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Cytoplasmic Binding Partners of the Platelet Integrin $\alpha_{IIb}\beta_3$

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1. ABSTRACT

Platelets function physiologically in mediating hemostasis, but are also associated with many pathological conditions, such as thrombosis, which can lead to myocardial infarction and/or stroke. Therefore, the study of platelet regulation and signaling has been of great interest and is necessary for generating effective anti-platelet therapeutics. One platelet signaling molecule of particular interest is the integrin $\alpha_{IIb}\beta_3$, which binds Fg and mediates platelet cross-linking. The integrin itself as well as cytoplasmic proteins that interact with $\alpha_{IIb}\beta_3$ have become potential targets for anti-platelet therapies. One such protein that has been shown to directly regulate $\alpha_{IIb}\beta_3$ function is calcium- and integrin-binding protein 1 (CIB1). CIB1 has been implicated in $\alpha_{IIb}\beta_3$ activation and outside-in signaling through the integrin. By increasing our understanding of CIB1 and other proteins that like it, associate with integrin $\alpha_{IIb}\beta_3$, and the signaling events that result from those interactions, we may bring ourselves closer to more effective therapies. In the present work, we explore known cytoplasmic binding partners of the integrin $\alpha_{IIb}\beta_3$ and their effect on $\alpha_{IIb}\beta_3$, focusing on CIB1.

2. INTRODUCTION

2.1. Historical Perspective

Platelets, small, anucleacted cells found circulating in the bloodstream, were first described anatomically and functionally by Bizzozero in the late 1800s (1). Bizzozero recognized the role of platelets in hemostasis by observing aggregation following vascular injury via intravital microscopy in guinea pigs. Around the same time, Osler recognized that platelets also played a role in pathological thrombosis, which can lead to myocardial infarction and/or stroke (1). The last century has witnessed leaps and bounds in the field of platelet science. It is now known what extracellular proteins platelets can bind to and through which receptors these interactions take place. It is understood that platelets need to become activated in order for adhesion to occur and that this activation causes distinct morphological changes. Numerous platelet agonists have been identified, both autocrine and paracrine. Our advanced understanding of platelet physiology and signaling has allowed us to postulate different treatments for platelet-related diseases.

2.2. Diseases Associated with Platelets

In the early 1900s two rare diseases were identified that corresponded to platelet defects in surface receptors GPIIb/GPIIIa (integrin $\alpha_{IIb}\beta_3$) and GP1b that resulted in the bleeding disorders Glanzmann thrombasthenia and Bernard-Soulier syndrome, respectively (1, 2). Another platelet-related disease is immune thrombocytopenia (ITP), which can arise naturally or can be drug-induced, and is a major side-effect of many current anti-platelet therapies (1, 3). Patients with ITP have abnormally low platelet counts because they are targeted and removed by the immune system. So, not only are diseases associated with unwanted platelet activation, heart attack and stroke, but also with defects in the platelet activation mechanism. The knowledge gained in the past century has greatly contributed to our understanding of platelet-related diseases and advances in disease treatment have since ensued. For example, aspirin is widely accepted as an anti-platelet therapy and can greatly lower the risk of heart attack and stroke. But what happens when a patient on aspirin therapy experiences trauma? The prolonged bleeding time associated with aspirin therapy could cause adverse effects. In order to design more effective, specific treatments and prevention strategies for platelet associated diseases, platelets need to be studied in greater depth.

2.3. Physiological Perspective

Unactivated platelets are normally circulating in a discoid state. Because of their small size, platelets travel closely to vessel walls under the physiological conditions of laminar flow where they can respond rapidly to signs of vascular damage. Damage to a vessel exposes extracellular matrix components, such as collagen, that interact with receptors on the discoid platelet and initiate platelet activation. Endothelial cells also release platelet agonists, such as von Willebrand factor (vWF) (4). Ultimately, platelet activation elicits a morphological change that causes the platelet to become spherical and extend filopodia. This spiky morphology enables platelets to spread over the area of vascular damage and initiate plug formation, contributing to hemostasis. Platelets also cross-link with neighboring platelets via the integrin $\alpha_{IIb}\beta_3$ which binds its bivalent ligand, fibrinogen (Fg). This platelet cross-linking is the basis of aggregation and is implicated in pathological thrombosis. Therefore, the integrin $\alpha_{IIb}\beta_3$ has become a favored target for antiplatelet therapies (5, 6). Current $\alpha_{IIb}\beta_3$ inhibitors commonly cause the severe side effect of immune thrombocytopenia and improved therapies are in demand (3).

3. THE PLATELET FIBRINOGEN RECEPTOR INTEGRIN $\alpha_{IIb}\beta_3$

3.1. Structure

Integrins are a family of type I transmembrane proteins that exist as αβ heterodimers and function as adhesive and bidirectional signaling molecules (6-11). There are three families of integrins found on platelets: β_1, β_2, and β_3, which account for a total of six platelet integrins: $\alpha_2\beta_1$ (collagen receptor), $\alpha_5\beta_1$ (thought to be the fibronectin receptor), $\alpha_6\beta_1$ (thought to be the laminin receptor), $\alpha_L\beta_2$ (intercellular adhesion molecule-1 (ICAM-1) receptor), $\alpha_{IIb}\beta_3$ (fibrinogen receptor), and $\alpha_v\beta_3$ (vitronectin receptor) (11-13). The most populous integrin on platelets (50-80,000 copies/platelet surface) is $\alpha_{IIb}\beta_3$, also known as glycoprotein GPIIb/IIIa, which is unique to the megakaryocytic lineage. Additional pools of integrin $\alpha_{IIb}\beta_3$ are located on the membranes of α-granules and the open canalicular system (14). Integrin $\alpha_{IIb}\beta_3$ is a calcium-dependent, noncovalent heterodimer. The α_{IIb} subunit consists of 1008 amino acids and the β_3 subunit consists of 762 residues (14). Both have large, globular extracellular domains and short cytoplasmic tails, 20 and 47 amino acids for α_{IIb} and β_3 subunits, respectively (14). Neither of these tails has any known enzymatic activity. Recent advances in the crystal structure of $\alpha_{IIb}\beta_3$ have tremendously enhanced our understanding of how this integrin functions, and it has been heralded as a prototype integrin.

3.2. Activation of $\alpha_{IIb}\beta_3$

On a circulating, discoid platelet the integrin $\alpha_{IIb}\beta_3$ is in a low-affinity state with a bent extracellular domain that is unable to bind soluble Fg. Upon platelet activation via extracellular stimuli, such as ADP, $\alpha_{IIb}\beta_3$ becomes activated via inside-out signaling, inducing a conformational change in the extracellular domain of the integrin, which increases its affinity for its ligand and allows it to bind soluble Fg (4, 15-23). Inside-out signaling involves cytoplasmic proteins interacting with the cytoplasmic tails of the integrin and inducing conformational changes in the extracellular domain. Integrin activation also allows for integrin clustering which increases its ligand avidity (14). The cytoplasmic tails of the integrin have a physical "hinge" or salt-bridge between the conserved α_{IIb} GFFKR and β_3 LLv-iHDR motifs that keeps them in a locked position (24, 25). Deletion of the conserved GFFKR domain in α_{IIb} converts the integrin to a constitutively active, ligand-binding state (26). The β_3 aspartic acid residue (D^{723}) is a similar displacement from the cytoplasmic side of the membrane as the α_{IIb} arginine residue (R^{995}), which suggests these two residues could

form the salt bridge (24). In fact, mutating either of these residues to an alanine causes integrin activation as seen by binding of PAC1, a monoclonal antibody specific for activated $\alpha_{IIb}\beta_3$ (24). Also, double reversal mutants, where the α_{IIb} arginine is converted to an aspartic acid and the β_3 aspartic acid is converted to an arginine, do not induce PAC1 binding, suggesting that the salt bridge is restored (24). Disruption of this salt bridge, however, causes integrin activation even in the absence of agonists, which suggests that cytoplasmic proteins function in inside-out activation of the integrin by physically disrupting the salt-bridge. One such cytoplasmic protein that has been implicated in this activation process is talin, which has been shown to disrupt the integrin α and β tails at the critical membrane-proximal region (27, 28).

