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Review Article

ROLE OF CIB1 IN PLATELET SIGNALING AND HEMOSTASIS

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ABSTRACT

Calcium- and integrin-binding protein 1 (CIB1) is a small ubiquitous Ca2+-binding protein of the helix-loop-helix EF-hand family. CIB1 has been widely expressed protein that interacts with a multitude of other target proteins, and has been involved in various cellular processes such as calcium signaling, cell differentiation, cell division, cell proliferation, cell migration, cell growth, thrombosis, angiogenesis, cardiac hypertrophy, adhesion, apoptosis, etc. Some of these binding partners include the serine/threonine kinases, p21-activated kinase 1 (PAK1), apoptosis signal-regulating kinase 1 (ASK1), and polo-like kinase 3 (PLK3). Structural and mutational studies indicate that CIB1 binds most or all of its partners via a well-defined hydrophobic cleft. Although CIB1 itself lacks known enzymatic activity, it supports the PI3K/AKT and MEK/ERK oncogenic signaling pathways, in part, by directly modulating enzymes in these pathways. The recent elucidation of the three-dimensional structure of CIB1, and biochemical analysis of its Ca2+, Mg2+ and target protein interactions has greatly enhanced our understanding of the protein's function, its mechanism of target protein binding, and its potential involvement in human disease. However, CIB1 was initially identified as a specific binding partner for platelet integrin aIIbb3 and has since been shown to bind several other platelet signaling proteins, implying an important role in hemostasis. In this review we briefly discuss about the role of CIB1 in hemostasis via platelet signaling.

Key words: CIB1, G-proteins; platelet signaling; Signal transduction; VWF; Thrombin; Collagen; ADP

INTRODUCTION

Platelet signaling was the initially identified role of CIB1 in 1997. There are three basic steps in the pathway of platelet signaling model which are initiation, extension and stabilization. Each of the steps is supported by signaling events within the platelet. Initiation occurs when moving platelets become tethered to and activated by collagen-von willebrand factor (VWF) complexes within the injured vessel wall. This produces a platelet monolayer that supports the subsequent adhesion of activated platelets to each other. Extension occurs when additional platelets adhere to the initial monolayer and become activated. Thrombin, ADP and thromboxane A2 (TxA2) play an important role in this step, activating platelets via cell surface receptors coupled to heterotrimeric G-proteins. Subsequent intracellular signaling activates integrin αIIbβ3 (glycoprotein (GP) IIb–IIIa in older literature) on the platelet surface, thereby enabling cohesion between platelets. Stabilization refers to the late events that help to consolidate the platelet plug and prevent premature disaggregation, in part by amplifying signaling within the platelet. Examples include outside-in signaling through integrins and contact-dependent signaling through receptors whose ligands are located on the surface of adjacent platelets. The net result is a hemostatic plug composed of activated platelets embedded within a cross-linked fibrin mesh, a structure stable enough to withstand the forces generated by flowing blood in the arterial circulation. This three stage model arises from studies on platelets from individuals with monogenic disorders of platelet function and from mouse models in which genes of interest have been knocked out. However, recent observations suggest that the model is overly simplistic in presenting platelet accumulation after injury as a linear, unstoppable and non-reversible series of events. In fact, there is now ample evidence for spatial as well as temporal heterogeneity within a growing hemostatic plug. [1-6] This means that, at any given time following injury there are fully activated platelets as well as minimally activated platelets, not all of which will inevitably become fully activated. Furthermore, with the passage of time, incorporated platelets draw closer together and many remain in stable contact with each other. This allows contact-dependent signaling to occur and produces a sheltered environment in which soluble molecules can accumulate. Thus, a more updated view of platelet activation needs to be less ordered than the three stage model, reflect differences in the extent of activation of individual platelets and incorporate the consequences of platelet-platelet interactions in a three dimensional space.

