MINIREVIEW

Platelet Integrin GPIIb/IIIa: Structure-Function Correlations. An Update and Lessons from Other Integrins[2] (44426)

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Abstract. **Glycoprotein (GP) IIb/IIIa complex (integrin $\alpha_{IIb}\beta_3$) is the most abundant platelet receptor. It serves as an inducible receptor for adhesive proteins and is the best-studied member of the integrin family. Its major global structural features have been elucidated mainly during the last decade. Since 1995, there has been a substantial increase in structural information on adhesion molecule domains. The crystal structures of isolated integrin I domains have been solved. Although a high resolution picture of a whole integrin molecule is not yet available, the crystal structures together with biochemical, mutagenesis and modeling data provide a useful framework for interpreting current experimental evidence on structure-function correlations of integrin molecules and for guiding further experiment. The aim of this minireview is to update a previous one summarizing recent (1995–98) functional and structural data of GPIIb/IIIa and other integrins in the perspective of an emerging model of the structure, and bidirectional signaling mechanism through, integrin $\alpha_{IIb}\beta_3$.** [P.S.E.B.M. 1999, Vol 222]

The integrins represent a large family of receptors constitutively expressed on the surface of all nucleated cells in the body. Integrin-mediated signals regulate cell-cell and cell-extracellular matrix interactions, which are important events in a wide variety of biological processes, such as embryonic development, morphogenesis, hemostasis and thrombosis, wound healing, immune system function, and metastasis (1). Integrin receptors are heterodimers consisting of a 120–180-kDa α-subunit noncovalently associated with a 90–110-kDa β-subunit. Both subunits are type-I membrane glycoproteins with a single membrane-spanning domain. At present, 8 β and 17 α subunits have been characterized (2), and these subunits combine in a restricted manner to form more than 22 different dimers, each of which exhibits a distinct ligand-binding profile.

The bulk of each integrin subunit is extracellular and contains an activation-dependent ligand-binding domain for extracellular matrix proteins, soluble macromolecules, or counter-receptors on the surface of apposed cells (3, 4). Integrins mediate both adhesion and bidirectional transmembrane signaling. Integrin function appears to be regulated by conformational changes and receptor clustering. Thus, the information flow that effects changes in the adhesive state of integrin receptors switching from a resting integrin to a ligand-binding competent receptor is regulated by intracellular events and is referred to as affinity modulation or inside-out signaling. Following receptor occupancy, the binding information is transduced across the

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plasma membrane in a process termed outside-in signaling that triggers integrin clustering (avidity modulation) and attachment to the membrane skeleton (5–8). The relative contribution of affinity and avidity modulation to ligand binding varies with the integrin type. Both processes play complementary roles in regulating $\alpha_{IIb}\beta_3$ activation (7).

Glycoprotein (GP) IIb/IIIa complex (integrin $\alpha_{IIb}\beta_3$) is the most abundant platelet adhesion receptor. Integrin $\alpha_{IIb}\beta_3$, a calcium-dependent heterodimer, is a receptor for fibrinogen, fibronectin, vitronectin, von Willebrand factor, and thrombospondin, and mediates platelet aggregation, firm adhesion, and spreading. Integrin $\alpha_{IIb}\beta_3$ is probably the most thoroughly studied integrin receptor and a paradigm molecule of the integrin protein family. Structure-function correlations of integrin $\alpha_{IIb}\beta_3$ have been the subject of recent reviews (3, 9). In addition, clues provided by studies from other integrin receptor systems have contributed to our understanding of the molecular basis of integrin-mediated bidirectional information transfer. These findings support the concept that integrins share common mechanisms for affinity modulation and regulated adhesion (1, 5–8, 10–15). Furthermore, integrin signaling pathways synergize with other receptor pathways to enhance or dampen signals elicited by each receptor (16). The aim of this review is to summarize structural data gathered from recent studies on GPIIb/IIIa and other integrins that may contribute to a general model of integrin function.

Inside-Out Signaling

Inside-out signaling appears to involve the propagation of conformational changes from the cytoplasmic domains of integrins to the extracellular ligand binding site in response to intracellular signaling events. Many studies have documented that the cytoplasmic tails of both integrin subunits are involved in modulation of the receptor activation state. Integrin cytoplasmic domains are plausible substrates for cellular kinases. Activation of platelet $\alpha_{IIb}\beta_3$ in response to thrombin is mediated by heterotrimeric G-proteins that appear to act through protein kinase C (PKC). Experimental evidence suggests that one of the mechanisms that regulates exposure/closure of ligand-binding sites on $\alpha_{IIb}\beta_3$ in response to activators of PKC is phosphorylation/dephosphorylation of a Ser/Thr residue in the β_3 subunit (17). On the other hand, the involvement of tyrosine phosphorylation in the enhancement of the binding affinity of integrin $\alpha_{IIb}\beta_3$ remains controversial. Hence, although tyrosine phosphorylation of the β_3 cytoplasmic domain (740 –762) has been shown to be required for integrin–cytoskeletal interactions (18), mutational studies strongly argue against a role of tyrosine phosphorylation in GPIIb/IIIa activation (19). Hers *et al.* (20) have reported that the relative contributions of Tyr and Ser/Thr kinases in $\alpha_{IIb}\beta_3$ exposure differ among different platelet aggregation agonists.

