Platelet cytoskeleton and its hemostatic role

Doris Cerecedo

Upon vascular injury, platelets adhere to the exposed extracellular matrix, which triggers the platelet activation and aggregation to form a hemostatic plug to seal the wound. All of these events involve dramatic changes in shape because of the cytoskeleton reorganization. The versatility of the cytoskeleton's main elements depends on the biochemical nature of the elements, as well as on the associated proteins that confer multiple functions within the cell. The list of these associated proteins grows actively, increasing our knowledge concerning the complexity of platelet cytoskeleton machinery. The present review evidences the recently described platelet proteins that promote characteristic modifications in their cytoskeleton organization, with special focus on the dystrophin–glycoprotein complex. ***Blood Coagul Fibrinolysis*** **24:798–808 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Platelets are small, heterogeneous, anucleated fragments derived from megakaryocytes in the bone marrow. Platelets are involved in inflammation, act against microbial infection, and promote tumor metastasis; however, their main role is stopping bleeding after the loss of integrity of a blood vessel. On exposure to damaged endothelium, platelets slow their speed and tether to the extracellular matrix (ECM) components; therefore, these thrombogenic proteins trigger platelet activation.

Resting platelet cytoskeleton is formed by filamentous actin (F-actin), a marginal ring of microtubules, intermediate filaments, and binding proteins. During the activation and adhesion process, cytoskeletal elements are reorganized; actin filaments form part of filopodia, lamellipodia, focal adhesions, stress fibers, and the contractile ring. Microtubule ring fragments disperse, whereas intermediate filaments are associated with the plasma membrane and the granulomere.

The dystrophin–glycoprotein complex (DGC) is a group of cytoskeletal proteins that establish communication between the ECM and the actin cytoskeleton. This complex was initially described in muscle cells. To date, the DGCs, composed of dystrophins and utrophins as central axes, have been described in kidney, lung, central nervous system, and liver. In blood tissue, their presence has been described in neutrophils and stem progenitor cells and, especially in platelets, both of these complexes are intimately associated with microfilaments, microtubules, and intermediate filaments, and participate in a very special manner during the adhesion process, forming stress fibers, focal adhesions, and granule trafficking. In addition, they are involved in the maintenance and organization of membranous systems, promoting their initial anchoring to the substrate. The purpose of this article was to review the evidence relating to the dynamism of the platelet cytoskeleton with an emphasis on its association with the DGC, discussing their feasible molecular interactions that mediate their participation in the hemostatic role of platelets.

Ultrastructure of resting, activated, and adhered platelets

The adhesion of circulating blood platelets to the subendothelial matrix that is exposed upon vessel wall injury represents the initial event of the hemostatic process required to limit hemorrhage. Recruitment of additional platelets into the growing thrombus allows platelets to aggregate, providing a hemostatic platelet plug that involves the elements of cytoskeletal reorganization, including cytoplasmic actin filaments and a membrane skeleton located immediately below the plasma membrane and that consists of both actin filaments and microtubules [1].

Platelet actin cytoskeleton

Actin is a highly conserved globular protein with a molecular weight of 42 kDa. In living cells, actin polymerizes to form F-actin with a mean diameter of approximately 8 nm, which is comprised of two helical protofilaments of globular actin (G-actin) subunits wound around each other, having a pseudo-repeat distance of 36 nm and comprising 14 monomers [2]. Upon activation, there is extensive rearrangement of the cytoskeleton; the proportion of total actin in filaments increases rapidly from 30 to 70% [3].

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Actin polymerization drives the extension of dynamic cellular structures such as filopodia and lamellipodia, thereby powering cell movement [4], whereas more stable, actin networks form static cellular structures such as stress fibers and contractile rings that function in cell adhesion and cell division, respectively [5,6].

Activation of platelet in suspension or spread on glass produces a reproducible sequence of the morphologic events such as rounding, filopodial projection, attachment, spreading, and, ultimately, contraction [7,8]. After the platelet adheres to the flat surface, its round shape changes, becoming spherical and slightly smaller as a result of a contractile event. Filopodia projection follows rounding (<1 min) by the burst of dynamic surface protrusions denominated pseudopodia, which can extend and retract; some of these pseudopodia remain as filopodia [9].

Filopodia are highly dynamic, actin-rich cell-surface protrusions that extend mainly from the periphery of the cell and aid cells to sense their external environment. Cdc42 is the well known factor that triggers filopodia formation [10], inducing actin nucleation at the barbed end of the filopodia, mediating cooperation between Wiskott–Aldrich syndrome protein (WASP)/neuronal WASP (N-WASP) and actin-related protein 2/3 (Arp2/3) [11]. Recently, it was established that Cdc42 also participates in platelet aggregation, determining granular secretion and demonstrating that Cdc42 is essential for normal platelet function [12].