Molecular Events:

Under steady state conditions, platelets circulate in an environment bordered largely by a continuous monolayer of endothelial cells. They move freely, but are quiescent. Once vascular injury has occurred, platelets are principally activated by locally exposed collagen, locally generated thrombin, platelet-derived thromboxane A2 (TxA2) and ADP that is either secreted from platelet dense granules or released from damaged cells. VWF serves as an essential accessory molecule. In the pre-injury state, VWF is found in plasma, within the vessel wall and in platelet α-granules. Additional VWF/collagen complexes form as collagen fibrils come into contact with plasma. Circulating erythrocytes facilitate adhesion to collagen by pushing platelets closer to the

vessel wall, allowing GP Ibα on the platelet surface to be snared by the VWF A1 domain. Once captured, the drivers for platelet activation include the receptors for collagen (GP VI) and VWF (GP Ibα), thrombin (PAR1 and PAR4), ADP (P2Y1 and P2Y12) and thromboxane A2 (TP) (**Fig. 1**). In general terms, agonist-initiated platelet activation begins with the activation of one of the phospholipase C (PLC) isoforms expressed in platelets. By hydrolyzing membrane phosphatidylinositol-4,5-bisphosphate (PIP2), PLC produces the second messenger inositol-1,4,5-trisphosphate (IP3) needed to raise the cytosolic Ca2+ concentration. This leads to integrin activation via a pathway that currently includes a Ca2+-dependent exchange factor (CalDAG-GEF), a switch (Rap1), an adaptor (RIAM), and proteins that interact directly with the integrin cytosolic domains (kindlin and talin)[7]. Which PLC isoform is activated depends on the agonist. Collagen activates PLCγ2 using a mechanism that depends on scaffold molecules and protein tyrosine kinases. Thrombin, ADP and TxA2 activate PLCβ using Gq as an intermediary. The rise in the cytosolic Ca2+ concentration that is triggered by most platelet agonists is essential for platelet activation. In resting platelets, the cytosolic free Ca2+ concentration is maintained at approximately 0.1 μM by limiting Ca2+ influx and pumping Ca2+ out of the cytosol either out across the plasma membrane or into the dense tubular system. In activated platelets, the Ca2+ concentration rises tenfold to >1 μM as Ca2+ pours back into the cytosol from two sources. The first is IP3-mediated release of Ca2+ from the platelet dense tubular system (DTS). The second is Ca2+ influx across the platelet plasma membrane, an event triggered when depletion of the DTS Ca2+ pool produces a conformational change in STIM1, a protein located in the DTS membrane. This conformational change promotes the binding of STIM1 to Orai1 in the plasma membrane allowing Ca2+ entry [8]. Ultimately, it is the binding of fibrinogen or another bivalent ligand to αIIbβ3 that enables platelets to stick to each other. Proteins that can substitute for fibrinogen include fibrin, VWF and fibronectin. Average expression levels of αIIbβ3 range from approximately 50,000 per cell on resting platelets to 80,000 on activated platelets. Mutations in αIIbβ3 that suppress its expression or function produce a bleeding disorder (Glanzmann's thrombasthenia) because platelets are unable to form stable aggregates. Antiplatelet agents such as Integrilin (eptifibatide) and ReoPro (abciximab) take advantage of this by blocking αIIbβ3.

