

Structural basis of transmembrane domain interactions in integrin signaling

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Cell surface receptors of the integrin family are pivotal to cell adhesion and migration. The activation state of heterodimeric $\alpha\beta$ integrins is correlated to the association state of the single-pass α and β transmembrane domains. The association of integrin $\alpha\text{IIb}\beta 3$ transmembrane domains, resulting in an inactive receptor, is characterized by the asymmetric arrangement of a straight (αIIb) and tilted ($\beta 3$) helix relative to the membrane in congruence to the dissociated structures. This allows for a continuous association interface centered on helix-helix glycine-packing and an unusual αIIb (GFF) structural motif that packs the conserved Phe-Phe residues against the $\beta 3$ transmembrane helix, enabling αIIb (D723) $\beta 3$ (R995) electrostatic interactions. The transmembrane complex is further stabilized by the inactive ectodomain, thereby coupling its association state to the ectodomain conformation. In combination with recently determined structures of an inactive integrin ectodomain and an activating talin/ β complex that overlap with the $\alpha\beta$ transmembrane complex, a comprehensive picture of integrin bi-directional transmembrane signaling has emerged.

which closely follows the first charged residue on the intracellular face, αIIb (K989), constrains the receptor to a default low affinity state.¹¹ Despite these efforts, an understanding of this sequence motif had not been reached until such time as the structure of the αIIb TM segment was determined.¹² In combination with the structure of the $\beta 3$ TM segment¹³ and available mutagenesis data,^{6,9,10,14,15} this has allowed the first correct prediction of the overall association of an integrin $\alpha\beta$ TM complex.¹² The predicted association was subsequently confirmed by the $\alpha\text{IIb}\beta 3$ complex structure determined in phospholipid bicelles,¹⁶ as well as by the report of a similar structure based on molecular modeling using disulfide-based structural constraints.¹⁷ In addition to the structures of the dissociated and associated $\alpha\beta$ TM domains, their membrane embedding was defined^{12,13,16,18,19} and it was experimentally recognized that, in the context of the native receptor, the TM complex is stabilized by the inactive, resting ectodomain.¹⁶ These advances in integrin membrane structural biology are complemented by the recent structures of a resting integrin ectodomain and an activating talin/ β cytosolic tail complex that overlap with the $\alpha\beta$ TM complex,^{20,21} allowing detailed insight into integrin bi-directional TM signaling.

The communication of biological signals across the plasma membrane is fundamental to cellular function. The ubiquitous family of integrin adhesion receptors exhibits the unusual ability to convey signals bi-directionally (outside-in and inside-out signaling), thereby controlling cell adhesion, migration and differentiation.¹⁻⁵ Integrins are Type I heterodimeric receptors that consist of large extracellular domains (>700 residues), single-pass transmembrane (TM) domains, and mostly short cytosolic tails (<70 residues). The activation state of heterodimeric integrins is correlated to the association state of the TM domains of their α and β subunits.⁶⁻¹⁰ TM dissociation initiated from the outside results in the transmittal of a signal into the cell, whereas dissociation originating on the inside results in activation of the integrin to bind ligands such as extracellular matrix proteins. The elucidation of the role of the TM domains in integrin-mediated adhesion and signaling has been the subject of extensive research efforts, perhaps commencing with the demonstration that the highly conserved GFFKR sequence motif of α subunits (Fig. 1),

Structure and Membrane Embedding of Integrin α and β Transmembrane Segments

In order to understand the structure, association and membrane embedding of the αIIb and $\beta 3$ TM segments, it is useful to first discuss their amino acid sequences, whose principal features are well conserved among the 18 α and 8 β human subunits.¹⁶ A stretch of 23 hydrophobic residues, commencing at αIIb (I966)/ $\beta 3$ (I693) on the extracellular side, is terminated prior to αIIb (K989)/ $\beta 3$ (K716) on the intracellular face, and is succeeded by four and five mostly hydrophobic residues, respectively (Fig. 1). Assuming α -helical conformation for the 23-residue hydrophobic segments preceding αIIb (K989)/ $\beta 3$ (K716), the conformation and membrane embedding of these four and five residue segments will govern the TM helix tilt and, thus, the default orientation at which αIIb and $\beta 3$ will face each other.

