Integrins: dynamic scaffolds for adhesion and signaling in platelets

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The major platelet integrin, αIIbβ3, is required for platelet interactions with proteins in plasma and the extracellular matrices (ECMs) that are essential for platelet adhesion and aggregation during hemostasis and arterial thrombosis. Ligand binding to αIIbβ3 is controlled by inside-out signals that modulate receptor conformation and clustering. In turn, ligand binding triggers outside-in signals through αIIbβ3 that, when disrupted, can cause a bleeding diathesis. In the past 5 years there has been an explosion of knowledge about the structure and function of αIIbβ3 and the related integrin, αVβ3. These developments are discussed here, and current models of bidirectional αIIbβ3 signaling are presented as frameworks for future investigations. An understanding that αIIbβ3 functions as a dynamic molecular scaffold for extracellular and intracellular proteins has translated into diagnostic and therapeutic insights relevant to hematology and cardiovascular medicine, and further advances can be anticipated. (Blood. 2004;104:1606-1615)

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Introduction

The platelet is a tightly regulated adhesion machine. Restrained in its functions while in the bloodstream, its adhesive, hemostatic, and proinflammatory capabilities are unleashed at sites of vessel injury to generate the primary hemostatic plug, catalyze fibrin formation, and supply soluble and membrane-bound factors that promote wound healing. While platelets can adhere to damaged endothelial cells, their principle adhesive surface is the extracellular matrix (ECM), which becomes exposed in injured vessels and offers a panoply of ligands for platelet adhesion receptors. Within this context, integrin adhesion receptors, and αIIbβ3 in particular, play critical roles in platelet function.

Integrins are heterodimeric (αβ) type I transmembrane receptors, each subunit typically containing a relatively large extracellular domain, a single-pass transmembrane domain, and a short cytoplasmic tail composed of 20 to 60 amino acids. Platelets express several integrins (αIIbβ3, also called glycoprotein IIb-IIIa [GPIIb-IIIa]; αVβ3; α2β1; α5β1; α6β1). Integrins are, in effect, “2-faced” receptors, one face oriented to the extracellular space and interactive with cognate ECM ligands and the other oriented to the cell interior and interactive with cytoplasmic proteins. Ligand binding to either face can trigger information transfer, or signaling, across the plasma membrane to “activate” cellular functions at the other face. Figure 1 illustrates this bidirectional signaling using αIIbβ3 as an example.

Basic research conducted in the past 3 decades on many facets of αIIbβ3 structure and function has led to remarkable breakthroughs culminating in the development of a chimeric anti-αIIbβ3 monoclonal antibody and small-molecule receptor antagonists now used parenterally to limit the formation of occlusive platelet thrombi in acute cardiovascular indications. On the other hand, clinical trials of oral αIIbβ3 antagonists have been disappointing and suggest that long-term extracellular blockade of ligand binding to αIIbβ3 might even be dangerous. Conceivably, then, further basic studies of this proven therapeutic target, and of αIIbβ3 signaling in particular, might lead to newer and better ways to diagnose, prevent, or treat arterial thrombosis or other consequences of αIIbβ3 dysfunction. The purpose of this review is to highlight recent experimental and conceptual advances in the integrin field that are particularly relevant to αIIbβ3 and platelets. It will draw from structural analyses of integrins and studies of human and mouse platelets, with the caution that platelets from these 2 species are similar but not identical. Several excellent general reviews of integrin signaling are also available.

Structural basis of β3 integrin signaling

Structure of the αIIbβ3 extracellular domain

Our first glimpse into αIIbβ3 structure was provided nearly 20 years ago when approximately 230-kDa αIIbβ3 complexes were purified from detergent-solubilized platelet membranes and visualized by electron microscopy. Rotary-shadowed, negatively stained images revealed an approximately 23-nm (230- Å) complex consisting of an approximately 8-nm (80- Å) globular head and 2 approximately 16-nm (160- Å) flexible stalks. In the absence of detergent, αIIbβ3 aggregated into rosettes that appeared to be contacting each other at the tips of their stalks (Figure 2A-B). Following the cloning of αIIb and β3 and using epitope-mapped antibodies, Weisel and colleagues correctly deduced that the stalks contain the C-terminal, transmembrane domain—containing segment of each subunit, and the globular head contains the N-terminal portions. These investigators also found that fibrinogen, von Willebrand factor (VWF), and fibronectin interact with the globular head (Figure 2C-D), thus identifying this domain as...
containing the ligand contact site. These findings were corroborated by the demonstration of fibrinogen binding to a recombinant αIIbβ3 head domain lacking the β3 stalk.19

