New explanations for old observations: marginal band coiling during platelet activation

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Summary. Blood platelets are tiny cell fragments derived from megakaryocytes. Their primary function is to control blood vessel integrity and ensure hemostasis if a vessel wall is damaged. Circulating quiescent platelets have a flat, discoid shape maintained by a circumferential microtubule bundle, called the marginal band (MB). In the case of injury platelets are activated and rapidly adopt a spherical shape due to microtubule motor-induced elongation and subsequent coiling of the MB. Platelet activation and shape change can be transient or become irreversible. This depends on the strength of the activation stimulus, which is translated into a cytoskeletal crosstalk between microtubules, their motors and the actomyosin cortex, ensuring stimulus-response coupling. Following microtubule motor-driven disc-to-sphere transition, a strong stimulus will lead to compression of the sphere through actomyosin cortex contraction. This will concentrate the granules in the center of the platelet and accelerate their exocytosis. Once granules are released, platelets have crossed the point of no return to irreversible activation. This review summarizes the current knowledge of the molecular mechanism leading to platelet shape change, with a special emphasis on microtubules, and refers to previously published observations, which have been essential for generating an integrated view of cytoskeletal rearrangements during platelet activation.

Keywords: actomyosin; blood platelets; cytoskeleton; microtubules; molecular motor proteins.

Introduction

Vertebrates have developed an efficient blood circulatory system to provide all parts of the body with nutrients and to evacuate cytotoxic products. They thereby expose themselves to life threatening danger if blood is lost by injury. Thus, during evolution a quality control and first aid repair system for blood vessels had to evolve, which has to respond to several essential criteria. First, the integrity of the whole circulatory system has to be constantly monitored. Second, vessel damage should give an alert signal to trigger use of a repair kit. Third, ideally the repair tools should be already in place at the site of injury. In non-mammalian vertebrates, nucleated thrombocytes are the specialized cell type able to fulfill this functional role. During evolution the cells involved in hemostasis have become more sophisticated [1]. Mammalian thrombocytes, called platelets, are generated by fragmentation of megakaryocytes [2]. They are tiny cell fragments devoid of a nucleus but equipped with all the necessary components for vessel repair, prefabricated and stored in the cytoplasm and in their granules. Most surprisingly, despite these fundamental differences between nucleated thrombocytes of lower vertebrates and mammalian platelets the principal features of cytoskeletal reorganizations during activation are conserved as discussed below.

Platelets circulate in the blood and control vessel integrity as small, discoid particles. Their small size and flat, discoid form allow them to pass through narrow spaces where large, round cells would get stuck. On vessel injury, platelets are activated and rapidly change shape. They become spherical and extend filopodia [3]. Blood flow simulations have shown that spherical platelets are transported more quickly to the vessel wall than disc-shaped platelets, giving them a better chance to adhere near the injured site [4]. They almost simultaneously secrete the content of their granules, releasing substances for the activation of surrounding platelets, cytokines for endothelial cells and coagulation factors.

An important field of investigation is the characterization of the molecular mechanisms leading to this rapid shape change during platelet activation and the
concomitant exocytosis of platelet granules. The precise regulation of these processes is also extensively studied, with the aim of developing new strategies against pathological thrombus formation.

The cytoskeleton of resting platelets

This section focuses on microtubules and actin filaments, because their reorganization is particularly important for the shape changes observed during platelet activation. Additionally, a detailed list of other structural components of the platelet cytoskeleton identified in the platelet proteome is shown in Table 1.

Microtubules

Microtubules are composed of heterodimers of α- and β-tubulin subunits, which polymerize in a head to tail fashion to form a protofilament (Fig. 1A). In most cells, 13 protofilaments assemble laterally to form a polar, hollow tube with the α-subunits exposed at one end (minus end) and the β-subunits at the other (plus end) [5]. Microtubule polymerization is mainly initiated at the centrosome, the major microtubule organizing center (MTOC) of animal cells, and subunit addition occurs essentially at the plus end, extending to the cell periphery. Microtubules alternate between growth and shrink phases, referred to as dynamic instability. The overall assembly rate depends on growth and shrink velocities as well as on the frequency of catastrophe (disassembly) and rescue (reassembly) events and the length of pausing periods and is highly regulated by several microtubule associated proteins (MAPs) [6]. At least six isoforms of α- and β-tubulins have been described, which are variably expressed in different tissues [5]. The main β isoform in platelets is the hematopoietic β1-tubulin [7]. Microtubule properties are not only influenced by their isoform composition, but also by post-translational modifications (only those