Apart from kinases and phosphatases, R-*ras*, a member of the small GTP-binding protein family, has been shown to activate integrins (21); however, the molecular basis of this regulation is not known.

An emerging concept is that the highly conserved membrane-proximal sequences of both integrin $\alpha(^{991}\text{GFFKR}^{995})$ and $\beta(^{717}\text{LLitiHDR}^{724})$ subunits lock the integrin receptor in a default low-affinity state (11, 15, 22, 23) (Fig. 1). The ability of the membrane-proximal portion of the integrin cytoplasmic domains to regulate integrin affinity may depend on an interaction between those favoring close association of the integrin subunits. Thus, it has been proposed that activation of GPIIb/IIIa by alanine substitutions of α R^{995} and βD^{723} may be ascribed to disruption of a salt bridge and the consequent release of a constraint that maintains the receptor in an off state (22). Other association mechanisms have been put forward, however. Haas and Plow (24) have provided evidence for the ability of the distal GPIIb cytoplasmic portion (^{999}PLEEDDEEGE^{1008}) and GPIIIa ^{721}IHDRKEFAKFEEERARAKWD^{740} to form a binary complex that stabilizes a complex-specific conformation, and a ternary complex with Tb^{3+}. Complex formation between the α_{IIb} and β_3 cytoplasmic tails involves their carboxyl- and amino-terminal aspects, respectively, and a structural model has been proposed (25). On the other hand, Melker *et al.* (26) have found evidence suggesting that the role of the GFFKR motif varies among different α subunits. These authors reported that the two Phe in the GFFKR motif of the integrin α6 A subunit are essential for heterodimerization.

The β_3 cytoplasmic domain ^{744}NPLY^{747} and Ser^{752} also appeared to be important in the regulation of the affinity state of $\alpha_{IIb}\beta_3$ since point mutations in these regions inhibit activation of the receptor (18) and generate a Glanzmann's thrombasthenia phenotype (3).

Interactions with intracellular partners may also contribute to the affinity modulation of integrins. Using the yeast two-hybrid system, several intracellular proteins have been identified that interact with the cytoplasmic domains of integrins (12). The calcium-binding protein calreticulin binds to synthetic peptides displaying the GFFKR motif (27). Its physiological relevance has been questioned, however, because this protein is generally localized in the endoplasmic reticulum, and a homologous protein, calnexin, serves as a chaperone during integrin subunit biosynthesis but does not associate with the mature integrin (1).

Integrin $\alpha_{IIb}\beta_3$ has been shown to form a stoichiometric association with CD9, an abundant platelet membrane protein that belongs to the tetraspan superfamily of four transmembrane domain-containing proteins (16), and the complex is present in resting platelet membranes (28). The CD9-$\alpha_{IIb}\beta_3$ association appears to involve hydrophobic interactions, suggesting that the proteins may interact *via* their transmembrane domains. Another intracellular regulatory protein for integrin $\alpha_{IIb}\beta_3$ is CIB, a 191-residue calcium-binding protein with sequence similarity to calcineurin B and calmodulin that binds specifically to the α_{IIb} cytoplas-