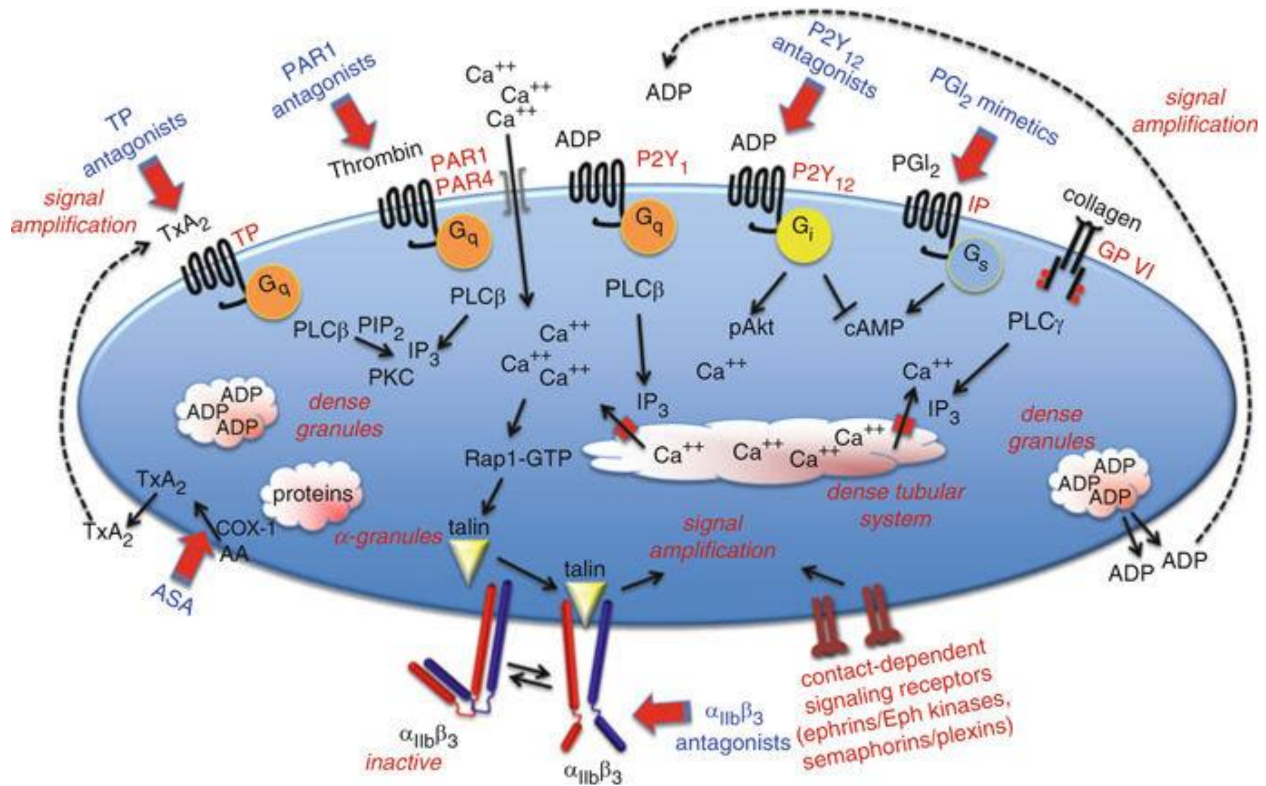

Figure 1: An overview of some of the pathways that support platelet activation. Targets for antiplatelet agents that are currently in clinical trials are indicated in blue. PLC (phospholipase C), PKC (protein kinase C), IP3 (inositol-1,4,5-trisphosphate), TxA2 (thromboxane A2), GP (glycoprotein), IP and TP, PGI2 and TxA2 receptors.

The Early Events of Platelet Activation:

Signaling within platelets begins with activation of receptors on the platelet surface by agonists such as collagen, thrombin, ADP, TxA2 and epinephrine. With the exception of collagen, each of these works through one or more members of the G protein coupled receptor super family. Some of the properties that are common to G protein coupled receptors (GPCRs) make them particularly well-suited for their tasks in platelets. Most bind their ligands with high affinity and each occupied receptor can activate multiple G proteins, amplifying the initial signal. In general, the GPCRs expressed on the platelet surface are present in low copy number, ranging from a few hundred (epinephrine receptors and P2Y1 ADP receptors) to a few thousand (PAR1) copies per cell. Their duration of signaling is subject to receptor internalization, receptor desensitization and the accelerated inactivation of G-proteins by members of the RGS (Regulators of G-protein Signaling) family. This multiplicity of mechanisms means that platelet activation can be tightly regulated even at its earliest stages. Although there is now growing evidence in cells other than platelets that GPCRs can signal by more than one mechanism, the events that have been described downstream of the GPCRs in platelets are mediated by heterotrimeric (αβγ)