The filopodia of activated platelets contain barbed-end actin assembly, whereas the structure of actin in lamella revealed that this is a tight, orthogonal branched network. However, prevention of the usual rise in cytosolic calcium accompanying platelet activation inhibited the formation of lamellipodial, but not filopodial, indicating that these actin structures are functionally and morphologically different [13].

The lamellipodium is a broad and flat network of actin filaments with many microns along its length and width, but only approximately 0.1–0.2 μm high [4,14]. Within the lamellipodium, the actin cytoskeleton forms a treadmilling dendritic array [15,16], in which the nascent filaments nucleate from Arp2/3 complexes, the major regulator of platelet actin dynamics and responsible for the formation of filopodia and lamellipodia [17]. ADF/cofilin action coupled with adenine triphosphate (ATP) hydrolysis facilitates actin filament disassembly, recycling the monomers to the leading edge. Actin filaments in the lamellipodium are oriented with their barbed ends toward the leading edge [15], and this arrangement is crucial for maintaining the lamellipodial structure. In-vivo experiments have shown that actin filament turnover comprises a critical step in the terminal phase of platelet formation, as well as in the maturation and sizing of early platelets for producing the homogeneous mature platelet population [18].

Lamellipodia can form from the lateral membrane between pseudopodia or from the sides of pseudopodia, extending slowly with frequent pauses accomplishing in the first 8–10 min. Further reorganization of the cytoskeleton occurs and adhesion plaque proteins, such as vinculin or vasodilator-stimulated phosphoprotein (VASP), are incorporated into the system [19,20]. Platelet adhesion to glass triggers the shape changes, which involve organization of actin filaments on four distinct actin filament structures that are formed at the following: filopodia, lamellipodia, a contractile ring, and stress fibers, each containing a different complement of actin-binding proteins [21].

Actin nodules represent a novel actin structure composed of punctate areas of actin, first observed at the onset of lamellipodia formation within platelets spread on multiple matrix proteins. It is proposed that they form during the early stages in actin polymerization and that they play an important role in driving the formation of later actin structures, including lamellipodia, and that they possibly contribute to stress fiber formation [22].

In platelets, as in many cells, the protrusive appendages consist of two overlapping, but molecularly and functionally distinct, actin networks denominated the lamellipodium and the lamella [23]. The lamellipodium assembles at the leading edge, but within a few microns disassembles, coupling to a second network, the lamella, where myosin contraction is integrated with substrate adhesion [24].

Platelets regulate their spreading and morphology depending on the environmental geometry affecting the reorganization of its cytoskeleton in response to the geometrical constraints of the microenvironment [25], as has been shown for nucleated mammalian cells [26]. This evidence shows that the molecular machinery for platelet adhesion and spreading is strikingly similar to that in other motile cells [27].

Finally, to mediate the subsequent contraction of the clot, platelet contraction reaches a steady state after 25 min, exerting total forces of approximately 34 nN [28]. To perform these clot contractions, further structural rearrangements occur, recruiting cytoplasmic and membrane proteins, promoting polymerization of actin, and organization of filaments into higher order structures such as stress fibers.

Stress fibers are composed of bundles of actin filaments and myosin II, and allow nonmuscle cells to apply contractile forces. Early work identified α-actinin and myosin as dynamically redistributed and segregating alternately on the actin of the cell body in fully spread platelets during the adhesion process [29], with RhoA as a crucial regulator of actin stress fiber formation [30]. There are other key elements working cooperatively: the PI-3 kinase/RhoA/mDia1 axis, which is a critical pathway

for coupling thrombin signaling to actin cytoskeletal remodeling during platelet spreading [31].

Integrins expressed in the platelets are the major class of the ECM receptors responsible for cell adhesion because they are both structurally and functionally linked with the actin cytoskeleton [32,33], as well as with integrin-associated proteins such as talin and vinculin [34–36]. Elegant studies in which the vinculin gene has been deleted has led to conclude that vinculin did not disturb the physiological responses depending in part on $\alpha_{IIb}\beta_3$ and F-actin, suggesting that other proteins can substitute or compensate for vinculin in its absence [37].

Integrin activation is achieved by a series of inside-out signaling events culminating in the binding of two key integrin activators: the talin head domain and kindling. Agonist-induced activation of platelet integrins is dependent on the talin–integrin interaction, which is necessary for fibrin clot retraction [38]. Kindlin is the most recently identified integrin activator; in platelets, kindlin-3 is the main functional isoform required for integrin-mediated responses binding to the β-cytoplasmic tails, which is needed for affinity modulation of the integrin [39,40]. Its absence causes severe bleeding and the immune disorder in human [41,42].

