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Structural Basis of Integrin Transmembrane Activation

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ABSTRACT

Integrins are cell adhesion receptors that transmit bidirectional signals across plasma membrane and are crucial for many biological functions. Recent structural studies of integrin transmembrane (TM) and cytoplasmic domains have shed light on their conformational changes during integrin activation. A structure of the resting state was solved based on Rosetta computational modeling and experimental data using intact integrins on mammalian cell surface. In this structure, the α_{IIb} GXXXG motif and their β_3 counterparts of the TM domains associate with ridge-in-groove packing, and the α_{IIb} GFFKR motif and the β_3 Lys-716 in the cytoplasmic segments play a critical role in the α/β association. Comparing this structure with the NMR structures of the monomeric α_{IIb} and β_3 (represented as active conformations), the α subunit helix remains similar after dissociation whereas β subunit helix is tilted by embedding additional 5–6 residues into the lipid bilayer. These conformational changes are critical for integrin activation and signaling across the plasma membrane. We thus propose a new model of integrin TM activation in which the recent NMR structure of the $\alpha_{IIb}\beta_3$ TM/cytoplasmic complex represents an intermediate or transient state, and the electrostatic interaction in the cytoplasmic region is important for priming the initial α/β association, but not absolutely necessary for the resting state. J. Cell. Biochem. 109: 447–452, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: INTEGRIN; CELL ADHESION; TRANSMEMBRANE SIGNALING

Integrins are a family of cell adhesion receptors that transmit bidirectional signals across the plasma membrane and regulate many biological functions, including cell differentiation, migration, growth, and survival. These receptors are heterodimeric transmembrane (TM) proteins containing two non-covalently associated α and β subunits, each with a large extracellular domain, a single spanning TM domain and a short cytoplasmic domain. The structures of the extracellular fragments of integrins $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ revealed a compact, V-shaped conformation, with each leg bent [Xiong et al., 2001, 2002; Zhu et al., 2008]. Increasing number of studies have together established that in the resting state, in which integrins exhibit low affinity for ligands, the extracellular domain exists in the bent conformation that is stabilized by specific α/β interfaces that exist in extracellular, TM and cytoplasmic domains (Fig. 1A), whereas priming and ligand binding induce a large-scale conformational rearrangement in which the integrin extends in the extracellular domains with two separated legs (Fig. 1B,C) [Takagi et al., 2002, 2003; Kim et al., 2003; Xiao et al., 2004; Luo et al., 2007; Zhu et al., 2007]. However, the mechanism of integrin signaling across the plasma membrane remained elusive until recently due to the fact that the structural information of TM and cytoplasmic domains was controversial. In the last few months, several papers have been published on the structures of the complex and isolated monomers of the integrin TM and cytoplasmic domains; consequently, these studies have greatly advanced our understanding of TM activation [Lau et al., 2009, 2008b; Zhu et al., 2009]. In this review, we focus on the current progress on the structural basis of integrin signaling across the plasma membrane.

DISULFIDE/ROSETTA STRUCTURE OF INTEGRIN TM AND CYTOPLASMIC DOMAINS IN THE RESTING STATE

TM HELIX–HELIX ASSOCIATION IN THE RESTING STATE

In the crystal structures of the full-length $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ ectodomains, integrins are in the bent conformation with the α and β subunit C-termini only a few angstroms apart [Xiong et al., 2001; Zhu et al., 2008], consistent with association of the α and β subunit TM domains. Many studies show that deletions or mutations in the α and β subunit TM and cytoplasmic domains, which are expected to destabilize α/β association, activate integrins [O'Toole et al., 1991, 1994; Hughes et al., 1996; Lu and Springer, 1997; Lu et al., 2001]. Specific interfaces on the integrin α and β subunit TM domains have been defined by several mutagenesis studies and