A number of early models of integrin structure, such as the one shown in the top left panel of Figure 3, were developed based on electron microscopic images, peptide and epitope mapping, photoaffinity and chemical cross-linking, and biochemical analyses of cysteine disulfide-bonding patterns. Portrayed were such structural features as (1) an αIIb subunit composed of an approximately 120-kDa heavy-chain disulfide bonded to an approximately 23-kDa light chain; (2) 4 αIIb calcium-binding domains; (3) a small N-terminal cysteine-rich domain in β3 attached by a disulfide bond to “cysteine-rich repeats” within the body of the molecule; and (4) a large, protease-sensitive “disulfide-bonded loop” within β3 bounded by residues 121 and 348 and containing the major ligand-binding sites.

Recent determination of the crystal structure of the extracellular segment of αVβ3 has provided a major advance.20 Remarkably, many of the structural and functional domains predicted in earlier models are recognizable in the 12 domains identified in the crystal, albeit with much higher resolution, and with some notable surprises. As shown in Figure 3, the 4 Ca²⁺-binding domains in αV are part of a β-propeller, the structure of which had been predicted.21 A large immunoglobulin-like “thigh” domain comprises the remainder of the αV subunit’s contribution to the integrin headpiece. In β3, the N-terminal cysteine-rich segment has become the plexin/
semaphorin/integrin (PSI) domain, while the former ligand-binding large disulfide-bonded loop emerges from a discontinuous, immunoglobulin-like hybrid domain in the form of an adhesive “I-like” or A domain. Finally, the previously observed αVβ3 stalk is now composed of 2 rigid “calf” modules, and the former cysteine-rich repeats of the β3 stalk have morphed into 4 endothelial growth factor (EGF)-like domains, which, together with a novel flowerlike structure termed the β-terminal domain (BTD), completes the stalk. Comparison of the predicted structures of αIIbβ3 with those actually found in the αVβ3 crystal can be found in Table 1.

Several groups have attempted to reconcile the αVβ3 crystal structure with electron microscopic images analyzed with refined methods. In one example, electron cryomicroscopy was used to derive a 2-nm (20-Å) resolution Fourier shell transformation density map of hydrated αIIbβ3 complexes frozen in a low-affinity, unliganded state. Taking liberties to introduce a few kinks into the flexible hinge regions within the 2 subunits, the authors were able to visually fit the 12 domains of αIIbβ3 into the extracellular region of their 3-dimensional contour map. They suggest that their model may represent the structure of αIIbβ3 as it exists in the surface membrane of resting platelets.

Structure of αIIbβ3 transmembrane and cytoplasmic domains

Since the short cytoplasmic tails of αIIb and β3 play key roles in signaling, much attention has focused on whether they have an ordered 3-dimensional structure and how they might interact with each other and with intracellular proteins. Vinogradova et al were the first to determine a nuclear magnetic resonance (NMR) structure for a membrane-anchored form of an isolated αIIb tail and found that it formed an N-terminal α-helix followed by a turn predicted to straighten out upon integrin activation. Ulmer et al employed NMR spectroscopy to analyze the αIIb and β3 cytoplasmic tails in aqueous solution and found them to be largely unstructured, with a tendency for the N-terminus of β3 to form a helix and the downstream NPLY motif to form a reverse turn that could support talin binding. Interestingly, neither these authors nor Li and colleagues could find any evidence for interaction between the αIIb and β3 tails themselves, despite the fact that the latter group expressed the tails in a relatively native state—that is, attached to their respective transmembrane domains buried in phospholipid. Rather, the transmembrane domains, which were present in a largely α-helical conformation, actually promoted the formation of homodimers and homotrimers. Based on the observation that certain mutations within the β3 transmembrane domain enhance the tendency to form αIIb-αIIb and β3-β3 homomeric associations, while at the same time conferring constitutive fibrinogen-binding activity, these workers proposed that homomeric transmembrane helix associations might drive subunit reshuffling to increase receptor clustering and avidity for ligand. On the other hand, cysteine scanning mutagenesis of the αIIb and β3 transmembrane domains in the context of the full-length integrin expressed in model cell systems suggests that interactions through a specific heterodimer interface help to maintain the default low-avidity state of the integrin. Furthermore, separation of the transmembrane helices is linked to an increase in αIIbβ3 affinity for ligands. Since a portion of the integrin residues involved in the transmission of bidirectional signals are embedded in the plasma membrane, the structure and function of the αIIbβ3 transmembrane domains clearly warrant further investigation.