For $\beta 3$, a 29-residue linear membrane-embedded helix, encompassing I693-I721, was revealed in phospholipid bicelle model membranes by NMR spectroscopy.¹³ This conformation embeds $\beta 3$ (K716) and indicates a pronounced TM helix tilt that minimizes the distance of K716's positive side chain $\epsilon\text{-NH}_3^+$ moiety to a negatively charged species such as a lipid's PO_4^- group; i.e.,

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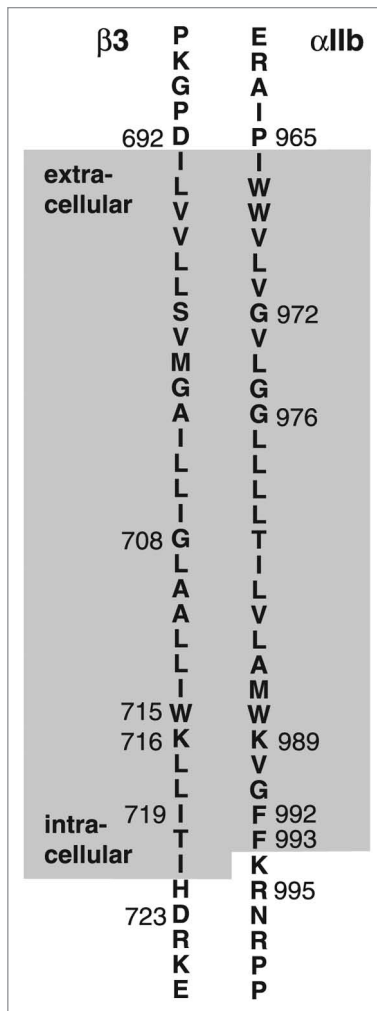


Figure 1. Amino acid sequence of integrin αIIb and $\beta 3$ transmembrane segments and flanking regions. Membrane-embedded residues^{12,13,16,18,19} are enclosed by a gray box. Residues 991–995 constitute the highly conserved GFFKR sequence motif of integrin α subunits.

$\beta 3$ (K716) points towards the cytosol (Fig. 2A). With 3.6 residues per helix turn, this points the most polar residue of the five-residue stretch, T720, towards the cytosol, whereas the remaining hydrophobic residues can orient their side chains mostly towards the membrane. An equivalent arrangement appears difficult for αIIb , which carries Phe residues at positions +3 and +4 from αIIb (K989) (Fig. 1), as do all 18 human α subunits.¹² With this realization, the unusual structure of the αIIb TM segment (Fig. 2A), determined in phospholipid bicelles by NMR,¹² is readily rationalized. A non-helical Gly-Phe-Phe conformation, with glycine efficiently abrogating helical tendencies and phenylalanine immersion in the membrane, is energetically preferable over a helical conformation wherein one phenylalanine invariably would have to face the cytosol. As a result, the αIIb TM segment can maintain a straight helix orientation in the membrane.¹² Thus, the dissociated $\alpha \text{IIb}/\beta 3$ TM segments face each other in a straight/tilted helix-helix arrangement (Fig. 2A). Despite the existence of relevant mutagenesis data⁶ and the plethora of proposed integrin TM signaling models, this arrangement had

been an overlooked possibility prior to the structural $\alpha \text{IIb}/\beta 3$ TM studies.^{12,13} Furthermore, the combination of the $\alpha \text{IIb}/\beta 3$ TM structures with TM mutagenesis data^{6,9,10,14,15} resulted in the first correct prediction of the structure of the associated $\alpha \text{IIb}/\beta 3$ TM state.¹²