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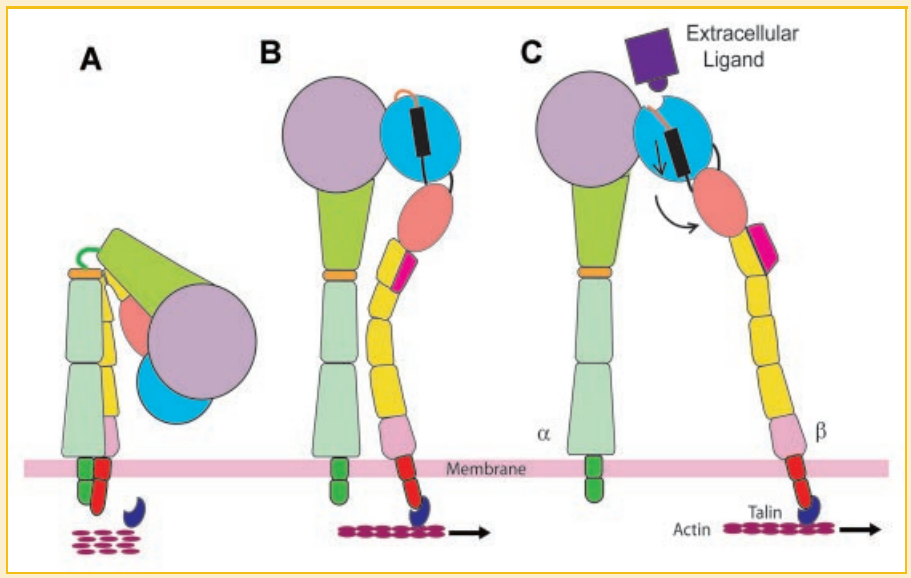

Fig. 1. Integrin conformational regulation. A: Cartoon drawing of integrin structure in the bent conformation. B,C: An extended model of $\alpha_{IIb}\beta_3$ by torsion at the α and β knees with closed (B) and open (C) headpiece. Assembled actin filaments bind integrin β subunit cytoplasmic domain through talin, and lateral forces transmitted by actin cytoskeleton cause integrin extension, leading high affinity for extracellular ligands.

disruption of these interactions results in activation [Luo et al., 2004, 2005; Li et al., 2005; Partridge et al., 2005]. A large number of papers have been published on how integrins associate in the membrane using mutagenesis data and computational modeling. Last year, a review article describes dozens of biophysical and modeling studies on the $\alpha_{IIb}\beta_3$ TM and cytoplasmic domains [Wegener and Campbell, 2008]. These models propose different orientations, crossing angles and crossing positions between the α and β TM helices [Wegener and Campbell, 2008]; however, it is unclear which one represents the real structure in physiological conditions.

Recently, a new developed method in combination of disulfide scanning with Rosetta computational modeling has been used to solve the structure of the integrin TM and cytoplasmic domains (denoted as the Disulfide/Rosetta structure) [Zhu et al., 2009]. Since the structure is obtained based on experimental data using intact integrins with the extracellular and cytoplasmic domains that regulate TM association on the mammalian cell surface, we believe that it most likely represents the physiological structure in the resting state. In this structure, α helices make up most of the TM domain (α_{IIb} residues 966–988 and β_3 residues 693–715) and cytoplasmic domain (α_{IIb} residues 989–998 and β_3 residues 716–737) except that the α_{IIb} helix terminates soon after the TM domain. From the exofacial region of the TM domain, two helices associate with ridge-in-groove packing along most of the interface (Fig. 2A), and then towards the cytoplasmic side of the membrane, the α_{IIb} and β_3 TM helices grow farther apart.