Platelet microtubule cytoskeleton

After their liberation from the megakaryocytes in the bone marrow, platelets circulate in the blood stream for about 10 days with a discoid shape whose size ranges from 1.5 to 4.5 μm [43]. This shape is because of a marginal ring of microtubules situated immediately beneath the plasma membrane [44–46].

The platelet marginal band is composed nearly entirely of microtubules. Microtubules are polymers of α-tubulin and β-tubulin dimers that associate into linear arrays called protofilaments, which laterally associate, forming the hollow rigid tubular structure characteristic of microtubules. The bulk of β-tubulin within the microtubule coil is composed of β1-tubulin, a divergent β-tubulin isoform exclusive to megakaryocytes and platelets [47,48].

Recently, in an elegant study by Patel-Hett *et al.* [49], microtubule dynamics was evaluated in living platelets and observed that the marginal band of microtubules is composed of multiple, bipolar dynamic microtubules arising from a single stable microtubule arranged in a coil. The dynamism of microtubules provides the platelet with the ability to alter its cytoskeleton during physiological processes, such as shrinking its coil during platelet aging and the formation of a radial microtubule array that reaches into the forming filopodia during activation and adhesion.

RanBP10 is a cytosolic protein with binding capacities for both β-tubulin and the small GTPase Ran; it decorates microtubule filaments in megakaryocytes and platelets [50], and might convert cytoplasmic Ran-GDP into Ran-GTP, providing information for microtubule nucleation or other filament properties [51]. In platelets, RanBP10 plays an essential role in hemostasis and in maintaining the microtubule dynamics with respect to both platelet shape and function [52].

Characterization and intracellular distribution of the dystrophin–glycoprotein complex

The Duchenne muscular dystrophy (*DMD*) gene, whose sequence comprises more than 79 exons, code for a 427-kDa skeletal muscle protein of 3685 residues named dystrophin (Dp427) [53]. Internal promoters in the *DMD* gene codify for the expression of short dystrophin products of 260 kDa (Dp260), 140 kDa (Dp140), 116 kDa (Dp116), and 71 kDa (Dp71) [54–58]. Dp71 (70–75 kDa) is the major dystrophin expressed in nonmuscle cells and its transcripts are alternatively spliced in exons 71–74 and in exon 78 to produce multiple products of 70–78 kDa. Differential splicing of Dp71d exon 78 produces at least two Dp71 isoforms: the Dp71d, which preserves the C-terminal end of Dp71, and the Dp71f (for Dp71 founder sequence), corresponding to the removal of exon 78. Two other transcripts, $Dp71\Delta_{110}{}^{a}$ and $Dp71\Delta1_{110}{}^{m}$, respectively, were characterized as the gene products resulting from alternative splicing at exons 71–74 and 78 [59]. Furthermore, the alternative gene codes for full-size utrophin Up400 (400 kDa), Up140 (140 kDa), and Up71 (70 kDa) [60,61]. Dystrophin is one of a number of large cytoskeleton-associated proteins that connect among various cytoskeletal elements, providing connections between a transmembrane complex known as the DGC and the underlying cytoskeleton [62]. The most established connection, and possibly the most important, is that connected to F-actin, but more recent evidence has been forthcoming of connections to membrane phospholipids, intermediate filaments, and microtubules [63–65]. The DGC is made up of dystroglycans, sarcoglycans, dystrobrevins, syntrophins, and sarcospan, which are grouped into three subcomplexes: the dystroglycan complex, the sarcoglycan complex, and the dystrobrevin/syntrophin complex [66,67].

The dystroglycan subcomplex consists of α-dystroglycan and β-dystroglycan subunits, which are encoded as a single polypeptide of 895 amino acids transcribed from the *DAG1* gene, which undergoes posttranslational proteolytic cleavage to yield the two noncovalently associated subunits [68]. α-Dystroglycan is an extensively glycosylated extracellular protein [67,69,70] that mediates binding to laminin and laminin G-like domains of perlecan, agrin, neurexin, and pikachurin [71–74], and proper glycosylation is essential to dystroglycan function [75,76]. β-Dystroglycan is a single-pass transmembrane protein with a largely unstructured amino-terminal extracellular domain that binds to the carboxy-terminal