3.3. Ligand Binding and Outside-in Signaling

After activation, the extended extracellular domain of $\alpha_{IIb}\beta_3$ becomes receptive to ligand binding. Fg has a bivalent structure that allows it to simultaneously bind two $\alpha_{IIb}\beta_3$ integrins with its γ chain sequence KQAGDV, and not the α chain RGD domains (Arg-Gly-Asp), on different platelets, *trans*, or on the same platelet, *cis* (29, 30). This *cis* binding may contribute to the process of integrin clustering. Activated $\alpha_{IIb}\beta_3$ may also bind vWF, fibronectin, and vitronectin via the RGD domains (10, 14, 18). This $\alpha_{IIb}\beta_3$-Fg- $\alpha_{IIb}\beta_3$ interaction is stabilized by the binding of a multivalent protein, vWF. The binding of Fg to $\alpha_{IIb}\beta_3$ induces outside-in signaling through the integrin, which contributes to irreversible platelet thrombus formation via outside-in signaling events (17). Such events include the mobilization of calcium via inositol-1,4,5-trisphosphate (IP3) generation, the activation of protein tyrosine kinases such as Src and focal adhesion kinase (FAK), and cytoskeletal rearrangements necessary for platelet spreading.

4. CYTOPLASMIC BINDING PARTNERS OF $\alpha_{IIb}\beta_3$

Many cytoplasmic proteins have been identified that interact with either or both of the cytoplasmic tails of $\alpha_{IIb}\beta_3$. These proteins are thought to participate in inside-out and outside-in signaling through the integrin. Signaling proteins have attracted attention because of their potential as targets for anti-platelet therapies. Some proteins thought to play a role in $\alpha_{IIb}\beta_3$ activation via cytoplasmic interactions with the α_{IIb} membrane-proximal sequence KVGFFKR include calreticulin, ancient ubiquitous protein 1 (Aup1), filamin, talin, ICln, protein phosphatase 1 (PP1c), and calcium- and integrin-binding protein 1 (CIB1) (31-37). Talin, Src, Shc, FAK, paxillin, β3-endonexin, α-actinin, filamin, skelemin, integrin-linked kinase (ILK), Grb2, receptor for activated C-kinase (RACK1), myosin, protein kinase C (PKCβ) and Syk are some of the cytoplasmic proteins known to interact with the β_3 cytoplasmic tail (19, 32, 35, 38-50). Some of these protein-integrin interactions have only been demonstrated *in vitro* and some that have been demonstrated *in vivo* still have yet to be demonstrated in platelets, although their expression has been detected.

4.1. Calreticulin

Calreticulin is a calcium binding protein that was originally isolated from skeletal sarcoplasmic reticulum and thought to function mainly as an endoplasmic reticulum chaperone protein (36, 61-64). Calreticulin contains three conserved domains, an N-terminal globular domain that binds zinc, a proline rich P-domain that binds calcium with high affinity, and the C-domain that binds calcium with low affinity (64). In the form where it is expressed on the surface of platelets, calreticulin is involved in mediating thrombospondin-induced cytoskeletal rearrangements. Intracellular calreticulin is known to interact with the highly conserved KXGFFKR sequence in integrin α subunits (36). However, it is debated whether calreticulin binds $\alpha_{IIb}\beta_3$ in platelets *in vivo*. One study showed that calreticulin remains localized in the granulomere in activated human platelets and does not co-immunoprecipitate with the integrin $\alpha_{IIb}\beta_3$ (61).

4.2. Ancient Ubiquitous Protein 1

Aup1, a ubiquitously expressed protein in human cells, was identified as a binding partner of $\alpha_{IIb}\beta_3$ via the yeast two-hybrid analysis using the α_{IIb} cytoplasmic tail as bait and the cDNA library from the thrombopoietin-dependent acute megakaryocytic leukemia-derived cell line, UT7/TPO (31). The protein-integrin interaction has a low affinity (K_d = 90 μM) and is postulated to be reversible. Binding assays demonstrate that Aup1 binds the conserved integrin α_{IIb} membrane-proximal sequence KVGFFKR. Mutation or deletion of this sequence induces integrin activation. The specific mutation F992A prevents Aup1 binding, suggesting that Aup1 may have a function in maintaining the low affinity state of the integrin (31).

4.3. Talin

Talin is a 225-285 kDa cytoplasmic protein that comprises greater than 3% of the total platelet protein (27, 28, 55-57, 65). It has a suggested role in establishing a connection between the actin cytoskeleton and extracellular environment because it is a cytoskeletal protein and has the ability to bind the cytoplasmic tails of membrane proteins like $\alpha_{IIb}\beta_3$. In resting platelets, talin distribution is diffuse in the cytoplasm, but upon activation, it becomes redistributed towards the periphery, most likely via a post-translational modification, namely phosphorylation (55). In fact, within 2 minutes of thrombin stimulation, the amount of phosphorylated talin has been shown to increase 4-fold over that of phosphorylated talin in resting platelets. The integrin $\alpha_{IIb}\beta_3$ is not required for this redistribution to the sub-membranous location as evidenced with platelets from Glanzmann thrombasthenia patients. These platelets lacked β_3, but still showed talin redistribution upon stimulation.

Talin binds both the α_{IIb} and β_3 cytoplasmic tails (32). This binding is thought to induce integrin activation via physically disrupting the salt bridge between the cytoplasmic tails, thereby transducing a conformational change across the plasma membrane, which ultimately affects the integrin's binding affinity. Talin is believed to be the final step in integrin activation. RNAi experiments against talin show a significant depreciation in the amount of integrin activation (27). Also, Förster resonance energy transfer (FRET) experiments demonstrate how talin lowers the FRET activity between the α_{IIb} and β_3 cytoplasmic tails,

indicating that talin is physically separating the two and induces integrin activation (56).

4.4. ICln

ICln was identified as a binding partner of $\alpha_{IIb}\beta_3$ via measured interactions of a high density protein expression array with biotin-tagged KVGFFKR (33). This protein is a 42 kDa chloride channel regulatory protein that is essential for regulation of cell volume. Deletion of ICln results in cell death. Interestingly, integrins have an implicated role in osmoregulation. Further studies in platelets using acyclovir, a pharmacological inhibitor of the ICln chloride channel, demonstrate a dose-dependent inhibition of platelet aggregation and integrin activation detected with PAC1. Significantly, a unique peptide sequence was identified that is found only in the ICln protein and the integrin β_3 cytoplasmic tail throughout the entire human genome, AKFEEE. Addition of a peptide corresponding to this domain to platelets induces a dose-dependent inhibition of platelet aggregation and integrin activation as well (33). However, it is unknown whether this affect is due to inhibition of ICln action, some other integrin binding protein being blocked, or some combination. Regardless, it suggests that this unique domain is important for integrin signaling.

4.5. Protein Phosphatase 1

The conserved membrane-proximal region of α_{IIb} encompasses the consensus-binding motif of PP1c, KVGF (37). It was found that PP1c, a serine/threonine protein phosphatase, does associate with $\alpha_{IIb}\beta_3$ in resting platelets. Ligand occupation of $\alpha_{IIb}\beta_3$ upon agonist stimulation causes PP1c to be released. Free PP1c can then participate in transient dephosphorylation events, such as the dephosphorylation of the PP1c substrate myosin light chain. Interestingly, the sequence to which PP1c binds overlaps the binding sequences of many other proteins that interact with $\alpha_{IIb}\beta_3$, so dissociation of PP1c may permit the association of other cytoplasmic proteins (37).

