The platelet integrin $\alpha_{IIb}\beta_3$ is required for platelet aggregation. Like other integrins, $\alpha_{IIb}\beta_3$ resides on cell surfaces in an equilibrium between inactive and active conformations. Recent experiments suggest that the shift between these conformations involves a global reorganization of the $\alpha_{IIb}\beta_3$ molecule and disruption of constraints imposed by the heteromeric association of the $\alpha_{IIb}$ and $\beta_3$ transmembrane and cytoplasmic domains. The biochemical, biophysical, and ultrastructural results that support this conclusion are discussed in this Review.
The platelet integrin αιιβ₃ is required for platelet aggregation. Like other integrins, αιιβ₃ resides on cell surfaces in an equilibrium between inactive and active conformations. Recent experiments suggest that the shift between these conformations involves a global reorganization of the αιιβ₃ molecule and disruption of constraints imposed by the heteromeric association of the αιι and β₃ transmembrane and cytoplasmic domains. The biochemical, biophysical, and ultrastructural results that support this conclusion are discussed in this Review.

Integrins are ubiquitous transmembrane α/β heterodimers that mediate diverse processes requiring cell-matrix and cell-cell interactions such as tissue migration during embryogenesis, cellular adhesion, cancer metastases, and lymphocyte helper and killer cell functions (1). Eighteen integrin α subunits and 8 integrin β subunits have been identified in mammals that combine to form 24 different heterodimers. The resulting heterodimers can then be grouped into subfamilies according to the identity of their β subunit (1). Platelets express 3 members of the β₃ subfamily (αιι, αι, and αιβ₃) that support platelet adhesion to the ECM proteins collagen, fibronectin, and laminin, respectively (2–5), and both members of the β₁ subfamily (αβ₁ and αβ₃β₅). Although αιβ₃ mediates platelet adhesion to osteopontin and vitronectin in vitro (6, 7), it is uncertain whether it plays a role in platelet function in vivo. By contrast, αιβ₃, a receptor for fibrinogen, vWF, fibronectin, and vitronectin, is absolutely required for platelet aggregation. Consequently, inherited abnormalities in αιβ₃ number or function preclude platelet aggregation.

A major advance in understanding the structure and function of αιιβ₃ resulted from the reports of crystal structures for the extracellular portions of αιιβ₃ (16) and the closely related integrin αιβ₃ (17). Xiong and coworkers prepared crystals of a presumably activated conformation of the αιβ₃ extracellular region grown in the presence of Ca²⁺ (17). Surprisingly, the crystals revealed that the head region was severely bent over 2 nearly parallel tails (Figure 1). When the structure was extended, its appearance and dimensions were consistent with rotary-shadowed EM images of αιιβ₃. The structure itself revealed that the amino terminus of αι was folded into a β-propeller configuration, followed by a “thigh” and 2 “calf” domains, constituting the extracellular portion of the αι stalk. The αι “knee” or “genu,” the site at which the head region bends, was located between the thigh and first calf domain. The β₃ head consists of a βA domain whose fold resembles that of integrin α subunit “I-domains” and contains a metal ion–dependent adhesion site (MIDAS) motif, as well as a hybrid domain whose fold is similar to that of f-set Ig domains. The interface between the αι β-propeller and the β₃ BA domain, the site at which the αι head interacts with the β₃ head, resembles the interface between the Gα and Gβ subunits of G proteins. The β₃ stalk consists of a PSI (plexin, semaphorin, integrin) domain, 4 tandem EGF repeats, and a unique carboxyterminal βTD domain. A cyclic Arg-Gly-Asp–containing (RGD-containing) pentapeptide, soaked into the crystal in the presence of Mn²⁺ (18), inserted into a crevice between the β-propeller and βA domains with the Arg side chain located in a groove on the upper surface of the propeller and the Asp carboxylate protruding into a cleft between loops on the βA surface, implying that the crevice constitutes at least a portion of the binding site for RGD-containing αιβ₁ ligands.