The structure of the αIIb TM segment is noteworthy for its rarity. There are only a few examples of a GFF turn motif in relation to an α -helix in water soluble proteins. A fine example is found within the structure of glycogen phosphorylase b (GPb) of *Oryctolagus cuniculus*²² (Fig. 2B). Reminiscent of a membrane-water interface, the GFF motif of GPb is localized at the protein surface-water interface (c.f. PDB entry 1a8i; residues 748–750). In membrane proteins, no direct homologue has thus far been reported to my knowledge; however, some similarities to a TM helix of cytochrome *c* oxidase from *Thermus thermophilus*,²³ and the β peptide (chain F) of the light-harvesting complex II from *Rps. acidophila*,²⁴ are discernible (Fig. 2C and D). Like αIIb , the helix of cytochrome *c* oxidase is followed by a left-handed turn initiated by N127 that localizes A129 at a similar position to αIIb (F992) (Fig. 2C). The free energy gain of an Ala compared to a Phe membrane insertion is obviously limited. For the β peptide of the light-harvesting complex II, a right-handed turn is induced by P38 that localizes W39 at a similar position to αIIb (F993). W39 and L40 are thus able to re-immerser into the membrane (Fig. 2D). Finally, it is noted that the VFFA sequence near the N-terminus of the C-terminal TM helix of the amyloid precursor protein (APP) also appears to be membrane immersed, albeit in helical conformation, probably residing along the membrane surface.²⁵

Structural Basis of Integrin $\alpha \text{IIb}/\beta 3$ Transmembrane Association

The NMR structure determination of the $\alpha \text{IIb}/\beta 3$ TM complex was carried out in bilayer-forming phospholipid bicelles²⁶ employing non-covalently associated TM segments to achieve physiological conditions and to avoid any bias of the association interface, respectively. The use of sophisticated isotope-labeling patterns and deuterated lipids allowed the observation of close side chain distances between αIIb and $\beta 3$, while avoiding the suppression of contaminating intramolecular protein and lipid signals at high lipid-to-protein ratios.¹⁶ Intersubunit distances were thus obtained along the entire dimerization interface (Fig. 3), which is characterized by protein backbone conformations that closely followed the dissociated αIIb and $\beta 3$ states.¹⁶ The association within the extracellular membrane leaflet is most conspicuously defined by the packing of aliphatic side chains against three glycine residues, αIIb (G972), αIIb (G976) and $\beta 3$ (G708), as is common for TM helix associations, defining an outer membrane clasp (OMC; Fig. 3). From the TM sequences alone (Fig. 1), such an arrangement would be difficult to deduce. However, remarkably accurate predictions of the OMC had been achieved previously by molecular modeling studies.^{27–29} Within the intracellular membrane leaflet, $\alpha \text{IIb}/\beta 3$ association defines a novel packing motif, referred to as the integrin inner membrane clasp (IMC; Fig. 3).¹⁶ Residues αIIb (F992) and αIIb (F993) pack