4.6. Shc

Shc was identified via phospho-peptide affinity column purification to be the primary protein to bind the phosphorylated cytoplasmic tail of β_3 (39). Tyrosine phosphorylation of the ICY domain of β_3, which contains two NXXY motifs separated by eleven amino acids, is known to be involved in outside-in signaling. Shc has two phosphotyrosine binding domains, SH2 and PTB, and itself becomes tyrosine phosphorylated upon LIBS antibody-induced aggregation, which further indicates that Shc is involved in the outside-in portion of the $\alpha_{IIb}\beta_3$ signaling pathway. LIBS antibodies induce Fg binding to $\alpha_{IIb}\beta_3$ via a conformational change in the integrin without agonist stimulation. Also, studies conducted with murine platelets that have a mutated β_3 (Y747F, Y759F) such that the β_3 cytoplasmic tail cannot be tyrosine-phosphorylated show that Shc does have highly elevated levels of phosphorylation compared to wild-type murine platelets when allowed to aggregate with thrombin as the agonist (39). However, the level of Shc phosphorylation in the aggregate lysates was 2.8-fold lower compared to the wild-type murine platelets.

4.7. Focal Adhesion Kinase

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that is central to cell signaling. FAK was found to bind a β_3 cytoplasmic domain peptide suggesting that it could associate with β_3 integrins *in vivo* (47). FAK has been shown to translocate to the cytoskeleton in platelets in an aggregation-dependent manner (66). Platelet aggregation induced by thrombin stimulation lead to FAK tyrosine phosphorylation. Also, PMA-induced activation of protein kinase C (PKC) caused FAK phosphorylation in platelets exposed to Fg. Immunoprecipitation studies show that FAK associates with Shc through Grb2 in thrombin stimulated platelets (66).

4.8. β_3-Endonexin

β_3-endonexin was isolated in 1995 via the yeast two-hybrid screen using β_3 cytoplasmic domains as bait (46). It consists of 111 amino acids and binds the β_3 cytoplasmic tail of $\alpha_{IIb}\beta_3$, specifically requiring the $N^{756}ITY$ sequence (67). Deletion or mutation of this sequence disrupts inside-out signaling through the integrin $\alpha_{IIb}\beta_3$ in both CHO cells and platelets (68). β_3-endonexin increases the affinity of $\alpha_{IIb}\beta_3$ for Fg, as seen in experiments measuring PAC1 binding in CHO cells expressing β_3-endonexin. These results suggest that β_3-endonexin binding to the cytoplasmic tail of the β_3 integrin promotes cell adhesion and spreading on immobilized fibrinogen.

4.9. Skelemin

Integrin association with cytoskeletal proteins is important for integrin-induced spreading. Skelemin, a 210 kDa protein belonging to a family of myosin-associated proteins, was identified as a binding partner of the cytoplasmic tail of β_3 via the yeast two-hybrid screen in 1998 (44). HA-tagged skelemin colocalizes with $\alpha_{IIb}\beta_3$ expressed in non-muscle CHO cells. Skelemin was also observed to colocalize with focal complexes in lamellipodia, but not focal adhesions, suggesting that the skelemin-β_3 association may be regulated and occur only in stages of lamellipodia extension (44).

4.10. Integrin-Linked Kinase

Integrin-linked kinase (ILK) co-immunoprecipitates with β_3 in activated platelets (43). Confocal images also reveal that in resting platelets, ILK is in the cytoplasm, but in activated platelets, ILK translocates to the membrane. An increase in ILK kinase activity and β_3 phosphorylation have also been demonstrated in activated platelets. It has also been demonstrated that inhibition of PI-3 kinase blocks ILK activity and decreases $\alpha_{IIb}\beta_3$ affinity. Therefore, it is suggested that ILK's association with $\alpha_{IIb}\beta_3$ is PI-3 kinase dependent.

4.11. Receptor for Activated C-Kinase

Receptor for activated C-kinase, RACK1, is a 36-kDa protein belonging to the RACK family of proteins, a group of PKC targeting proteins (38). RACK1 binds directly to the β_3 tail *in vitro*. RACK1 co-immunoprecipitates with β_3 in platelets plated on BSA and fibrinogen, suggesting that it could be constitutively associated with the integrin (38). RACK1 has also been shown to directly bind PKCβ (69). These interactions

suggest that RACK1 modulates the PKCβ- $\alpha_{IIb}\beta_3$ interaction.

4.12. Protein Kinase Cβ

Platelets express at least eight isoforms of PKC. The role of PKCs in agonist-induced regulation of $\alpha_{IIb}\beta_3$ in platelets has been largely based on the fact that phorbol esters, like PMA, have stimulatory effects and PKC inhibitors have inhibitory effects on platelet aggregation. In 2005, it was found that out of all the PKC isoforms expressed in platelets, only PKCβ associates with $\alpha_{IIb}\beta_3$ upon fibrinogen binding (38). This association is directed through the β_3 cytoplasmic tail and results in elevated PKC activity. Furthermore, this association is seen to occur at focal complexes and lamellipodia edges, suggesting involvement in outside-in signaling (38).

4.13. The Src Family Kinases

Src constitutively associates with $\alpha_{IIb}\beta_3$ in platelets (28, 41, 50, 70). Adhesion of platelets to fibrinogen results in a direct association of Syk with β_3, a dissociation of Csk from Src, and a 35-fold increase in Src activity. Csk is associated with unactivated $\alpha_{IIb}\beta_3$ and functions in keeping Src inactive by phosphorylating tyrosine 529. Platelet activation leads to protein-tyrosine phosphatase (PTP)-1B recruitment to this inhibitory complex to dephosphorylate tyrosine 529, and it has been shown to be required for Csk dissociation (70). Src is then phosphorylated on tyrosine 418 which constitutes the active kinase. Syk is a downstream signaling protein of Src and upstream of cytoskeleton regulatory proteins (41). Platelet activation leads to Syk association with Src. Inhibitors of Src inhibit Syk phosphorylation and result in poorly spread platelets. Syk null platelets spread poorly on fibrinogen suggesting Syk is necessary for platelet spreading.

5. CALCIUM- AND INTEGRIN-BINDING PROTEIN 1

Expression of an $\alpha_{IIb}\beta_3$ mutant in CHO cells where the α_{IIb} cytoplasmic tail was deleted resulted in a constitutively active integrin (26). To identify proteins that interact with α_{IIb} and possibly regulate it, a yeast-2-hybrid screen was conducted using the α_{IIb} cytoplasmic tail as bait. In 1997, calcium- and integrin-binding protein, CIB, was identified via this yeast-2-hybrid screen (34). It was later determined that the isolated CIB was the founding member of a family of CIB proteins and was termed CIB1. RT-PCR analysis of platelet RNA revealed the presence of CIB1. CIB1 is conserved on the amino acid sequence level among species (71). Since its initial isolation, CIB1 has been identified in many other cell types including, but not limited to, megakaryocytic cells, heart, pancreas, liver, skeletal muscle, kidney, lung, placenta, and brain (72).

5.1. Structural Insights

CIB1 contains 191 amino acids and has a predicted molecular weight of 21.7 kDa (34). CIB1 contains 4 EF-hand motifs, the 2 EF-hands in the C-terminal domain being functional, and is N-terminally myristoylated (34, 73). Recently, the crystal structure of CIB1 has been solved at 2.3 Å resolution by our lab and one other (74, 75). When we superimpose our structure with the Gentry et al structure, it appears that CIB1 consists of 2 globular domains separated by a flexible linker region (74). Since its initial isolation, CIB1's interaction with $\alpha_{IIb}\beta_3$ has been measured with numerous techniques (51-53, 72, 76).