Table 1 Structural constituents of the platelet cytoskeleton. Members of different cytoskeletal families identified in a proteomic study by Burkhart et al. [21] are listed and the relative expression levels are indicated according to the color code shown below the table (no color in the case of unavailable copy number estimations)
detected on platelet microtubules will be discussed in more detail here) [8]. With time long-lived microtubules become modified by both lysine 40 acetylation and detyrosination of the C-terminus of the \(\alpha\)-subunit (Fig. 1A). Lysine acetylation is catalyzed by the tubulin acetyltransferase, \(\alpha\)-TAT1 [9,10], while the carboxy-terminal tyrosine (Y) residue can be removed. Both modifications are reversible and the reverse reaction takes place on the free tubulin dimer rapidly after microtubule depolymerization. The acetyl-group is removed by the major tubulin deacetylase, HDAC6 [11], while the tyrosine residue is added by the tubulin tyrosine ligase, TTL [12]. Thus, newly polymerizing microtubules have tyrosinated \(\alpha\)-subunits and lack acetylation, because they are formed from this pool of ‘remodeled’ free heterodimers [8].

In the resting platelet about half of the total tubulin content is in the polymerized state forming several microtubules organized in a peripheral ring structure called the marginal band (MB) (Fig. 1B) [13]. Platelets don’t have a MTOC and microtubules are nucleated from \(\gamma\)-tubulin seeds within the MB [14]. The MB is responsible for the

![Fig. 1. The microtubule part of the platelet cytoskeleton. (A) General composition of microtubules: heterodimers of \(\alpha\)- and \(\beta\)-tubulin subunits (red boxes) assemble head to tail into protofilaments, which associate laterally to form a polar tube with \(\alpha\)-tubulin exposed at one end (called the minus end) and \(\beta\)-tubulin at the other (plus end). Post-translational modifications detected on platelet microtubules are indicated on the structure of the tubulin dimer generated with the Protein Model Portal database (http://proteinmodelportal.org) [97] using the human sequences TUBA1C and TUBB1. Lysine 40 of \(\alpha\)-tubulin can be acetylated (K40) and the carboxy-terminal tyrosine (Y) residue can be removed. (B) Microtubule organization in a resting platelet: several anti-parallel microtubules form the marginal band (MB) essential to maintain the discoid shape of quiescent platelets (transmission image in the upper part, tubulin-stained MB in the middle and the schematic organization of the MB in the lower image with the black box corresponding to part of a microtubule shown in the black box in A). (C) Microtubule organization in an activated platelet: the MB is coiled, inducing the spherical shape of activating platelets (transmission image in the upper part, tubulin-stained MB in the middle, schematic organization of MB in the lower part with the black box corresponding to part of a microtubule shown in the black box in A).
discoïd resting shape because platelets become spherical when microtubules are depolymerized by nocodazole, colchicine, vincristine or cold treatment [15]. Interestingly, platelets deficient or mutated in the hematopoietic β1-tubulin isoform have an altered organization of the MB and a spherical shape [16,17]. Besides these genetic alterations directly affecting microtubules, there are several other inherited diseases characterized by pathologically abnormal platelets with altered MBs as reviewed by Thon and Italiano [18]. The MB surrounds the platelet organelles and has a length of about 9 µm [14,19,20]. It is composed of long-lived, acetylated/detyrosinated microtubules and 8–12 dynamic/tyrosinated microtubules, which polymerize in both directions within the MB. The actively polymerizing microtubules have been visualized by Patel-Hett et al. [14] in living platelets using the green fluorescent protein (GFP) fused to the plus-end binding protein EB3, which associates specifically with growing microtubule ends. It is not completely clear whether there are 8–12 microtubules acetylated near their minus ends and decorated at their growing ends with end-binding proteins or whether there are a few stable, acetylated microtubules that co-exist with 8–12 shorter, growing microtubules.