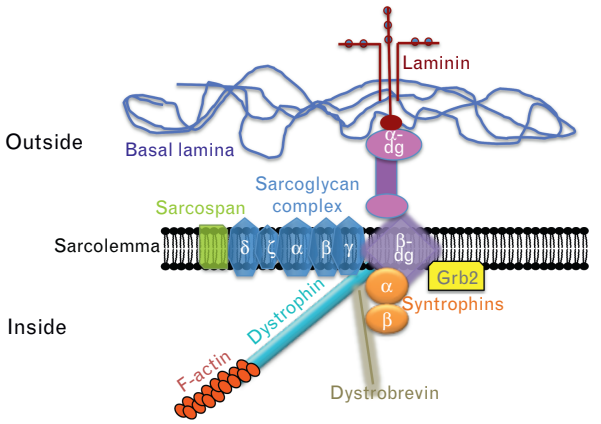
globular domain of α-dystroglycan [77], and its carboxy-terminal cytoplasmic domain binds dystrophin [78]. Transmembrane localization of dystroglycan constitutes a link between the intracellular actin cytoskeleton and the ECM, and clearly defines dystroglycan as an adhesion molecule. Although dystroglycan possesses no apparent intrinsic ability for transducing adhesion-mediated signals, adhesion-like receptors, such as integrins, both inside-out and outside-in signaling, are mediated via signaling molecules that associate with its adhesion receptor. The cytoplasmic domain of β-dystroglycan is associated with rapsyn and the Ras/MAPK signaling pathway through the adapter protein Grb2 [79–81]. The sarcoglycan complex is composed of α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, and δ-sarcoglycan isoforms, each encoded by a separate gene [82–84], and sarcospan [85]. All sarcoglycans are single-pass transmembrane glycoproteins with long extracellular domains and relatively short cytoplasmic domains. Sarcospan encodes four transmembrane-spanning segments that are homologous to the tetraspanin family of proteins [85], which are thought to mediate interactions among transmembrane proteins.

Dystrobrevins and syntrophins are two families of cytoplasmic proteins encoded by multiple genes expressed in a tissue-specific manner [86–88] that interact directly with dystrophin [89–91]. The syntrophin family of proteins comprises five members: α-syntrophin; β1 and β2 syntrophin, and γ1 and γ2 syntrophin [92]. All syntrophins share one domain unique to syntrophins: a postsynaptic density protein of 95 kDa (PDZ) domain and two pleckstrin homology domains, suggesting their role as adaptor proteins involved in anchoring cell signaling molecules to the plasma membrane [93].

The syntrophin unique domain and the carboxy-terminal pleckstrin homology domain interact with the extreme carboxy terminus of dystrophin [90]. Dystrobrevins share significant sequence homology with the carboxy-terminal domains of dystrophin [88,94]. Two dystrobrevin genes encode multiple isoforms expressed in a wide array of tissues: α-dystrobrevins, expressed predominantly in the skeletal muscle with α-dystrobrevin-1 localizing to the neuromuscular junction [94–96] and α-dystrobrevin-2 distributed throughout the sarcolemma [97]. In addition to their independent interactions with dystrophin, syntrophins and dystrobrevins directly to bind each other [98], it has been suggested that two syntrophin molecules associate with each DGC through independent interactions with dystrophin and dystrobrevin (Fig. 1). β-Dystrobrevin is expressed in nonmuscle tissues and associates with dystrophin, Dp71, utrophin, and with different syntrophins participating in cellular polarization [99].

In 1995, Earnest *et al.* [100] demonstrated the presence of utrophin (Up400) in platelets as part of the membrane

Fig. 1



Schematic diagram of the dystrophin–glycoprotein complex (DGC) in the skeletal muscle. Dystrophin is a linker between the subsarcolemmal cytoskeleton and the extracellular matrix (ECM). Dystrophin (or its homolog, utrophin) is associated with the dystroglycan complex, the sarcoglycans–sarcospan complex, and the dystrobrevin/syntrophin complex. The sarcoglycan–sarcospan complex comprises the sarcoglycans (α, β, γ, and δ) and sarcospan. α-Dystroglycan (α-Dg) binds to laminin-2 in the ECM and β-dystroglycan (β-Dg) in the sarcolemma; β-Dg binds to the dystrophin, completing the link between actin cytoskeleton and the ECM.

skeleton, participating in an integrin-induced reorganization of the cytoskeleton and in transmembrane signaling that occurs as a consequence of integrin–ligand interactions.

Although platelets do not express full-length dystrophin, short dystrophins have been detected. In 2002, Austin *et al.* [101] provided evidence that $Dp71\Delta_{110}$ is expressed in human platelets as a component of the platelet membrane cytoskeleton, and that it is redistributed in integrin-induced reorganization of the cytoskeleton during thrombin-induced platelet activation. It was stated that $Dp71\Delta_{110}$ mediate cytoskeletal reorganization and signaling in thrombin-stimulated platelet adhesion, because adhesion of Dp71-deficient platelets from mdx^{3cv} mice was significantly reduced compared with platelets from wild-type mice.