It has been found that GXXXG motifs are common among many TM proteins. This motif is critical to form homodimeric interface in glycophorin A [Popot and Engelman, 2000], and has been proposed to mediate similar associations in many other membrane proteins consisting of two-helix bundles. The similar motifs are also found in both integrin α and β TM domains, and considered to get involved either in integrin homodimeric interaction [Li et al., 2001, 2003; Gottschalk and Kessler, 2004], or in heterodimeric association [Adair and Yeager, 2002; Gottschalk et al., 2002; Schneider and Engelman, 2004; Gottschalk, 2005; Zhu et al., 2009]. In the Disulfide/Rosetta structure [Zhu et al., 2009], Gly-972 and Gly-976 of the GVLGG motif in α_{IIb} closely associate with β_3. However, unlike other assays [Schneider and Engelman, 2004], Ser-699 and Ala-703 in the β_3 SVMGA sequence point away from the interface instead of interacting with α_{IIb} Gly-972 and Gly-976, respectively. The G972-VLG-G976 motif in α_{IIb} and its β_3 counterpart contribute to ridge-in-groove packing, which has been found to be a common packing mode between two adjacent α helices [Chothia et al., 1981]. The ridge on α_{IIb} subunit is formed by residues W968, G972, G976, and L980, and the groove on β_3 subunit is by residues V696 and L697, V700 and M701, I704 and L705, and G708 and L709 (Fig. 2A). This interhelical interface is consistent with a series of mutational studies which showed that the most activating, and hence structurally disruptive mutations within the TM domains, are of the three interfacial Gly residues, α_{IIb} Gly-972 and Gly-976, and β_3 Gly-708 [Luo et al., 2005; Partridge et al., 2005; Zhu et al., 2009]. By contrast, mutations of α_{IIb} Gly-975 and β_3 Gly-702, which are not interfacial, are not activating.

ASSOCIATION OF THE INTEGRIN CYTOPLASMIC DOMAINS IN THE RESTING STATE

The cytoplasmic domains also play a critical role on integrin regulation. Membrane-proximal cytoplasmic region of integrins contains GFFKR and LLXXXHDRRE in α and β subunits respectively have been shown to be structurally and functionally important [Calderwood, 2004]. The highly conserved GFFKR motif has a structural role in TM association and thus is functionally important. Mutation of any of the residues in this motif activates integrins by destabilizing association of the α and β subunit TM domains [O'Toole et al., 1994; Hughes et al., 1996; Lu and Springer, 1997; Lu