In contrast to the above studies, several others have found evidence that integrin cytoplasmic tails can and do interact with each other, and in some cases the nature of the interaction appears to change as a consequence of inside-out activation. A tightly packed approximately 3-nm (30-Å) long cylindrical rod was observed in the region of the αIIbβ3 transmembrane domain by electron cryomicroscopy, corresponding to a pair of tightly packed, parallel, right-handed α-helical coils. The cytoplasmic domains could be seen extending from the transmembrane α-helix as a single, cohesive, heart-shaped density of approximately 7 kDa. Two recent NMR structures also show extensive, although somewhat contradictory, interactions between αIIb and β3 cytoplasmic tails near the membrane-proximal interface of each tail. The latter structure showed intersubunit contacts composed of hydrophobic and hydrostatic interactions mediated by amino acid residues highly conserved among integrins. A recent study has shown that in the presence of membrane-mimetic micelles, the cytoplasmic faces of the αIIb and β3 cytoplasmic tails and the NPLY region of β3 become embedded in the membrane, a conformation that differs

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<tr>
<th>Subunit</th>
<th>Old Properties/function</th>
<th>New Properties/function</th>
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<tr>
<td>α</td>
<td>Cat-binding domain</td>
<td>ζ-propeller</td>
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<td>Known for many years to require μM levels of Ca²⁺ to maintain structural integrity, this 7-bladed, propeller-like domain contributes to the integrin head and forms the major site of contact with the ζ subunit.</td>
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<td>β</td>
<td>Heavy-β-chain cleavage site</td>
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<td>Evident only upon reduced SDS-PAGE, posttranslational cleavage of the α subunit takes place within the Golgi apparatus but appears inconsequential for integrin structure or function.</td>
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<td>αIIb</td>
<td>N-terminal cysteine-rich domain</td>
<td>PSI domain</td>
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<td>Flexible, immunogenic domain composed of residues 1-54, including 3 disulfide bonds and 1 &quot;extra&quot; cysteine that couples the N-terminus to the bent-hinge region of the integrin; harbors the clinically important PIII alloantigen at Leu153.</td>
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<td>β3</td>
<td>Chymotrypsin-resistant domain</td>
<td>Hybrid domain</td>
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<td>Immunoglobulin-like domain composed of discontinuous residues 55-108 and 353-434. The ligand-binding &quot;A&quot; domain emerges from the 2 ζ-sheets that comprise this domain (see below).</td>
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<td>αIIb</td>
<td>Large disulfide-bonded loop</td>
<td>ζA domain</td>
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<td>Residues 109-352. The ligand-binding domain that, together with the αIIb ζ-propeller, forms nearly half of the previously visualized integrin head. Antibodies that bind this domain can inhibit ligand binding; easily removed from the rest of the molecule by protease cleavage at flanking residues 121 and 348. The resulting 66-kDa subunit still contains the PIII epitope but can no longer bind ligand.</td>
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<td>β3</td>
<td>Cysteine-rich repeats</td>
<td>EGF repeats</td>
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<td>Protease-resistant stalk composed of 4 repeating EGF domains that may broker integrin conformational changes.</td>
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NA indicates not applicable; and SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.
importantly from that observed in a strictly aqueous environment. Furthermore, the binding of purified talin to β3 caused unclamping of the tails and changes in tail-membrane interactions (Figure 4A). These in vitro results are largely consistent with fluorescence resonance energy transfer (FRET) studies of green fluorescence protein (GFP)–tagged αL and yellow fluorescent protein (YFP)–tagged β2 subunits in living cells. Based on changes in FRET, the cytoplasmic tails were calculated to be close to each other in resting cells but become separated by up to 10 nm (100 Å) following either ligand binding to αLβ2 or agonist-induced cellular activation. Overall, these data provide compelling evidence that inter-subunit cytoplasmic tail associations, and possibly heterodimeric transmembrane associations, function to maintain the αIIbβ3 complex in a resting, nonadhesive conformation, while disruption of these interactions causes separation of the tails and propagated changes in the extracellular domains to increase αIIbβ3 affinity.

