MINIREVIEW

Structures of Integrin Domains and Concerted Conformational Changes in the Bidirectional Signaling Mechanism of $\alpha_{IIb}\beta_3$

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Integrins are heterodimeric type I transmembrane cell-adhesive receptors whose affinity for ligands is regulated by tertiary and quaternary conformational changes that are transmitted from the cytoplasmic tails to the extracellular ectodomains during the transition from the inactive to the active state. Receptor occupancy initiates further structural alterations that transduce signals across the plasma membrane and result in receptor clustering and recruitment of signaling molecules and cytoskeletal rearrangements at the integrin's cytoplasmic domains. The large distance between the intracellular cytoplasmic domains and the ligand-binding site, which in an extended conformation spans more that 200 Å, imposes a complex mechanism of interdomain communication for the bidirectional information flow across the plasma membrane. Significant progress has recently been made in elucidating the crystal and electron microscopy structures of integrin ectodomains in its unliganded and liganded states, and the nuclear magnetic resonance solution structures of stalk domains and the cytoplasmic tails. These structures revealed the location of sites that are functionally important and provided the basis for defining new models of integrin activation and signaling through bidirectional conformational changes, and for understanding the structural basis of the cation-dependent ligand-binding specificity of integrins. Platelet integrin $\alpha_{IIb}\beta_3$ has served as a paradigm for many aspects of the structure and function of integrins The aim of this minireview is to combine recent structural and biochemical studies on integrin receptors that converge into a model of the tertiary and quaternary conformational changes in $\alpha_{IIb}\beta_3$ and other homologous integrins that propagate inside-out and outside-in signals. Exp Biol Med 229:732–744, 2004.

Key words: integrins; conformational changes; bidirectional signaling; $\alpha_{IIb}\beta_3$

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Human $\alpha_{IIb}\beta_3$, also termed glycoprotein (GP) IIb/IIIa, is a major and inducible platelet type I membrane-spanning receptor for adhesive proteins, and one of the best characterized members of the large family of eukaryotic cell-surface, cation-dependent heterodimeric adhesion receptors called the integrins (1). On the surface of circulating, resting platelets, integrin $\alpha_{IIb}\beta_3$ exists in a nonadhesive state for ligands such as fibrinogen, von Willebrand factor (vWf), fibronectin, vitronectin, and thrombospondin. As a consequence of platelet activation triggered by platelet agonists such as thrombin, produced from the activated coagulation cascade upon vascular injury, integrin $\alpha_{IIb}\beta_3$ rapidly (<1 sec) switches from a low-affinity to a high-affinity ligand-binding conformation of its ectodomains through conformational changes initiated by intracellular events referred to as affinity modulation, priming, or "inside-out" signaling (2–4), which converge on the C-terminal cytoplasmic tails of the integrin subunits. Activated platelets arrest by interacting with exposed subendothelial extracellular matrix proteins through various surface receptors (GPIb/IX/V complex–vWf, integrin $\alpha_2\beta_1$, and GPVI-collagen), express binding surfaces for activated coagulation factors, and form aggregates through the simultaneous binding of dimeric fibrinogen molecules to activated integrin $\alpha_{IIb}\beta_3$ receptors on adjacent platelets. Following receptor occupancy, the binding information is transduced across the plasma membrane in a process coined "outside-in" signaling (3, 4), which triggers entropy-driven $\alpha_{IIb}\beta_3$ clustering (5). Transmembrane domain–mediated oligomerization of the integrin $\alpha_{IIb}\beta_3$ (6) promotes and stabilizes the integrin-mediated association between extracellular matrix proteins and the tightly structured intracellular membrane skeleton ("avidity regulation," Ref. 7) and provides the physical link for the retraction of the fibrin clot and the arrest of bleeding.

The crucial importance of $\alpha_{IIb}\beta_3$ in primary hemostasis is underscored by the occurrence of a genetic bleeding

disorder, Glanzmann thrombastenia (8), caused by missense, nonsense, and frameshift point mutations and exon skipping in the α_{IIb} and β_3 genes that result in functional abnormalities, or prevention of surface expression of integrin $\alpha_{IIb}\beta_3$, or both. On the other hand, nonphysiological platelet activation often leads to thrombosis, and to date, the only approved anti-integrin therapy targets the platelet-specific $\alpha_{IIb}\beta_3$ integrin (9, 10).

Platelet integrin $\alpha_{IIb}\beta_3$ has served as a paradigm for many aspects of the structure and function of integrins without an I-domain. Although the molecular basis for the bidirectional conformational changes associated with integrin receptor function modulation are still elusive, the recent determination of the x-ray crystal structure of the extracellular domains of integrin $\alpha_v\beta_3$, unligated, and in complex with a cyclic RGD peptide (11, 12); electron microscopy and image analysis of integrins $\alpha_{IIb}\beta_3$ (13) and $\alpha_5\beta_1$ (unligated and in complex with a fibronectin fragment; Ref. 14); x-ray scattering of the head region of $\alpha_5\beta_1$ in an inactive state and complexed with a fragment of fibronectin (15); and the nuclear magnetic resonance (NMR) solution structures of the Cys-rich module 3 of the integrin β2 subunit (16), and of the cytoplasmic tails of integrin $\alpha_{IIb}\beta_3$ subunits (17–20) have provided "snapshots" of integrin domains in defined conformations, and these have enabled several decades of biochemical work to be put into context and are giving valuable insights into the tertiary and quaternary conformational changes that occur during the transition from the inactive to the active state. Several excellent recent reviews have been published on the structure and the conformational regulation of integrin function (3, 4, 7, 20–28). The unexpected bent conformation found in the crystal lattices of the first x-ray structures of the unliganded and RGD-bound extracellular fragment of integrin $\alpha_v\beta_3$ (11, 12) corroborated structure predictions that were made for functional regions of the α and β subunits; namely, the β-propeller and the A(I)-like domain, respectively (reviewed in Ref. 29), provided a first glimpse into the structural ground of cation-dependent integrin-ligand interaction, but also raised more questions than they have answered. In particular, the crystal structures suggested that novel models of integrin activation need to be invoked. The impact of the structural studies has stimulated a number of elegant biochemical and protein engineering investigations to address the dynamic regulatory mechanisms of integrin function in the framework of the three-dimensional structure. The aim of this minireview is to correlate the available structural information with current biochemical, mutagenesis, and modeling data. The author apologizes to colleagues whose work may have been omitted; however, this manuscript is an update of previously published reviews (1, 30), and thus primary literature referred to in these papers or that has been compiled in recent reviews will not be cited here.

The Three-Dimensional Structure of $\alpha_v\beta_3$ as a Model for $\alpha_{IIb}\beta_3$

Molecular models of the human platelet integrin $\alpha_{IIb}\beta_3$ have been developed utilizing the crystal structure of the ectodomain of the homologous integrin $\alpha_v\beta_3$ (31), and by docking this structure into the 20-Å resolution map of detergent-solubilized molecules visualized by electron cryomicroscopy (Fig.1, left; Ref. 13). As expected from the high homology between integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, which share the β subunit and exhibit ~40% amino acid sequence identity (50% homology) between their α subunits (Swiss-Prot accession codes: α_{IIb}, P08514; α_v, P06756) (http://expasy.org), the crystal structure of the integrin $\alpha_v\beta_3$ could be easily accommodated into the electron cryomicroscopy map of full-length $\alpha_{IIb}\beta_3$ with only small hinge movements at linker regions between domains. Hence, it seems plausible to assume that structure-function correlations of integrin $\alpha_v\beta_3$ are mirrored in $\alpha_{IIb}\beta_3$.