**Actin filaments**

Another important part of the cytoskeleton is composed of actin filaments, polar, ropelike polymers of globular actin subunits. Actin is the most abundant protein in platelets [21]. In resting platelets about 60% is stored in the monomeric globular form (G-actin) [22], while filamentous actin (F-actin) is mostly localized beneath the plasma membrane [23]. These cortical actin fibers are in close association with another part of the platelet cytoskeleton formed by a 2D-network of spectrin molecules [24]. The main spectrin isoforms in platelets are the αII and βII subunits, which associate to form hetero-tetramers [25]. The submembranous organization of the cytoskeleton appears to be particularly important in platelets for the stabilization and flexibility of the membrane cortex [26]. In fact, much of the plasma membrane in resting platelets is invaginated. This peculiar membrane organization constitutes the open canicular system and is clearly visible in cryo-electron tomography [27]. It is thought to serve as a membrane reservoir for rapid spreading during platelet activation.

**The cytoskeleton during platelet activation**

Resting platelets circulate in the blood for about 5–10 days and may be cleared in the spleen or liver without ever having rearranged their cytoskeleton [28]. However, when a vessel is damaged, platelets adhere to the exposed extracellular matrix (ECM) and rapidly change from the discoid to a spherical shape due to a dramatic reorganization of their cytoskeleton [3]. They then spread on the ECM and release substances into the bloodstream to activate surrounding platelets, which undergo a similar disc-to-sphere transition, this time in suspension [29]. In pathological situations, platelets may be activated by shear stress through a stenosed blood vessel [30] or by adhesion to an atherosclerotic plaque [31]. Whatever the activation stimulus (mechanical shear stress, adhesion to the ECM or soluble agonists), it is the strength of the signal that determines whether platelets are only transiently activated (just undergoing disc-to-sphere transition) or release their granules to become irreversibly activated. The strength of the activation stimulus depends on the number and type of agonists binding to their corresponding receptors at the plasma membrane of a given platelet. Although initially different, most of the signaling events triggered by individual agonist/receptor interactions ultimately converge in common reactions, for instance a rise in intracellular calcium, an early, essential step during platelet activation [32]. The speed of activation is also strongly dependent on the strength of the stimulus. At agonist concentrations used in standard aggregation assays, the kinetics of the cytoskeletal reorganizations are extremely fast, precluding the elucidation of the early steps during platelet activation leading to the spherical shape. Recently, new light has been shed on the molecular mechanism by analyzing platelets present in freshly drawn blood, which are transiently activated due to the mechanical stress during blood sampling [20]. This mechanical activation stimulus is strong enough to promote and maintain the round, spherical shape for several minutes but is sufficiently mild to allow platelets to go back to the resting state.

**Microtubules at early steps of platelet activation**

Tubulin immunofluorescence studies have revealed that transiently activated platelets, present in freshly drawn blood, have elongated MBs in a three-dimensional coiled form, like the seam of a tennis ball, giving them their characteristic spherical shape (Fig. 1C) [20]. These observations are in agreement with earlier studies showing that microtubule depolymerization is not necessary for platelets to attain a spherical shape during activation [33]. Without an additional stimulus, coiled MBs relax to the flat resting state after a recovery period (Fig. 2A). In a resting platelet population, only a small percentage of sporadically activated platelets with coiled MBs can be observed. Cross-sections of such platelets have been illustrated by Xu and Afzelius [34] using electron microscopy (Fig. 3A). Their interpretation was, however, that there were two perpendicular microtubule bundles (Fig. 3B) instead of one bundle in the coiled conformation (Fig. 3C). In transiently activated platelets with strongly coiled MBs, the curvature of the microtubule bundle becomes critical for newly polymerizing microtubules within the bundle. The growing microtubules cannot follow the coiled bundle anymore and diverge from the original path by switching to the opposite side of the coiled...
Fig. 2. Typical examples of MBs in platelets at different activation states. (A) Weak, transient activation: a short, weak activation stimulus (indicated in grey) leads to elongation (indicated in blue) and a slight distortion of the MB (green ring). Without further stimulation, slightly coiled MBs relax to the resting state. (B) Strong, transient activation: an enhanced activation stimulus leads to a strongly coiled MB. Newly polymerizing microtubules within the coiled MB diverge from the coiled path by switching to the opposite side of the coiled structure, ultimately forming a new flat bundle (new path in red). When the activation stimulus is not maintained, the coiled part will depolymerize and leave the flat bundle as the new resting MB. (C) Irreversible activation: a strong or long-lasting stimulus leads to compression of the strongly coiled MB (indicated in pink) and the opposite sides of the coiled structure become closer. Thus the newly polymerizing microtubules, which shortcut the coiled MB, have a shorter way to go, compared with the situation in B. This leads to the formation of a smaller microtubule ring in irreversibly activated platelets.