Conformational changes associated with β3 integrin activation

Several observations indicate that the extracellular domains of αIIbβ3 and αVβ3 must undergo conformational transitions upon cellular activation or ligand binding. First, activation of platelets or endothelial cells induces the binding of arginine-glycine-aspartic acid (RGD)–containing antibody Fab fragments to αIIbβ3 and αVβ3, respectively. In the case of αIIbβ3, Fab binding requires discontinuous regions of the αIIb β-propeller and the β3 A domain. Second, when platelets are activated by agonists, a change in FRET is observed between fluorophore-conjugated antibodies bound to αIIb and β3. Finally, anti-αIIb or anti-β3 antibodies of the ligand-induced binding sites (LIBS) type preferentially recognize the ligand-occupied form of the integrin, and they in turn increase receptor affinity. In terms of primary amino acid sequence, the epitopes for many of these LIBS antibodies are located a relatively long distance from the ligand-binding headpiece, suggesting that the integrin is subject to long-range conformational changes.

Differences in αVβ3 structure have been visualized in negatively stained electron microscopic images of the integrin in apparent low- and high-affinity states. The low-affinity, unliganded form was present as a compact, V-shaped structure highly reminiscent of the bent conformation found in the crystal structure of αVβ3 (Figure 3), becoming extended upon ligand binding into the “head + 2 tails” configuration previously visualized by other investigators (eg, Figure 2). This transition has been described as analogous to a “switchblade-like” movement such that the extended form may represent the ligand-bound high-affinity receptor. However, a less drastic change may be all that is required for initial transformation of β3 integrins from low- to high-affinity state. Coincided the “deadbolt” model, inside-out signals are postulated to transmit conformational changes through the transmembrane helices into the immediately proximal βTD. The CD loop of the βTD, which in resting integrins acts like a deadbolt to pin the F/α7 loop of the βA domain forward to obstruct contact with macromolecular ligands (Figure 4B), then moves out of the way, allowing the F/α7 loop to swing away from the ligand contact site, making it available for productive, high-affinity ligand binding.

Experimental evidence that displacement of the F/α7 loop is involved in ligand binding comes from studies of αLβ2, where mutational shortening of the β2 α-helix by approximately one turn resulted in a constitutively active receptor. One of the most attractive features of this model is that larger-scale structural changes are not required to convert β3 integrins into high-affinity receptors. Furthermore, the model does not preclude switchblade-like straightening of the bent integrin or separation of the cytoplasmic tails, both of which are likely to promote outside-in signaling in response to ligand binding. Additional studies are required to rigorously test and refine existing models of integrin activation and, in particular, to fully understand coordinated transitions among the integrin extracellular, transmembrane, and cytoplasmic domains, molecular movements that will likely affect outside-in as well as inside-out signaling.

Integrin clustering

In addition to conformational changes, cell activation promotes the lateral mobility and clustering of integrins within the plane of the plasma membrane. Initially, small oligomers or “microclusters” below the resolution of the light microscope may contribute to “avidity” or “valency” regulation of ligand binding. Microclustering in native membranes or living cells has been difficult to study, but new techniques are beginning to open up this area of investigation. The αIIbβ3 clustering may be promoted by several mechanisms, including the binding of multivalent ligands, ligand self-association, lateral interactions of integrins with other membrane proteins, reversible integrin linkages to the actin cytoskeleton, and homomeric interactions of the transmembrane domains. Integrin conformational change and clustering are not mutually exclusive; they are complementary and may even be mechanistically linked. Each may be involved in different aspects of bidirectional signaling. For example, conformational change seems to be the dominant way in which ligand binding to αIIbβ3 and αVβ3 is regulated, while clustering is
Biochemical basis of β3 integrin signaling

Regulation of inside-out signaling: excitatory and inhibitory agonist receptors

Binding of adhesive ligands to αIIbβ3 can be triggered by soluble agonists, such as adenosine diphosphate (ADP), thrombin, epinephrine, and thromboxane A2, which engage cognate G-protein–coupled receptors. In addition, certain platelet adhesion receptors, notably GPIb-IX-V (the primary receptor for VWF), GPVI (collagen), α2β1 (collagen), and even αIIbβ3, can trigger activation signals when bound to and clustered by ECM ligands. The relative contribution of soluble and ECM stimuli to inside-out signaling likely varies with flow conditions and other circumstances of vascular injury. For example, GPIb-IX-V function is most relevant under conditions of high shear typical of the arteriolar and capillary circulations and in stenotic arteries. An important but under-studied process is inhibition or reversal of ligand binding to αIIbβ3. Activation of αIIbβ3 is negatively regulated in a complex manner by cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP), generated by interaction of platelets with prostacyclin (PG12) and nitric oxide (NO), respectively, and by an endothelial cell ecto-ADPase (CD39). In mouse platelets, the excitatory function of GPVI and GPVI3 can be triggered by associated FcR.