The crystal structure of unliganded integrin $\alpha_v\beta_3$ at 3.1 Å resolution (PDB code 1JV2, accessible at http://www.rcsb.org; Ref. 11) and that of $\alpha_v\beta_3$ with bound cyclic RGD pentapeptide (obtained by soaking the ligand in the presence of Mn^{2+} into preexisting crystals) at 3.2 Å resolution (PDB code 1L5G; Ref. 12) both contain all the extracellular residues, with the exception of α_v 839–867 and 957, and β_3 1–54 (the plexin-semaphorin-integrin [PSI] domain), 435–531, and 691–692, and revealed 12 domains, 4 in the α subunit and 8 in the β subunit (Fig. 1). The two subunits assemble into a globular (90 × 60 × 45 $Å^3$) "head" built by the NH_2-terminal seven-bladed β-propeller domain of α_v (residues 1–438) and the β_3 A domain (residues 109–352), a six-stranded β sheet surrounded by eight helices Rossmann-fold looping out from an Ig-like "hybrid" domain (β_3 residues 55–108 and 353–432). As predicted, a metal ion-dependent adhesion site (MIDAS) motif, formed by the side chains of Asp119, Ser121, Ser123, Glu220, and Asp251, occupies a crevice at the top of the central β strand. The geometry of the metal coordination site is similar to that of the α1- and α2-subunit A-domains. However, a metal ion was not clearly visible in β3 MIDAS of unliganded $\alpha_v\beta_3$, but one was present in the $\alpha_v\beta_3$–RGD complex structure. The MIDAS site is flanked by two other cation-binding motifs, ADMIDAS (i.e., adjacent to MIDAS) formed by the carbonyl oxygens of Ser123 and Met335, and by the side chains of Asp126 and Asp127, which are occupied in $\alpha_v\beta_3$ with and without bound ligand; and LIMBS (i.e., ligand-induced metal-binding site), which is formed by the carboxylate oxygen of Glu220, the side chains of Asp158, Asn215, and Asp217, and the carbonyl oxygens of Asp217 and Pro219), which was occupied only when the peptide ligand was present. Metal ions regulate integrin adhesiveness, acting as effectors, promoting ligand binding; as an antagonist, inhibiting ligand binding; and as selectors, by changing the ligand binding specificity (reviewed in Ref.

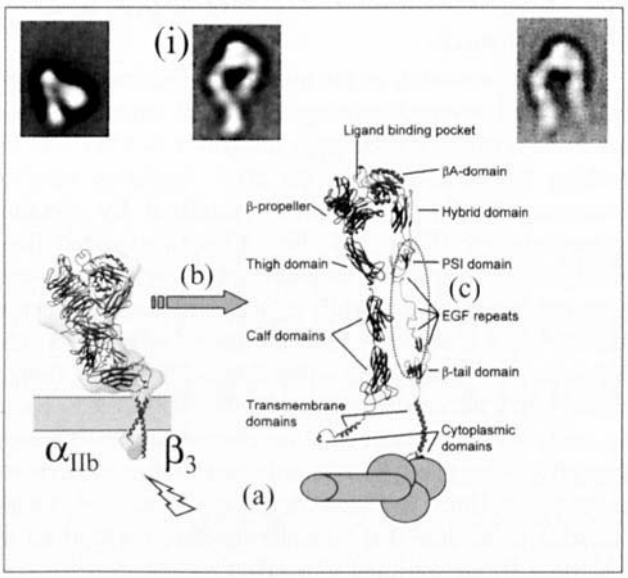

Figure 1. Schematic representation of the conformational transition from the bent to the extended structure associated with affinity regulation of integrin $\alpha_{IIb}\beta_3$. The panels at the upper corners show electron micrographs of the bent (left panel) and the extended (right panel) conformations of integrin $\alpha_v\beta_3$ (25). The ribbon diagrams portray a docking model of the x-ray structure of the $\alpha_v\beta_3$ integrin ectodomain into the map of $\alpha_{IIb}\beta_3$ defined by electron microscopy (left; Ref. 13) and an artificially straightened model of the integrin (right; Ref. 11) to which the transmembrane and cytoplasmic domains (20) have been pasted. During "inside-out" signaling (a), cytosolic proteins (talin, or β3-endonexin, or both) bind to the membrane-distal β_3 tail of the bent (inactive) integrin structure, disrupting thereby the membrane-proximal inhibitory α-β tail interaction, and triggering the spatial separation of the α_{IIb} and β_3 cytoplasmic domains followed by transmission of structural changes across the plasma membrane, which lead to the extended, high-affinity ligand-binding conformation of the integrin (b). The shift from the low-affinity to the high-affinity structure may involve a number of intermediate conformational states (25) such as the extended conformation with crossed headpiece (i), none of which are depicted here for clarity. (c) The β_3 stalk region, where the overwhelming majority of activation epitopes and naturally occurring mutations that cause activation of integrin $\alpha_{IIb}\beta_3$ have been mapped, is encircled in the extended integrin conformation.

32). Using surface plasmon resonance, the β_3 integrins were shown to contain two classes of ion-binding sites. One class, LC (for the ligand-competent site), must be occupied for the ligand to bind, while the second class, I, (for the inhibitory site) displays specificity for Ca^{2+}, is allosterically linked to the ligand-binding pocket, and exerts the inhibitory effect of high Ca^{2+} concentration on ligand binding by increasing the rate of ligand dissociation. The metal ion of the MIDAS motif directly coordinates the side chain of an acidic residue that is characteristic of all integrin ligands, and may correspond to the LC site. ADMIDAS and LIMBS appear to act as negative (I site) and positive regulatory sites, respectively (33). In the $\alpha_4\beta_1$ integrin, the MIDAS metal ion alternates between two coordination geometries, one stabilized by ADMIDAS occupation by Ca^{2+} that mediates low-affinity interactions during rolling adhesion, and another stabilized by LIMBS occupation that mediates high-affinity interaction and which is necessary for firm adhesion. In line with this evidence, the ADMIDAS plays a role in stabilizing the active conformation of integrin $\alpha_5\beta_1$, and mutations of ADMIDAS residues also perturb the transduction of outside-in signaling (34). Thus, as discussed below, the coordination between the LC, MIDAS, and I cation binding sites appears to represent a key regulator of the ligand-binding event.

In agreement with previous biochemical data, a divalent cation (Ca^{2+} or Mn^{2+}, depending on the crystallization buffer) was found coordinated at each of the four predicted Ca^{2+}-binding repeats located in hairpin loops in blades 4–7 at the base of the β-propeller. Each of the four Ca^{2+}-binding loops span a nine-residue segment with the consensus sequence DhD/NxD/NGhxD (where h = hydrophobic residue and x = any amino acid), with the Ca^{2+} being usually coordinated by oxygen atoms from the side chains of residues 1, 3, 5, and 9 and the carbonyl oxygen of residue 7. The four metal-coordinating loops are linked by a network of contacts and may help to make rigid the interface between the β-propeller and the thigh domain. These metal binding sites lay opposite to the αβ interface, which harbors the ligand binding surface (see below). This topology is in agreement with domain mapping by limited proteolysis and does not support reports that have suggested that the α subunit Ca^{2+}-binding sites may interact directly with ligands (1).