5.2. Binding of CIB1 to $\alpha_{IIb}\beta_3$ – *in vitro*

CIB1 has sequence similarity to calcineurin B and calmodulin, 58% and 56% respectively, which allowed for construction of a homology-based CIB1 structure (25, 51). This predicted structure reveals the α_{IIb} binding site on calcium-bound CIB1 to be in the C-terminus and its ideal area to bind α_{IIb} would be in the membrane-proximal region (25). *In vitro* studies confirm these predicted results. The CIB1- $\alpha_{IIb}\beta_3$ interaction has also been measured with intrinsic tryptophan fluorescence (ITF) (51). Interestingly, not a single tryptophan is present in the 191 amino acids that comprise CIB1. The α_{IIb} cytoplasmic tail has a tryptophan residue at position 988, so CIB1 binding to α_{IIb} peptides can be characterized by ITF of α_{IIb} upon addition of CIB1. The ITF of CIB1-α_{IIb} is 75% greater than α_{IIb} alone. Various α_{IIb} peptides have been titrated with CIB1 and it has been demonstrated that α_{IIb} peptides lacking the C-terminus have the same ITF spectra as full-length, wild type α_{IIb} cytoplasmic tail. This suggests the site of CIB1-α_{IIb} interaction is at the N-terminal membrane-proximal region of the α_{IIb} cytoplasmic tail. Peptide mutational analysis was conducted to determine the minimal sequence on α_{IIb} necessary for CIB1 binding to be Leu^{983}-Arg^{997}. The interface on CIB1 that could potentially interact with this region was determined to be a C-terminal hydrophobic pocket that could become exposed in the calcium-bound structure, as determined by homology analysis (51). Peptides corresponding to the C-terminus of CIB1 inhibit CIB1 binding to GST-α_{IIb} cytoplasmic tail as shown via an *in vitro* binding assay (52). Because CIB1 preferentially binds to α_{IIb} and not other α subunits or β_3, it was originally predicted to be unlikely that CIB1 would bind to the conserved α subunit GFFKR membrane-proximal domain because this domain is necessary for $\alpha_{IIb}\beta_3$ dimerization, but this prediction seems to have been proven otherwise (34, 76).

5.3. Binding of CIB1 to $\alpha_{IIb}\beta_3$- *in vivo*

The colocalization of CIB1 and $\alpha_{IIb}\beta_3$ has also been visualized with immunofluorescence (53). In platelets, immunoprecipitation of CIB1 brings down α_{IIb} in platelets plated on BSA and increasingly so in platelets plated on immobilized Fg (53). HEL cells, full-length CIB1 with a C-terminal FLAG tag co-immunoprecipitates with the α_{IIb} subunit (52). A yeast 2-hybrid analysis was conducted using deletion fragments of CIB1 as bait to identify a more confined region for the CIB1- $\alpha_{IIb}\beta_3$ interaction (52). It was found that CIB1 fragments lacking the C-terminus failed to interact with the αIIb cytoplasmic tail while fragments containing this region showed an interaction. This indicates that the region of CIB1 responsible for the interaction with α_{IIb} is in the C-terminus.

5.4. CIB1 Binds Calcium

The CIB1- $\alpha_{IIb}\beta_3$ interaction was also shown to be calcium-dependent by isothermal titration calorimetry

(ITC) and calcium-dependent pull-down assays (52, 72). However, a GST-CIB1 fusion protein retains $\alpha_{IIb}\beta_3$ in liquid phase binding assays regardless of Ca^{2+} presence (76). Surface plasmon resonance (SPR) showed no difference in α_{IIb} peptide binding to GST-CIB1 in the presence or absence of Ca^{2+} (76). However, Shock et al have argued that ITC is a more accurate technique because they were able to use full-length CIB1 and not a GST modified CIB1. In addition, the K_d value obtained via ITC falls within the range of accuracy by ITC. They suggest that the GST-CIB1 fusion protein may be conformationally altered or not be as flexible as full-length CIB1. They also argue that SPR has many pitfalls (72). It is now clear that CIB1 is able to bind $\alpha_{IIb}\beta_3$ in both the presence and absence of calcium, but the K_d for calcium-bound CIB1 is approximately 100-fold lower than for calcium-unbound CIB1.

The structure of CIB1 suggests that it could be a member of the Ca^{2+}-myristoyl switch family of proteins (77, 78). In this family of proteins, the myristoyl group, in the absence of Ca^{2+}, is sequestered in a deep hydrophobic box within the protein where it is held down by residues contributed by the EF hands. Introduction of Ca^{2+} leads to unclamping of these EF hand residues and extrusion of the myristoyl group. The hydrophobic acyl chain is then free to interact with the membrane, thereby localizing the protein to the membrane. This model seems to fit for CIB1 because it has also been reported that CIB1 colocalization with $\alpha_{IIb}\beta_3$ is Ca^{2+} dependent. Ca^{2+} concentrations in an average resting platelet are in the order of 100 nM and increase to around 10 μM in activated platelets, of which local concentrations could be even higher (52). So, an increase in intracellular Ca^{2+} concentration upon agonist stimulation could lead to an increase in CIB1 binding to $\alpha_{IIb}\beta_3$ by localizing CIB1 to the platelet membrane via the Ca^{2+}-myristoyl switch mechanism. However, this mechanism is still debated because some publications report a Ca^{2+}-independent association of CIB1 with $\alpha_{IIb}\beta_3$ (76, 79). A recent paper suggests a novel binding mode wherein the C-terminus of Ca^{2+}-bound CIB1 determines binding specificity to the α_{IIb} cytoplasmic domain (79). The C-terminal extension binds transiently and is then displaced, revealing a hydrophobic pocket between the two C-terminal EF hands that binds α_{IIb}. Solving the structure of α_{IIb}-bound CIB1 is ongoing and is expected to clear the uncertainty.

6. THE PHYSIOLOGICAL ROLE OF CIB1 IN REGULATING $\alpha_{IIb}\beta_3$ FUNCTION

It is well established that CIB1 and $\alpha_{IIb}\beta_3$ directly associate with each other, but what is the physiological relevance of this interaction between CIB1 and the integrin $\alpha_{IIb}\beta_3$?

6.1. The Role of CIB1 in Megakaryocytes

It has been argued that CIB1 is an inhibitor of $\alpha_{IIb}\beta_3$ activation (80). In this study, however, megakaryocytes were utilized in lieu of platelets because they can be directly genetically manipulated. A CIB1-EGFP construct inhibited thrombin receptor activating peptide (TRAP) induced fibrinogen binding to megakaryocytes. Since CIB1 binds p21 associated kinase (PAK1), a CIB1 mutant was constructed that retained its PAK1 binding ability, but could not bind α_{IIb}, CIB1 F173A-EGFP. The mutant failed to inhibit PAR4 peptide induced activation of $\alpha_{IIb}\beta_3$, suggesting that the agonist induced inhibition of $\alpha_{IIb}\beta_3$ via CIB1 is direct. However, this data was collected completely in megakaryocytes and this calls into question whether or not megakaryocyte biology can be indicative of platelet biology. Megakaryocytes, like platelets, express $\alpha_{IIb}\beta_3$, but the function of $\alpha_{IIb}\beta_3$ in megakaryocytes is to uptake fibrinogen for α-granule storage (81). The nucleic acid and protein composition of megakaryocytes differs greatly from that of platelets and agonist stimulation does not reliably induce $\alpha_{IIb}\beta_3$ activation in this unique cell type. So the behavior of the integrin in megakaryocytic studies may not be able to be generalized to how it would behave under the same conditions in platelets.

6.2. The Role of CIB1 in Inside-Out Signaling

CIB1 appears to be necessary for platelet activation. Addition of a palmitoylated peptide corresponding to the C-terminus of CIB1 (amino acids 179-188) to intact platelets inhibited $\alpha_{IIb}\beta_3$ activation upon ADP stimulation as monitored with PAC1 binding (52). Contrasting results have shown that CIB1 does not stimulate $\alpha_{IIb}\beta_3$ activation via $\alpha_{IIb}\beta_3$-CIB1 liquid phase binding assay and immunoprecipitation with PAC1 (76). However, Tsuboi has argued that these methods are less sensitive than the former and any change in integrin activation in the presence of CIB1 could have been missed. It was also predicted that CIB1 is necessary for platelet aggregation (52). Binding of radiolabeled Fg to $\alpha_{IIb}\beta_3$ increases in a dose-dependent manner following the addition of GST-CIB1. A peptide corresponding to amino acids 179-188 in the C-terminus of CIB1 competes with CIB1 for binding to $\alpha_{IIb}\beta_3$, and Fg binding upon addition of this peptide is decreased (52). Platelets incubated with a myristoylated version of this peptide showed no PAC1 binding when stimulated with ADP. The myristoylation of the peptide allows it to cross the membrane and enter the platelet and this was confirmed by tagging the peptide with FITC.