Nonstandard abbreviations used: GpA, glycoporphin A; MIDAS, metal ion–dependent adhesion site; PSI, plexin, semaphorin, integrin; RGD, Arg-Gly-Asp; TM, transmembrane.

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Ligand binding to αIIbβ3
Fibrinogen, the major αIIbβ3 ligand, is composed of pairs of α, β, and γ chains folded into 3 nodular domains. Although peptides corresponding to either the carboxyterminal 10–15 amino acids of the γ chain (20) or the 2 α chain RGD motifs inhibit fibrinogen binding to αIIIβ1 (21), only the γ chain sequence is required for fibrinogen binding to αIIbβ3 (22). Nonetheless, RGD-based peptides and peptidomimetics inhibit αIIbβ3 function in vitro and are clinically effective antagonists of αIIbβ3 function in vivo (23). The structural basis for these observations is not entirely clear, but competitive binding measurements indicate that γ chain and RGD peptides cannot bind to αIIbβ3 at the same time (24), implying that RGD peptides inhibit fibrinogen binding by preventing the interaction of the γ chain with αIIbβ3.

Ligand binding to αIIbβ3 involves specific regions of the amino-terminal portions of both αIIb and β3. In the crystal structure of the αIIbβ3 head domain, ligand binds to a “specificity-determining” loop in the β3 domain and to a “cap” composed of 4 loops on the upper surface of the αIIb-β-propeller domain (16). The αIIb-β-propeller results from the folding of 7 contiguous aminoterminal repeats (17, 25). Each blade of the propeller is formed from 4 antiparallel β strands located in each repeat; loops connecting the strands are located on either the upper or the lower surface of the propeller. A number of naturally occurring and laboratory-induced mutations distributed between αIIb residues 145 and 224 and located in loops on the upper surface of the propeller impair αIIbβ3 function, implying that these residues interact with ligand (26–28). Further, Kamata et al. replaced each of the 27 loops in the αIIb propeller with the corresponding loops from αv or α3 (29). They found that 8 replacements, all located on the upper surface of the second, third, and fourth repeats, abrogated fibrinogen binding to αIIbβ3, suggesting that fibrinogen binds to the upper surface of the propeller in a region centered around the third repeat. Previous chemical cross-linking experiments suggested that the fibrinogen γ chain binds to αIIb in the vicinity of its second calmodulin-like motif near amino acids 294–314 (30), but these residues are located on the lower surface of the propeller and are unlikely to interact with ligands such as fibrinogen (16). It is noteworthy that ligand binding itself induces conformational changes in αIIbβ3, most often detected by the appearance of neoepitopes for mAbs. In fact, such ligand-induced changes or LIBSs (ligand-induced binding sites) may be responsible for the immune-mediated thrombocytopenia associated with the clinical use of αIIbβ3 antagonists (31).

Ligand binding to αIIbβ3 requires divalent cations (32). Eight divalent cation-binding sites were identified in the αIIbβ3 crystal structure (17, 18). Four were located in the αv-β-propeller domain, 1 at the α, γ genu, and 3 in the β3, βA domain, but only those located in the βA domain appeared to participate in ligand binding. In the absence of ligand, only the βA MIDAS (adjacent to the metal ion–dependent adhesion site) motif was occupied, but when Mn2+ and a cyclic RGD ligand were present, each of the βA sites contained a cation. One site was the βA MIDAS, Mn6+ present at this site was in direct contact with ligand. A second Mn2+, located 6 Å from the MIDAS, was bound to a site designated ligand-induced metal-binding site (LIMBS), but the cation at this site did not interact with ligand. It had been postulated that Mn6+ induces integrin activation by antagonizing inhibitory effects of Ca2+ (33), but the αIIbβ3 crystal structure suggests that cations bound to the MIDAS and LIMBS motifs act by stabilizing the ligand-occupied conformation of the βA domain (18).