The "head" sits on two "legs," one from each subunit, which correspond to the rod-like tails (160 Å × 20 Å) observed in electron micrographies (Fig.1). The α_v tail is composed of a C2-set Ig-like "thigh" domain (residues 439–592) and a "calf" module formed of two similar β-sandwich domains named calf-1 (residues 602–738) and calf-2 (residues 739–956). A metal ion was found at the thigh–calf-1 interface. The calf-2 domain includes a disordered loop (Gln839–Gly867), at which several α subunits (including α_v and α_{IIb}) are proteolytically processed (after α_v Arg860/α_{IIb} Arg856) to generate disulfide-bonded N-terminal (extracellular) heavy and C-terminal (type I membrane-spanning) light chains. The β_3 tail is formed by four consecutive EGF-like domains, the first two of which (encompassing residues 453–529) are poorly visible in the electron density map, whereas EGF-3 (residues 532–562) and EGF-4 (residues 563–605) form an extended structure, followed by a novel β tail domain (βTD, residues 606–690). The structure of the EGF-3 module of the integrin β_2 has been solved by NMR (16) (PDB code 1L3Y). The disulfide bond connectivity of this structure (1–5, 2–4, 3–6, and 7–8) is identical to the pattern of $\alpha_v\beta_3$ EGF-4, but it differs strikingly from the assignment reported in the $\alpha_v\beta_3$ EGF-3 module (3–5, 4–6, and 7–8). However, it must be noticed that the first 10 residues of $\alpha_v\beta_3$ EGF-3, including the first and the second cysteine residues, were not well resolved in the 3.1-Å resolution $\alpha_v\beta_3$ crystal structure, suggesting that the discrepancy could be due to incorrect backbone tracing.

The Subunit Interface and the Ligand-Binding Surface

The major continuous intersubunit contact is found in the head with two smaller and discontinuous interfaces between the legs (for details about intrasubunit and intersubunit contacts in the crystal structure of unliganded integrin $\alpha_v\beta_3$, see Table 1 in Ref. 28). The presence of 7–8 cations in the crystal structure of unliganded/RGD-bound $\alpha_v\beta_3$ is in agreement with previous biochemical results (35). However, the location of these Ca^{2+}/Mn^{2+} ions in the integrin structure contradicts the longstanding hypothesis of the existence of a high-affinity Ca^{2+}-coordination site bridging the two subunits into a Ca^{2+}-dependent heterodimer (36). Instead, residue Arg261 from the βA domain protrudes into the center of the β-propeller channel, and is caged into place by two rings of predominantly aromatic amino acids contributed by the seven repeats of the propeller. The side chain of residues forming the lower ring, Phe21, Phe159, Tyr224, Phe278, and Tyr406 contact Arg261 directly, whereas residues Tyr18, Trp93, Tyr221, Tyr275, and Ser403 of the upper ring interact with side chains in the lower ring to provide a hydrophobic surface for residues of the 3_{10}-helix flanking Arg261. The βA domain-propeller interface has a number of additional contacts and buries a surface area of about 1620 $Å^2$.

The structure of the $\alpha_v\beta_3$–RGD complex (12) revealed limited quaternary rearrangements in the integrin head region. The interface between βA and the α_v β-propeller undergoes a small change, with the two domains moving closer together at the peptide-binding site. The cyclic pentapeptide inserts into a crevice between the propeller and the βA domains. The RGD sequence makes the main contacts with the integrin, and each residue participates extensively in the interaction. The Arg and Asp side chains point in opposite directions, exclusively contacting the propeller and the βA domains, respectively. The Arg side chain inserts into a narrow groove at the top of the propeller formed by loops D3A3 and D4A4, and its guanidinium group is held in place by a bidentate salt bridge to Asp218 and by an additional salt bridge to Asp150. The hydrophobic portion of the Arg side chain is sandwiched between the side chains of a_v residues Tyr178 and Ala215. On the other hand, one of the Asp carboxylate oxygens contacts a Mn^{2+} ion at MIDAS (in a similar way as a collagen glutamate forms a direct bond to the Mn^{2+} ion of the $\alpha_2\beta_1$ I-domain; PDB code 1DZI; Ref. 37), and the second carboxylate oxygen forms hydrogen bonds with the backbone amides of Tyr122 and Asn215. The middle glycine residue of the RGD motif lies in the interface between the α and the β subunits, and makes several hydrophobic interactions in α_v (i.e., with the carbonyl oxygen of Arg216).

The mechanism of RGD binding confirms many biochemical studies, indicating that the ligand-binding pocket of RGD-dependent, non-αI–domain-containing integrins (including $\alpha_{IIb}\beta_3$) consists of portions of both the α and the β subunits (29, 30). In addition, the present $\alpha_v\beta_3$-RGD structure does not corroborate a plethora of studies that located ligand-binding sites at the β-propeller metal-binding loops (1, 29, 30). The structural data also provide an explanation for the conservation of an acidic residue in integrin ligands, and the absolute dependence of ligand binding on divalent cations. In addition, that integrin receptors that share a β subunit bind ligands with differing specificities suggests a role for residues in the α subunit in determining ligand specificity. In line with this hypothesis, the specificity of ligand recognition by integrin $\alpha_5\beta_1$ has been mapped to amino acid sequences in loops within the second and third blades of the α subunit β-propeller (38, 39), and residues within a prominent loop in blade 3, corresponding to Tyr204–Gln214, participate in binding to the synergy sequence (15). The RGD and synergy sites lie on the same face of the fibronectin molecule separated by ~35 Å. The existence of an extended integrin binding surface across the top of the head region is supported from the structure of an integrin $\alpha_5\beta_1$–Fn6-10 complex deduced from solution x-ray scattering and site-directed mutagenesis (15).

Platelet $\alpha_{IIb}\beta_3$ is a promiscuous receptor for adhesive ligands; namely, fibrinogen, vWf, fibronectin, and vitronectin; all three of which may employ their RGD sites for integrin binding in a way that has been seen in the crystal structure of the RGD-$\alpha_v\beta_3$ complex (12). There is also evidence for an interaction of $\alpha_{IIb}\beta_3$ with the synergy region of fibronectin (40). On the other hand, the KGD-containing disintegrin barbourin (isolated from the venom of the southern pigmy rattlesnake, *Sistrurus miliarius barbouri*) inhibits the $\alpha_{IIb}\beta_3$ integrin's high degree of selectivity (41). Inspection of the $\alpha_v\beta_3$-RGD structure suggests that formation of the salt bridge to Asp218 would not be possible if the ligand Arg is substituted by the shorter Lys side chain. However, the lack of conservation of the α_v propeller's RGD-binding residues, which in α_{IIb} are Phe218, W150, Tyr178, and Pro215, and the existence of an eight-residue insertion in the α_{IIb} D3A3 loop, may allow the formation of an alternative recognition site for the KGD sequence.