In 2005, these previous studies were complemented with the reporting of the expression of two Dp71 isoforms (Dp71d and Dp71f), of the Utrophin isoform Up71, and of three dystrophin-associated proteins (DAPs): α-dystrobrevin-1 (α1-Db), α-dystrobrevin-2 (α2-Db), and β-dystrobrevin (β-Db), as well as their co-distribution with actin filaments in resting and in activated platelets by adhesion to glass or by exposure to thrombin. In addition, the distribution of $Dp71d/Dp71\Delta_{110}{}^{m}$, Up400/Up71, and DAP was established. The presence of two DGCs composed of short dystrophins ($Dp71d/Dp71\Delta_{110}{}^{m}$) and utrophins (Up400/Up71) that were associated with actin-based structures suggests their

participation in cytoskeleton remodeling, adhesion to substrate, and granule centralization [102].

Platelet cytoskeleton with deficient short dystrophins

Utrophin is an autosomal homolog of dystrophin that can also bind to proteins of the DGC [60,103]. Dystrophin and utrophin share 74% similarity at the amino acid level and have very similar domain structures [104]. Utrophin is expressed in place of dystrophin in fetal muscle, but in adult myofibers, it is confined to the neuromuscular and myotendinous junctions. Thus, utrophin upregulation is an attractive therapeutic approach for DMD. Studies in mdx mice, a model for DMD, have shown that utrophin, when overexpressed in myofibers by viral vector-mediated delivery or by transgenic means, can compensate for the absence of dystrophin, restoring normal muscle function [105,106]. Preclinical investigations attempting to upregulate utrophin have been conducted; however, no utrophin upregulation therapy is available yet for clinical use in patients with DMD.

Previous studies in patients with DMD have shown normal plasmatic coagulation, a slight, but not significant, increase of bleeding time, as well as a marked reduction of the expression of glycoprotein GPIV [107,108]. However, other authors have concluded that the blood loss was not because of direct defects in platelets, but rather because of vascular smooth muscle dysfunction [109]. Platelets isolated from *mdx*^{3cv} showed a diminution of adhesion to collagen in response to thrombin, suggesting that in major surgery, platelet function deficiencies in adhesion and aggregation were evidenced by the absence of the Dp71Δ^{110} isoform in platelets [101].

In a retrospective study, Turturro *et al.* [110] also described increased blood loss with the retention of normal platelet function, suggesting that impaired vessel reactivity caused the hemostatic dysfunction in patients with DMD. In 2008, Labarque *et al.* [111] showed a tendency toward increased bleeding, attributable to dysfunctional Dp71 in the cytoskeleton of DMD platelets. The disorganized dystrophin-containing cytoskeleton in the platelets of patients with DMD results in increased blood loss because of decreased collagen response, enhanced Gsα expression, and inducible Gs hyperfunction promoted by the natural release of prostacyclin during a surgical procedure.

Analysis of dystrophin gene expression and function has been aided by the studies in mice with dystrophin gene mutations (*mdx*) [112,113]. The majority of dystrophin studies to date have been carried out with the original *mdx* mutant, which contains a premature stop codon in exon 23 [112], different from that in 60–70% of mutations in the human dystrophin gene, which corresponded to deletions or duplications [114]. Four newer strains of *mdx* mice have been described *mdx*$^{2cv-5cv}$, which were generated with N-ethylnitrosourea (ENU) chemical mutagenesis [113]. All of the strains were found to have point mutations; the *mdx*^{3cv} allele arises from a mutant splice acceptor site in intron 65 [115], rendering the unique animal model that failed to express the Dp71 isoforms of dystrophin (71 kDa) normally found in brain and in many other nonmuscle tissues including platelets; therefore, this system directly reflects the effects of the absence of Dp71 isoforms. Cellular distribution of DP71d/Dp71Δ_{110}^{m}, utrophins, and DAP in adhered platelets from dystrophic mdx^{3cv} mice was evaluated during the adhesion process. The absence of Dp71 isoforms in platelets from this animal model disrupted DAP expression and distribution without modifying the platelet morphology displayed during the adhesion. Utrophins were found to be upregulated and in association with DAPs to form a protein complex that might compensate for the absence of Dp71 in *mdx*^{3cv} platelets [116].