G-proteins. Human platelets express ten members of the Gs, Gi, Gq and G12 families. This includes at least one Gs, four Gi (Gi1, Gi2, Gi3 and Gz), three Gq family members (Gq, G11 and G16) and two G12 family members (G12 and G13). As will be discussed below, much has been learned about the role of G-proteins in platelets through studies on mice in which the genes encoding one or more forms of Gα have been disrupted

Signaling Responses by Selected Platelet Agonists:

Platelet Activation by Collagen:

Four distinct collagen receptors have been identified on human and mouse platelets. Two bind directly to collagen (α2β1 and GP VI); the other two bind to collagen via VWF (αIIbβ3 and GP Ibα). Of these, GP VI is the most potent signaling receptor [9]. The structure of the GP VI extracellular domain places it in the immunoglobulin super family. Its ability to generate signals rests on its constitutive association with the ITAM-containing Fc receptor γ-chain (FcRγ). Loss of FcRγ affects collagen signaling in part because of loss of a necessary signaling element and in part because FcRγ is required for GP VI to reach the platelet surface. The α2β1 integrin also appears to be necessary for an optimal interaction with collagen, supporting adhesion to collagen and acting as a source of integrin-dependent signaling after engagement [10, 11]. However, this appears to require an initial wave of signaling that activates α2β1, much as the fibrinogen receptor, αIIbβ3 is activated by signaling within platelets. Human platelets with reduced expression of α2β1 have impaired collagen responses, as do mouse platelets that lack β1 integrins when the ability of these platelets to bind to collagen is tested at high shear [12]. Signaling through GP VI can be studied in isolation with the snake venom protein, convulxin, or with synthetic "collagen-related" peptides (CRP), both of which bind to GP VI, but not to other collagen receptors. According to current models, collagen causes clustering of GP VI. Polymerization of soluble collagen and clustering of GP VI/FcRγ complexes contribute to the lag that is commonly observed when collagen is added to platelets in an aggregometer. This leads to the phosphorylation of FcRγ by Src family tyrosine kinases associated with a proline-rich domain in GP VI [13]. Phosphorylation creates an ITAM motif that is recognized by the tandem SH2 domains of Syk. Association of Syk with the GP VI/FcRγ-chain complex activates Syk and leads to the phosphorylation and activation of PLCγ2. Loss of Syk impairs collagen responses [14]. PLCγ2 hydrolyzes PIP2, raising the cytosolic Ca2+ concentration and indirectly triggering Ca2+ influx across the platelet plasma membrane. The changes in the cytosolic Ca2+ concentration that occur when platelets adhere to collagen under flow can be visualized in real time [15, 16].

Platelet Activation by ADP:

ADP is stored in platelet dense granules and released upon platelet activation. It is also released from damaged cells at sites of vascular injury, serving as an autocrine and paracrine stimulus for recruiting additional platelets and stabilizing the hemostatic plug. Aggregation studies performed ex vivo show that all of the other platelet agonists are dependent to some extent on released ADP to elicit maximal platelet aggregation, although this dependence varies with the agonist and is dose-related. When added to platelets in vitro, ADP