Figure 1
Ribbon diagram of the structure of the extracellular portion of αIIbβ3. (A) Bent conformation of αIIbβ3 as it was present in the crystal. (B) Extension of the structure to reveal its domains. Adapted with permission from Annual Review of Cell and Developmental Biology (97).

Regulation of αIIbβ3 ligand-binding activity
Integrins reside on cell surfaces in an equilibrium between inactive and active conformations (34). In experiments where the cytoplasmic domains of αIIbβ3 and αvβ3 were replaced by acidic and basic peptides (35, 36), purified integrins were inactive when their stalks were in proximity and active when the stalks were farther apart. This was corroborated by measurements of fluorescence resonance energy transfer (FRET) efficiency between cyan and yellow fluorescent proteins fused to the cytoplasmic domains of αv and β3 expressed in K562 cells (37). FRET efficiency decreased when αIIbβ3 interacted with immobilized or soluble ligand, implying that bidirectional signaling resulted from the coupling of conformational changes in the αIIbβ3 extracellular domain to the spatial separation of the αI and β3 cytoplasmic domains, a result consistent with EM images of αIIbβ3 in which scissor-like movements of the αIIb and β3 stalks differentiate active and inactive molecules (19).

Nonetheless, the relationship of these observations to the αIIbβ3 and αvβ3 crystal structures is controversial. Takagi et al., supported by negatively stained EM images of active and inactive integrins, suggested that the bent conformation of αIIbβ3 in crystals corresponds to low-affinity αIIbβ3 and the shift to a high-affinity conformation occurs when the integrin undergoes a global reorganization characterized by a “switchblade-like” opening to an extended structure and scissor-like separation of the α and β subunit stalks (34). Xiong et al., however, suggested that the bent conformation resulted from flexibility at the αv and β3 genua and from crystal contacts not likely to occur in nature (17). This possibility was supported by cryo-EM reconstructions of intact inactive αIIbβ3 molecules, which revealed a collapsed but unbound structure consisting of a large globular head and an L-shaped stalk whose axis was rotated approximately 60° with respect to the head and was
connected at an angle of approximately 90° to a rod containing the TM domains of the integrin (Figure 2A) (38). They also suggested that extension at the “knees” may be a post-ligand-binding “outside-in” signaling event and that the transition of αβ3 from its inactive to its active conformation results when the CD loop of the β3 βTD domain moves away from the βδ backbone, allowing the latter to assume its active conformation (39). How to reconcile each of these models with the rotary-shadowed EM images of demonstrably inactive and active αβinβ3 shown in Figure 2, B and C, is not obvious.

The αIIb and β3 cytoplasmic domains constrain αinβ3 function

Cytoplasmic domain sequences, most convincingly demonstrated for conserved membrane-proximal sequences, constrain integrins in their low-affinity (inactive) conformations. Thus, truncation of the αIIb cytoplasmic domain at Gly991 or the β3 cytoplasmic domain at Leu717 or deletion of the conserved membrane-proximal αIIb GFFKR or β3 LLITHD motifs (Table 1) shifts αinβ3 to its active state (40). Similarly, constitutive αinβ3 function can be induced by replacement of αIIb residue Arg992 or Arg995 or β3 residue D723 with alanine, whereas heterodimers containing simultaneous R995→D and D723→R substitutions are inactive (41). These observations led to the suggestion that the membrane-proximal sequences form an activation-constraining “clasp,” an essential feature of which is a salt bridge between αIIb and β3. Paradoxically, replacing the αIIb cytoplasmic domain with the cytoplasmic domain of αδ, αδκδ, or αδλδ, each of which contains a GFFKR motif, activates αinβ3 (40). This implies that additional cytoplasmic domain sequences modulate αinβ3 function, consistent with the inhibitory effects observed for the β3 mutation Ser752Pro (42), β3 truncation at Arg724 (43), and mutations involving the β3 sequences EFAKFEEE, NPLY, and NITY (44–46) and the αIIb sequence Pro998/Pro999 (47, 48).