Signaling intermediates that link agonist receptors to αIIbβ3

Receptors couple to second messengers such as Ca2+, cyclic nucleotides, and products of phospholipases and tyrosine kinases. A major gap remains in how second messengers effect functional changes in αIIbβ3. For example, protein kinase C (PKC), phosphatidylinositol 3–kinase (PI 3-kinase), and Rap1b have been implicated as intermediates in promoting inside-out signaling, but the identities and activities of the relevant effectors of these enzymes remain to be determined. Rap1b serves as a timely case in point.

RAP1L is a Rap1-GTP–binding protein that is expressed in platelets, MnCl2 or LIBS antibodies convert RAP1L to a high-affinity conformation of purified αIIbβ3. In platelets, MnCl2 or LIBS antibodies convert αIIbβ3 into a high-affinity conformation. Some have speculated that certain αIIbβ3 antagonists or soluble CD40 ligand may exert similar effects in vivo. Reducing agents such as dithiothreitol can activate purified or platelet αIIbβ3, as can mutation of single cysteines within the β3 EGF repeats. The platelet surface and β3 integrins in particular are reported to possess thiol isomerase activity, leading to the proposition that disulfide exchange may help to regulate αIIbβ3 activation. Also, a pool of αIIbβ3 may form complexes with CD9 (a tetrarasin) or CD47 (a thrombospondin receptor). The relationship between CD47 and αIIbβ3 appears particularly complex. Specific peptides from thrombospondin can bind to CD47 and activate platelet G proteins, providing one way for CD47 to regulate αIIbβ3. In addition, platelet CD47 can interact with receptors in activated endothelial cells, leading to αIIbβ3 activation. Finally, the extracellular portion of CD47 bound to a thrombospondin peptide can directly modulate αIIbβ3 activation state in Chinese hamster ovary (CHO) cells. Despite these observations, no platelet aggregation abnormalities have been reported in CD47-deficient mice.

Evidence to date indicates that any role for extracellular or transmembrane molecules in affinity modulation is secondary to αIIbβ3 regulation by intracellular proteins, and in particular talin, which engage the integrin cytoplasmic tails. Talin1 is an approximately 270-kDa antiparallel dimer composed of an approximately 50-kDa N-terminal FERM domain, which contains F1-3 subdomains and assumes a phosphotyrosine-binding (PTB) domain–like fold, and an approximately 220-kDa C-terminal rod domain (Figure 5). F2-3 contains a major binding site for integrin β3 cytoplasmic tails and several other proteins, including type I phosphatidylinositol phosphate kinase (PIP1), an enzyme responsible for generating the lipid second messenger, PIP2. The rod domain, separated from the FERM domain by a calpain cleavage site, contains the major binding sites for F-actin.
Platelet adhesion to fibrinogen or VWF triggers morphologic changes ranging from filopodial and lamellipodial extension to full spreading. As in nucleated cells, these changes are mediated by effectors of the Rho GTPases, cdc42, Rac1, and Rho A. The morphologic changes are associated with dynamic modifications of the actin cytoskeleton that affect the polymerization state and organization of actin. αIbβ3 participates in this process by nucleating signaling complexes at adhesion sites that regulate actin. Platelets and αIbβ3-expressing CHO cells adherent to fibrinogen have frequently been used as model systems to study this process. In both cases, outside-in signaling occurs in a discrete pattern whereby ligand binding initiates integrin clustering and assembly of a nascent signaling complex proximal to the cytoplasmic tails of αIbβ3, followed by the growth of a larger actin-based signaling complex.