Of interest, CIB1 forms a complex with Wiskott-Aldrich syndrome protein (WASP) when platelets are activated wherein they bind N-terminal to N-terminal (54). Blocking this complex formation with CIB1 N-terminal fragments reduces $\alpha_{IIb}\beta_3$ affinity for fibrinogen and inhibits the bent-to-extended conformational change of the extracellular domain of the integrin as determined by lack of PAC1 binding. These results suggest that the WASP-CIB1 interaction is important for platelet $\alpha_{IIb}\beta_3$ activation via inside-out signaling.

6.3. The Role of CIB1 in Outside-In Signaling

Platelets activated with thrombin, collagen, the calcium ionophore A23187, or PMA under stirring

Table 1. Proteins that have been demonstrated to bind cytoplasmic tails of $\alpha_{IIb}\beta_3$

Protein	Tail it Binds	Binding Site	Inside-out/Outside-in Signaling	Reference
Calreticulin	α	KXGFFKR	Outside-in	36
Ancient Ubiquitous Protein 1 (Aup1)	α	KVGFFKR	Possibly Inside-out	31
ICln	α	KVGFFKR	Possibly Inside-out	33
Protein Phosphatase 1 (PP1c)	α	KVGF	Outside-in	37
Calcium- and Integrin-Binding Protein 1 (CIB1)	α	L983-R997	Inside-out/Outside-in	34, 51-54
Talin	α and β	H722-K738 β	Inside-out	27, 55-57
Filamin	β	NPXY	Outside-in	58
α-actinin	β	FAKFEEERAR	Outside-in	42, 58
Skelemin	β	WKLLITIHDRK	Outside-in	44
Integrin-Linked Kinase (ILK)	β	Not Identified	Outside-in	43
Grb2	β	D740-T762	Outside-in	19
Receptor for Activated C-Kinase (RACK1)	β	Membrane-proximal	Outside-in	45
Myosin	β	D740-T762	Outside-in	49
Protein Kinase C (PKC β)	β	Not Identified	Outside-in	38
Syk	β	R734-T762	Outside-in	48
Src	β	YRGT	Outside-in	50, 59, 60
Shc	β	ICY Domain	Outside-in	39

conditions and allowed to aggregate showed a significant increase in the level of CIB1 that co-sedimented with the actin cytoskeleton, indicating that upon platelet activation, CIB1 localizes to the cytoskeleton (72). This translocation could be blocked by pre-incubation with a RGDW peptide that blocks Fg binding to $\alpha_{IIb}\beta_3$. Addition of a monoclonal antibody against CIB1, UN7.79, halts platelet spreading by 95% on immobilized Fg (53). Platelets are arrested in the spiky morphology, suggesting CIB1 is necessary for the spiky to spread transition. Addition of recombinant CIB1 protein rescues the spread phenotype, suggesting the CIB1-$\alpha_{IIb}\beta_3$ interaction is necessary for platelet spreading on immobilized fibrinogen. Also, addition of an α_{IIb} peptide to compete with the binding of CIB1 to α_{IIb} halts platelet spreading at the spiky morphological stage. Addition of exogenous ADP to these arrested platelets recovers the spread morphology, suggesting that the CIB1- $\alpha_{IIb}\beta_3$ interaction is necessary for the granular secretion of ADP that is required for platelet spreading (53).

6.4 . Other CIB1 Interactions

CIB1 has also been shown to bind many other cytoplasmic proteins including but not limited to presenilin-2, polo-like kinase 2 (Plk2), polo-like kinase 3 (Plk3), DNA-PK, PAK1, FAK, FEZ1, Rac3, EDD, factor VIII, caspase 2S, Pax3, telomerase, NBR1, 6-16, and WASP (54, 71, 73, 82-92). Some of these important protein-protein interactions will be elaborated.

CIB1 has been shown to bind PAK1 and stimulate PAK1 autophosphorylation (84). CIB1 overexpression in REF52 cells plated on fibronectin resulted in a loss of focal adhesions, membrane ruffling, CIB1 localization to areas of increased actin dynamics, and a decrease in cell spreading. siRNA knockdown of CIB1 resulted in a 3-5 fold increase in cell migration. However, it has also been shown via wound healing assays that overexpression of CIB1 in CHO cells plated on fibronectin increases cell migration, spreading, and focal adhesions (71). Interestingly though, CIB1 does not directly interact with the fibronectin receptor, $\alpha_5\beta_1$, and thus the CIB1 mediated alterations in cell motility must be through some other downstream component. A dominant-negative FAK, FRNK, overexpressing cell culture shows the same migratory phenotype of control cells, but when FRNK and CIB1 are overexpressed together, the previously seen CIB1 induced cell migration is inhibited (71). This suggests that CIB1 modulates CHO cell migration on fibronectin via FAK. In fact, CIB1 co-immumoprecipitates FAK in CHO cells and platelets, increasingly so in platelets when plated on immobilized fibrinogen. This suggests that CIB1 and FAK binding results from outside-in signaling and that CIB1 can modulate platelet spreading on immobilized fibrinogen via FAK regulation.

CIB1 has also been shown to interact with activated Rac3, but not Rac1 or Rac2 (86). CIB1 expression in CHO cells plated on fibrinogen resulted in increased spreading 130%. The amount of CIB1 associated with activated Rac3 (V12Rac3) also increased. In the presence of V12Rac3, the amount of CIB1 that associates with the cytoskeleton increased. Also, constitutively active Rac3 increases adherence through $\alpha_{IIb}\beta_3$, while co-expression with CIB1 further increases adhesion. These results are interesting, but the expression of Rac3 in platelets has yet to be determined.

An interesting association shown with CIB1 is that with the IP3 receptor (IP3R) calcium release channel, as reported in PC12 cells (93). CIB1 binds the IP3R within the IP3 binding domain in a calcium-dependent manner. In the absence of IP3, CIB1 is capable of directly activating the channel and causing calcium release. However, if the cells were pre-exposed to CIB1, subsequent activation by IP3 was reduced. Overall, overexpression of CIB1 reduces calcium release through the IP3R, suggesting that CIB1 could play a role in channel gating via inhibition of IP3 stimulation of the IP3R. This CIB1-IP3R interaction has not been demonstrated in platelets as yet.

7. SUMMARY

Platelets function physiologically in hemostasis, but also pathologically in thrombosis. The study of platelet regulation is necessary for generating effective anti-platelet therapeutics. One platelet signaling molecule of particular

interest is the integrin $\alpha_{IIb}\beta_3$, which binds Fg and is required for platelet aggregation. The integrin itself as well as proteins that interact with $\alpha_{IIb}\beta_3$ have become targets for anti-platelet therapies. One such protein that has been shown to directly regulate $\alpha_{IIb}\beta_3$ function is calcium- and integrin-binding protein 1 (CIB1). CIB1 has been implicated in $\alpha_{IIb}\beta_3$ activation and outside-in signaling through the integrin. By increasing our understanding of CIB1 and other proteins that like it, associate with integrin $\alpha_{IIb}\beta_3$, and the signaling events that result from those interactions, we may bring ourselves closer to more effective therapies.