Fig. 3. Cross-sectioned microtubule bundles in a human platelet. (A) Transmission electron microscopy of a platelet in freshly drawn blood with microtubules cross-sectioned at four locations as indicated by red arrowheads (original figure 5 from [34] reprinted with permission from Elsevier). (B) A possible explanation for the cross-sectioned microtubule bundles observed in A: two perpendicular rings are cross-sectioned; section plane indicated in blue. (C) An alternative explanation for the cross-sectioned microtubule bundles observed in A: a coiled ring is cross-sectioned at the section plane indicated in blue.

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necessary for physiological platelet activation [20]. Platelets with such a microtubule organization have been described by Behnke and Forer in a population of freshly prepared platelets [35]. When the activation stimulus is not maintained, these platelets can still return to the resting state by disassembly of the coiled part, leaving the flat microtubule bundle in the periphery as the new resting MB (Fig. 2B). This new MB is formed of the pool of deacetylated, tyrosinated α-tubulin subunits (mentioned above) and may be gradually reacetylated and detyrosinated with time. The heterogeneity of MB acetylation within a resting platelet population [14] could thus be explained by recent transient activation events.

Coiled MBs are not only observed in transiently activated platelets. In fact, MB coiling is always observed as an early step during platelet activation for all activation pathways so far tested [20]. For instance, when platelets are activated by contact with a glass surface, the MB coils just before platelet spreading. White and colleagues may have been the first to observe twisted marginal bands after contact of platelets with a glass surface [36]. MB coiling is also seen when platelets are activated in suspension with very low-dose agonists, such as 5–10 μM arachidonic acid, 25–100 nM ADP or 0.01–0.02 U mL⁻¹ thrombin [20]. At these threshold concentrations, which are close to physiological concentrations [37–39], the steps leading to platelet activation are slow enough to be studied over time and MBs reorganize from the flat, resting to the coiled state within 30–60 s. This time period is similar to the time needed for MB coiling during platelet spreading on a glass surface and suggests that this might be the time necessary for physiological platelet activation [20].

**Actin filaments during early steps of platelet activation**

An early step during platelet activation is the formation of membrane protrusions or tethers observed on discoid platelets under flow conditions. This process is actin polymerization independent [40]. In contrast, once disc-to-sphere transition is completed, the polymerization of actin filaments and their association into bundles drive the extension of filopodia on the surface of spherical platelets, leading to the spiny sphere morphology [41]. Incubation of platelets at low temperatures leads also to actin assembly, formation of filopodia and an activated state of platelets [42]. It has been suggested that this facilitated activation may be important when platelets have to activate at low temperatures in external wounds, while a more precisely controlled activation mechanism at internal body temperatures may reduce the risk of thrombosis [43].