β-Dystroglycan and cell adhesion

In general, adhesion morphology and dynamics vary considerably among different cell types. These differences might arise from isoform composition, intrinsic differences in actin organization, or cell-type-dependent variations in the molecular composition of the adhesions. For a cell, dystroglycan represents a connection protein between the ECM and the cytoskeleton, as well as a signal transducer, it has been difficult to discriminate between their mechanical and signaling functions, and it is very probable that these are indivisible.

A commonly known mechanism for regulating adhesion-dependent processes is tyrosine phosphorylation, and in humans, phosphorylation of Y892 in β-dystroglycan represents an important molecular switch to control its function in podosomes and focal adhesions [117].

Peripheral membrane localization of dystroglycan confers a scaffold role for the components of the actin signaling machinery to generate actin-based structures such as filopodia and microvilli by local activation of Cdc42 at the membrane [118].

Links between the dystroglycan and integrin adhesion systems have been implied both at the signaling and at the mechano-structural level [119–121]. Dystroglycan is clearly present in focal adhesions, interacting indirectly with vinculin through vinexin and contributing to focal adhesion stability and turnover, thus affecting the cell migration [122].

Platelets display focal adhesions, as well as stress fibers, to contract and facilitate the expulsion of growth and procoagulant factors contained in the granules and to constrict the clot; in full-spread platelets, microfilament bundles in association with other cytoskeleton proteins are anchored in the focal contacts.

The recent studies in migrating cells suggest that coordination and direct physical interaction of microtubules and the actin network modulate adhesion development [123]. In platelets, a feasible association has been proposed between these two cytoskeletal systems, as well as the participation of the DGC, as part of the focal adhesion complex. β-Dystroglycan was found to act as an interplay protein between actin and microtubules, and additional communication between these two cytoskeleton networks was maintained through the proteins of the focal adhesion complex [124].

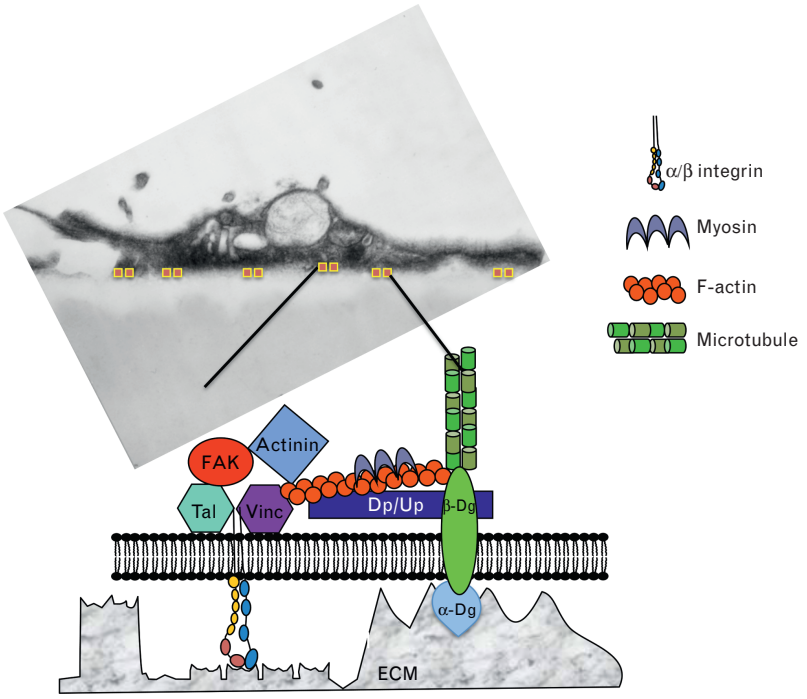
Additionally, the participation of DGC made up of short dystrophins (Dp71d/Dp71Δ_{110}^{m}) and utrophins (Up400/Up71), their potential association with integrin β-1 fraction, and the focal adhesion system (α-actinin, vinculin, and talin) were evaluated during the platelet adhesion process. It was shown that DGC composed of short dystrophins participated in stress fiber assembly and in the centralization of cytoplasmic granules, whereas DGC composed of utrophins participated in assembling and regulating focal adhesions, incorporating focal adhesive kinase (FAK) into the complex. The simultaneous presence of dystrophin and utrophin complexes indicates complementary structural and signaling mechanisms to the actin network during the adhesion process [125] (Fig. 2).

The platelet cytoskeleton and granule secretion

Dynamic reorganization of the platelet cytoskeleton triggers a signaling cascade that results in the secretion of adhesive proteins and platelet agonists from their granules. Four types of secretory organelles have been identified in platelets, based on their ultrastructure and selective protein composition: α-granules, dense granules, multivesicular bodies, and lysosomes [126,127]. α-Granules develop from the budding vesicles in the Golgi complex within megakaryocytes, where they transform into multivesicular bodies, fuse with endocytic vesicles, and are thought to be a common precursor of both α and dense granules. α-Granule subpopulations can be distinguished on the basis of morphology in spherical and tubular granule population [128]. α-Granules exhibit heterogeneous and spatial protein packaging, cargo type [129–131], in response to agonists [132–134] and microenvironmental factors, such as temperature, which selectively regulate the release of their proteins [135].