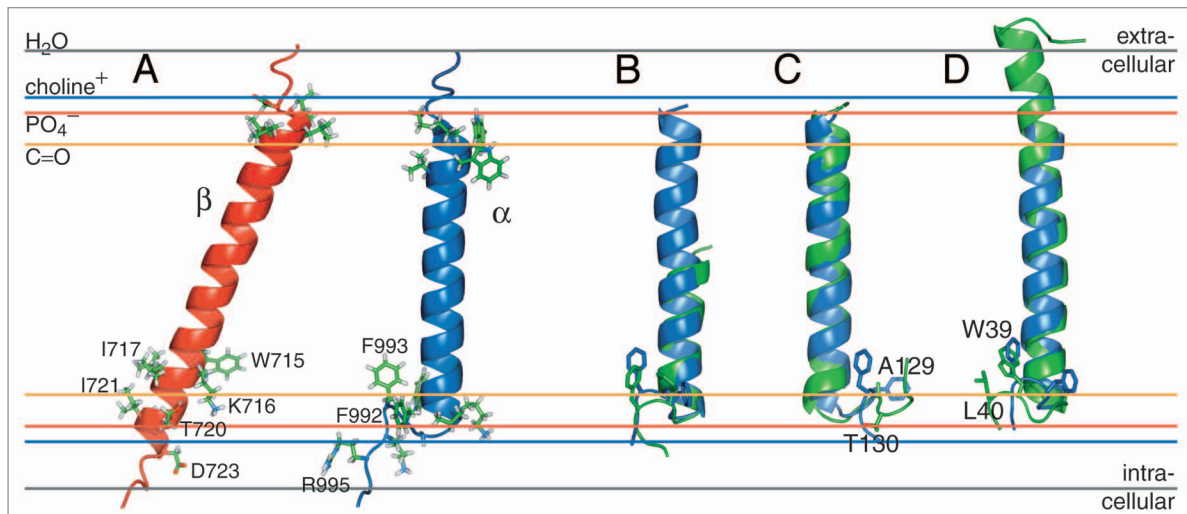
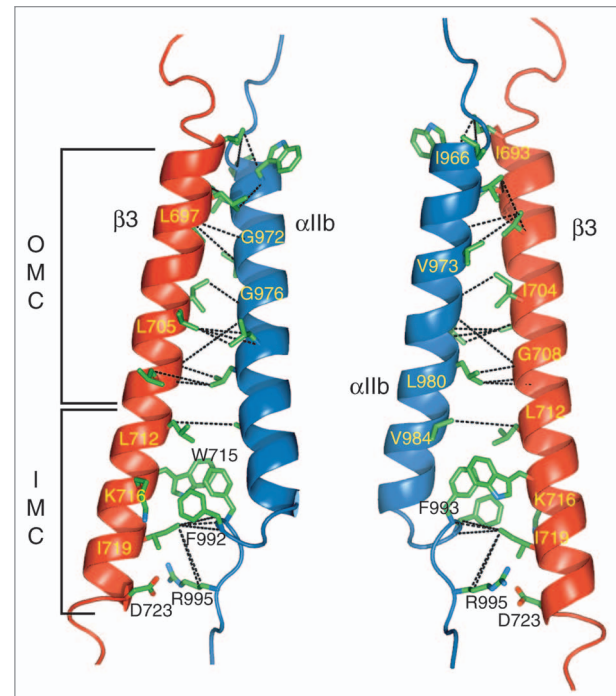


Figure 2. NMR structures of the individual integrin α IIb and β 3 transmembrane segments. (A) Structures of the monomeric α IIb and β 3 TM segments (PDB entries 2k1a and 2rmz, respectively) and their estimated membrane embedding.^{12,13,18,19} Selected side chains are shown in ball-and-stick representation. (B) An α IIb-homologous GFF structural motif is found in glycogen phosphorylase b (GPb; PDB entry 1a8i;²² residues 748–750). The α IIb TM segment and pertinent GPb helix are shown in blue and green, respectively. (C) For α IIb (blue), structural homology was found for a TM helix of cytochrome c oxidase (PDB entry 1xme, chain A), shown in green. The side chains of α IIb(F992–F993) and 1xme-A (A129–T130) are shown in ball-and-stick representation. F992 and A129 are structurally homologous. (D) The β peptide from the light-harvesting protein B-800/850 (homologous PDB entries 2fkw, chain S; 1nkz, chain F; and 1kzu, chain B also exhibits homology to α IIb. Shown is the structural alignment of integrin α IIb(P965–R995), in blue, with chain F of 1nkz, in green. The side chains of α IIb(F992–F993) and 1nkz-F(W39–L40) are shown in ball-and-stick representation. F993 and W39 are structurally homologous. The experimentally determined average positions of the lipid carbonyl, phosphate and choline groups (16, 20 and 22 Å from the center of the membrane) are depicted for 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine.⁵⁴ The bulk water phase is assumed to be fully established at 28 Å.⁵⁵