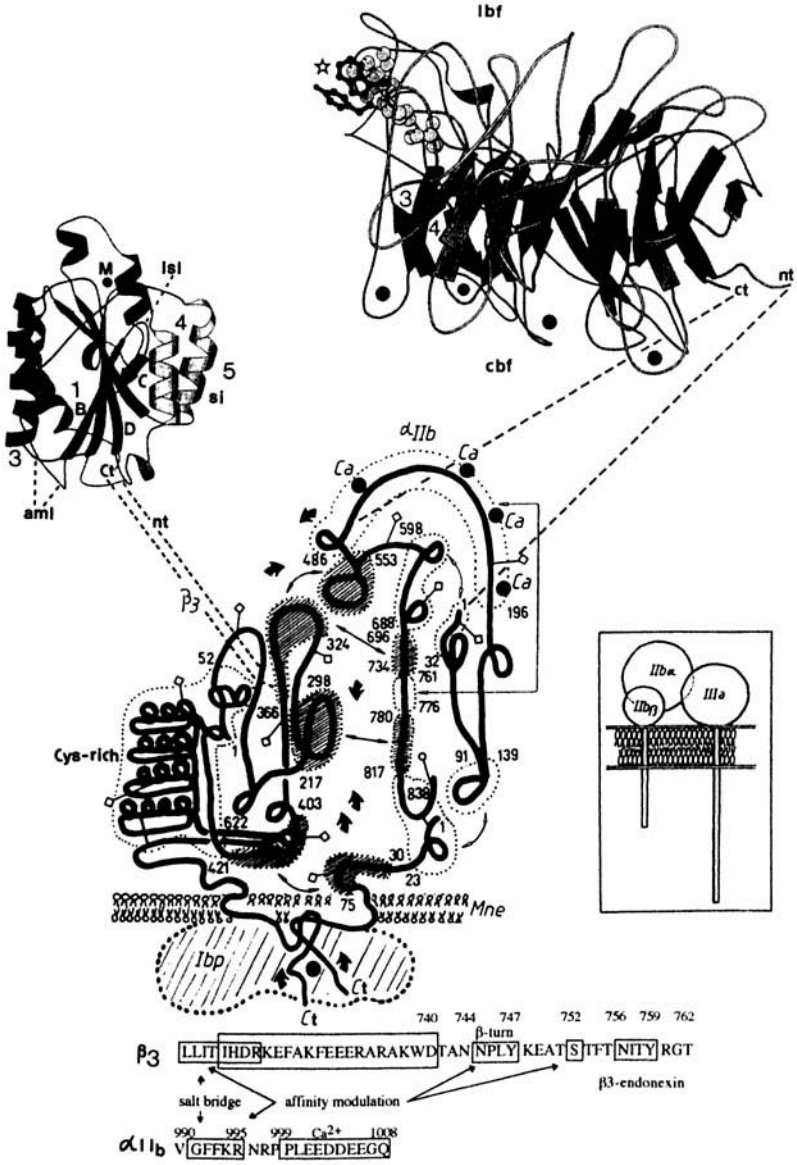

Figure 1. A working model for integrin $\alpha_{IIb}\beta_3$. Cartoon of intra- (open) and inter- (shadowed) molecular domain connectivities in nonactivated integrin $\alpha_{IIb}\beta_3$ (3, 43). This figure is not to scale. The thin arrows indicate interdomain associations. N- and O-glycosylation sites are depicted as –❑ and –○, respectively. lbp, putative intracellular integrin-binding protein; Mne, platelet plasma membrane; Ca, calcium-binding site with calcium (●) bound; Ct, C-terminal; Cys-rich, cysteine-rich domain of β_3. Thick arrows indicate conformational changes that propagate information back and forth between the cytoplasmic and the ligand-binding domains of $\alpha_{IIb}\beta_3$. The amino acid sequences of the cytoplasmic tails of the subunits are depicted below the $\alpha_{IIb}\beta_3$ model. Regions involved in affinity modulation of the integrin receptor and binding to β3-endonexin are boxed. A proposed calcium-binding domain within the C-terminal region of α_{IIb} is also boxed and labelled Ca^{2+}. The structures of the proposed I domain of β_3 (residues 110–350) and the predicted β-propeller domain of α_{IIb} (residues 15–452) are shown at the upper left and right sides of the figure. The models are based on the x-ray crystal structure of the I domain of α_2 (pdb accession code 1AOX) and on the theoretical model of the Mac-1 (α_M) β-propeller (pdb accession code 1A8X), and were rendered with MOLSCRIPT. Selected α-helices 3, 4 and 5 and β-strands B, C, and D are labeled, and structural features linked to integrin function are highlighted: **M**, a putative cation (●) in the MIDAS site of the GPIIIa I-domain. Unlike the α subunit, direct evidence for metal binding to this region of β subunits has not been provided; lsl, ligand-specificity loop; aml, affinity modulatory loops; si, subunit interface. In the β-propeller model, the ligand binding face (lbf) is located on the upper region of the domain. Residues of αIIb, α5, and α4, which have been implicated in ligand binding and are clustered in loops between the W2 and W3 and W3 and W4 repeats, are depicted in ball-and-stick or space-filling models, and this region is labeled with a star. The ligand-binding region of α subunits may include residues from additional loops. The calcium-binding face (cbf), depicted here with four calcium ions (●) bound, lies on the lower part of the β-propeller and may play a structural rather than a ligand-binding role. ct, C-terminal; nt, N-terminal. Insert, cartoon of isolated $\alpha_{IIb}\beta_3$, based on an electron microscopic image of the integrin, revealing that the extracellular (N-terminal) portions of the subunits fold into a globular head (about 8 × 10 × 10 nm^3) that is connected to the membrane by two rod-like segments extending 12–16 nm from one side.

mic domain (29). Calcineurin B and calmodulin are known regulatory proteins of various enzymes and membrane proteins.