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9. REFERENCES

1. Coller, B. S.: A Brief and Highly Selective History of Ideas about Platelets in Health and Disease. In: Platelets. Ed: A. D. Michelson. Elsevier Science, San Diego (2002)

2. Nurden, A. T.: Glanzmann thrombasthenia. *Orphanet J Rare Dis*, 1, 10 (2006)

3. Aster, R. H.: Immune thrombocytopenia caused by glycoprotein IIb/IIIa inhibitors. *Chest*, 127, 53S-59S (2005)

4. Mannel, D. N. & G. E. Grau: Role of platelet adhesion in homeostasis and immunopathology. *Mol Pathol*, 50, 175-85 (1997)

5. Coller, B. S.: Blockade of platelet GPIIb/IIIa receptors as an antithrombotic strategy. *Circulation*, 92, 2373-80 (1995)

6. Topol, E. J., T. V. Byzova & E. F. Plow: Platelet GPIIb-IIIa blockers. *Lancet*, 353, 227-31 (1999)

7. Xiao, T., J. Takagi, B. S. Coller, J. H. Wang & T. A. Springer: Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature*, 432, 59-67 (2004)

8. Shattil, S. J. & M. H. Ginsberg: Perspectives series: cell adhesion in vascular biology. Integrin signaling in vascular biology. *J Clin Invest*, 100, 1-5 (1997)

9. Shattil, S. J.: Signaling through platelet integrin alpha IIb beta 3: inside-out, outside-in, and sideways. *Thromb Haemost*, 82, 318-25 (1999)

10. Calvete, J. J.: On the structure and function of platelet integrin alpha IIb beta 3, the fibrinogen receptor. *Proc Soc Exp Biol Med*, 208, 346-60 (1995)

11. Clemetson, K. J.: Platelet Receptors. In: Platelets. Ed: A. D. Michelson. Elsevier Science, San Diego (2002)

12. Salas, A., M. Shimaoka, U. Phan, M. Kim & T. A. Springer: Transition from rolling to firm adhesion can be mimicked by extension of integrin alphaLbeta2 in an intermediate affinity state. *J Biol Chem*, 281, 10876-82 (2006)

13. Ostermann, G., L. Fraemohs, T. Baltus, A. Schober, M. Lietz, A. Zernecke, E. A. Liehn & C. Weber: Involvement of JAM-A in mononuclear cell recruitment on inflamed or atherosclerotic endothelium: inhibition by soluble JAM-A. *Arterioscler Thromb Vasc Biol*, 25, 729-35 (2005)

14. Hato, T., Ginsberg, M. H., and Shattil, S. J.: Integrin alphaIIbbeta3. In: Platelets. Ed: A. D. Michelson. Elsevier Science, San Diego (2002)

15. Bennett, J. S.: Structure and function of the platelet integrin alphaIIbbeta3. *J Clin Invest*, 115, 3363-9 (2005)

16. Clemetson, K. J.: Platelet activation: signal transduction via membrane receptors. *Thromb Haemost*, 74, 111-6 (1995)

17. Hawiger, J.: Mechanisms involved in platelet vessel wall interaction. *Thromb Haemost*, 74, 369-72 (1995)

18. Kouns, W. C., C. F. Fox, W. J. Lamoreaux, L. B. Coons & L. K. Jennings: The effect of glycoprotein IIb-IIIa receptor occupancy on the cytoskeleton of resting and activated platelets. *J Biol Chem*, 266, 13891-900 (1991)

19. Law, D. A., L. Nannizzi-Alaimo & D. R. Phillips: Outside-in integrin signal transduction. Alpha IIb beta 3-(GP IIb IIIa) tyrosine phosphorylation induced by platelet aggregation. *J Biol Chem*, 271, 10811-5 (1996)

20. Parise, L. V.: Integrin alpha (IIb)beta (3) signaling in platelet adhesion and aggregation. *Curr Opin Cell Biol*, 11, 597-601 (1999)

21. Payrastre, B., K. Missy, C. Trumel, S. Bodin, M. Plantavid & H. Chap: The integrin alpha IIb/beta 3 in human platelet signal transduction. *Biochem Pharmacol*, 60, 1069-74 (2000)

22. Stouffer, G. A. & S. S. Smyth: Effects of thrombin on interactions between beta3-integrins and extracellular matrix in platelets and vascular cells. *Arterioscler Thromb Vasc Biol*, 23, 1971-8 (2003)

23. Shattil, S. J., H. Kashiwagi & N. Pampori: Integrin signaling: the platelet paradigm. *Blood*, 91, 2645-57 (1998)

24. Hughes, P. E., F. Diaz-Gonzalez, L. Leong, C. Wu, J. A. McDonald, S. J. Shattil & M. H. Ginsberg: Breaking the integrin hinge. A defined structural constraint regulates integrin signaling. *J Biol Chem*, 271, 6571-4 (1996)

25. Hwang, P. M. & H. J. Vogel: Structures of the platelet calcium- and integrin-binding protein and the alphaIIb-integrin cytoplasmic domain suggest a mechanism for calcium-regulated recognition; homology modelling and NMR studies. *J Mol Recognit*, 13, 83-92 (2000)

26. O'Toole, T. E., D. Mandelman, J. Forsyth, S. J. Shattil, E. F. Plow & M. H. Ginsberg: Modulation of the affinity of integrin alpha IIb beta 3 (GPIIb-IIIa) by the cytoplasmic domain of alpha IIb. *Science*, 254, 845-7 (1991)

27. Tadokoro, S., S. J. Shattil, K. Eto, V. Tai, R. C. Liddington, J. M. de Pereda, M. H. Ginsberg & D. A. Calderwood: Talin binding to integrin beta tails: a final common step in integrin activation. *Science*, 302, 103-6 (2003)

28. Ginsberg, M. H., A. Partridge & S. J. Shattil: Integrin regulation. *Curr Opin Cell Biol*, 17, 509-16 (2005)

29. Kloczewiak, M., S. Timmons, M. A. Bednarek, M. Sakon & J. Hawiger: Platelet receptor recognition domain on the gamma chain of human fibrinogen and its synthetic peptide analogues. *Biochemistry*, 28, 2915-9 (1989)

30. Kloczewiak, M., S. Timmons, T. J. Lukas & J. Hawiger: Platelet receptor recognition site on human fibrinogen. Synthesis and structure-function relationship of peptides corresponding to the carboxy-terminal segment of the gamma chain. *Biochemistry*, 23, 1767-74 (1984)

31. Kato, A., N. Kawamata, K. Tamayose, M. Egashira, R. Miura, T. Fujimura, K. Murayama & K. Oshimi: Ancient ubiquitous protein 1 binds to the conserved membrane-proximal sequence of the cytoplasmic tail of the integrin alpha subunits that plays a crucial role in the inside-out signaling of alpha IIbbeta 3. *J Biol Chem*, 277, 28934-41 (2002)

32. Knezevic, I., T. M. Leisner & S. C. Lam: Direct binding of the platelet integrin alphaIIbbeta3 (GPIIb-IIIa) to talin. Evidence that interaction is mediated through the cytoplasmic domains of both alphaIIb and beta3. *J Biol Chem*, 271, 16416-21 (1996)

33. Larkin, D., D. Murphy, D. F. Reilly, M. Cahill, E. Sattler, P. Harriott, D. J. Cahill & N. Moran: ICln, a novel integrin alphaIIbbeta3-associated protein, functionally regulates platelet activation. *J Biol Chem*, 279, 27286-93 (2004)

34. Naik, U. P., P. M. Patel & L. V. Parise: Identification of a novel calcium-binding protein that interacts with the integrin alphaIIb cytoplasmic domain. *J Biol Chem*, 272, 4651-4 (1997)

35. Pfaff, M., S. Liu, D. J. Erle & M. H. Ginsberg: Integrin beta cytoplasmic domains differentially bind to cytoskeletal proteins. *J Biol Chem*, 273, 6104-9 (1998)

36. Rojiani, M. V., B. B. Finlay, V. Gray & S. Dedhar: In vitro interaction of a polypeptide homologous to human Ro/SS-A antigen (calreticulin) with a highly conserved amino acid sequence in the cytoplasmic domain of integrin alpha subunits. *Biochemistry*, 30, 9859-66 (1991)