causes TxA2 formation, protein phosphorylation, increase in cytosolic Ca2+, shape change, aggregation and secretion. It also inhibits cAMP formation. These responses are half-maximal at approximately 1 μM ADP. However, even at high concentrations, ADP is a comparatively weak activator of PLC, its utility as a platelet agonist resting more upon its ability to activate other pathways. Human and mouse platelets express two distinct GPCRs for ADP, denoted P2Y1 and P2Y12. P2Y1 receptors couple to Gq. P2Y12 receptors couple to Gi family members other than Gz. Optimal activation of platelets by ADP requires activation of both receptors. When P2Y1 is blocked or deleted, ADP is still able to inhibit cAMP formation, but its ability to cause an increase in cytosolic Ca2+, shape change and aggregation is greatly impaired, as it is in platelets from mice that lack Gqα. P2Y1−/− mice have a minimal increase in bleeding time and show some resistance to thromboembolic mortality following injection of ADP, but no predisposition to spontaneous hemorrhage. Primary responses to platelet agonists other than ADP are unaffected and when combined with serotonin, which is a weak stimulus for PLC in platelets, ADP can still cause aggregation of P2Y1−/− platelets. Taken together, these results show that platelet P2Y1 receptors are coupled to Gqα and responsible for activation of PLC. P2Y1 receptors can also activate Rac and the Rac effector, p21-activated kinase (PAK), but do not appear to be coupled to Gi family members. As had been predicted by inhibitor studies and by the phenotype of a patient lacking functional P2Y12, platelets from P2Y12−/− mice do not aggregate normally in response to ADP [17]. P2Y12−/− platelets retain P2Y1-associated responses, including shape change and PLC activation, but lack the ability to inhibit cAMP formation in response to ADP. The Gi family member associated with P2Y12 appears to be primarily Gi2, since platelets from Gi2α−/− mice have an impaired response to ADP [1, 18], while those lacking Gi3α or Gzα do not [1, 19]. Conversely, expression of a Gi2α variant that is resistant to the inhibitory effects of RGS proteins produces a gain of function in mouse platelets stimulated with ADP [20].

Platelet Activation by Thrombin:

Platelet responses to thrombin are mediated by members of the protease-activated receptor (PAR) family of GPCRs. There are four members of this family, three of which (PAR1, PAR3 and PAR4) can be activated by thrombin. PAR1 and PAR4 are expressed on human platelets; mouse platelets express PAR3 and PAR4. Receptor activation occurs when thrombin cleaves the extended N-terminus of each of these receptors, exposing a new N-terminus that serves as a tethered ligand [21]. Synthetic peptides based on the sequence of the tethered ligand domain of PAR1 and PAR4 are able to activate the receptors, mimicking at least some of the actions of thrombin. While human PAR3 has been shown to signal in response to thrombin in transfected cells, PAR3 on mouse platelets appears to primarily serve to facilitate cleavage of PAR4 rather than to generate signals on its own [22]. Thrombin is able to activate platelets at concentrations as low as 0.1 nM. Although other platelet agonists can also cause phosphoinositide hydrolysis, none appear to be as efficiently coupled to phospholipase C as thrombin. Within seconds of the addition of thrombin, the cytosolic Ca2+ concentration increases tenfold, triggering downstream Ca2+-dependent events, including the activation of phospholipase A2. Thrombin also activates Rho, leading to rearrangement of the actin cytoskeleton and shape change,

responses that are greatly reduced or absent in mouse platelets that lack G13α. Finally, thrombin is able to inhibit adenylyl cyclase activity in human platelets, either directly (via a Gi family member) or indirectly (via released ADP) [23].

Platelet Activation by Epinephrine:

Compared to thrombin, epinephrine is a weak activator of human platelets. Nonetheless, there are reports of human families in which a mild bleeding disorder is associated with impaired epinephrine-induced aggregation and reduced numbers of catecholamine receptors. Epinephrine responses in platelets are mediated by α2A-adrenergic receptors [24, 25]. In both mice and humans, epinephrine is able to potentiate the effects of other agonists. Potentiation is usually attributed to the ability of epinephrine to inhibit cAMP formation, but there are clearly other effects as well. Epinephrine has no detectable direct effect on phospholipase C and does not cause shape change, although it can trigger phosphoinositide hydrolysis indirectly by stimulating TxA2 formation. These results suggest that platelet α2A-adrenergic receptors are coupled to Gi family members, but not Gq or G12 family members. Knockout studies show that epinephrine responses in mouse platelets are abolished when Gzα expression is abolished, while loss of Gi2α or Gi3α has no effect. Gz also appears to be responsible for the ability of epinephrine to activate Rap1B [1, 26].