Figure 3. NMR structure of the integrin α IIb β 3 transmembrane complex. The locations of the outer and inner membrane clasps, OMC and IMC, respectively, are indicated. The structure illustrates the inter-subunit distance (NOE) restraints used to calculate the α IIb β 3 TM complex.¹⁶ For clarity, interproton NOE connectivities are denoted by dotted black lines between the carbon atoms that are covalently bonded to the hydrogen nuclei, giving rise to the NOEs. The structure shown is the average structure of an ensemble of 20 structures calculated without an α IIb(R995)- β 3(D723) structural constraint. It exhibits a negligible backbone r.m.s.d. of 0.32 Å to the average structure calculated with such a restraint (PDB entry 2k9j). The depicted orientations are related by a rotation of $\sim 180^\circ$ about the y axis.



into a groove formed by β 3(L712), β 3(W715), β 3(K716) and β 3(I719). In addition to hydrophobic interactions, electrostatic contributions arising from β 3(W715/ ϵ -NH)- α IIb(F993/CO) and β 3(K716/ ϵ -NH₃⁺)- α IIb(F992/CO) interactions would be compatible with the obtained structural ensemble and permissive side chain rotamers. However, competing interactions with lipid molecules may lead K716 to again prefer to fully neutralize its positive charge by engaging a lipid molecule. The NMR intersubunit distance restraints juxtapose positively charged α IIb(R995) on average to negatively charged β 3(D723), enabling the formation of a mutually beneficial electrostatic interaction that completes the IMC (Fig. 3). Based on the strongly dissociating effects of altering either residue to alanine in the context of phospholipid bicelles and CHO membranes,¹⁶ coupled with the ability of R \leftrightarrow D charge reversal variants to maintain an inactive receptor,⁶ it is appropriate to favor ensemble structures that exhibit

a close α IIb(R995)- β 3(D723) side chain distance¹⁶ to represent the fully associated α IIb β 3 TM complex. The lipid embedding of the α IIb β 3 TM complex was found essentially unchanged to its constituent monomeric peptides (Fig. 2A),¹⁶ showing the absence of an intrinsic tendency to alter its membrane topology.