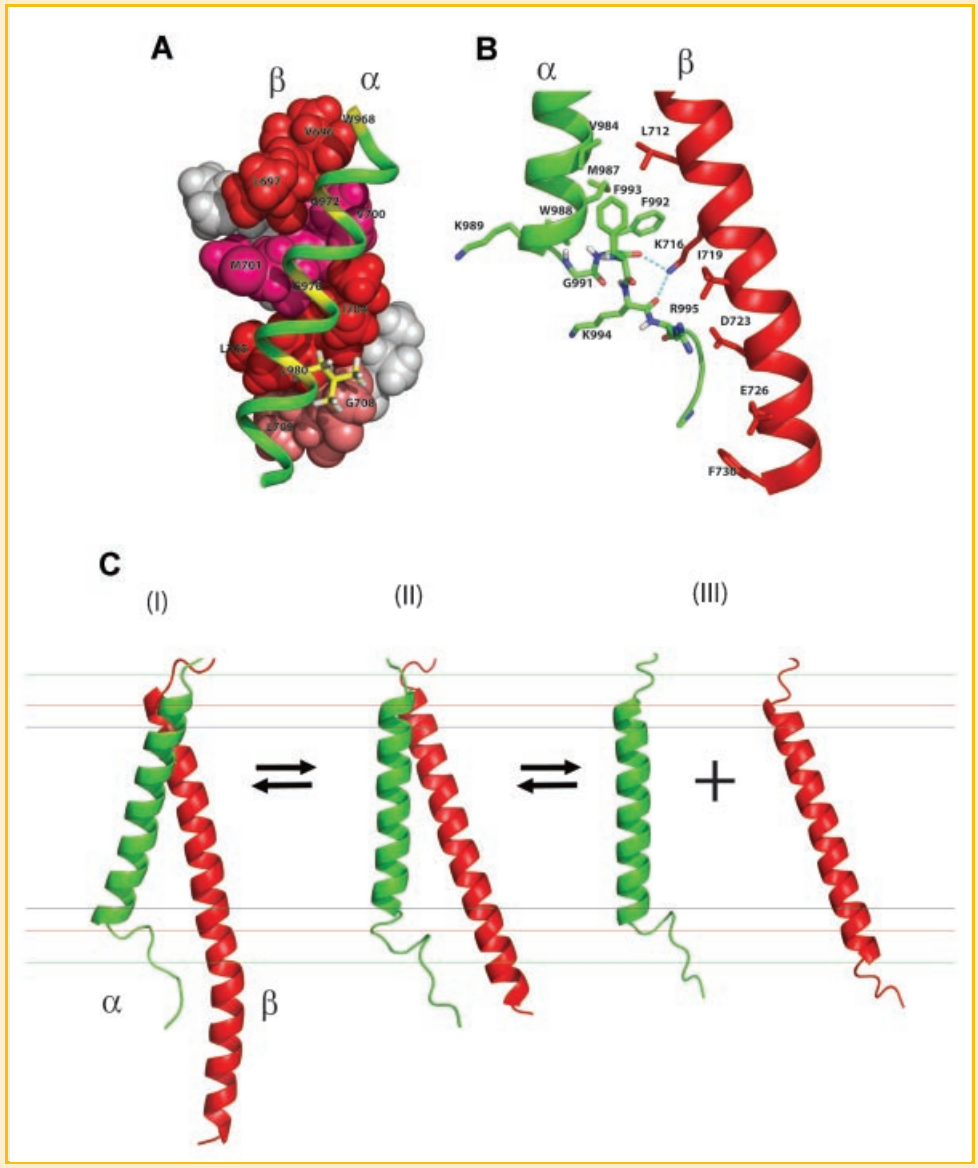

Fig. 2. The Disulfide/Rosetta structure of integrin TM and cytoplasmic regions and model of integrin TM activation. A: The interface between two associating TM domains on the cell surface. The ridge on α_{IIb} subunit is formed by residues W968, G972, G976, and L980, shown in yellow in cartoon representation, and the groove on β_3 subunit is by residues V696 and L697, V700 and M701, I704 and L705, and G708 and L709 which are shown in sphere representation. B: Cytoplasmic fragment association of integrin α_{IIb} and β_3 subunits in the Disulfide/Rosetta structure. C: Predicted model of integrin TM activation. (I) The resting state represented as Disulfide/Rosetta structure; (II) the "intermediate" or "transient" state represented as NMR structure of integrin $\alpha_{IIb}\beta_3$ TM and cytoplasmic domain peptides (PDB ID 2K9J); (III) the activated state represented as monomeric α_{IIb} and β_3 NMR structures (PDB ID codes 2K1A and 2RMZ). The outer bounds of the hydrophobic, interface, and polar region of the membrane are shown as black, red, and green lines, respectively.

et al., 2001]. In addition, a salt bridge between Arg-995 of the α_{IIb} GFFKR motif and Asp-723 of β_3 has been suggested to stabilize the resting state [Hughes et al., 1996]. However, previous NMR studies on the cytoplasmic domains including the GFFKR motif have yielded widely different α/β complex structure, or a lack of association [Ulmer et al., 2001; Vinogradova et al., 2002, 2004; Weljie et al., 2002].

The recent Disulfide/Rosetta structure [Zhu et al., 2009] provided a clear picture on how α and β subunit cytoplasmic domains interact and associate since it was obtained from the intact receptor in the mammalian cell membrane under physiological conditions. The α_{IIb} TM α-helix extends beyond the 23-residue TM hydrophobic segment through Lys-989 and Val-990, and then Gly-991 of GFFKR is a turn which changes the TM right-handed α-helix to left-handed one, making Phe-992 and Phe-993 sit in the interface of α_{IIb} and β_3 at the membrane/cytoplasm interface (Fig. 2B). Phe-993 is located in the center of two helices where it contacts with β_3 subunit Leu-712 and Lys-716 and is surrounded by Trp-988, Met-987, and Phe-992 from α_{IIb}. Both sidechains of the Phe residues orient towards the hydrophobic core of the membrane. A salt bridge between α_{IIb} Arg-995 and β_3 Asp-723 was proposed previously based on mutagenesis data as mentioned above [Hughes et al.,

1996]. In the structure, Arg-995 is close to both Asp-723 and Glu-726, consistent with this electrostatic interaction. However, there are a variety of different conformations of the sidechains of Arg-995 and Asp-723, indicating that this salt bridge is not absolutely necessary for the association. Some other computational modeling studies place such great emphasis on this electrostatic interaction that the resulting models are likely biased.