**Microtubules in irreversibly activated platelets**

A strong stimulus leads to granule release and thus irreversible activation, which is also characterized by a reduction in size of the spiny sphere platelets. The successive conversion from discoid platelets to large spheres and smaller spiny spheres during platelet activation has been observed in 1982 by Deranleau et al. [44] using a turbidimetric assay. It is now known that in these smaller spheres the coiled MB becomes compressed (Fig. 2C). As in transiently activated platelets with strongly coiled MBs, the newly polymerizing microtubules short-cut the coiled bundle to form a new flat ring. Because the coiled bundle is compressed, the newly formed microtubules have a smaller distance to go, which diminishes the size of the new, flat microtubule ring (Fig. 2C). Again, the old coiled and acetylated part is depolymerized, giving the de-acetylase HDAC6 access to its substrate for the rapid tubulin deacetylation observed during platelet activation [45,46]. Disassembly of the coiled part leaves the new, smaller microtubule ring in the center of the small spiny sphere platelets. Several investigators have described this smaller microtubule ring, but it has previously been thought to be generated by centripetal constriction of the resting MB, a process called ‘internal contraction’ [47,48].

**Actin filaments in irreversibly activated platelets**

Irreversibly activated platelets in suspension extend a high number of filopodia through an actin polymerization process, resulting in an increase of the cell surface area and redistribution of fibrinogen receptors. This enhances the platelet binding capacity for fibrinogen and fibrin strands for the formation of a hemostatic plug [49]. Filopodia also form in surface activated platelets before actin filaments organize into an orthogonal network, leading to the formation of lamellipodia and ultimately to fully spread platelets [50,51]. A recent study describes a close interplay between actin fibers, microtubules and an intermediate filament network in spread platelets. The observed intermediate filaments are composed of vimentin and desmin, which are type III intermediate filament proteins forming elastic homopolymeric fibers [52]. Their ability to adapt their rigidity according to a deforming stress [53] suggests that they might also have an important role to play in the maintenance of the resting platelet shape, as proposed by Tablin and Taube [54].

**The role of molecular motors in cytoskeletal rearrangements during platelet activation**

In eukaryotic cells three types of motor proteins are involved in cargo transport, cell motility, cell division and cytoskeletal reorganization for cell shape adaptation. All use ATP as an energy source for force generation. Myosin motors act on actin filaments, while dynein and kinesin motors are responsible for microtubule-based processes. Members of all three motor types are expressed in platelets and the rapid and profound reorganization of
the cytoskeleton during platelet activation relies on the precise coordination of all three motors.

**Myosins**

The myosin superfamily is subdivided into at least 17 classes. Class II or conventional myosins are the most studied and include the skeletal and cardiac muscle myosins as well as the smooth muscle and non-muscle myosins [55]. Conventional myosins are composed of two heavy chains with N-terminal motor domains. Two different light chains are associated with each heavy chain to regulate their stability and catalytic activity. Several myosin molecules assemble into bipolar filaments, which can pull together actin filaments of opposing polarity, resulting for instance in muscle or actomyosin cortex contraction [56]. It has long been thought that only the non-muscle myosin heavy chain IIA is expressed in platelets [57] and it has been shown that deletion or mutation of its gene (MYH9) is associated with macrothrombocytopenia and bleeding disorders [58]. While myosin IIA is indeed the most abundant myosin isof orm in platelets, it has become clear that other conventional class II isoforms, as well as several unconventional myosins, are present in platelets, as detected in several proteomic studies (see Table 2).

**Kinesins**

The kinesin superfamily is comprised of 45 kinesin genes, which can be classified into 14 subfamilies [59]. The position of the motor domain determines the walking direction of the motor along the microtubule. Most kinesins are microtubule plus-end directed motors formed of two

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**Table 2** Motor proteins in platelets. Motor proteins of the dynein, kinesin and myosin families detected in different proteomic studies [21,98–100]. Relative expression levels have been extracted from the study by Burkhart et al. [21] and are indicated according to the color code shown below the table (no color in the case of unavailable copy number estimations).
polypeptide chains with motor domains at their amino-terminus and light chains may be associated. Seven plus-end directed motors have been detected in the platelet proteome (see Table 2), two of which can form homotetramers (kinesin 5/Kif11/Eg5 and kinesin 12/Kif15) and can therefore cross-link and slide anti-parallel microtubules. In addition, one kinesin with a carboxy-terminal motor domain walking to the minus end (kinesin 14B/KifC3) and a relatively high copy number of a kinesin with a central motor domain and depolymerizing activity (kinesin 13/Kif2A) have been detected in platelets (see Table 2). Besides being demonstrated in proteomic studies, kinesin heavy chains have also been evidenced by Western blot in mature platelets using pan-anti-kinesin heavy chain antibodies [20,60].