Interaction between the αIIb and β3 cytoplasmic domains has been studied experimentally using peptides dissolved in aqueous buffer or anchored to phospholipid micelles via amineterminal myristoylation. Using terbium luminescence and electrospray ionization mass spectroscopy, Haas and Plow observed the formation of a cation-containing complex involving αIIb residues 999–1,008 and β3 residues 721–740 (49). Similarly, Vallar et al. used surface plasmon resonance to detect a weak (Kd ~50 μM) KGFFKR-dependent, calcium-stabilized complex between soluble αIIb cytoplasmic domain and immobilized β3 cytoplasmic domain peptides (50). Further, Weljie et al. determined an NMR structure for a heterodimer that formed at low ionic strength between an 11-residue GFFKR-containing αIIb peptide and a 25-residue LLITHD-containing β3 peptide (51). They identified 2 conformers differing in the conformation of the β3 backbone: one had an elongated β3 structure; the other was bent back at D723–A728, causing the peptide to adopt a closed L shape. Nonetheless, both conformers were predominantly helical with significant hydrophobic interactions between V990 and F993 of αIIb and L717–I721 of β3. Although there was no NMR evidence of an R995–D723 salt bridge, modeling suggested that a salt bridge was possible if the β3 backbone was elongated. Vinogradova et al. also used NMR to characterize complexes between full-length αIIbβ3 cytoplasmic domain peptides (48, 52, 53). Despite low affinity, they identified interfaces for the complexes that included hydrophobic and electrostatic interactions between membrane-proximal helices (Figure 3A) (52). When the experiments were repeated in the presence of diphasphocholine micelles, αIIb residues 989–993 and β3 residues 716–721 were embedded in lipid and there was interaction between β3 residues 741 and 747 and micelle lipid (53). Talin binding to β3 disrupted the complex of αIIb with β3 as well as β3 interaction with lipid (Figure 3B). On the other hand, Li et al. were unable to detect heteromeric interaction between proteins corresponding to the αIIb and β3 TM and cytoplasmic domains in diphasphocholine micelles at physiologic salt concentrations using a number of biophysical techniques, perhaps because heteromeric interaction is substantially weaker than homomeric interaction (54). Similarly, Ulmer et al. did not detect heteromeric

Table 1

<table>
<thead>
<tr>
<th>Amino acid sequences of the TM and cytoplasmic domains of αIIb and β3</th>
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<tbody>
<tr>
<td><strong>TM domains</strong></td>
<td></td>
</tr>
<tr>
<td>αIIb</td>
<td>W988LVGVLGGLLTLITLVMW998</td>
</tr>
<tr>
<td>β3</td>
<td>L988LVLVSVMGAILGLAILLW995</td>
</tr>
<tr>
<td><strong>Cytoplasmic domains</strong></td>
<td></td>
</tr>
<tr>
<td>αIIb</td>
<td>K898GFFKRNRPPLEDDEEGE</td>
</tr>
<tr>
<td>β3</td>
<td>K898LLITHDRLKEFKEFEEARAARAKWDHANPPLYKEATSTFTNTYRGT782</td>
</tr>
</tbody>
</table>

The amino acids in the αIIb and β3 cytoplasmic domains are designated in the single-letter code; the subscript numbers correspond to the position of the preceding amino acid in the sequence for mature αIIb and β3 (98, 99). The αIIb GxxxG motif, β3 G708, the membrane-proximal regions of both cytoplasmic domains, and both β3 NxxY motifs are underlined.
interactions of αIIb with β3 in an NMR analysis of a coiled-coil construct containing the αIIb and β3 cytoplasmic domains (55).