Platelet Activation by TxA2:

When added to platelets in vitro, stable thromboxane analogs such as U46619 cause shape change, aggregation, secretion, phosphoinositide hydrolysis, protein phosphorylation and an increase in cytosolic Ca2+, while having little if any direct effect on cAMP formation. Similar responses are seen when platelets are incubated with exogenous arachidonate. Once formed, TxA2 can diffuse across the plasma membrane and activate other platelets (Fig. 1) [27]. Like secreted ADP, release of TxA2 amplifies the initial stimulus for platelet activation and helps to recruit additional platelets. This process is limited by the brief half-life of TxA2 in solution, helping to confine the spread of platelet activation to the original area of injury. Loss of Gqα abolishes U46619-induced IP3 formation and changes in cytosolic Ca2+, but does not prevent shape change. Loss of G13α abolishes TxA2-induced shape change [28]. In cells other than platelets, TPα and TPβ have been shown to couple to Gi family members [29], however, in platelets the inhibitory effects of U46619 on cAMP formation appear to be mediated by secreted ADP. These observations have previously been interpreted to mean that platelet TxA2 receptors are coupled to Gq and G12/13, but not to Gi family members. However, the gain of function recently observed in mouse platelets carrying an RGS protein resistant Gi2α variant suggests that this is still an open issue [20]. TP−/− mice have a prolonged bleeding time. Their platelets are unable to aggregate in response to TxA2 agonists and show delayed aggregation with collagen, presumably reflecting the role of TxA2 in platelet responses to collagen [30]. The most compelling case for the contribution of TxA2 signaling in human platelets comes from the successful use of aspirin as an antiplatelet agent. When added to platelets in vitro, aspirin abolishes TxA2 generation (Fig. 1). It also blocks platelet activation by arachidonate and impairs

responses to thrombin and ADP. The defect in thrombin responses appears as a shift in the dose/response curve, indicating that TxA2 generation is supportive of platelet activation by thrombin, but not essential.

Some of the Later Events in Platelet Activation:

As platelet activation in response to injury proceeds in vivo, previously mobile platelets come into increasingly stable contact with each other, eventually with sufficient stability and proximity that molecules on the surface of one platelet can interact directly with molecules on the surface of adjacent platelets. Although in theory this can occur anywhere within a growing hemostatic plug or thrombus, it is likely to occur most readily in the thrombus core where platelets appear to be closest together. Stable cohesive contacts between platelets require engagement of αIIbβ3 with one of its ligands, after which inward-directed (i.e., outside-in) signaling occurs through the integrin and through other molecules that can then engage with their counterparts in trans. Some of these are primarily signal-generating events that affect platelet activation and thrombus stability. Others serve primarily to help form contacts between platelets and create a protected space in which soluble molecules, including agonists, can accumulate.

Hemostasis:

Platelet aggregation and spreading at the site of a vascular injury are essential for the arrest of bleeding, and both processes are mediated by integrin aIIbb3. In unstimulated platelets the aIIbb3 integrin is maintained in an inactive conformation through interactions between the aIIb and b3 cytoplasmic and transmembrane (TM) domain [31, 32]. One mechanism of "insideout" integrin activation occurs when the cytoplasmic protein talin binds to the b3 [33] and possibly aIIb cytoplasmic domain, dissociating the interaction between them, and inducing a conformational change that renders the extracellular domains capable of binding to soluble fibrinogen (Fg). Fg cross-linking of adjacent platelets results in the formation of a blood clot and generates additional "outside-in" signals through aIIbb3 that are necessary for full platelet aggregation and spreading [34]. CIB1 binds specifically to the integrin aIIb subunit, but not other integrin a or b subunits [35], suggesting a specific role in platelet signaling rather than general integrin regulation. The similar affinity for synthetic aIIb peptides in the presence of Ca2+ or resting cellular Mg2+ concentrations, suggests that binding is probably not governed by Ca2+ stimuli in vivo. Nevertheless, the interaction has been shown to have both a positive and negative influence on aIIbb3 activation, as well as a role in more downstream platelet signaling events **(Fig. 2**). In one report it was shown that CIB1 could stimulate Fg-binding to aIIbb3 in vitro, and that a synthetic competing CIB1 peptide could block agonist-induced activation by CIB1 in platelets [36]. Thus it was concluded that CIB1 might be capable of converting aIIbb3 from an inactive to an active conformation during inside-out signaling. However, in another study the over expression of CIB1 in megakaryocytes (platelet precursors) was shown to inhibit agonist-induced integrin activation, whereas lowering endogenous CIB1 levels had the opposite effect [37]. The authors further demonstrated that CIB1 could directly compete with talin for aIIb, implying that CIB1 might limit the extent of talin-induced aIIbb3 activation in resting platelets.