**Dyneins**

Dynein motors are large multisubunit complexes and the largest subunit or heavy chain contains the motor domain. There are nine types of heavy chains; seven are found in axonemal dynein complexes, which are responsible for cilia/flagellar motility. The cytoplasmic dynein 1 heavy chain forms motors that are implicated in most cytoplasmic transport processes, and the cytoplasmic dynein 2 heavy chain is essential for intra-cilia or flagellar transport processes (IFT). The cytoplasmic dyneins are composed of two heavy chains and each heavy chain is associated with an intermediate, a light-intermediate and several light chains. Dyneins are microtubule minus-end directed motors [61]. Surprisingly, the kinesin and myosin motor domains are quite similar, while the dynein motor domain is composed of six ATP-binding sites characteristic of the AAA (ATPases Associated with diverse cellular Activities) protein family. Besides cargo transport along microtubule tracks, dynein can also move whole microtubules using two possible configurations. Dynein anchored to a cellular compartment can move a microtubule by walking with both motor domains to the minus end. Alternatively, dynein can slide adjacent anti-parallel microtubules by binding to each microtubule with one of its motor domains and each motor walks to the respective minus end [62]. The presence of cytoplasmic dynein in platelets has been documented by Western blot and immunofluorescence [20,60,63]. Furthermore, besides the cytoplasmic dynein 1 heavy chain several other dynein subunits, including the cytoplasmic dynein 2 heavy chain as well as axonemal dynein heavy chains, have been identified in global studies of the platelet proteome (see Table 2). The functional relevance of axonemal and ITF motors in platelets has still to be investigated.

**Coordinated motor actions in platelets**

The reorganization of the cytoskeleton during platelet activation, described in the previous sections, necessitates the precise coordination of motor actions. An equilibrium of antagonistic microtubule motors of the kinesin and dynein families keeps the MB in its resting state. Once kinesin actions are inhibited (either through drug treatment or following an activation stimulus), dynein slides microtubules apart, which leads to MB extension [20]. This process is similar to dynein-mediated microtubule bundle extensions in megakaryocytes during proplatelet production [60]. The limited available space and/or the weakening of the extended bundle induce the three-dimensional twist of the MB resulting in the disc-to-sphere transition of platelets during activation (Figs 2 and 4). Within the microtubule motors expressed in platelets (see Table 2), it is most probably cytoplasmic dynein 1 that is responsible for MB elongation. It is less clear which kinesin motor could counterbalance its actions. The force balance is not strictly antagonistic because dynein inhibition can prevent elongation but has otherwise no impact on the resting MB [20]. Thus the potential candidate should be a plus-end directed kinesin able to cross-link microtubules rather than sliding them apart in the opposite direction (Fig. 4). Good candidates might be kinesin 5/Eg5 or kinesin 12/Kif15, two plus-end motors with microtubule cross-linking properties, known to antagonize dynein actions within mitotic spindles [64,65].

What could the spatial and molecular organization be, allowing dynein to slide microtubules apart? Dynein-driven sliding of microtubules within a circular bundle would just make them slip around the cell periphery unless the motor is anchored somewhere with respect to the microtubules. The fact that MB coiling cannot be induced when the actin cytoskeleton is depolymerized [19,20,66], may suggest that dynein is anchored to the actin cortex, which could serve as a support for dynein-mediated microtubule sliding. Indeed, a study by Nakata and Hirokawa in 1987 describing the actin and microtubule organization in resting platelets supports this view because several cortical actin filaments in contact with the plasma membrane run parallel to the microtubules and protein cross-links (maybe motors) are observed between the two polymers [67]. This has led the authors to suggest at the time that: ‘actin filaments could have a direct influence on the movement of microtubules via the cross-links and vice versa’ [67]. Cortical-anchored dynein has already been shown to push or pull on microtubules in nucleated cells. In axons for instance, cortical dynein can slide microtubules to promote axonal growth [68].