Whereas ILK (integrin-linked kinase) binds to the β_1, β_2, and β_3 cytoplamic domains (12), proteins that bind specifically to individual integrin β cytoplasmic domains include ICAP, a β1 integrin cytoplasmic domain-associated protein-1 (12); cytohesin (which binds to β2) (12); and β3-

endonexin, a novel 111-amino acid polypeptide that interacts selectively with the cytoplasmic sequence ^{756}NITY^{759} of the β_3 integrin (30). The functional relevance of any of these integrin-associated proteins awaits further definition. Furthermore, the evidence suggests that multiple pathways lead to the activation of integrins.

The short cytoplasmic tails of α_{IIb} and β_3 associate between themselves and with other intracellular proteins in a specific and complex manner that is critical for heterodimer assembly and maintenance of the latent state of the integrin. Whereas the membrane-proximal GFFKR is conserved in the α cytoplasmic domains, the β cytoplasmic sequences are more variable, suggesting that the precise cytoplasmic associations may vary depending on the subunit composition of the heterodimeric integrin.

Piecing Together the Puzzle: Structures of Integrin Ligands and of Integrin Ligand Binding Sites. *Integrin ligands.* The majority of integrin ligands are either cell-surface molecules of the immunoglobulin (Ig) superfamily or large, modular extracellular molecules. Since 1995, there has been a substantial increase in the structural information on adhesion molecules. The structure of isolated domains of modular integrin-binding proteins has been solved by NMR or x-ray crystallography (31–33). These structures reveal features important for integrin recognition. In particular, an invariant carboxylate, implicated in adhesion, is prominently displayed usually on a distinctive loop (between β-strands C and D of the Ig domains of VCAM-1 (34, 35) and MadCAM-1 (36)) but also on a β-strand (ICAM-1 (37, 38) and ICAM-2 (39)). The RGD motif involved in the binding of non-Ig ligands (i.e., fibronectin and snake venom disintegrins) to their integrin receptors is also prominently displayed on loop regions of various integrin-binding proteins (3, 40). The RGD motif shares little structural similarity with the integrin-binding motif in VCAM, MadCAM, or ICAM molecules other than the exposure of the aspartic acid residue. In fact, all integrin ligands possess a critical carboxylate (usually an aspartate but also a glutamate) as a key feature of their integrin-binding motifs. These structures, together with mutational data and the crystal structures of integrin I domains (see below) provide insights into how these molecules function as integrin ligands. Current knowledge is consistent with the concept that, upon binding, the critical carboxylate of the ligand completes the coordination sphere of a metal ion in the ligand-binding site of the receptor. However, the cation-carboxylate bond may not account for the total binding energy. The rest of the energy as well as the ligand specificity may arise from further interactions between complementary surfaces of the integrin and the ligand.

Integrin ligand-binding sites. Both, the α and the β subunits of integrin contribute to the ligand-binding capability of integrin $\alpha_{IIb}\beta_3$. Although a recombinant soluble form of GPIIb/IIIa, which assumes an active, ligand-binding conformation has been produced (41), the three-dimensional structure of the heterodimer (or of any other integrin) has not been solved. Therefore, our understanding of its structure and molecular determinants of ligand binding are incomplete. Thus, it is not clear whether the α and β subunit ligand-binding regions define an extended binding surface exhibiting subsites for different ligand structural determinants, or are arranged into discrete nontopologically related binding pockets. It also remains unclear whether the relative disposition of α and β subunit binding sites is conserved in different heterodimers. The extended-α/β-binding-site hypothesis is supported by studies showing that photoactivatable RGD and fibrinogen γ-chain peptides, which bind, respectively, to sites in β_3 and α_{IIb}, crosslink to regions of both $\alpha_{IIb}\beta_3$ subunits (3). On the other hand, the nontopologically related-binding-surfaces hypothesis is favored by experimental evidence showing that the α and β subunits of integrin $\alpha_5\beta_1$ bind simultaneously to the synergy region (PHSRN sequence in the 9th type III repeat) and to the RGD motif (in the 10th type III repeat) of fibronectin (42), respectively. The x-ray crystal structure of a recombinant fibronectin fragment spanning F7–F10 type III repeats reveals that both binding sites are located 35 Å apart on the same face of the molecule (40). A scale model of the fibronectin/integrin interaction (see Fig. 5 in Ref. 40) shows that the synergy region and the RGD loop are easily accessible to the globular head of a single integrin molecule.

Structural studies are only available for the ligand-binding I domains of the α subunits of $\alpha_M\beta_2$, $\alpha_L\beta_2$, and $\alpha_2\beta_1$, although a wealth of information exists about residues and epitopes of both integrin subunits conferring biological activity. Clues for understanding the structure and function of the prototypic platelet integrin $\alpha_{IIb}\beta_3$ published before 1995 have been reviewed (3, 9, 43). More recent biochemical, structural, and modeling data, which provide a good framework for further studies, are summarized below.