Initiation of outside-in signaling

Among the earliest detectable biochemical responses of platelets to fibrinogen binding is activation of Src and Syk protein tyrosine kinases. Occupancy of αIbβ3 by fibrinogen causes integrin microclustering, which appears necessary for this tyrosine kinase activation. Although some monovalent ligands promote αIbβ3 homo-oligomerization in detergent solution, there is no unequivocal evidence yet that they do so or trigger outside-in signaling in vivo. Soluble CD40 ligand can bind and induce outside-in signaling through αIbβ3, but it is a trimer, suggesting even in this case that clustering of αIbβ3 is required.

Components of a nascent αIbβ3 signaling complex have been identified in platelets and CHO cells based on their ability to coimmunoprecipitate with αIbβ3 or to become rapidly tyrosine phosphorylated by integrin-associated Src or Syk, even in the presence of inhibitors of actin polymerization. Bypassed by studies with purified proteins as well as by detection of specific protein-protein interactions in living cells, the following sequence of events for assembly of the αIbβ3 signaling complex can be envisioned. (1) Src kinases constitutively bound to the β3 cytoplasmic tail become activated when fibrinogen engages and clusters αIbβ3. (Figure 6), (2) Syk is recruited to the β3 tail and activated by Src. (3) Src and/or Syk phosphorylate

Figure 5. Domain structure of talin, a key protein in regulation of inside-out integrin signaling. Location of binding sites for other proteins is depicted.

Figure 6. Model for αIbβ3 regulation of Src. (Left) According to current structural models, family kinases are membrane associated and maintained in a "clamped," inactive state through intramolecular interactions between the SH2 domain and a C-terminal phosphotyrosine motif at Tyr529 (Y529), and the SH3 domain and a polyproline motif in the linker region between the SH2 domain and the N-lobe of the catalytic domain. (Middle) In platelets, a pool of c-Src (and several other Src family kinases) is constitutively bound to αIbβ3 through interaction of the β3 cytoplasmic tail with the SH3 domain. This may maintain Src in a partially clamped, primed state but not yet fully active, in part because Tyr529 remains phosphorylated by integrin-associated Csk. (Right) Upon αIbβ3 ligation, Src becomes clustered and Csk dissociates from the integrin complex. The net result is dephosphorylation of Tyr529 by an unidentified tyrosine phosphatase and autophosphorylation of Tyr418 (Y418) in the Src activation loop. Consequently, Src is now unclamped and fully active to phosphorylate downstream effectors. From Arias-Salgado et al and Obergfell et al with permission.
substrates, including SLP-76, ADAP and c-Cbl (molecular adaptors), and Vav (a Rac GTPase), that are implicated in signaling to the actin cytoskeleton.\textsuperscript{118-120} 

**Propagation of outside-in signaling**

As the nascent complex assembles, many additional proteins are recruited that are capable of influencing actin dynamics and reorganization. These include Rac, Nck (an adapter), PAK, PI 3-kinase, and vasodilator-stimulated phosphoprotein (VASP), an actin-bundling protein (Figure 7). Although not all components of the signaling network have been identified, 3 proteins warrant particular discussion here because each is tyrosine phosphorylated by Src and/or Syk during platelet aggregation and spreading and each probably helps to morph nascent complexes into actin-based complexes. These proteins are β3 itself, phospholipase Cγ, and α-actinin. Phosphorylation of the β3 cytoplasmic tail at residues 747 and 759 may enhance post–ligand-binding events by generating docking sites for SH2-containing protein (Shc), an adapter in Ras signaling, and myosin, a motor protein involved in clot retraction and stabilization of platelet aggregates.\textsuperscript{121,122} Mice in which these tyrosines have been mutated to phenylalanine exhibit rebleeding from tail wounds and subtle defects in clot retraction and platelet aggregation.\textsuperscript{123}

Phospholipase Cγ is a substrate of Src and Bruton tyrosine kinase (Btk) kinases, and its activation by tyrosine phosphorylation downstream of αIbb3 generates some of the diacylglycerol and inositol phosphate (IP3) needed for maximal platelet aggregation and spreading.\textsuperscript{113} Diacylglycerol and IP3-dependent Ca\textsuperscript{2+} fluxes activate conventional and novel isoforms of PKC as previously in the context of inside-out signaling. Preliminary studies show that certain PKC isoforms coimmunoprecipitate with αIbb3 under some conditions, and broad-spectrum PKC inhibitors block platelet spreading on fibrinogen (Churito Buensuceso, Alessandra Soriani, Achim Obergfell, Koji Eto, and S.J.S., unpublished observations, January 2004). Thus, some integrin-associated proteins may regulate both phases of αIbb3 signaling.