Proteins that interact with the αIIb and β3 cytoplasmic domains

Proteins have been identified, most often using yeast 2-hybrid screens, that bind to the cytoplasmic domains of integrin α and β subunits. These proteins include CIB (calcium- and integrin-binding protein) (56), Aup1 (ancient ubiquitous protein 1) (57), ICln (a chloride channel regulatory protein) (58), and PP1c (the catalytic subunit of protein phosphatase 1) (59), each of which binds to the membrane-proximal αIIb sequence KVGGFKR. However, because a substantial portion of this sequence is likely embedded in the plasma membrane (60, 61), the physiologic importance of these interactions is uncertain. Proteins that interact with the β3 cytoplasmic domain include the cytoskeletal proteins talin, α-actinin, filamin, myosin, and skelemin; various members of the Src family of kinases; the kinases integrin-linked kinase (ILK), Syk, and Shc; the adapter Grb2; the scaffold RACK1; CD98 (62); and those that bind to integrins with lesser affinity (69). The talin head contains an approximately 300-residue FERM (four-point-one, ezrin, radixin, moesin) domain that folds into F1, F2, and F3 subdomains (69). F2 and F3 bind to the β3 cytoplasmic domain, although the affinity of F3 binding is substantially greater (70). A crystal structure for a fusion protein composed of the F2 and F3 subdomains and a contiguous aminoterminal peptide corresponding to the midportion of the β3 cytoplasmic domain, including its NPLY motif, revealed that the interaction of the β3 peptide with F3 was mainly hydrophobic and that NPLY interacted with F3 in a manner that resembled that of canonical PTB domain ligands (71). However, studies using NMR also revealed that F3 and F2-F3 interact with the membrane-proximal region of the β3 cytoplasmic domain (71, 72), consistent with previous observations that talin binds to peptides corresponding to this portion of β3 (73).

Overexpressing the talin head domain in αIIbβ3-expressing CHO cells induces αIIbβ3 activation (74), either directly because talin disrupts the clasp between αIIb and β3 (Figure 3B) or indirectly via conformational changes induced by F3 binding to the β3 NPLY motif (70). Conversely, reducing talin expression using short hairpin RNAs decreases ligand binding to αIIbβ3 in CHO cells and in ES cell-derived agonist-stimulated megakaryocytes (75). Taken together, these results imply that talin binding to the β3 cytoplasmic domain may be a final step in αIIbβ3 activation. Nonetheless, how talin binding to the β3 cytoplasmic domain is regulated remains to be determined. The integrin-binding domain in intact talin appears to be masked (76). Although the enzyme calpain can cleave talin, releasing its head domain (77), calpain activation in platelets is a relatively late step after platelet stimulation (78) and would be unlikely to contribute to integrin-activating inside-out signaling. On the other hand, talin binds to membrane-associated phosphoinositol 4,5-bisphosphate, inducing a conformational change that enables it to bind to the β3 cytoplasmic domain (79). By analogy, talin binding to phosphoinositol 4,5-bisphosphate may enable it to bind to β3.

Regulation of αIIbβ3 function by TM domain interaction

TM domain–mediated protein oligomerization is a common mechanism for the assembly of membrane proteins and regula-
membranes (84). Subsequently, Li et al. reported that facilitation of homomeric and heteromeric association in bacterial cytoplasmic domains to search for interactions constraining activation and clustering remains controversial (89, 90), but there is compelling evidence that heterodimeric interactions constrain consistent with the presence of a unique responding to formation of disulfide bonds with a helical periodicity in a region corresponding to the αIIb cytoplasmic domain helices, respectively.

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Figure 5

Diagram illustrating the “push-pull” hypothesis for regulation of the αIIbβ3 activation state. The white and blue cylinders represent the αIIb TM and membrane-proximal cytoplasmic domain helices, respectively. The red and green cylinders represent the β3 TM and membrane-proximal cytoplasmic domain helices, respectively.