Integrin I domains. Seven of the 17 integrin α subunits contain a 200-amino-acid-inserted domain, referred to as the I (or A) domain. It shows homology to the collagen-binding A domains of von Willebrand factor and is involved in ligand binding (44). The crystal structures of the I domains of α_M (45), α_L (46), and α_2 (47) have been reported. The I domains adopt the dinucleotide-binding (Rossmann) fold with a central parallel β-sheet surrounded on both sides by α-helices (Fig. 1). The domain also contains a metal-binding site (called the metal-ion dependent adhesion site or MIDAS) at the top of the β-sheet which is critical for its adhesive function. In the crystal structures, a magnesium ion is coordinated (directly or through water molecules) by five oxygenated residues: the sequence DxSxS plus noncontiguous aspartate and threonine residues. In the crystal structure of the α_M I domain, the sixth coordination site is occupied by a carboxylate oxygen atom from a neighboring I domain, and the authors proposed that under physiological conditions, this site might be provided by the conserved acidic residue of integrin ligands (45) (see the docking model of the I domain of LFA-1 with domain D1 of

ICAM-1 in Fig. 5 of Ref. 38). Furthermore, the DxSxS motif is present in all known integrin β subunits, and there is growing evidence for the functional importance of this motif in ligand binding by β_1 and β_3 integrins (3, 48–50). Relevant to this point, Chen *et al.* (51) using chemical cross-linking have identified the binding site for an LDV-based inhibitor of $\alpha_4\beta_1$ within residues 130–146 of α_4, a region that contains the DxSxS motif.

Lee *et al.* (45) have proposed that the ligand-binding domain of integrin β subunits may adopt an I-domain-like fold, and a structure prediction appears to support this hypothesis (52). The proposed region in GPIIIa corresponds to residues 110–350 (Fig. 1). Interestingly, when the sequence CTSEQNC of β_1 was replaced by the corresponding sequence of β_3 ^{177}CYDMKTTC^{184} (where cysteines 177 and 184 are linked by a disulfide bond in GPIIIa (3, 43)), the ligand specificity switched from that of $\alpha_v\beta_1$ to that of $\alpha_v\beta_3$ (53). In the I domain-like model, this highly divergent sequence among integrin β subunits is located in a loop structure between β-strands B and C on the same side of the domain and near the MIDAS motif (labeled "lsl" for ligand specificity loop, in Fig. 1). The results suggest that the predicted loop structure is a key determinant of integrin ligand specificity. On the other hand, regions 217–298 and 324–366 of GPIIIa form part of the subunit interface in the $\alpha_{IIb}\beta_3$ heterodimer (3, 43) (Fig. 1). In the proposed I-domain fold these regions would correspond to α4-βD-α5 and α6-α7, respectively. The α4, α5, and α6 are located on the same side of the domain (labeled "si" for subunit interaction, in Fig. 1), in line with the proposed structure-function correlation model. Moreover, the region around GPIIIa Arg^{214} (located in the loop βD-α4) has been involved in the conformational change(s) leading to the activation-dependent exposure of the ligand-binding site (3), and the region of GPIIIa 217–231 (loop βD-α4 and N-terminal part of helix α4) is cryptic in resting GPIIb/IIIa and becomes exposed following platelet activation (3). Using synthetic peptides, McDowall *et al.* (54) have shown that peptides covering the α4-βD loop, the βD-α5 loop, and helix α5 of the I domain of α_L define an area involved in a conformational change necessary for conversion to the high-affinity state of integrin $\alpha_L\beta_2$. Moreover, the region of β_1 207–218 (which corresponds to GPIIIa 198–209 and encompasses the loop between βC and helix α3 in the I domain model) (Fig. 1) contains epitopes for both activating and inhibitory antibodies (3, 50). Similarly, Zhang and Plow (55) have reported that two short and spatially proximal epitopes of the I domain of $\alpha_M\beta_2$(loop α1-βB and loop βC-α3 and N-terminal region of α3, labeled "aml" for affinity modulatory loop in Fig. 1) are involved in the modulation of the adhesive activity of this receptor. Hence, in the proposed fold, these structural elements lay on the same side of the domain (front face in Fig. 1), suggesting that the MIDAS motif, the ligand-specificity B-C loop, the subunit interface (helices α4-α5), and the affinity modulatory region(loops α1-βB and βC-α3) are topologically related.

Mapping the Ligand Binding Site in Non-I-Domain α Subunits. A number of potential ligand interactive sites have been identified in non-I-domain-containing integrin α subunits, mainly α_{IIb}, α_4, and α_5. Domain-swapping experiments indicate that the amino-terminal one-third (residues 1–334) of α_{IIb} defines the ligand recognition specificity of integrin $\alpha_{IIb}\beta_3$ (56). Similarly, recombinant α_5(229–448) appears to constitute the minimal domain of $\alpha_5\beta_1$ for fibronectin recognition (57). The ligand-binding domain of α_4 has been narrowed down to the N-terminal portion (residues 108–268 (58), 152–203 (59), and ^{181}GAPGSSYWTG^{190} (60)) of the molecule by mapping the epitopes for function-blocking antibodies and alanine-scanning mutagenesis. This sequence is relatively well conserved among integrin α subunits. Mutations to alanine of residues G^{184}, Tyr^{189}, Tyr^{190}, Phe^{191}, and Gly^{193} within the homologous region of GPIIb(^{184}GAPGGYYFLG^{193}) significantly blocked binding of $\alpha_{IIb}\beta_3$ to soluble fibrinogen and, in contrast to most of natural GPIIb mutations occurring in Glanzmann's thrombasthenic platelets, did not affect cell surface expression of $\alpha_{IIb}\beta_3$ (61).