The most important physiological ligand of $\alpha_{IIb}\beta_3$ is fibrinogen, a dimeric molecule (42) whose major $\alpha_{IIb}\beta_3$-recognition site resides in the C-terminal region of its γ-chain (^{400}HHLGGAKQAGDV^{411}). Cross-linking and surface plasmon resonance studies have suggested the presence of two distinct, allosterically linked ligand-binding pockets in $\alpha_{IIb}\beta_3$ for RGD and the fibrinogen γ-chain peptide (29, 30). Loop swapping and Ala-scanning mutagenesis have identified 30 amino acid residues that are critical for fibrinogen binding to integrin $\alpha_{IIb}\beta_3$, which are clustered in eight loops in repeats 2–4, and at the boundary between repeats 4 and 5 of the α_{IIb} β-propeller (43). These regions of the propeller are involved in αβ heterodimer association (see Fig. 5 in Ref. 11) and include the three nonconserved loop

regions between α_v and α_{IIb} (which in human α_{IIb} are residues 71–85, 114–125, and 148–164). However, many of the identified residues are cryptic in the $\alpha_{IIb}\beta_3$ modeled structure (31), indicating that changes in these positions may impair function by affecting the tertiary structure of the integrin domain. On the other hand, replacement by alanine of Gln111, His112, or Asn114 in a surface-exposed loop of repeat W2 of the α_{IIb}-propeller abolished binding of fibrinogen induced by different activation modes, and CHO cells stably expressing recombinant (Q111A, H112A, or N114A), mutated integrin did not exhibit $\alpha_{IIb}\beta_3$-mediated adhesion to fibrinogen (44). Residues Asp158 and Asn215, located in the spatially contiguous loops βB-βC and βC′-α3, respectively, of the β_3 A-domain at the heterodimer interface, also appear to be critical for fibrinogen binding to activated $\alpha_{IIb}\beta_3$ (45). These amino acids flank the specificity loop 159–188 located between β-strands B and C of the β_3 A-domain and defined by the disulfide bond Cys177–Cys184. These regions of β_3, along with the α_{IIb} nonconserved loops, form a continuous surface at the top of the integrin headpiece located in a strategic position for ligand binding (Fig. 2).

Electron micrographies of $\alpha_{IIb}\beta_3$-fibrinogen complexes (46) can now be interpreted by docking the model of $\alpha_{IIb}\beta_3$ based on the crystal structure of the straightened $\alpha_v\beta_3$ integrin and the crystal structure of fibrinogen (42) into the molecular envelope determined by electron microscopy. In accordance with biochemical data discussed above, the model of the complex indicates that the fibrinogen molecule binds through its distal (D)-domain (which harbors the γ-chain recognition site) on top of the integrin head region at the interface between the α subunit β-propeller and the β subunit A-domain (Fig. 2). However, for an atomic definition of the integrin-ligand interface and of the molecular basis of ligand-recognition specificity, key targets for future high-resolution crystallographic studies may include $\alpha_{IIb}\beta_3$-fibrinogen (i.e., fragments) complexes.

Integrin Conformational States

The crystal structure of the unliganded integrin, as well as that of the $\alpha_v\beta_3$-RGD complex, exhibit a pronounced bend of 135° at the thigh–calf-1 interface, termed the genu (Fig. 1). Although it was initially suggested that the V-shaped genuflexed arrangement could be the combined result of intrinsic integrin molecular flexibility and crystal packing forces favoring the bent structure and unlikely to be present on cell surfaces (11), recent electron microscopic images in conjunction with physicochemical measurements, mutational introduction of disulfides, and ligand binding to $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins showed that the highly bent integrin conformation may be physiological and represents the low-affinity ligand binding state (25, 47). It has also been suggested that the peptide–$\alpha_v\beta_3$ crystal complex may represent the active integrin conformation (12). However, peptide antagonists, including small molecules such as the disintegrin eristostatin from the venom of *Eristocophis macmahoni,* bind with the same affinity to resting and activated platelets (48). These ligands exhibit diffusion-limited kinetics and may bind to the nonactivated (low-affinity) integrin conformation. On the other hand, in the bent (inactive) conformation, the ligand-binding headpiece is folded back over the largely parallel legs of the integrin subunits, facing toward the membrane, a topology unfavorable for binding to large adhesive ligands. However, high-affinity binding of integrins to physiological ligands requires a conformational change in the receptors, and the switch from the low-affinity to the high-affinity state. Activation-dependent unmasking of the integrin's ligand-binding site appears to be linked to tertiary and switchblade-like quaternary structure rearrangements from the bent to the straightened morphology (Fig. 1) as observed in negative-staining studies of $\alpha_{IIb}\beta_3$ reconstituted into lipid vesicles (49) in which the integrin receptor is positioned >200 Å above the membrane surface (25, 26).

Inside-Out Signaling

Cells regulate integrin functions through spatial and temporal control of the receptor affinity for extracellular ligands. The switch from the low-affinity to the high-affinity state is tightly controlled and triggered naturally by inside-out signals that release intracellular constraints on the cytoplasmic tails of the integrin subunits (Fig. 1). As a result, conformational changes are transmitted to the extracellular domains of the receptor and enhance the propensity to bind ligand efficiency. The current view is that the β subunit tail is the principal site for binding of cytoskeletal proteins and signaling molecules, whereas the α subunit tail has a regulatory role.

Although the precise intracellular events underlying the activation process are still incompletely characterized, there is convincing evidence that the membrane-proximal sequences of both subunits of $\alpha_{IIb}\beta_3$ interact with each other through a salt bridge between α_{IIb} Arg995 and β_3 Asp723, and lock the integrin receptor in a default, low-affinity state (Fig. 1; Ref. 1). The more distant regions of the β subunit tail regulate activation through interactions with signaling proteins that might disrupt the membrane-proximal interaction, whereas the membrane-distal α subunit region regulates in a cell-type-specific manner the conformation of the β tail and its association with activator proteins (Ref. 50 and references cited therein).

Recent NMR studies of the structure and dynamics of synthetic C-terminal peptides of integrin $\alpha_{IIb}\beta_3$ provide structural details of how the cytoplasmic tails mediate inside-out signaling. Thus, the solution structure of the cytoplasmic tail complex from α_{IIb} (Lys989–Glu1008) and β_3 (Lys716–Thr762) has been reported (PDB code 1M8O; Ref. 20). The structure of the bound α_{IIb} tail, which is very similar to that of the free α_{IIb} tail (17), exhibits a helical conformation that terminates at Pro998. A turn following