The sliding of microtubules to extend the MB and induce coiling is expected to require high force generation. Although dynein is a weak motor, it appears particularly well suited for this task. When working in a team, dynein motors have the exceptional capacity to develop high forces collectively through the establishment of catch-bonds, which strengthen with increasing load [69]. The exerted forces are likely to be proportional to the strength of the activation stimulus and must somehow be
sense by the actomyosin cortex. When the stimulus is strong enough to induce irreversible activation, myosin becomes activated, leading to the contraction of the cortex and compression of the coiled MB (Figs 2C and 4). Non-muscle myosin II is implicated in this process because its inhibition strongly reduces internal contraction of platelets during activation [19,20,70]. Most importantly, myosin itself may be able to sense the developed forces because an intrinsic mechanosensor function has been attributed to non-muscle myosin II [71,72]. In fact, adhering cells actomyosin-dependent force build-up is determined by substrate rigidity. The adjusted contractile response is similar to the adaptive response of a muscle to load and influences cell polarization and reorients migration towards stiffer regions [72]. Within platelets, MB extension and coiling may lead to an enhanced ‘rigidity’ of the plasma membrane, which could be sensed by the myosin motor, leading to a ‘spring-like’ force accumulation. The trigger to release these forces via actomyosin contraction could be rupture of dynein-cortex or dynein-microtubule interactions once the load becomes too important. Load-dependent detachment of dynein from the cortex has already been described in yeast cells [73]. An advantage of combining sensor and response functions within the same molecule (myosin IIA in this case) is that actions are faster compared with indirect mechanochanical signaling through local molecular stretch sensors [71]. Thus, the degree of actomyosin contraction could be the rapid, adaptive response to the strength of the activation signal. This may explain how the cytoskeletal crosstalk during platelet activation is established to ensure stimulus-response coupling.

**Consequences of the cytoskeletal crosstalk in platelets**

Electron microscopic studies have shown that granules are concentrated in the center of activating platelets, where they are surrounded by the smaller microtubule ring [33,48]. The granules are densely packed for rapid secretion of their content into the extracellular space by fusion with the plasma membrane of the open canalicular system traversing the entire platelet [27,74]. The close packaging...
is a prerequisite for compound exocytosis, which is characterized by the fusion of vesicles not only with the plasma membrane but also between individual vesicles, leading to the almost immediate release of the whole vesicle pool [75,76]. It is tempting to hypothesize that MB coiling, its compression via actomyosin contraction and the formation of a smaller microtubule ring is a particularly efficient mechanism to concentrate the granules in the platelet center. Indeed, reduced actomyosin contractibility, observed after activation of RhoA-deficient platelets, is correlated with an impairment of shape change and a reduction of granule secretion [77]. Thus, the efficiency of actomyosin contraction influences the extent of vesicle release and appears therefore to determine whether platelet activation is transient or becomes irreversible.

A similar MB coiling process, depending on microtubule and cortical actin/myosin/spectrin-based forces, has been shown to determine mature platelet size. In fact, MB extension in preplatelets, which are large discoid platelet precursors, leads to MB coiling. The resulting figure ‘8’ or barbell-shaped proplatelets can either return to the discoid preplatelet shape or separate into two mature platelets [18,78].