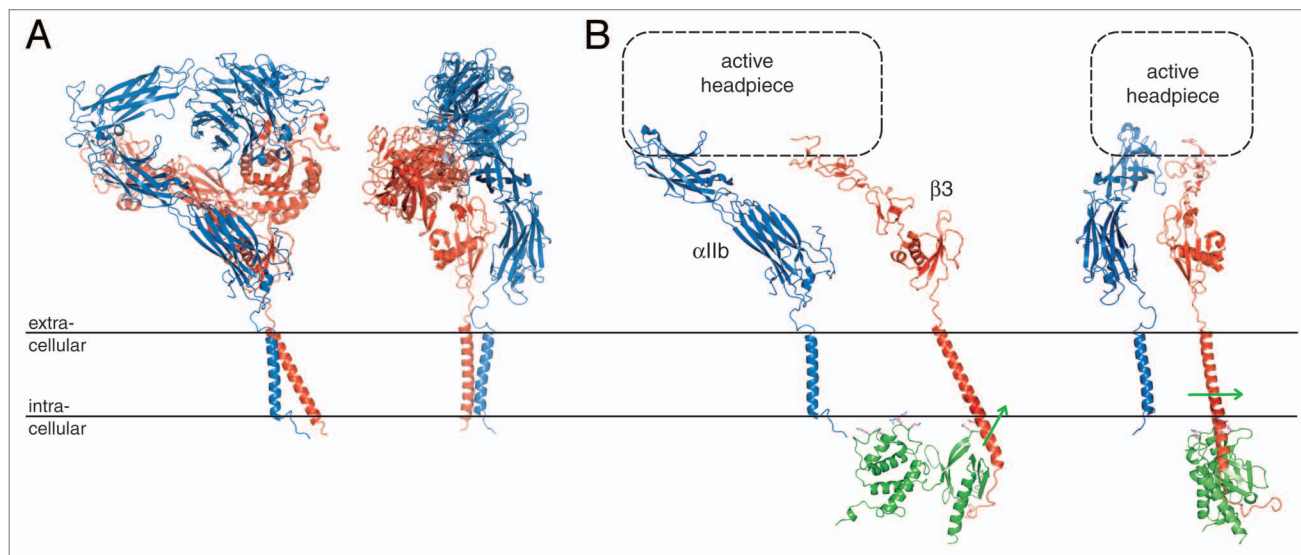


Figure 4. Integrin bi-directional signaling. (A) Model of a resting integrin receptor. The α V β 3 ectodomain crystal structure was fused to an α V β 3 TM model based on the α I**IIb β 3 TM complex NMR structure.^{16,20} The α and β subunits are shown in blue and red, respectively. (B) Model of an active integrin conformation. The α (Cal β -1/2) and β (TD/IE2-4) domains of the integrin ectodomain³⁷ are shown dissociated with a schematic active headpiece. The dissociated β TM segment was overlaid with the activating talin2/ β 1D crystal structure (PDB entry 3g9w). Talin2 is shown in green and the direction of its effected β TM segment reorientation²¹ is indicated by green arrows. Two orientations, which are related by a rotation of $\sim 90^\circ$ about the y axis, are shown for both models.**

For the NMR structure determinations of the associated and dissociated integrin α I**IIb β 3 TM states,^{12,13,16} care was taken to ensure a physiological lipid environment. It was shown that an asymmetric lipid bilayer distribution is not essential to a study of the β 3 TM segment;¹³ the aggregation states of the bicelle-embedded α I**IIb** and β 3 peptides were assessed to be predominantly monomeric;^{12,13} the invariance of the structures to changes in lipid composition was confirmed;^{12,13,16} and the comparison of membrane substitutes for β 3 revealed the superiority of phospholipid bicelles over dodecylphosphocholine (DPC) micelles.¹³ This is also an extension of careful earlier work which revealed that, when fused to a coiled-coil construct as TM helix substitute, the GFFKR motif of α I**IIb** failed to specifically interact with β 3,³⁰ providing an early indication that GFFKR does not have a tendency to adopt helical conformation, as had been reported in some later studies.³¹⁻³³ In particular, the recent report of an α I**IIb β 3 TM structure, performed in 50%/50% CD₃CN/H₂O solution,³³ lacked basic physiological conditions (e.g., a lipid-water interface), and invoked severe distortions in helical geometry of both α I**IIb** and β 3 to juxtapose α I**IIb**(R995)- β 3(D723). Since platelet membranes contain a lipid composition³⁴ that would be difficult to completely reproduce in vitro, extensive mutagenesis data in mammalian cells were also useful in further confirming the asymmetry of the α I**IIb β 3 association encoded by the IMC.^{6,16,17,35}******

Subsequent to the publication of the α I**IIb** and β 3 TM structures,^{12,13} modeling predictions for the IMC improved conspicuously.^{17,36} A particularly exhaustive study was based on α I**IIb**- β 3 disulfide cross-linking efficiencies fed into an extension of the Rosetta sparse-data sampling approach.¹⁷ In this approach, the overall IMC arrangement was predicted with relative consistency,

but with quite a considerable degree of heterogeneity, perhaps owing to the absence of any experimental restraints on its central α I**IIb**(GFF) motif. Notably, out of 5,000 Rosetta solutions, only 52 structures were ultimately selected;¹⁷ for membrane protein modeling approaches to become independent tools of structure determination, a more robust convergence of structures appears to be desirable.