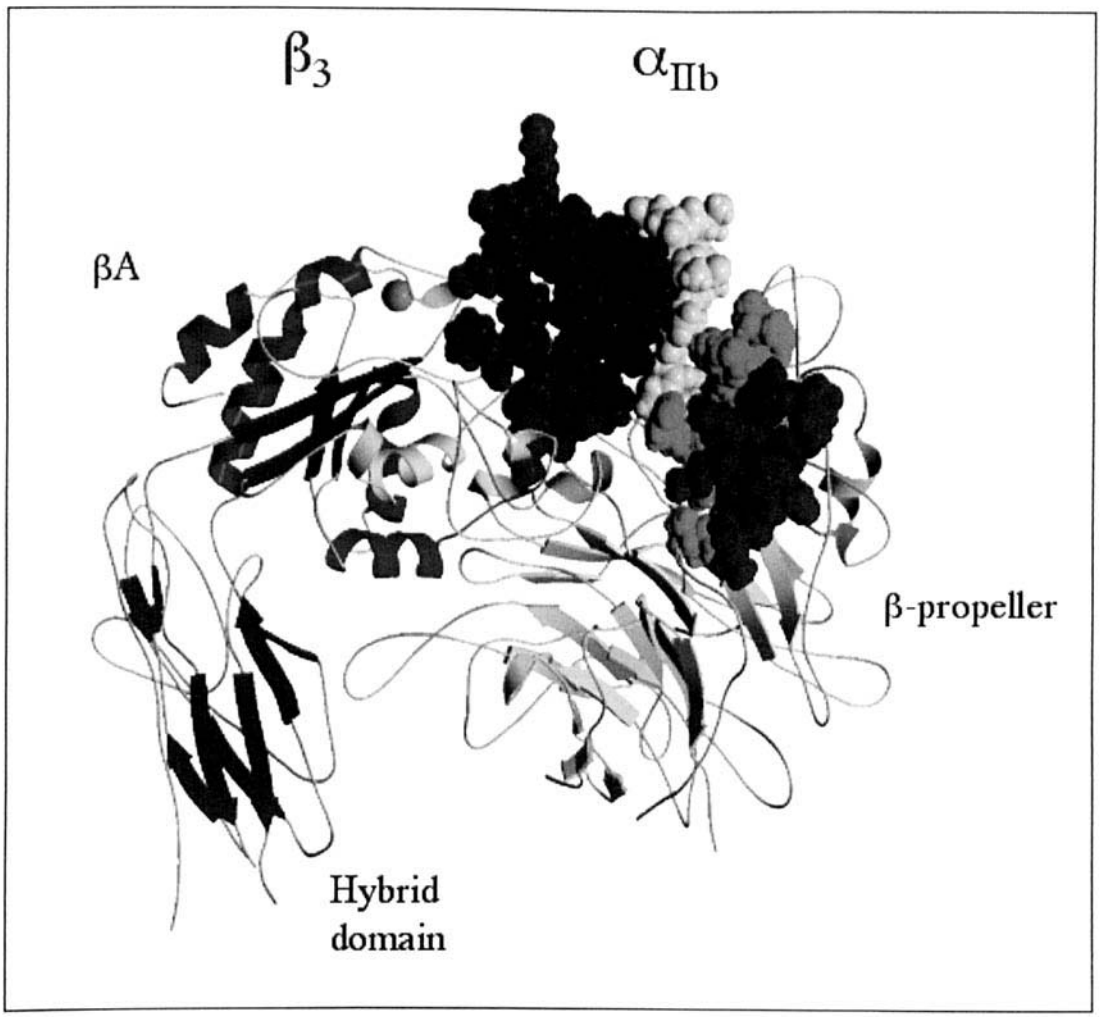

Figure 2. The $\alpha_{IIb}\beta_3$ ligand binding region. A ribbon model of the $\alpha_{IIb}\beta_3$ integrin head domains was built using the coordinates provided by Dr. Bradley P. Feuston (31). A similar three-dimensional model has been developed by Filizola *et al.* (86). The nonconserved loops between α_{IIb} and α_v, where critical residues for fibrinogen binding to activated $\alpha_{IIb}\beta_3$ have been mapped, and the β_3 specificity-determining loop are highlighted in the space-filling model, and colored magenta (β_3 residues 159–188)), red (α_{IIb} 71–85), green (α_{IIb} 114–125), and yellow (α_{IIb} 148–164). The divalent ion at the βA MIDAS site is shown as a brown sphere.

the helix allows the acidic C-terminal loop to fold back and interact with the positively charged N-terminal region. The N-terminal stretch Lys716–Lys738 of the bound β3 peptide also folds into a helical structure, whereas the C-terminal part is disordered, and the 744–747 sequence (NPLY) has a propensity to form a turn. Both structures dimerize through adjacent helices, and this interaction is stabilized by hydrophobic contacts between residues α_{IIb} Val990-β_3 L718, α_{IIb} Val990-β_3 I719, α_{IIb} Phe922-β_3 I721, and α_{IIb} Phe922-β_3 H722, and electrostatic interactions involving the side chains of the following pairs: α_{IIb} Arg995-β_3 H722, α_{IIb} Arg995-β_3 D723, and α_{IIb} Arg995-β_3 Glu726.

RNA interference (RNAi) knockdown of talin expression has revealed that talin binding to integrin β tails plays a crucial role in a final, common step of integrin activation (Fig. 1; Ref. 51). Talin, a major cytoskeletal actin-binding protein that colocalizes with activated integrins, is an antiparallel homodimer of 270 kDa subunits built by an N-terminal 50-kDa globular head, which contains the major integrin-binding site, and a 220-kDa C-terminal rod. Talin head subdomains F2 and F3 bind specifically to the integrin β_3 tail, and expression of the talin 96-residue F3 phosphotyrosine binding (PTB)-like module, but not F2, activates $\alpha_{IIb}\beta_3$ integrin (52). The x-ray crystal structure of talin F3-engaging residues 739–750 of the β_3 tail (PDB accession code 1MK7; Ref. 53) revealed that W739 interacts with an F3 pocket, whereas residues ^{740}DTA^{742} form a β strand that augments the β sheet of F3, and residues ^{744}NPLY^{747} form a reverse turn with Y747 pointing into an acidic and hydrophobic pocket. The talin head domain prevented detection of the membrane-proximal interaction between α_{IIb} and β_3 tail peptides by NMR (20) and reduced the fluorescence resonance energy transfer (FRET) between fluorophore-tagged α and β integrin subunits in living cells (54), providing further support that binding of the talin PTB F3 domain to the membrane-distal β tail might mediate integrin activation through disruption of the membrane-proximal inhibitory α-β tail interaction.

The structural data also explain previous reports showing that point mutations within the β_3 tail talin-binding residues suppressed integrin activation (1).

β_3-endonexin and AUP-1 (i.e., ancient ubiquitous protein 1) represent other regulators of integrin $\alpha_{IIb}\beta_3$ activation (Ref. 55 and references cited therein). β_3-endonexin binds specifically to β_3 ^{756}NITY^{759} and may cooperate with talin during $\alpha_{IIb}\beta_3$ integrin activation in platelets. The mutation Ser752Pro in human β_3 caused Glanzmann thrombasthenia and also impaired the ability of the cytoplasmic domain to bind to β3-endonexin (1). Potential mechanisms regulating talin-mediated integrin activation have been put forward (see Fig. 3 in Ref. 50), and may involve its cleavage by calpain. On the other hand, AUP-1 binds to the conserved membrane proximal sequence ^{989}KVGFFKR^{995} of α_{IIb} and has been reported to play a crucial role in the inside-out signaling of $\alpha_{IIb}\beta_3$. However, how cellular signaling pathways control integrin activation by intracellular proteins must remain conjectural.

Propagation of the inside-out activation signal involves the spatial separation (up to 14 nm) of the α and β cytoplasmic domains (54, 56) followed by transmission of this conformational change across the plasma membrane (Fig. 1). Modeling of the transmembrane domains in the electron cryomicroscopy map of the integrin $\alpha_{IIb}\beta_3$ revealed that they are most likely packed in a crossed helix structure in the inactive state (13). A previously proposed "hinge" or "scissor" movement of the subunits for integrin conformational regulation has been ruled out by analysis of negatively stained electron microscopy with image averaging, coupled to hydrodynamic and surface plasmon resonance of integrin $\alpha_v\beta_3$ .in different activation states (25), and FRET-based measurements (54). These studies showed, in different intermediate and extended integrin conformers (see Fig. 6 in Ref. 25), activation-dependent, large-scale cytoplasmic domain rearrangement consistent with separation of the transmembrane domains. The emerging hypothesis is that unclasping of the cytoplasmic tail regions may destabilize the interdomain interactions in the bent (inactive) integrin structure leading to a shift to the extended, high-affinity conformation (Fig. 1).

