philipp, 2000). Comparison of the number of MKs or platelets in various venous or arteriolar beds also favored the hypothesis of the lungs being an important site of platelet release (Howell and Donahue, 1937; Kallinikos-Maniatis, 1969; Trowbridge et al., 1982). More recently, intravital videomicroscopic observations within the lungs of living mice unambiguously showed nPPT remodeling occurring in real time within the pulmonary vasculature, including whole MKs (Lefrançais et al., 2017). The origin of MKs in the lung circulation is still to be determined. It could be mature MKs entering the sinusoid vessels or immature progenitors emerging from BM (Howell and Donahue, 1937). The lungs might provide these cells with a favorable microenvironment to end maturation and with hemodynamic forces to release platelets (Ouzegdouh et al., 2018). These findings, nevertheless, raise the question of how platelet counts evolve in patients suffering from lung disease. Although few data are available, they were difficult to interpret due to the inflammatory profile of such patients, who are prone to thrombotic events (Biljak et al., 2011). What seems clear is that experimental or pathological conditions, such as inflammation, thrombocytopenia rebound, THPO treatment and myelogenous leukemia – all of which are known to increase the number or activity of MKs in the BM – will also increase the number of whole MKs present in the lungs (Léon et al., 2012; Yamauchi and Shimamura, 1994; Zucker-Franklin and Philipp, 2000); a fact that might be associated with lung fibrosis (Aschoff, 1893; Thachil, 2009). Furthermore, the lungs might not be the only capillary bed capable to fragment nPPT and/or MKs into platelets as increased MK numbers have also been reported in kidneys of patients presenting with increased MKs within BM and lungs (Broghamer and Weakley-Jones, 1981). Hence, although the BM is the main site for MK maturation and nPPT formation, further remodeling is required in the downstream circulation to release bona fide platelets.

Conclusions and future perspectives

Our knowledge of the highly complex platelet formation process has considerably increased in recent years. Technological advances in single-cell isolation and their follow-up after transplantation have allowed us to revise the long established hierarchical model of megakaryopoiesis. With regard to the final stage of the process, intravital imaging has revealed differences between *in vivo* and *in vitro* mechanisms of PPT formation. Nevertheless, more than a century after identification of the MK as the precursor cell for platelets, many questions still remain to be answered with regard to the mechanisms that regulate the full maturation of MKs, their polarization, and the signals that initiate cPPT and nPPT, as well as the events that control the final platelet remodeling. Further characterization of the different factors that act within the native environment will contribute to ameliorate treatments of thrombocytopenic patients. Moreover, identification of mechanisms that occur in cell culture will allow to optimize *in vitro* platelet production for transfusion, which now appears feasible in the long term.

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Competing interests

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Supplementary information

Supplementary information available online at
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Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at https://jcs.biologists.org/lookup/doi/10.1242/jcs.244731.supplemental

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