The β_3 continues as an α-helix beyond the membrane into cytoplasmic segments (residues 716–732). β_3 residue Lys-716 has the most important role in the α_{IIb} interface. The aliphatic portion of the Lys-716 sidechain is in the interface with the α_{IIb} GFFKR motif and remarkably, the Lys-716 ε-amino group hydrogen bonds to the α_{IIb} backbone (Fig. 2B). Mutations of Lys-716 to any other residues activate integrins for ligand binding [Zhu et al., 2009]. This Lys-716 is structurally critical for α/β association as well as for the tilting of the dissociated β TM and cytoplasmic helix in the plasma membrane.

Previous NMR structures of the complex between the α_{IIb} and β_3 juxtamembrane/cytoplasmic domains in aqueous media have yielded conflicting results, and none of them resemble the Disulfide/Rosetta structure. Since they were constructed by cytoplasmic segments without TM and ectodomains, it is not surprising that the unique backbone reversal of the Phe residues in GFFKR motif was missing in these constructs. Furthermore, it has been shown that in intact cells when the GFFKR motif is deleted, the association between the α and β TM segments is also missing [Luo et al., 2004]. These findings together demonstrate cooperativity between these segments for folding and assembly, consistent with their intimate interaction in the Disulfide/Rosetta structure [Zhu et al., 2009].

NMR STRUCTURE OF INTEGRIN TM AND CYTOPLASMIC DOMAINS IN THE αβ COMPLEX CONFORMATION

It is interesting that the similar interhelical interface was also found in the recent NMR structure of integrin $\alpha_{IIb}\beta_3$ TM and cytoplasmic domain peptides [Lau et al., 2009]. The structure was determined in the presence of phospholipid bicelles. The GVLGG motif in α_{IIb} and its β_3 counterpart associate, consistent with the Disulfide/Rosetta structure. However, the NMR structure has several substantial differences in comparison with the Disulfide/Rosetta structure. First, the β_3 helix ends at residue Asp-723 and thus is significantly shorter. It is unclear at present whether this structural difference is physiological relevant, and whether it is important during integrin signaling. Second, the TM helices cross within their exofacial region at an angle of 25° and are asymmetric (α is straight and β is tilt) within membrane in the NMR structure. As a result, the β_3 helix that is embedded in the lipid membrane is 29 residues instead of 23. This helix is tilted almost identically to the dissociated monomeric β_3 helix, as discussed below. Third, the interactions between the α and β subunit C-terminal TM region and cytoplasmic domains are different. In NMR structure, α_{IIb} (F992–F993) contacts with β_3 W715 and I719. In addition, Lys-716 is less important in this structure due to the lack of association between Lys-716 and Phe residues in GFFKR motif.

The NMR structure was solved using an artificial hydrogen-bond constraint between the α_{IIb}(R995)–β_3(D723). The presence of the salt bridge was based on the fact that mutations of either residue affected the helix–helix interaction as monitored by NMR. In the Disulfide/Rosetta structure, the α_{IIb} Arg-995 and β_3 Asp-723 are close, but only about 20% of the representative structures are within hydrogen bonding distance, indicating that this salt bridge is not absolutely necessary for the association. Thus the C-terminal region of the NMR complex structure might be over-constrained. We suggest that this electrostatic interaction is important for the priming of α/β interaction. After forming more stable α/β interaction, the salt bridge is not critical for further stabilization. So the NMR structure might represent an intermediate or ''transient'' state between the physiological resting state (represented as the Disulfide/Rosetta structure) and the dissociated active state (represented as the NMR structure of the isolated monomers as discussed below) (Fig. 2C). It is interesting that the NMR structures of the complex have almost identical structures and angles with the membrane to the isolated monomers, and there were substantial amounts of α_{IIb} and β_3 monomers present in the solution used for determining the NMR complex structures. These observations confirm our hypothesis that the NMR structure of the complex is an intermediate or ''transient'' state.