The heterogeneity of α-granules is also based on the expression of vesicle-associated membrane proteins (VAMPs), which have been demonstrated to function in platelet granule secretion. VAMP-7 moves to the periphery during spreading, in contrast to the granules

Fig. 2

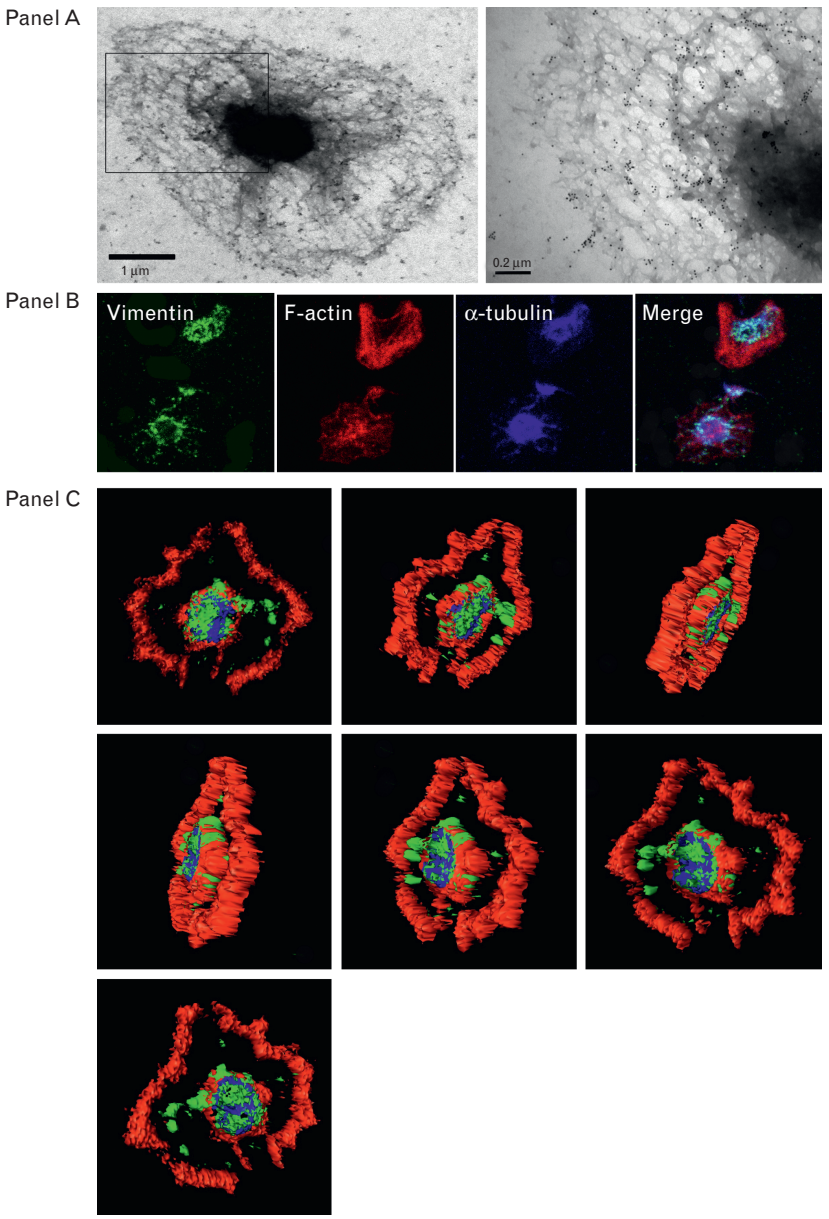


Schematic diagram of some platelet cytoskeleton proteins. Focal adhesion (magnified at the bottom of the figure) clusters the α, β integrin receptors and induces recruitment of the focal adhesion proteins vinculin (Vin), talin (Tal), and α-actinin (α-act), which connect directly with microfilaments and short dystrophins (Dp71) and indirectly with microtubules and intermediate filaments. The adhesion complex activates integrin-associated signaling cascades, including focal adhesion kinase (FAK). Dystroglycan plays a scaffold role, modulating the cytoplasmic protein kinases, and is in close association with integrin β1.

expressing VAMP-3 or VAMP-8, which are maintained at the central granulomere of spread platelets [136].