α-actinin is a homodimeric actin-binding protein that localizes to integrin adhesion sites. The nonmuscle isoform found in platelets contains binding sites for vinculin, zyxin, and the membrane-proximal portions of β1, β2, and β3 integrin cytoplasmic tails.\textsuperscript{124} Overexpression of full-length α-actinin in fibroblasts leads to stabilization of adhesion sites, while integrin-binding fragments disrupt actin stress fibers, focal adhesions, and mechanotransduction.\textsuperscript{125} α-actinin becomes tyrosine phosphorylated on a single N-terminal residue in response to platelet aggregation or spreading.\textsuperscript{126} Phosphorylation may be mediated by focal adhesion kinase (FAK), whose own activation is dependent on actin polymerization following platelet costimulation through agonist and αIbb3 receptors. In a model system, tyrosine phosphorylation of α-actinin reduced its cosedimentation with F-actin.\textsuperscript{126} Therefore, phosphorylation of α-actinin during later stages of outside-in signaling might regulate αIbb3 linkages with actin and the assembly/disassembly of actin-based signaling complexes.

Many facets of outside-in αIbb3 signaling remain to be explored. What are the identities and functions of the phosphatases that counterbalance the effects of the protein and lipid kinases that operate in integrin signaling? What are the roles in platelets of other proteins, such as skelemin and integrin-linked kinase, reported to interact with the αIIb or β3 cytoplasmic tails in model systems?\textsuperscript{120-127,128} Disassembly of αIbb3-based signaling complexes may be required to achieve full platelet spreading or to limit platelet adhesion and aggregation to the hemostatic plug. How is disassembly regulated? Perhaps it involves αIbb3-dependent activation of FAK and its effectors.\textsuperscript{129,130} Perhaps it involves specific protein or lipid phosphatases or proteases like calpain, which cleave β3, Src, and other proteins upon platelet aggregation. Do the other integrins in platelets engage in bidirectional signaling? Recent investigations of αVβ3 and α2β1 suggest that they do.\textsuperscript{53,131-133} How are they regulated?

**Perspective**

αIbb3 was identified as the platelet fibrinogen receptor over 25 years ago. Ensuing investigations of αIbb3 and its relative, αVβ3, have provided many key insights about integrin structure and function. Some of these have led to improvements in clinical practice, most notably the use of parental αIbb3 antagonists to prevent arterial thrombosis. Future studies of αIbb3 signaling promise to yield additional information of clinical relevance. For example, while deficiency of αIbb3 is rare, specific defects in αIbb3-related signal transduction may account for many incompletely characterized bleeding disorders associated with defects in

Figure 7. Cartoon depicting portions of the signaling network linking αIbb3 to actin polymerization and reorganization. The insert provides a key to some of the modules or domains within the proteins that mediate or regulate protein functions and/or interactions. Domain abbreviations: CH, calponin homology; P–Tyr, phosphotyrosine; PTB, phosphotyrosine binding; PH, pleckstrin homology; WH, WASP homology; and VH, verprolin homology. WIP indicates WASP-interacting protein; PLCγ, phospholipase Cγ. The figure is offered solely to provide a visual context for the discussion in the text of early phases of outside-in signaling. No attempt is made to show all proteins involved or all interactions of a given protein, and important signaling cross-talk between αIbb3 and other platelet receptors is not depicted.\textsuperscript{2} See Bearer et al,\textsuperscript{109} Hartwig et al,\textsuperscript{111} and Calderwood et al\textsuperscript{124} for reviews of integrin-dependent actin dynamics and organization in platelets.
platelet aggregation. Moreover, currently available antplatelet drugs, such as aspirin and clopidogrel, work in effect by dampening inside-out signaling to $\alpha_{IIb}\beta_3$. Although bidirectional $\alpha_{IIb}\beta_3$ signaling is complex, novel orally active drugs may eventually be developed that target specific facets of this process. More generally, progress in integrin research can also be anticipated in several other areas of interest to hematologists, among them elucidation of the relationships between integrin polymorphisms, platelet function, and thrombotic risk; the pathogenesis of immune thrombocytopenias; the role of $\alpha_{IIb}\beta_3$ in hematopoietic stem cells; and the development of drugs that modulate integrin signaling in inflammatory diseases and cancer. Stay tuned.

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