NMR STRUCTURES OF THE INTEGRIN TM AND CYTOPLASMIC DOMAINS IN THE ACTIVE STATE

Rearrangements of integrin domains are necessary for integrin activation. Several different models of the integrin TM and cytoplasmic domain conformational changes have been proposed, including separation, hinging and piston-like motions [Williams et al., 1994; Gottschalk et al., 2002; Wegener and Campbell, 2008]. Accumulated data tend to support separation of α and β TM and cytoplasmic domains in integrin activation. First, ectodomain-TM domain linkers are flexible, indicating that the large-scale movement (like separation) of TM domain is necessary to trigger conformational change of ectodomain [Zhu et al., 2008]. FRET studies suggested that unclasping of TM and cytoplasmic domains is required for integrin signal transduction [Kim et al., 2003]. Mutational studies showed that disrupting the association between α and β TM and cytoplasmic domains resulted in integrin activation by increasing affinity for extracellular ligand binding [Lu et al., 2001; Partridge et al., 2005]. By contrast, integrins remained in the inactive low-affinity state when two legs were exchanged or cytoplasmic domains were replaced with acidic and basic peptides or an artificial clasp [Lu et al., 2001; Takagi et al., 2001]. Disulfide scanning study also confirmed TM domain separation but not hinging or piston-like motions as the mechanism of TM signaling [Luo et al., 2004]. Together, all these experiments demonstrated that separation of α and β subunits in this region is necessary for integrin signaling. Indeed, this mechanism was further verified by the fact that introducing disulfide bonds to block α and β dissociation

locked the receptor in the resting state, whereas mutating interface residues to leucine that disrupts α and β association in TM region resulted in activation [Luo et al., 2004, 2005].

The NMR structures of isolated α_{IIb} and β_3 TM/cytoplasmic domain fragments were resolved in phospholipid bicelle [Lau et al., 2008a,b]. In contrast to findings that similar α_{IIb} and β_3 constructs formed homomultimers in detergents, they found that these subunits were monomeric in the more bilayer-like bicelle environment. These structures are believed to represent the physiologically active state in which the TM and cytoplasmic domains are in the dissociated monomeric conformation. By comparing these dissociated monomeric α_{IIb} and β_3 structures with the Disulfide/Rosetta structure and NMR structure of the complex, we are now able to elucidate the conformational change of the TM and cytoplasmic domains during integrin activation.

The isolated α_{IIb} and β_3 TM/cytoplasmic NMR structures are similar with the Disulfide/Rosetta structure. The α_{IIb} GFFKR packing is almost the same in the two structures. The only significant difference of the α_{IIb} in this region is the tilt between the TM and justamembrane segments. This tilt brings Phe-993 in monomeric α_{IIb} 2 Å closer to Val-984 so that the van der Waal contact is formed between them. In the $\alpha_{IIb}\beta_3$ complex, β_3 Leu-712 interacts with α_{IIb} Val-984, and there is no room for Phe-993 to come that close. Thus, some conformational adjustments must occur between the associated and dissociated states of the α_{IIb} subunit.