The platelet cytoskeleton interacts with the select soluble N-ethyl-maleimide-sensitive factor activating protein receptor (SNARE), whereas actin polymerization facilitates α-granule release [137]; on evaluating the kinetics of granule secretion, as well as the degree of secretion, it was determined that platelets treated with actin-disrupting agents inhibit α-granule secretion, but stimulate dense granule secretion [138]. In adhered platelets, it was suggested that actin filaments and microtubules contribute to α-granule and dense granule mobilization in adhered platelets, identifying α-dystrobrevins as part of the platelet transport machinery in close association with ubiquitous kinesin heavy chain (UKHC) [139],

Fig. 3



Platelet distribution of intermediate filaments. (Panel A) Representative electron micrographs of the detergent-insoluble platelet cytoskeleton. Platelets were viewed by the immunoelectron microscopy to localize vimentin in the detergent-insoluble cytoskeletons of adhered platelets. (Panel A, right). High magnification of the granulomere, cytoplasm, and platelet membrane, observing vimentin distribution in these structures. (Panel B). Adhered platelets processed by immunofluorescence staining of microfilaments labeled with tetramethylrhodamine (TRITC)-phalloidin, microtubules labeled with Alexa 633 secondary antibody, and vimentin labeled with a fluorescein isothiocyanate-conjugated (FITC) secondary antibody. (Panel C). Three-dimensional (3D) reconstruction of microfilaments, microtubules, and intermediate filaments of the lower adhered platelet on glass shown in Panel B performed with AMIRA V5.3.2 software.

a motor protein that has been described as participating in the transport of cellular components in neurons [140].

Single-platelet amperometric measurements performed on surface-adherent platelets revealed that F-actin, but not the microtubule coil, regulates platelet dense-body granule secretion. These measurements suggested that F-actin acts as a physical barrier to platelet dense-body granule secretion [141].

Participation of desmin and vimentin in the adhesion process

Intermediate filaments represent one of the main cytoskeletal systems and are one of the most stable components found in vertebrate cells. Intermediate filaments are highly dynamic structures and reorganize by phosphorylation, glycosylation, and transglutamination [142,143]; they participate in organelle distribution [144,145], signal transduction [142], cell polarity [146], and gene regulation [147–149].

Intermediate filaments have a dual structure, with a central conserved domain, in contrast with highly variable head and tail domains, which play a crucial role in intermediate filaments assembly; they interact with various cytoplasmic proteins including other cytoskeletal components, conferring to the cell type different and specific functions [150,151].

The three major filaments of the cytoskeleton, microfilaments, microtubules, and intermediate filaments, are linked by the protein, plectin. Plectin is a member of the plakin family of cytolinkers, which includes the desmosomal desmoplakin and which also binds to integrins and cadherins [152]. Plectin distribution is not affected in desmin knockout mice, but in plectin knockout mice, the amounts of vinculin and spectrin are reduced. Intermediate filaments appear to be transported throughout the cell associated with microtubules and microfilaments via cytoplasmic dynein [153]; the majority of intermediate filaments, including desmin, together with microtubules, support and position the organelles [154–156].

Tablin and Taube [157] in 1987 suggested the presence of a 58-kDa vimentin-like protein that was associated with the microtubule coil and the plasma membrane, which thus may help to maintain the resting platelet's discoid shape. In adhered platelets, the presence of desmin and vimentin and their association with DAPs, as well as with microfilaments and microtubules, through plectin have been described. A pharmacological approach has evaluated the participation of vimentin and desmin in granule trafficking. Additionally, the results have suggested that the three cytoskeleton networks (microfilaments, microtubules, and intermediate filaments) modulate platelet membranous system organization [158] (Fig. 3).

Conclusion

Platelets are key elements to avoid bleeding; in developing their task, they undergo dramatic morphological changes, thus becoming one of the best models for studying cytoskeletal remodeling. To achieve their function, platelets follow consecutive events including a self-amplifying process promoted by several proteins stored in their own granules, with granule extrusion contents the platelet's priority.

Apparently, the mechanical and physiological functions of microfilaments, microtubules, and intermediate filaments in platelets are not exclusive. The cytoskeleton could play concomitant structural and regulatory roles; thus, it would be impossible to seek out a single function for each cytoskeletal element. However, there is experimental evidence that represents crucial information on the complexity of these cytoskeletal cell fragments, whose versatility is because of the association of diverse associated proteins with the main cytoskeletal components. To date, there are many proteins that have been described as part of the platelet cytoskeleton, whereas others appear to be redundant in terms of completing the function.

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Conflicts of interest

There are no conflicts of interest.

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