Potential regulatory mechanisms of motor activities in platelets

All agonist/receptor interactions independent of the triggered signal transduction pathway will lead to a rise in intracellular calcium concentrations, an early event necessary for platelet activation [79]. Multiple cellular reactions are regulated by calcium but it is worthwhile to mention that a rise in intracellular calcium may directly perturb the activity balance of the two antagonistic microtubule motors and allow dynein to override kinesin to slide microtubules apart, eventually leading to actomyosin contraction. Indeed, calcium tends to reduce the activity of kinesin motors while increasing dynein activity [80–82]. This sequence of events is in agreement with studies reporting that an intracellular calcium rise is responsible for the initial onset of shape change and Rho-kinase activity (and thus actomyosin contraction) is important for the maintenance and the amplitude of the response [83–85]. Phosphorylation of dynein could be an additional way to regulate the activity of this motor because it has been shown that dynein subunits become phosphorylated during platelet activation [63]. The myosin-mediated contractility of the cell cortex is also regulated by phosphorylation. Two sites in the regulatory myosin light chain are differentially phosphorylated, which appears to determine the degree of contraction [83]. Another post-translational modification, which may regulate the kinetics of MB coiling, is tubulin acetylation. Microtubules are heavily acetylated in flat and coiled MBs and deacetylation is rapid once platelets are activated [45,46]. It has been shown that the affinity of dynein for microtubules and the efficiency of its action are enhanced when microtubules are acetylated [86]. Because rapid platelet activation can be vital, it is tempting to speculate that microtubule acetylation may help to speed up the dynein-mediated sliding of microtubules and thus the coiling and activation process. This hypothesis is supported by the faster spreading capacity of platelets with hyperacetylated MBs described previously [45]. In this context it is interesting to note that MBs in avian erythrocytes are not acetylated, but in contrast to platelets, rapid reorganization of their cytoskeleton is unnecessary because they keep their discoid shape permanently [87].

Similarities of cytoskeleton rearrangements in platelets and nucleated cells

Platelets are an ideal model system to study shape maintenance, shape change and the regulation of these processes in the absence of a nucleus and centrosomes and without any influence of the cell cycle and cell polarity. The principal mechanistic features should, however, be applicable to nucleated cells. In support of this notion, highly similar microtubule coils have been described upon activation of fish thrombocytes, which are large, nucleated cells with a

![Fig. 5. Erythrocytes of a mollusk (blood clam) stained with anti-tubulin antibodies. (A) Normal, flat cell. (B–D) Spherical cells after shape transformation. (E) Diagram of the basic marginal band deformation pattern; bar 10 μm (original Figure 9 from [90] reprinted with permission from the Marine Biological Laboratory, Woods Hole, MA). Inserts in A and C show marginal bands in platelets; bar 2 μm.](https://example.com/fig5.jpg)
discoid shape in the resting state [88]. Similarly, amoebocytes of the horseshoe crab, which are flat ellipsoid clotting cells in the hemolymph, have twisting MBs when activated [89]. Another surprising example is nucleated erythrocytes of mollusks, which have an ellipsoid morphology and a MB formed by centrosomal organized microtubules. In contrast to vertebrate erythrocytes, which keep their discoid shape permanently, these cells undergo disc-to-sphere transition upon collection of the hemolymph and coiled MBs are observed in the spherical cells (Fig. 5) [90]. It is worth emphasizing that despite the extreme difference in cell size the MB coil in these nucleated cells appears similar to the MB coil in platelets (compare Fig. 5A, C with the inserts in 5A, C illustrating the size difference between MBs in erythrocytes of mollusks and MBs in platelets). The fact that MB coiling is observed in various nucleated cells of lower vertebrates, arthropods and mollusks indicates that it is an evolutionary conserved process used by different cell types to adapt cell shape. Thus, microtubules and their motors have to be considered as important players for global shape changes in addition to the actomyosin cytoskeleton previously thought to play the primordial role. Interestingly, smaller cell shape changes in subcellular domains of nucleated cells appear to follow similar principles. It has been shown that microtubule bending in the periphery of epithelial cells is dependent on microtubule motor actions and not on actomyosin contraction or tubulin polymerization [91]. Similarly, cortical dynein can generate microtubule movements along the plasma membrane to drive shape changes in the periphery of the cells independent of the actin cytoskeleton [92].

Perspectives

Besides providing major contributions to answer fundamental cell biological questions, the detailed knowledge of the sequence of events leading to platelet activation is primordial for the development of new therapies for the treatment of bleeding or thrombotic diseases. In addition, an increasing number of studies suggest that besides their primary role in hemostasis, activated platelets may have a major impact in pathologies such as cancer, inflammation, infection and neurological disorders [93,94] and appear to promote liver regeneration and tissue repair [95,96]. Continued efforts to unravel mechanisms contributing to the platelet activation process are therefore well worth the time invested.

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Disclosure of Conflict of Interest

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