The dissociated β_3 TM domain solved by NMR appeared to be a 30-residue (I693–H722) linear α-helix extended into the cytoplasm, and instead of the 23 TM residues in the Disulfide/Rosetta complex structure, 29 residues (I693–I721) appeared to be embedded in the bicelle core. The embedding of these residues did not change when lipid with different chain length was used, indicating that it is the energetically favorable state of the monomeric β_3 helix in lipid environment [Lau et al., 2008b]. This result is consistent with the boundary determination by glycosylation mapping studies in which monomeric β_1 TM domain was used. The border between the β_1 TM and cytoplasmic domain was found to be I721 [Stefansson et al., 2004], which corresponds to the β_3 I721. In the NMR structure, β_3 Lys-716 is followed by a 5-residue hydrophobic segment (L717LITI721), and the continuous helix spanning the TM and justamembrane segments could undergo a substantial tilt in the membrane, with snorkeling of the Lys-716 sidechain into the polar environment [Lau et al., 2008b]. By contrast, the β_3 helix embedded in lipid membrane in the Disulfide/Rosetta complex structure is significantly shorter, suggesting that after dissociating from the α_{IIb} helix, the β_3 helix is tilted due to inserting of 5–6 additional hydrophobic residues to the hydrophobic lipid environment. The tilting of the β_3 helix may be important for integrin activation and signaling. By contrast, in the NMR structure of the TM/cytoplasmic complex the tilting angle of the β_3 helix in the membrane is identical to the monomeric helix, suggesting that before the full association of the α and β TM helices, there is no substantial conformational change of the two helices, and the initial association may be primed by the electrostatic interaction between the α_{IIb} Arg-995 and β_3 Asp-723. Thus, the integrin TM/cytoplasmic signaling involves equilibrium of three states: a resting state, an intermediate or transient state, and an active state (Fig. 2C).

INTEGRIN ACTIVATION ACROSS THE PLASMA MEMBRANE

The inside-out activation of integrins involves binding of intracellular proteins to integrin cytoplasmic domains, leading to switchblade-like conformational change of integrin extracellular domains (Fig. 1). As described above, this process is coupled with following conformational changes of the integrin TM and cytoplasmic domains: (1) separation of two TM and cytoplasmic helices; (2) following separation, α subunit helix maintains the similar structure, whereas the β subunit helix is tilted by inserting 5–6 additional residues into the hydrophobic lipid membrane core. Obviously, binding of integrin TM and cytoplasmic domains to intracellular proteins that induces separation will result in integrin ligand binding. For one typical example, talin, an important intracellular protein, binds to NPXY motif at β_3 residues 744–747 through their FERM domains as well as to more membrane-proximal β_3 regions, including Phe-727 and Phe-730 [Wegener et al., 2007]. The Disulfide/Rosetta structure shows that these residues face the α_{IIb} cytoplasmic tail and thus the binding of talin to this membrane proximal region activates integrins by interfering with the interface between the α and β cytoplasmic domains. Indeed, talin clashes with α_{IIb} residues that interact with the β_3 α-helix in the superposition of the talin β_3 and $\alpha_{IIb}\beta_3$ complexes. Thus, binding of talin to β_3 could lead to the α_{IIb} and β_3 TM and cytoplasmic domain separation by perturbing its association with α_{IIb}.

In addition to the above mechanism, another possible way for integrin activation may come from force transmission from the actin cytoskeleton [Zhu et al., 2008]. Ligand binding by integrins can be activated by force applied to the actin cytoskeleton that binds to the β cytoplasmic domain. As described above, during integrin activation, α subunit TM and cytoplasmic domains assume similar structure to that in the complex. It is interesting that in the recent crystallography structure of integrin $\alpha_{IIb}\beta_3$ ectodomains, the bottom of the α_{IIb} subunit calf-2 domain is hydrophobic [Zhu et al., 2008]. It is possible that there are interactions between the plasma membrane with the calf-2 domain's broad base and unstructured loops, and this interaction will create friction to resist the movement of the β TM domain. Lateral force exerted on the β cytoplasmic domain by actin polymerization and contraction will pull the β-leg away from the α-leg and further open the headpiece with the hybrid domain swung out laterally in the direction of the pulling force (Fig. 1). This extended open conformation has high affinity for ligand. Application of lateral force would tilt the membrane-embedded segments in the plane of the membrane, and affect the equilibrium between formation and dissociation of the $\alpha_{IIb}\beta_3$ membrane complex. Thus the tilting of the β subunit TM domain may play an important role on integrin activation by stabilizing the dissociated helix in the membrane and facilitating force-induced conformational change. However, the significance of the tilting of the β helix in integrin outside-in signaling remains unclear. It will be of great interest to understand the structural basis of the binding of

intracellular signaling molecules to the β integrin TM/cytoplasmic tail in the lipid environment.

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