The ability of homomeric TM helix interactions to induce αIIbβ3 activation and clustering remains controversial (89, 90), but there is compelling evidence that heterodimeric interactions constrain αIIbβ3 in a low-affinity state. By simultaneously scanning the αIIb and β3 TM helices with cysteine residues, Luo et al. detected the formation of disulfide bonds with a helical periodicity in a region corresponding to αIIb residues 966–974 and β3 residues 693–702, consistent with the presence of a unique αIIbβ3 TM heterodimer (91).

They also scanned the αIIb and β3 helices with leucines, confirming that mutation of the αIIb GxxxG motif induces αIIbβ3 activation (90). Partridge et al. used random mutagenesis of the β3 TM and cytoplasmic domains to search for interactions constraining the αIIbβ3 activation (92). They detected 12 activating mutations in the membrane-proximal cytoplasmic domain and 13 activating mutations in the β3 TM helix. Nine of the latter were predicted to shorten the helix, perhaps activating αIIbβ3 by altering the tilt of the helix in the membrane (Figure 4). The remaining mutations were located in the carboxyterminal half of the helix and were postulated to activate αIIbβ3 by disrupting the packing of an αIIbβ3 TM heterodimer.

Despite the biochemical evidence supporting the presence of αIIbβ3 and β3 TM domain oligomers, their existence has not been confirmed by NMR spectroscopy or x-ray crystallography because of difficulty in obtaining high-resolution structures for TM proteins using these techniques. However, computational methods have been used to construct TM domain models incorporating the constraints imposed by mutational data. Based on cryo-EM images (Figure 2A), Adair and Yeager proposed that the TM domains of inactive αIIbβ3 associate in a parallel α-helical coiled coil (38). Using the R995-D723 salt bridge as the primary constraint, they found that a right-handed coiled coil based on the GpA TM dimer (93) placed more conserved residues in the helix-helix interface than a coiled coil based on the canonical left-handed leucine zipper. Gottschalk and coworkers proposed that the αIIb and β3 TM helices remain in close contact in the activated state and that the helix-helix interface is a GpA-like structure containing the αIIb, S699xxxA703 motifs (94). Moreover, simulated annealing and molecular dynamics supported a model in which the αIIb and β3 TM domains interact weakly in a right-handed coiled coil when the integrin is in its low-affinity conformation (95). Subsequently, in order to account for both aminoterminal and carboxyterminal restraints, Gottschalk proposed that the αIIbβ3 TM and membrane-proximal cytoplasmic domains form a right-handed coiled coil in which the helices interact over their entire length, placing the αIIb GxxxG motif, but not β3, S699xxxA703, in the helix-helix interface (96). By contrast, Luo et al. used their disulfide cross-linking data to construct a model based on the GpA TM dimer; however, in this model, the αIIb GxxxG-like motif corresponded to residues 968–972, rather than 972–975 (91). DeGrado and coworkers used a Monte Carlo–simulated annealing algorithm to obtain atomic models for an αIIb TM homodimer (86) and an αIIbβ3 heterodimer (87). In each case, a family of structures was found that satisfied mutational constraints. For the αIIb homodimer, all structures had right-handed crossing angles ranging from 40° to 60°, but with an interface rotated by 50° relative to the GpA homodimer. In the case of the αIIbβ3 heterodimer, initial docking identified local minima with both right- and left-handed crossing angles. However, the right-handed structures had lower energies and more extensive interactions, and the αIIb GxxxG motif was in intimate contact with the β3 TM domain. Lastly, Partridge et al., using a Monte Carlo simulation, obtained 2 structures for an αIIbβ3 TM heterodimer with helix packing near either the amino or the carboxyl terminus of the helices, respectively; of the 2 models, carboxyterminal helix packing was more consistent with their mutational data (92). It is obvious that there is wide disparity among these models, making it clear that obtaining actual structures for αIIb and β3 TM domain hetero- and homo-oligomers will be the next major advance in our understanding of the structural basis for the regulation of platelet integrin function.
platelet activation. J. Biol. Chem. 279:27286–27293.