The loop of GPIIb ^{147}RGNTLSRIYVENDFSWDKRY^{166} defined by the disulfide bond between Cys^{146}-Cys^{167} has also been implicated in the ligand binding function of $\alpha_{IIb}\beta_3$ (62). Alanine substitution of each of the oxygenated residues within this loop did not significantly affect surface expression of $\alpha_{IIb}\beta_3$. Only the Asp^{163}/Ala mutation abolished the ligand binding function of the integrin, indicating that this residue might be critical for ligand binding. The involvement of this loop in ligand binding is also supported by the report that a double mutation in α_5 (S^{156}G/W^{157}S, which corresponds to^{156}YV^{157} in α_{IIb}) blocked the interaction of a peptide ligand to $\alpha_5\beta_1$ (63).

Interestingly, although some reports have suggested that α-chain Ca^{2+}-binding sites may interact directly with ligands (3, 9, 43), the putative ligand-binding region of α_4 does not include any of its calcium binding repeats. In agreement with this conclusion, Pujades *et al.* (64) showed that mutations of the three α_4 "EF-hand" Ca^{2+}-binding sites had no effect on binding of soluble monovalent or bivalent VCAM-1 to $\alpha_4\beta_1$. In the same line of evidence, Niewiarowska *et al.* (65) used peptide-specific antibodies as probes of the topology of the Ca^{2+}-binding motifs of $\alpha_{IIb}\beta_3$ and concluded that all four Ca^{2+}-binding sites are partially exposed and are not in close vicinity of the ligand recognition domain. Moreover, in agreement with domain mapping by limited proteolysis of GPIIb/IIIa (3, 43), mutational studies indicate that the calcium-binding domains of GPIIb are not necessary for complex formation with GPIIIa (66). Analysis of platelet Glanzmann thrombasthenic phenotypes linked to mutations in the calcium-binding domains of GPIIb (67, 68) demonstrate their importance in maintaining the proper $\alpha_{IIb}\beta_3$ conformation required for intracellular transport of the heterodimer to the cell surface. Thus, the Ca^{2+}-binding domains may play a structural rather than a ligand-binding role.

A 4-fold helix-loop-helix (HLH) arrangement for the calcium-binding domain in the α_L integrin subunit has been proposed (69). On the other hand, the N-terminal approximately 440 amino acids of non-I-domain integrin α subunits are predicted to fold into a β-propeller domain built by seven four-stranded β-sheets arranged in a torus around a pseudosymmetry axis (70). In this model, the Ca^{2+}-binding sites of α_{IIb} are at the lower part of the β-propeller domain model in loop regions joining β-strands 1 and 2 of repeats W4, W5, W6, and W7, as depicted in Figure 1. The ligand-binding site is hypothesized to lie in the upper surface of the domain (70). The HLH and the β-propeller domain model are clearly mutually incompatible with regards to the structure of the calcium-binding sites.

In the β-propeller domain model, residues G^{184}, Tyr^{189}, Tyr^{190}, Phe^{191}, and Gly^{193} of α_{IIb}, and the homologous residues Tyr^{187}, Trp^{188}, and Gly^{191} of α_4, whose mutation to Ala affects cell adhesion to fibrinogen (61) and to VCAM-1 and CS-1 (59), respectively, are clustered in a loop between the W3 and W4 repeats of the predicted β-propeller structure (Fig. 1). The Cys^{146}-Cys^{167} disulfide bridge is in the loop joining the fourth β-strand of W2 and the first β-strand of W3. These two loops are adjacent in the proposed β-propeller model (Fig. 1). Although the experimental evidence is limited (50, 71), the proposed models may be useful for interpreting current data and guiding further experiments.

Subunit Association. Domain mapping of GPIIb/IIIa by limited proteolysis indicated the existence of complex intra- and intermolecular connectivities in the heterodimer (3) (Fig. 1). Regions of GPIIIa including residues 217–298 and 324–366 (α4-βD-α5 and α6-α7 in the proposed I-domain fold), together with regions of the C-terminal half of the GPIIb heavy chain (486–553; 696–734; and 780–817) and the membrane-proximal part of the GPIIb light chain (residues 30–75), form part of the subunit interface in the $\alpha_{IIb}\beta_3$ heterodimer (3, 43). In addition, the amino- and C-terminal portions of GPIIb have been shown to be in close association in resting $\alpha_{IIb}\beta_3$ (see Figs. 1 and 2 in Ref. 3). In agreement with this low-resolution domain model, Huang *et al.* (72) have shown that folding of the proposed I domain of β_2 is not complete until after association with α_L, suggesting that the β_2 domain may be intimately associated with the α subunit. Also in agreement with the domain connectivity model, is the report that the Ca^{2+}-binding domains are not involved in heterodimer formation and that regions amino-terminal to the calcium-binding domains of GPIIb (1– ~200) play a role in integrin subunit association (66).