A Switchblade-like Model for Integrin Activation and Signal Transmission Through the Stalk Region

The stalk region provides the crucial link for coupling activation-dependent structural alterations from the cytoplasmic and transmembrane domains with the structural transitions within the integrin's ligand-binding pocket that are brought about by inside-out signaling. Hence, many monoclonal antibodies recognizing epitopes that become exposed upon integrin activation, or that activate integrins upon binding, map to stalk regions of the β_3 subunit, particularly to the PSI domain and to the EGF-like repeats 2–4 (1, 3, 29). The β_3 stalk region contributes about 70% of the solvent-accessible surface area that is buried in the headpiece-stalk interface in the integrin's bent structure. Superposition of the NMR structure of β2 EGF-like modules 2 and 3 on the crystal structure of $\alpha_v\beta_3$ (where the PSI domain and the EGF-like modules 1 and 2 and 27% of EGF-3 are not visible) allowed the orientation of functionally important residues of EGF-like module 3 (PDB code 1L3Y) to be visualized. Residues participating in activation epitopes (KIM127 in β_2 EGF-2, and CBR LFA-1/2 and MEM48 in EGF-3) are buried in the bent conformation but become unmasked in the extended integrin conformation (Fig. 1c; Ref. 16). Therefore, it has been proposed that activation triggers a switchblade-like opening motion that extends the ligand-binding headpiece of the integrin heterodimer more than 100 Å away from the plasma membrane (16, 29).

Mutation T562N in β_3, which results in a constitutively active $\alpha_{IIb}\beta_3$ receptor (57), creates an N-glycosylation site at the center of the interaction between the EGF-like modules 3 and 4 and the hybrid domain, which may break the headpiece-tailpiece interface that stabilizes the high-affinity extended conformation. In addition, a number of naturally occurring cysteine mutations in the cysteine-rich region of β_3 subunit have been identified in patients with Glanzmann thrombasthenia. In the PSI domain, natural or artificially introduced Cys5Ala mutations that disrupt the long-range disulfide bond linking the PSI domain to the top of the EGF-1 module in the β_3 subunit and result in a constitutively active integrin (58). Also disrupting the Cys663-Cys687 disulfide bond within the βTD domain constitutively activated $\alpha_{IIb}\beta_3$ (59). Other naturally occurring cysteine mutations that cause a Glanzmann thrombasthenia phenotype include Cys457Tyr, Cys506Tyr, Cys508Tyr, Cys542-Tyr, Cys560Arg, Cys560Phe, and Cys598Tyr (Ref. 60 and references cited therein). All these mutations, except Cys508Tyr, caused reduced β_3 surface expression in platelets. In addition, the mutations C560F and C560R (in EGF-3; Refs. 61 and 62) and C598Y (in EGF-4; Ref. 60) induced activation of integrin $\alpha_{IIb}\beta_3$. Cys560 participates in an interdomain disulfide bond linking EGF-3 and EGF-4, and Cys598 forms an intra-EGF-4 domain disulfide bridge. Disruption of these linkages may disturb the interdomain interfaces EGF-3/EGF-4 and EGF-4/β tail domain, loosening the headpiece-tailpiece interface. Because the C560R and C598Y mutations have been identified in patients with defective platelet aggregation, the thrombasthenic phenotype can only be explained by the low expression level of the mutant integrin.

The activation-sensitive epitope recognized by the anti-β_3 antibody D3GP3 maps to the region the EGF-1/hybrid domain interface (63), and the EGF-4/β tail domain interface is recognized by the activating anti-β_2 antibody KIM185 (64). The fact that activating antibodies bind to epitopes that are hidden in the headpiece-tailpiece interface of the integrin's resting conformation indicates the occurrence of breathing movements at this interface. Polypeptide flexibility is supported by the structural disorder of this

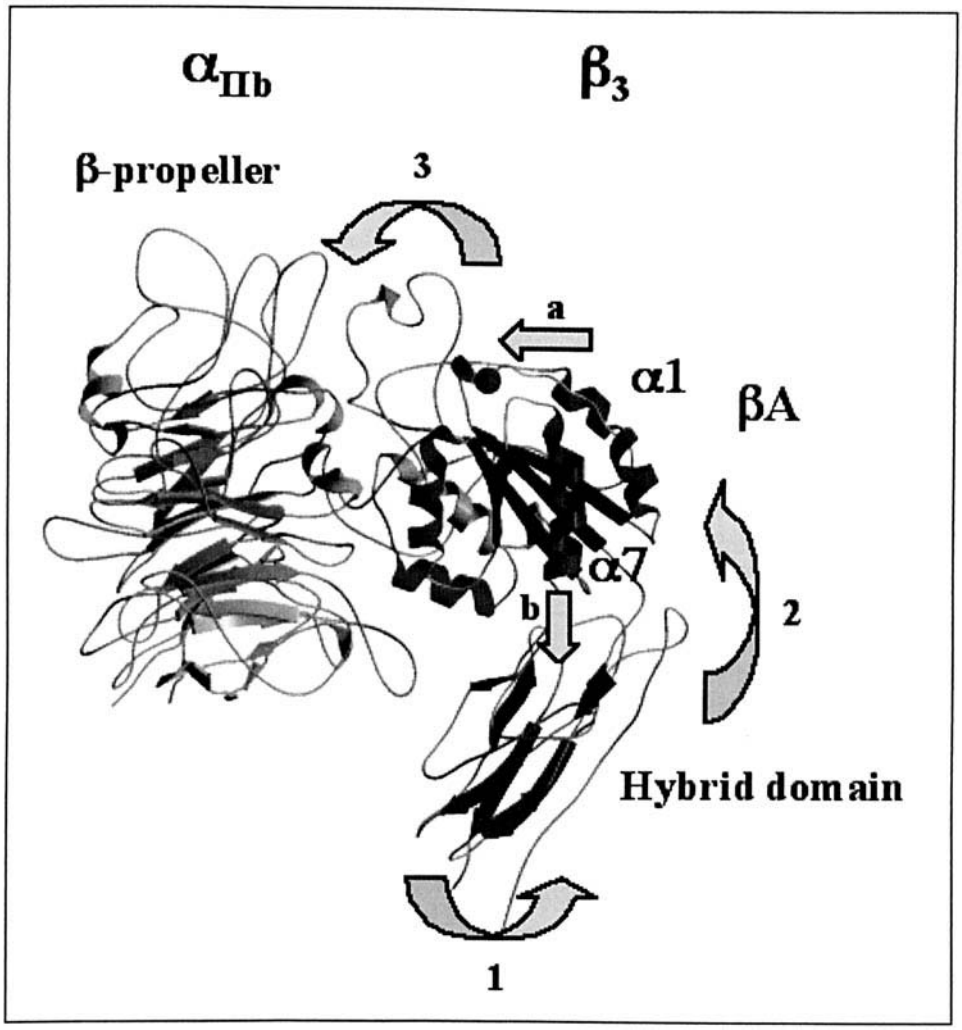

Figure 3. Conformational changes in the βA domain associated with signal transduction. A ribbon model of the $\alpha_{IIb}\beta_3$ integrin head domains was built using the coordinates provided by Dr. Bradley P. Feuston (31). The model is displayed in the same orientation as in Figure 1 and rotated 180° relative to the view in Figure 2. Disruption of the tailpiece-headpiece interface (arrow 1) enables the hybrid domain to swing out with respect to the βA domain (arrow 2), facilitating the downward movement of the α7 helix (b), which is coupled to a movement of the MIDAS loops (arrow 3) and helix α1 (b), β6-α7 loop reshaping, and activation of the βA domain. The secondary structural element that undergo topological rearrangement associated with receptor activation are colored blue (β strands) and red (α helices). The divalent ion at the βA MIDAS site is shown as a magenta sphere. The swing-out motion of the hybrid domain also appears to represent a crucial event of the outside-in signaling mechanism, and thus quaternary changes of this domain may underlay the transmission of signals to and from the ligand-binding site.

region in the crystal structure of integrin $\alpha_v\beta_3$ (11, 12). Thus, upon temporary exposure, binding of an antibody would prevent rebending of the receptor and shift the equilibrium toward the extended conformer.