These apparently contradictory results suggest that CIB1 might have a unique effect on aIIbb3 activation in different cell types or under different conditions or that CIB1 is influenced by additional factors during inside-out signaling. Whatever the effect of CIB1 on inside-out signaling may be, it is apparent that CIB1 can associate with aIIbb3 subsequent to integrin activation. In fact, experiments performed both in vitro and in vivo have shown that CIB1 binds preferentially to the active conformation of aIIbb3. The reason for this increased association is not known, but could be due to conformational changes in aIIbb3 which displace important CIB1-binding residues out of the membrane and into the cytoplasm [38]. Naik and Naik have reported that the interaction between CIB1 and aIIb subsequent to integrin activation is necessary to control the release of ADP from platelet granules [39]. Secreted ADP signals through platelet P2Y1 and P2Y12 receptors to sustain platelet activation and regulate platelet spreading [39, 40] suggesting that CIB1 might be important for clot stabilization and growth. Furthermore, a recent study reported that CIB1 could recruit WASP to aIIbb3 following agonist stimulation and enhance platelet adhesion most likely by concentrating the actin polymerization activity of WASP at the aIIbb3 sites [40]. Changes in actin dynamics are well known to drive platelet shape change during aggregation and spreading, and both CIB1 and aIIbb3 associate with the actin cytoskeleton (and each other) following platelet activation [39, 41] Therefore, CIB1 might play a key role in outside-in signaling by concentrating and associating the actin cytoskeleton at the aIIbb3-rich regions of the platelet.