The α subunit β-propeller and the β subunit I domain of integrins $\alpha_M\beta_2$ and $\alpha_L\beta_2$ may associate, since both are dependent on heterodimer formation for folding (72, 73). The region 599–718 of α_M (450–570 in α_{IIb}) that follows the β-propeller domain is predicted to fold into a structurally independent 6–7 β-stranded domain, and the following subregion, from residues 725–729 (573–577 in α_{IIb}) may directly associate with the β_2 subunit, or its conformation may depend on associations elsewhere with β_2 (73). The involvement of regions of the C-terminal half of the α subunit in the formation of functional heterodimers is also suggested by a study showing that a Glanzmann thrombasthenic phenotype is associated with deletion of amino acids ^{817}Leu-Asn^{826} of the GPIIb heavy chain and insertion of eight different residues (74).

Conformational Changes. The structural basis of the activation-dependent conformational change and the structural changes that propagate information back and forth between the cytoplasmic tails and the ligand-binding site in the globular head remain elusive. Both relative movements of domains (quaternary structure changes) that unmask the ligand-binding site and conformational changes within the ligand-binding domain (tertiary structure changes) that alter the properties of the ligand binding pocket may operate within integrins. Hence, the observation that the interaction of monoclonal antibodies with the extracellular, membrane-proximal region of GPIIIa triggered fibrinogen binding to the globular head of integrin $\alpha_{IIb}\beta_3$, proved that a long-range conformational change can be propagated along the integrin (75). Similarly, activating monoclonal antibodies bind to the cysteine-rich region of β1 integrin (76, 77). Fluorescence resonance energy transfer studies have indicated that platelet activation correlates with a change in the relative orientation of the integrin $\alpha_{IIb}\beta_3$ subunits (78). Comparison of the limited proteolysis degradation patterns of the non-RGD-binding (off state) and the RGD-binding (activated state) of integrin $\alpha_{IIb}\beta_3$ indicates the existence of dramatic interdomain movements involving regions covering helices α4 and α5 and the βD-α4 loop of the proposed β subunit I domain and the N-terminal half (including the β-propeller domain) of the α subunit (79). A conformational shift of α_5 relative to β_1 has also been reported for integrin $\alpha_5\beta_1$ (80, 81). However, whereas Tsuchida *et al.* (80) interpret their results as an indication of relocation of the α_5 subunit to uncover the cysteine-rich region of β_1, Mould *et al.* (81), suggest that the conformational change results in exposure of ligand-binding sites near the interface between the α subunit β-propeller and the β subunit I domain. Another model has been presented by Loftus and Liddington (42). In this model, the β_3 I domain sits on top of the α_{IIb} β-propeller in the low-affinity state of the integrin, sterically blocking access to the ligand-binding site of α_{IIb}. Receptor activation involves release of the I domain constraint leading to exposure of the ligand-binding site in α_{IIb} (42). Although experimental confirmation of any of the models needs resolution of the structures of integrins in different affinity states, the different models converge to suggest that the extracellular domains of the α and the β subunits change their relative reorientation along the subunit interface during a quaternary structure conformational transition of the integrin receptor.

Tertiary structure changes within the ligand-binding domain of the β_2 subunit are also indicated by the report that helices α4 and α5 and the βD-α4 loop of the I domain

of LFA-1 appear to be involved in a conformational change leading to high-affinity/avidity binding to ICAM-1 (54). Comparison of two crystal forms of the I domain of α_M with bound Mg^{2+} (closed conformation) and Mn^{2+} (open conformation) revealed a changed in metal coordination that was linked to a large (10-Å) shift of the C-terminal helix, the burial of two Phe residues into the hydrophobic core of the Mn^{2+} form, a direct T^{209}-Mn^{2+} bond, and the presence of a glutamate side chain from a neighboring molecule in the MIDAS site (82). This led to the interpretation that the different structures may represent different affinity states of the I domain (82). However, the significance of this finding remains controversial. On the one hand, crystallographic studies of CD11a (α_L) I domain in the absence or presence of bound Mn^{2+} ion did not show major structural arrangements in the metal-binding site (83), and Baldwin *et al.* (84) have reported that addition of cations Mn^{2+}, Mg^{2+}, and Cd^{2+} to the metal-free I domain of CD11b (α_M) did not induce conformational changes in the crystalline environment. The authors (84) suggest that the proposed active conformation (82) is likely to be a construct artifact. On the other hand, Li *et al.* (85) used F302 W and T209 A mutants to show that the MIDAS site includes conformationally sensitive residues that undergo significant shifts in the open versus closed structures. They suggest that stabilization of the open structure is independent of the nature of the metal ligand and that the open conformation may represent the physiologically active form.