Thiol-modifying reagents such as dithiothreitol can induce high-affinity ligand binding to integrin $\alpha_{IIb}\beta_3$, and recent evidence suggests that this integrin might be a target for redox modulation by a Mn^{2+}-induced allosteric endogenous thiol isomerase activity (65). The putative redox site has been tentatively assigned to the cysteine-rich core comprising the EGF-like modules, and the mechanism of integrin activation would involve the reduction of one or two disulfide bonds within this β_3 region (66). Kamata *et al.* (67) have reported that mutating the cysteines at positions 435, 460, 462, 473, 495, 508, 521, 523, 536, 542, 549, 560, 575, 581, 588, 601, and 687 clustered within the EGF and βTD domains of β_3 induced the binding fibrinogen to $\alpha_{IIb}\beta_3$, and that disrupting a single disulfide bond is enough to fully activate $\alpha_{IIb}\beta_3$. In the same line of evidence, an altered glutathione redox environment potentiated platelet aggregation and increased sulfhydryl labeling in the β subunit of $\alpha_{IIb}\beta_3$ (68). The concept of a redox mechanism of integrin activation seems to be in apparent contradiction with the current models of integrin structure (in which all cysteine residues are engaged in disulfide bond formation) and activation. Hence, the precise location of the putative redox site, the hypothesized functional role for $\alpha_{IIb}\beta_3$-endogenous thiol isomerase activity, and the definition of a structural model for the redox modulation by disulfide bond reduction of the ligand-competent conformation of integrin $\alpha_{IIb}\beta_3$ require further work.

Structural Rearrangements in the βA-Domain

The βA domain is inserted into the hybrid domain, to which it is attached by both its amino terminus and its carboxy-terminal α7 helix (11). The orientation between the βA and the hybrid domains appears to be critical for converting the switchblade global conformational transition into local βA intradomain structural alterations that regulate receptor affinity. The switchblade-like opening motion appears to trigger conformational changes in the integrin ligand-binding pocket that correlate with conversion of the receptor from an inactive to an active state. Initially, comparison of the tertiary structures of the α_2 I-domain–collagen hexapeptide Gly-Phe-hydroxyproline(Hyp)-Gly-Glu-Arg (GFOGER) complex with that of the unoccupied α_2 I-domain revealed changes that provided an insight into the process of receptor activation (69, 70). The principal coformational changes occur in three regions and are initiated by a movement of the MIDAS loops and helix α1, which approaches MIDAS, thereby permitting contacts with both MIDAS cation and ligand through Ser121, Tyr122, and Ser123. MIDAS loops 1 and 3 undergo a radical rearrangement that triggers a reorganization of the C-helix and the α6 helix, and a large shift of the C-terminal helix α7, which moves axially by 10 Å, pulling the β6-α7 loop away from the ADMIDAS. The ADMIDAS moves in concert with helix α1 because it is primarily coordinated by α1 residues. This changes its coordination sphere, with the consequent loss of the coordination to Met335 in the β6-α7 loop with the 2.6-Å displacement of the ADMIDAS metal ion toward the LIMBS, resulting in the formation of a direct bond with Thr221 (which was coordinated to a water molecule in the unoccupied structure), a loss of coordination to Asp254, and a Gly255 peptide bond flip of 180° so that its Cα moves about 4 Å from the metal ion, allowing a new water-mediated coordination to Glu256. This structural reorganization within the I-domain reoriented the side chains of Tyr157 and His258 in such a way that they create a complementary surface that was able to fit into grooves in the collagen helix. The crystal structure of a mutationally stabilized active conformation of the αL I-domain in the absence of ligand (71), along with the ability to detect activation-induced structural alterations near the MIDAS (72) and the α7 helix (73) using conformation-sensitive antibodies in intact cells in the absence of ligand, demonstrated that the observed conformational changes within the I-domain crystal structures that are associated with the transition to the high-affinity receptor conformation can form independent of ligand or a ligand mimetic lattice contact.

In support of the notion that subtle changes in metal coordination in the βA-domain of non-αI–domain-containing integrins are also directly linked to extensive secondary and tertiary changes that create a complementary surface for ligand binding, structural changes have been observed upon the binding of a cyclic RGD peptide to the preformed integrin $\alpha_v\beta_3$ crystal that are in the direction of those observed in the αI-domains, although they appear to be limited, probably because of restraints imposed by lattice contacts (12). The movement of the α1 helix of βA appears to be the central event, and most of the remaining structural changes within the βA domain can be viewed as indirectly caused by the shift of α1 (12, 28). βA loop 1 shifts by 2.6 Å toward loop 2, and this movement is accompanied by a change in the coordination of the ADMIDAS metal ion, such that it no longer links the α1 and α7 helices (Fig. 3). In addition, the invariant Glu220 side chain from loop 2, which prevents occupation of MIDAS in unliganded $\alpha_v\beta_3$, changes its position to coordinate a metal at the novel LIMBS, allowing the occupation of MIDAS with a metal ion coordinated by residues from loops 1 (Asp119-X-Ser121-X-Ser123) and 2. Likewise, cation-binding sites regulate ligand binding in $\alpha_5\beta_1$, mainly through effects on conformation of the head region, rather than on straightening (34), and in this integrin the ADMIDAS is involved in transduction of a conformational change from the MIDAS through the C-terminal helix region of the βA domain to the underlying hybrid domain (34).

The inward movement of the α1 helix of βA appears to be necessary for integrin activation (74). Hence, the mutationally engineered disulfide bonds within the βA domain of $\alpha_{IIb}\beta_3$ V332C/M335C (designed to displace the β6-α7 loop downward) and T329C/A347C (designed to hold the β6 strand and α7 helix together near the end of the α7 helix) locked the receptor, respectively, into a high-affinity and a low-affinity conformation (75). Moreover, four-residue deletions that shorten by about one turn the βA α7 helix produced constitutively high-affinity mutant integrins (76), strongly supporting the view that C-terminal α7 helix displacement *per se*, rather than specific interactions of α7 helix residues with other βA-domain residues, regulates activation.

Outside-In Signaling

Ligand binding by integrins transduces signals into cells. The binding event exhibits multistep kinetics, with conversion to a higher-affinity receptor within about 10 sec after ligand binding (7). Ligand-dependent, postadhesion events result in enhanced adhesive strength (avidity augmentation) either by accumulation of receptors in the substrate contact region or changes in integrin conformation, or both. The binding of fibrinogen and other ligands to activated integrin $\alpha_{IIb}\beta_3$ causes conformational changes linked to the expression of neoepitopes called ligand-induced binding sites (LIBS). Strikingly, integrin activation can likewise be triggered from either its tails or its head, and thus the tertiary changes seen in the $\alpha_v\beta_3$-RGD structure may mirror those induced by outside-in or inside-out signals. It is conceivable that ligand binding triggers an alteration in the positioning of the βA α1 helix, and that this change is linked to a movement in the α7 helix region and a

swing of the hybrid domain away from the αβ interface, allowing the propagation of the ligand-binding–induced conformational change downstream to the integrin legs. Hence, the hybrid domain motion would provide the conduit for the transduction of signals to and from the head region (77). Ligand binding might thus reinforce and stabilize the conformational changes associated with activation (Fig. 3). In support of this notion, the binding of ligand to integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$ in solution resulted in a dramatic structural alteration to an extended conformation in which the hybrid domain swings outward by about 80° (14, 25). Opening of the hybrid-βA domain interface of $\alpha_5\beta_1$ and $\alpha_{IIb}\beta_3$ by mutationally introducing an N-glycosylation site into it, also leads to constitutive high-affinity ligand-binding affinity by both integrins (78). In addition, the anti-LIBS monoclonal antibodies 15/7 and HUTS-4 map to a region of the β_1 hybrid domain that faces the α_5 subunit β-propeller that becomes exposed upon movement of the hybrid domain away from the α subunit interface caused by a shift of the βA α7 helix (77).