Mechanism of Bi-Directional Integrin α I**IIb** β 3 Transmembrane Signaling

Just two short years ago, integrin transmembrane signaling had been a little understood, perhaps even misunderstood, area of integrin biology. In addition to the novel information regarding the TM segments discussed above, the recently determined structures of a resting integrin ectodomain, and of an activating talin/ β cytosolic tail complex that overlap with the TM structures,^{20,21} have profoundly changed this scenario. The C-terminal extension of the original, monumental α V β 3 ectodomain crystal structure³⁷ has provided an excellent fusion partner for an α V β 3 TM model based on the α I**IIb** β 3 TM complex structure,^{16,20} thus yielding a model of a complete, resting integrin (Fig. 4A). Another recently determined ectodomain structure of integrin α I**IIb** β 3,³⁸ which succeeded the original α V β 3 structure by seven years, incorporates a non-physiological disulfide bond at the membrane interface. Based upon the ability of the ectodomain to stabilize the association of a TM complex that carries destabilizing mutations, a mutual thermodynamic coupling of the resting ectodomain conformation and TM complex has been demonstrated.¹⁶ The structures of the resting α V β 3 and α I**IIb** β 3 ectodomains reveal a defined, well-packed membrane-proximal

$\alpha\beta$ stalk interface (Fig. 4A), which is further demonstrated by the findings of a thorough mutagenesis study³⁹ that can serve to verify such a stabilization. Thus, outside-in signaling appears to be based on ectodomain rearrangements that disrupt the $\alpha\beta$ stalk interface, which, in turn, destabilizes the TM complex.¹⁶ An ensuing dissociation of the TM complex and accompanying separation of the cytoplasmic tails may indeed occur.^{7,8} It is also noted that Mn²⁺-mediated activation does not require separation of the $\alpha\beta$ TM and cytoplasmic segments.^{7,15}

In contrast to the invariably symmetric arrangement of homodimeric TM complexes,⁴⁰⁻⁴³ the asymmetric nature of heterodimeric integrins allows for two distinct association motifs (OMC and IMC), perhaps reflective of their ability to signal outside-in as well as inside-out. Moreover, the extension of the IMC into the lipid headgroup region allows for direct structural interferences by ligands that are capable of integrin activation. Binding of the F3 domain from the talin N-terminal head domain to integrin β tails is sufficient for integrin activation⁴⁴ (i.e., dissociation of the TM complex); however, additional talin domains contribute to activation.⁴⁵ Detailed insight into talin-mediated activation has emerged from a recent talin2/ β 1D cytosolic tail complex structure and its biochemical characterization.²¹ In addition to direct competition for the conserved α IIB(R995)- β 3(D723) salt bridge,^{6,21,46,47} a membrane orientation patch (MOP) on the F2 domain has been identified that can effect a reorientation of the β TM helix tilt as a consequence of talin interaction with the intracellular membrane surface (Fig. 4B). The altered helix tilt creates a mismatch between the orientation of α IIB and β 3 found within their complex,²¹ thereby favoring the dissociated state. In turn, the dissociation of the TM complex destabilizes the membrane-proximal $\alpha\beta$ stalk interface, thereby initiating a

sequence of structural rearrangements that results in an active, adhesive receptor. There are two principal models for this rearrangement. The switchblade model suggests an extension of the extracellular domains,⁴⁸ whereas, in the deadbolt model, the ectodomain retains a compact structure.⁴⁹ Electron microscopy provides a range of compact and extended ectodomain structures,³⁸ but techniques such as fluorescent lifetime imaging microscopy (FLIM) and cryoelectron tomography suggest the absence of ectodomain extension as a direct consequence of integrin activation.^{20,50}

Outlook

Having established the structural basis of α IIB β 3 integrin TM signaling, a deeper understanding of integrin TM subunit associations in other integrin receptors is felt to be desirable. Promising work in this area has focused on integrin α LB2,^{51,52} and an initial mutagenesis study¹⁶ suggests varying affinities between different subunit combinations. From a therapeutic standpoint, the design of peptides, or the discovery of small molecules that can compete for native $\alpha\beta$ TM interactions and thereby modulate the receptor's activation state,⁵³ are of further interest.

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