Outside-In Signaling The binding of fibrinogen and other ligands to activated GPIIb/IIIa causes conformational changes linked to the expression of neoepitopes called LIBS (ligand-induced binding sites) (3, 9, 43). Regions of GPIIIa downstream of the proposed I domain and the C-terminal part of GPIIb have been shown to exhibit LIBS. A current model (3) suggests that LIBS may arise as a consequence of subtle changes in the relative orientation of the integrin subunits and that the C-terminal halves of the molecules may be involved in receptor clustering and outside-in signaling. Considerable amounts of evidence point to a role for the short cytoplasmic tails of the integrin subunits in driving the nucleation of large intracellular complexes containing both cytoskeletal and catalytic signaling proteins. The reactions that initiate and propagate the assembly, as well as the architecture of the intracellular macromolecular complexes, are the subject of intense research. A detailed description is beyond the scope of this paper; however, excellent review articles have been published recently (7, 8).

Available experimental evidence suggests that the β subunit cytoplasmic tail contains the molecular determinants for cytoskeletal attachment of ligated and clustered $\alpha_{IIb}\beta_3$. In the resting integrin, the α subunit tail blocks the cytoskeletal interaction with the β tail, and ligand binding appears to remove this block (3, 12). Active regions of the β_3 cytoplasmic domain for cytoskeletal interactions include ^{727}FAKFEEE^{733} (binding site for α-actinin), ^{744}NPLYKEAT^{751}, and ^{757}ITYR^{760} (86). Direct binding of platelet integrin $\alpha_{IIb}\beta_3$ to talin has been reported. This interaction appears to be mediated through a conformational epitope of the cytoplasmic domains of both α_{IIb} and β_3 (87).

Activation of *syk* and *src* are early events of the signaling pathway through ligated and clustered $\alpha_{IIb}\beta_3$ in platelets. Full spreading or aggregation is associated with a cascade of tyrosine phosphorylations of a number of proteins. β_3 becomes phosphorylated on tyrosine residues in response to thrombin-induced platelet aggregation (88), and a peptide corresponding to residues ^{740}DTANNPLYKEATSTFTNITYRGT^{762} of the β_3 cytoplasmic domain was capable of binding the signaling proteins SHC (β_3 peptide monophosphorylated at Tyr^{759}) and GRB2, when the β_3 peptide was phosphorylated at both Tyr^{747} and Tyr^{759}. The data suggest that tyrosine phosphorylation may recruit phosphotyrosine-binding molecules, thereby initiating signaling pathways. In addition, the highly conserved ^{744}NPLY^{747} in the C-terminal segment of the β_3 cytoplasmic domain was found to be required for triggering autophosphorylation of the focal adhesion kinase, FAK (89). The structural integrity of the motif rather than its phosphorylation status appears to be important for β_3-mediated cytoskeleton reorganization and tyrosine phosphorylation of FAK (90). This suggests that the NPLY motif may modulate the binding of a protein required for integrin-mediated FAK phosphorylation. On the other hand, the phosphorylation status of Tyr^{747} is important for optimal β_3 postligand binding events (90).

A mutation in the β_3 cytoplasmic tail ($Ser^{752} \rightarrow$Pro), known to inhibit activation of $\alpha_{IIb}\beta_3$ in a variant o Glanzmann's thrombasthenia, also inhibited the ability of the β_3 cytoplasmic domain to signal FAK phosphorylation (89), failed to bind to GRB2, and showed reduced binding with SHC (86), and profoundly affected cell spreading and β(3) localization into focal contacts (91). The mutant GPIIb/GPIIIa$^{Ser752\rightarrow Pro}$ also had reduced ability to bind β3 endonexin (30), suggesting that a single mutation can block both inside-out and outside-in GPIIb/IIIa signaling due to the inability of the mutant integrin to bind cytoplasmic proteins. Since Ser^{752}Ala substitution mostly restores normal integrin functions, the structure-disrupting presence of Pro may be responsible for the receptor's loss of function (91).

Activation and tyrosine-397 autophosphorylation of FAK (7) provides a docking site for SH2 domains of *src* family kinases, and possibly PI3 kinase. *Src* phosphorylates FAK at additional Tyr residues, generating an anchor site for the adapter protein GRB2. The C-terminal domain of FAK also contains Pro-rich motifs that bind to SH3 domains enabling the recruitment of more adapters and signaling molecules and linking integrin engagement with downstream signaling events, including the *ras* and MAP kinase cascades.

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