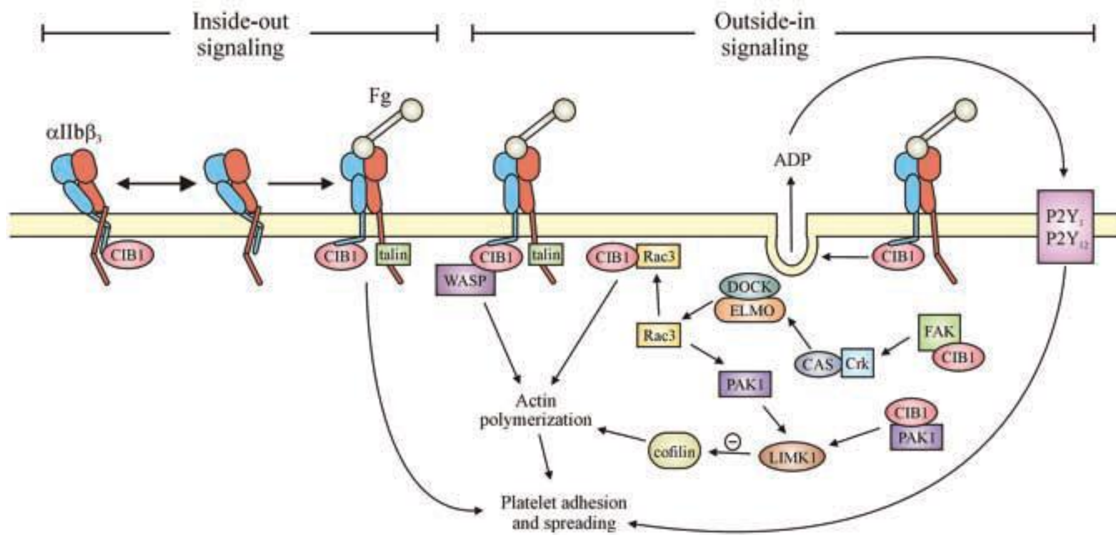

Figure 2: Model for the role of CIB1 in platelet signaling

In addition to aIIbb3 and WASP, CIB1 can bind focal adhesion kinase (FAK) in platelets adhering to Fg. FAK signaling through the effector proteins CAS/Crk and DOCK/ELMO leads to activation of Rho family kinases, which in turn bind to their targets and stimulate actin polymerization, lamellipodium formation and cell migration [42]. Over-expression of CIB1 in CHO cells could up-regulate FAK activity and increase cell migration, suggesting that CIB1 is an important regulator of FAK-stimulated pathways. CIB1 also binds to the activated

(GTP-bound) Rho family member Rac3, but not other Rac isoforms, and together CIB1/Rac3 were found to have a positive effect on aIIbb3-mediated fibroblast adhesion and spreading on Fg. Therefore, CIB1 might regulate platelet adhesion and spreading through FAK activation, and a subsequent interaction with Rac3. Leisner et al found that CIB1 could also co-immunoprecipitate PAK1 from platelet lysates and could activate the kinase activity of PAK1 in vitro and in vivo in a Ca2+-dependent manner [43]. However, in contrast to FAK and Rac3, PAK1 activation inhibited the spreading of model cell lines, likely through PAK1-LIMK1-dependent phosphorylation and inactivation of cofilin, which inhibits cofilin's ability to polymerize actin. Rho GTPases including Rac3 can also activate PAK1 [44]. Therefore, the differential regulation of FAK, Rac3 and PAK1 by CIB1 could provide precise control over actin cytoskeletal changes and platelet spreading during outside-in signaling. This regulatory function for CIB1 likely extends to other cell types as well since FAK, Rac3 and PAK1 are also each widely expressed and regulate cell signaling pathways outside of platelets.

CONCLUSION

CIB1 has been known to have broader functions in various cellular processes but its role in platelet signaling and hemostasis has always been outstanding. From this brief review we have come to know that, platelet signaling occurs in very three basic steps that is initiation, extension and stabilization. Platelets continuously circulate freely in endothelial environment of the vessels where, following injury, they are exposed to collagen, locally generated thrombin, platelet-derived thromboxane A2 (TxA2) and ADP, that is, either secreted from platelet dense granules or released from damaged cells. VWF, found in plasma, forms complexes with collagen with the help of erythrocytes. In general terms, agonist-initiated platelet activation begins with the activation of one of the phospholipase C (PLC) isoforms expressed in platelets. By hydrolyzing membrane phosphatidylinositol-4,5-bisphosphate (PIP2), PLC produces the second messenger inositol-1,4,5-trisphosphate (IP3) needed to raise the cytosolic Ca2+ concentration. This leads to integrin activation via a pathway that currently includes a Ca2+-dependent exchange factor, a switch, an adaptor, and proteins that interact directly with the integrin cytosolic domains. Collagen activates PLCγ2 using a mechanism that depends on scaffold molecules and protein tyrosine kinases. Thrombin, ADP and TxA2 activate PLCβ using Gq as an intermediary. In resting platelets, the cytosolic free Ca2+ concentration is maintained by limiting Ca2+ influx and pumping Ca2+ out of the cytosol either out across the plasma membrane or into the dense tubular system. In activated platelets, the Ca2+ concentration rises tenfold and Ca2+ pours back into the cytosol from two sources, then this event produces a conformational change in STIM1, a protein located in the DTS membrane. This conformational change promotes the binding of STIM1 to receptor in the plasma membrane allowing Ca2+ entry. Ultimately, it is the binding of fibrinogen or another bivalent ligand to αIIbβ3 that enables platelets to stick to each other. This shows the role of CIB1 in platelet signaling is very important.

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