37. Vijayan, K. V., Y. Liu, T. T. Li & P. F. Bray: Protein phosphatase 1 associates with the integrin alphaIIb subunit and regulates signaling. *J Biol Chem*, 279, 33039-42 (2004)

38. Buensuceso, C. S., A. Obergfell, A. Soriani, K. Eto, W. B. Kiosses, E. G. Arias-Salgado, T. Kawakami & S. J. Shattil: Regulation of outside-in signaling in platelets by integrin-associated protein kinase C beta. *J Biol Chem*, 280, 644-53 (2005)

39. Cowan, K. J., D. A. Law & D. R. Phillips: Identification of shc as the primary protein binding to the tyrosine-phosphorylated beta 3 subunit of alpha IIbbeta 3 during outside-in integrin platelet signaling. *J Biol Chem*, 275, 36423-9 (2000)

40. Lin, S. Y., S. Raval, Z. Zhang, M. Deverill, K. A. Siminovitch, D. R. Branch & B. Haimovich: The protein-tyrosine phosphatase SHP-1 regulates the phosphorylation of alpha-actinin. *J Biol Chem*, 279, 25755-64 (2004)

41. Obergfell, A., K. Eto, A. Mocsai, C. Buensuceso, S. L. Moores, J. S. Brugge, C. A. Lowell & S. J. Shattil: Coordinate interactions of Csk, Src, and Syk kinases with [alpha]IIb[beta]3 initiate integrin signaling to the cytoskeleton. *J Cell Biol*, 157, 265-75 (2002)

42. Otey, C. A., G. B. Vasquez, K. Burridge & B. W. Erickson: Mapping of the alpha-actinin binding site within the beta 1 integrin cytoplasmic domain. *J Biol Chem*, 268, 21193-7 (1993)

43. Pasquet, J. M., M. Noury & A. T. Nurden: Evidence that the platelet integrin alphaIIb beta3 is regulated by the integrin-linked kinase, ILK, in a PI3-kinase dependent pathway. *Thromb Haemost*, 88, 115-22 (2002)

44. Reddy, K. B., P. Gascard, M. G. Price, E. V. Negrescu & J. E. Fox: Identification of an interaction between the m-band protein skelemin and beta-integrin subunits. Colocalization of a skelemin-like protein with beta1- and beta3-integrins in non-muscle cells. *J Biol Chem*, 273, 35039-47 (1998)

45. Rodriguez, M. M., D. Ron, K. Touhara, C. H. Chen & D. Mochly-Rosen: RACK1, a protein kinase C anchoring protein, coordinates the binding of activated protein kinase C and select pleckstrin homology domains in vitro. *Biochemistry*, 38, 13787-94 (1999)

46. Shattil, S. J., T. O'Toole, M. Eigenthaler, V. Thon, M. Williams, B. M. Babior & M. H. Ginsberg: Beta 3-endonexin, a novel polypeptide that interacts specifically with the cytoplasmic tail of the integrin beta 3 subunit. *J Cell Biol*, 131, 807-16 (1995)

47. Schaller, M. D., C. A. Otey, J. D. Hildebrand & J. T. Parsons: Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. *J Cell Biol*, 130, 1181-7 (1995)

48. Woodside, D. G., A. Obergfell, L. Leng, J. L. Wilsbacher, C. K. Miranti, J. S. Brugge, S. J. Shattil & M. H.
Ginsberg: Activation of Syk protein tyrosine kinase through interaction with integrin beta cytoplasmic domains. *Curr Biol*, 11, 1799-804 (2001)

49. Puszkin, E. G., E. A. Mauss & M. B. Zucker: Assembly and GPIIIa content of cytoskeletal core in platelets agglutinated with bovine von Willebrand factor. *Blood*, 76, 1572-9 (1990)

50. Arias-Salgado, E. G., F. Haj, C. Dubois, B. Moran, A. Kasirer-Friede, B. C. Furie, B. Furie, B. G. Neel & S. J. Shattil: PTP-1B is an essential positive regulator of platelet integrin signaling. *J Cell Biol*, 170, 837-45 (2005)

51. Barry, W. T., C. Boudignon-Proudhon, D. D. Shock, A. McFadden, J. M. Weiss, J. Sondek & L. V. Parise: Molecular basis of CIB binding to the integrin alpha IIb cytoplasmic domain. *J Biol Chem*, 277, 28877-83 (2002)

52. Tsuboi, S.: Calcium integrin-binding protein activates platelet integrin alpha IIbbeta 3. *J Biol Chem*, 277, 1919-23 (2002)

53. Naik, U. P. & M. U. Naik: Association of CIB with GPIIb/IIIa during outside-in signaling is required for platelet spreading on fibrinogen. *Blood*, 102, 1355-62 (2003)

54. Tsuboi, S., S. Nonoyama & H. D. Ochs: Wiskott-Aldrich syndrome protein is involved in alphaIIb beta3-mediated cell adhesion. *EMBO Rep*, 7, 506-11 (2006)

55. Bertagnolli, M. E., S. J. Locke, M. E. Hensler, P. F. Bray & M. C. Beckerle: Talin distribution and phosphorylation in thrombin-activated platelets. *J Cell Sci*, 106 (Pt 4), 1189-99 (1993)

56. Tremuth, L., S. Kreis, C. Melchior, J. Hoebeke, P. Ronde, S. Plancon, K. Takeda & N. Kieffer: A fluorescence cell biology approach to map the second integrin-binding site of talin to a 130-amino acid sequence within the rod domain. *J Biol Chem*, 279, 22258-66 (2004)

57. Yan, B., D. A. Calderwood, B. Yaspan & M. H. Ginsberg: Calpain cleavage promotes talin binding to the beta 3 integrin cytoplasmic domain. *J Biol Chem*, 276, 28164-70 (2001)

58. Calderwood, D. A., S. J. Shattil & M. H. Ginsberg: Integrins and actin filaments: reciprocal regulation of cell adhesion and signaling. *J Biol Chem*, 275, 22607-10 (2000)

59. Kralisz, U. & C. S. Cierniewski: Association of pp60c-src with alpha IIb beta 3 in resting platelets. *Biochem Mol Biol Int*, 45, 735-43 (1998)

60. Dorahy, D. J., M. C. Berndt & G. F. Burns: Capture by chemical crosslinkers provides evidence that integrin alpha IIb beta 3 forms a complex with protein tyrosine kinases in intact platelets. *Biochem J*, 309 (Pt 2), 481-90 (1995)

61. Reilly, D., D. Larkin, M. Devocelle, D. J. Fitzgerald & N. Moran: Calreticulin-independent regulation of the platelet integrin alphaIIbbeta3 by the KVGFFKR alphaIIb-cytoplasmic motif. *Platelets*, 15, 43-54 (2004)

62. Leung-Hagesteijn, C. Y., K. Milankov, M. Michalak, J. Wilkins & S. Dedhar: Cell attachment to extracellular matrix substrates is inhibited upon downregulation of expression of calreticulin, an intracellular integrin alpha-subunit-binding protein. *J Cell Sci*, 107 (Pt 3), 589-600 (1994)

63. Ghebrehiwet, B. & E. I. Peerschke: cC1q-R (calreticulin) and gC1q-R/p33: ubiquitously expressed multi-ligand binding cellular proteins involved in inflammation and infection. *Mol Immunol*, 41, 173-83 (2004)

64. Goicoechea, S., M. A. Pallero, P. Eggleton, M. Michalak & J. E. Murphy-Ullrich: The anti-adhesive activity of thrombospondin is mediated by the N-terminal domain of cell surface calreticulin. *J Biol Chem*, 277, 37219-28 (2002)

65. Liu, S., D. A. Calderwood & M. H. Ginsberg: Integrin cytoplasmic domain-binding proteins. *J Cell Sci*, 113 (Pt 20), 3563-71 (2000)