Regions spread along the C-terminal halves of α_{IIb} and β_3 have been shown to exhibit LIBS expression, implying the occurrence of a long-range conformational propagation mechanism of outside-in signaling. The activation-sensitive anti-β_3 monoclonal antibody D3GP3 maps to the region spanning EGF-like 1 and the N- and C-terminal junctions with the hybrid and EGF-2 domains, respectively, and the EGF-4/βTD interface is recognized by the anti-β_3 LIBS2 antibody (22). Likewise, there are sites within the Calf-2 and the βTD domains of the $\alpha_5\beta_1$ integrin that bind activating monoclonal antibodies (79). Ligand binding to $\alpha_{IIb}\beta_3$ on platelets also exposes the $LIBS_{cyt}1$ epitope localized to the ^{994}KRNRPPLEED^{1003} within the intracellular tail of α_{IIb} (80), and the calcium and integrin-binding protein CIB, an α_{IIb}-specific ligand (81) that binds to the activated conformation of $\alpha_{IIb}\beta_3$ through the membrane-proximal cytoplasmic region Leu983-Arg997 (82), appears to be a player of the postreceptor occupancy event.

Emerging evidence has implicated outside-in signaling in stabilization of the integrin activated, open conformation, allowing the separated legs to promote integrin clustering and the cytoplasmic domains to interact with cytoskeletal and signaling proteins. During platelet aggregation, the β_3 subunit becomes phosphorylated on Thr753, causing Shc and myosin to interact with the β_3 cytoplasmic tail (83). Platelets from mice lacking the β_3 tyrosine phosphorylation motif formed defective aggregates and poorly retracted clots. The mutation Ser752Pro in human β_3, in addition to causing Glanzmann thrombasthenia and impairing the binding to β3-endonexin, inhibited the ability of the cytoplasmic domain of β_3 to signal phosphorylation of FAK, and showed reduced recruitment of Shc (1). Thus, the single Ser752Pro mutation can block the mechanisms of both inside-out and outside-in signaling by integrin $\alpha_{IIb}\beta_3$, suggesting that phosphorylation of β_3 may be a mechanism by which integrin function is regulated (83), and that this region of β_3 located between the talin-binding sequence ^{744}NPLY^{747} and the β3–endonexin-recognition motif ^{756}NITY^{759} may play, like the hybrid domain, a crucial role in the bidirectional signal transduction of the integrin.

Specific nonreceptor tyrosine kinases have been implicated in $\alpha_{IIb}\beta_3$ outside-in signaling (84). Platelet adhesion to fibrinogen stimulated both the activation of Syk and the direct association of Syk with the cytoplasmic tail of β_3 (85). Recent studies (84) have established that Src and its regulatory kinase, Csk, are constitutively associated with $\alpha_{IIb}\beta_3$ in resting platelets, and that upon fibrinogen binding and $\alpha_{IIb}\beta_3$ clustering, Csk dissociates from $\alpha_{IIb}\beta_3$, and Src becomes activated. The activity of Src is required for $\alpha_{IIb}\beta_3$-dependent tyrosine phosphorylation of Syk. Syk activation precedes actin polymerization, and the Syk substrates, the Rac exchange factor Vav-1 and the molecular adaptor SLP-76, have been implicated in cytoskeletal regulation downstream of integrins (Ref. 84 and references cited therein). Thus, the outside-in signaling in platelets is initiated by the sequential activation of Src and Syk, providing a molecular basis for signal generation from $\alpha_{IIb}\beta_3$ to the actin cytoskeleton (84). A more complete understanding of the outside-in signaling mechanism will require further biochemical and structural characterization of integrin–signaling protein complexes.

Naturally Occurring Mutations

The genes coding for human α_{IIb} and β_3 (both in chromosome 17q21.32) are highly polymorphic. Molecular genetic analyses carried out worldwide have identified many single nucleotide mutations within the genes of both subunits of the human integrin $\alpha_{IIb}\beta_3$ that lead to amino acid polymorphisms. These polymorphisms are associated with either Glanzmann thrombasthenia or with the expression of alloantigens (HPA). Glanzmann thrombasthenia is an inherited disorder in which quantitative or occasionally qualitative defects of $\alpha_{IIb}\beta_3$ results in platelets being unable to aggregate, causing a moderate (type II, 5%–15% of functional $\alpha_{IIb}\beta_3$) to severe (type I, <5% functional $\alpha_{IIb}\beta_3$) bleeding syndrome. Platelet alloantigen systems are involved in neonatal alloimmune thrombocytopenia, post-transfusion purpura, and refractoriness to platelet transfusions. A list of the human platelet alloantigens is available at http://www.uniklinikum-giessen.de/immunologie/hkroll/HPAs.htm and a database of mutations within the α and the β subunits of integrin $\alpha_{IIb}\beta_3$ causing Glanzmann thrombasthenia can be consulted at http://sinaicentral.mssm.edu/intranet/research/glanzmann/menu. The crystal structure of the extracellular domains of $\alpha_v\beta_3$ provides the basis for understanding these naturally occurring polymorphism-associated platelet phenotypes in the context of current mechanistic models of $\alpha_{IIb}\beta_3$.

Most of the human platelet alloantigens reside on β_3, and only two alloantigens have been reported on α_{IIb}.

Residues associated with the expression of HPAs are surface-exposed and distributed throughout the entire structure of β_3 (PLA, PSI domain; Mo and La, hybrid domain; Yuk [= Pen] and Duv, βA domain; Tu, EGF1/2 loop; Sr, Gro, and Oe, βTD) and on the C-terminal Calf-2 module of α_{IIb} (Bak and Max). On the other hand, gene defects leading to Glanzmann thrombasthenia phenotypes include nonsense mutations and stop codons; gene deletions, insertions, or inversions; alternative RNA splicing sites; and missense mutations. Among the latter, most of the single amino acid substitutions within α_{IIb} are located in the β-propeller domain (including the Ca^{2+}-binding regions) and produce destabilizing effects on the structure of the transcript, preventing heterodimer maturation, or the processing of the integrin necessary for surface expression, or both. On the other hand, missense mutations in β_3 map mainly to the βA domain, and produce both markedly decreased expression of $\alpha_{IIb}\beta_3$ and abrogation of $\alpha_{IIb}\beta_3$ function without affecting its surface expression (1, 8). Other Glanzmann thrombasthenia mutations within β_3 that are linked to reduced platelet-surface levels of $\alpha_{IIb}\beta_3$ affect the correct disulfide bond pattern of the hybrid and EGF-like domains. Furthermore, Glanzmann thrombasthenia phenotypes associated with mutations in the cytoplasmic tails of α_{IIb} (Arg995Gln within the β_3-interacting GFFKR sequence) and β_3 (Ser752Pro in the β3-endonexin-binding region) impair, respectively, the surface expression and the bidirectional signaling of $\alpha_{IIb}\beta_3$.

Concluding Remarks

Significant progress has recently been made to unravel the complex, bidirectional signaling mechanism underlying integrin $\alpha_{IIb}\beta_3$ function into the context of a structural model. The emerging picture points to the α subunit as a regulator of the integrin's affinity state and ligand-binding specificity, whereas the β subunit appears to function as a bidirectional molecular device.

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