66. Ohmori, T., Y. Yatomi, N. Asazuma, K. Satoh & Y. Ozaki: Involvement of proline-rich tyrosine kinase 2 in platelet activation: tyrosine phosphorylation mostly dependent on alphaIIbbeta3 integrin and protein kinase C, translocation to the cytoskeleton and association with Shc through Grb2. *Biochem J*, 347, 561-9 (2000)

67. Eigenthaler, M., L. Hofferer, S. J. Shattil & M. H. Ginsberg: A conserved sequence motif in the integrin beta3 cytoplasmic domain is required for its specific interaction with beta3-endonexin. *J Biol Chem*, 272, 7693-8 (1997)

68. Kashiwagi, H., M. A. Schwartz, M. Eigenthaler, K. A. Davis, M. H. Ginsberg & S. J. Shattil: Affinity modulation of platelet integrin alphaIIbbeta3 by beta3-endonexin, a selective binding partner of the beta3 integrin cytoplasmic tail. *J Cell Biol*, 137, 1433-43 (1997)

69. Ron, D., C. H. Chen, J. Caldwell, L. Jamieson, E. Orr & D. Mochly-Rosen: Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins. *Proc Natl Acad Sci U S A*, 91, 839-43 (1994)

70. Arias-Salgado, E. G., S. Lizano, S. Sarkar, J. S. Brugge, M. H. Ginsberg & S. J. Shattil: Src kinase activation by direct interaction with the integrin beta cytoplasmic domain. *Proc Natl Acad Sci U S A*, 100, 13298-302 (2003)

71. Naik, M. U. & U. P. Naik: Calcium-and integrin-binding protein regulates focal adhesion kinase activity

during platelet spreading on immobilized fibrinogen. *Blood*, 102, 3629-36 (2003)

72. Shock, D. D., U. P. Naik, J. E. Brittain, S. K. Alahari, J. Sondek & L. V. Parise: Calcium-dependent properties of CIB binding to the integrin alphaIIb cytoplasmic domain and translocation to the platelet cytoskeleton. *Biochem J*, 342 Pt 3, 729-35 (1999)

73. Stabler, S. M., L. L. Ostrowski, S. M. Janicki & M. J. Monteiro: A myristoylated calcium-binding protein that preferentially interacts with the Alzheimer's disease presenilin 2 protein. *J Cell Biol*, 145, 1277-92 (1999)

74. Blamey, C. J., C. Ceccarelli, U. P. Naik & B. J. Bahnson: The crystal structure of calcium- and integrin-binding protein 1: insights into redox regulated functions. *Protein Sci*, 14, 1214-21 (2005)

75. Gentry, H. R., A. U. Singer, L. Betts, C. Yang, J. D. Ferrara, J. Sondek & L. V. Parise: Structural and biochemical characterization of CIB1 delineates a new family of EF-hand-containing proteins. *J Biol Chem*, 280, 8407-15 (2005)

76. Vallar, L., C. Melchior, S. Plancon, H. Drobecq, G. Lippens, V. Regnault & N. Kieffer: Divalent cations differentially regulate integrin alphaIIb cytoplasmic tail binding to beta3 and to calcium- and integrin-binding protein. *J Biol Chem*, 274, 17257-66 (1999)

77. Ames, J. B., R. Ishima, T. Tanaka, J. I. Gordon, L. Stryer & M. Ikura: Molecular mechanics of calcium-myristoyl switches. *Nature*, 389, 198-202 (1997)

78. Meyer, T. & J. D. York: Calcium-myristoyl switches turn on new lights. *Nat Cell Biol*, 1, E93-5 (1999)

79. Yamniuk, A. P., H. Ishida & H. J. Vogel: The interaction between CIB1 and the alpha IIb integrin cytoplasmic domain involves a novel C-terminal displacement mechanism. *J Biol Chem* (2006)

80. Yuan, W., T. M. Leisner, A. W. McFadden, Z. Wang, M. K. Larson, S. Clark, C. Boudignon-Proudhon, S. C. Lam & L. V. Parise: CIB1 is an endogenous inhibitor of agonist-induced integrin alphaIIbbeta3 activation. *J Cell Biol*, 172, 169-75 (2006)

81. Shattil, S. J. & A. D. Leavitt: All in the family: primary megakaryocytes for studies of platelet alphaIIbbeta3 signaling. *Thromb Haemost*, 86, 259-65 (2001)

82. Kauselmann, G., M. Weiler, P. Wulff, S. Jessberger, U. Konietzko, J. Scafidi, U. Staubli, J. Bereiter-Hahn, K. Strebhardt & D. Kuhl: The polo-like protein kinases Fnk and Snk associate with a Ca (2+)- and integrin-binding protein and are regulated dynamically with synaptic plasticity. *Embo J*, 18, 5528-39 (1999)

83. Wu, X. & M. R. Lieber: Interaction between DNA-dependent protein kinase and a novel protein, KIP. *Mutat Res*, 385, 13-20 (1997)

84. Leisner, T. M., M. Liu, Z. M. Jaffer, J. Chernoff & L. V. Parise: Essential role of CIB1 in regulating PAK1 activation and cell migration. *J Cell Biol*, 170, 465-76 (2005)

85. Whitehouse, C., J. Chambers, K. Howe, M. Cobourne, P. Sharpe & E. Solomon: NBR1 interacts with fasciculation and elongation protein zeta-1 (FEZ1) and calcium and integrin binding protein (CIB) and shows developmentally restricted expression in the neural tube. *Eur J Biochem*, 269, 538-45 (2002)

86. Haataja, L., V. Kaartinen, J. Groffen & N. Heisterkamp: The small GTPase Rac3 interacts with the integrin-binding protein CIB and promotes integrin alpha (IIb)beta (3)-mediated adhesion and spreading. *J Biol Chem*, 277, 8321-8 (2002)

87. Henderson, M. J., A. J. Russell, S. Hird, M. Munoz, J. L. Clancy, G. M. Lehrbach, S. T. Calanni, D. A. Jans, R. L. Sutherland & C. K. Watts: EDD, the human hyperplastic discs protein, has a role in progesterone receptor coactivation and potential involvement in DNA damage response. *J Biol Chem*, 277, 26468-78 (2002)

88. Hollenbach, A. D., C. J. McPherson, I. Lagutina & G. Grosveld: The EF-hand calcium-binding protein calmyrin inhibits the transcriptional and DNA-binding activity of Pax3. *Biochim Biophys Acta*, 1574, 321-8 (2002)

89. Ito, A., T. Uehara & Y. Nomura: Isolation of Ich-1S (caspase-2S)-binding protein that partially inhibits caspase activity. *FEBS Lett*, 470, 360-4 (2000)

90. Fang, X., C. Chen, Q. Wang, J. Gu & C. Chi: The interaction of the calcium- and integrin-binding protein (CIBP) with the coagulation factor VIII. *Thromb Res*, 102, 177-85 (2001)

91. Lee, G. E., E. Y. Yu, C. H. Cho, J. Lee, M. T. Muller & I. K. Chung: DNA-protein kinase catalytic subunit-interacting protein KIP binds telomerase by interacting with human telomerase reverse transcriptase. *J Biol Chem*, 279, 34750-5 (2004)

92. Tahara, E., Jr., H. Tahara, M. Kanno, K. Naka, Y. Takeda, T. Matsuzaki, R. Yamazaki, H. Ishihara, W. Yasui, J. C. Barrett, T. Ide & E. Tahara: G1P3, an interferon inducible gene 6-16, is expressed in gastric cancers and inhibits mitochondrial-mediated apoptosis in gastric cancer cell line TMK-1 cell. *Cancer Immunol Immunother*, 54, 729-40 (2005)

93. White, C., J. Yang, M. J. Monteiro & J. K. Foskett: CIB1, a Ubiquitously Expressed Ca2+-binding Protein Ligand of the InsP3 Receptor Ca2+ Release Channel. *J Biol Chem*, 281, 20825-20833 (2006)

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