Signal Transduction

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The Transmembrane Structure of Integrin αIIbβ3: Significance for Signal Transduction**

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The formation of multicellular organisms requires concerted action by cells, which alter their adhesive and migratory behaviors. Cell adhesion and migration are tightly regulated by intra- and extracellular signals, which are conveyed through the cellular membrane by specialized receptors known as integrins.[1] Integrins are the starting point of a variety of signaling cascades and are involved in a multitude of physiological events important to multicellular organism morphogenesis, ranging from cell adhesion to migration, apoptosis, and angiogenesis as well as pathophysiological behaviors such as those found in cancer metastasis.[2] The transmembrane (TM) domains of integrins are at the center of integrin signaling.[3] Recently, a structure of the TM domains of the αIIbβ3 integrin has been reported that sheds light on the signal transduction mechanism of integrins.[4–7]

Integrins are essential TM proteins that couple the extracellular matrix to the cytoskeleton. They consist of noncovalently bound heterodimers, in which each subunit (α and β) contains one TM helix.[8] With eighteen α and eight β subunit types identified, there are 24 known distinct heterodimer combinations with partially overlapping yet specific function.[9]

Cells regulate their integrin-mediated adhesion through a variety of mechanisms on different time scales. On the slower time scale, expression patterns are altered by external signals, such as growth factors.[10] On faster time scales, integrins can be redistributed (by clustering or recycling) on the cellular surface,[11] change their intracellular attachment state to the cytoskeleton[12] as exemplified by different lateral mobilities of integrins on a single cell,[13] or alter the affinity state for their extracellular ligand (integrin activation).[14] Changes in the affinity state are correlated with integrin conformational changes,[14] which act as one potential mechanism to relay signals either from the extracellular to the intracellular space (outside-in signaling) or vice versa (inside-out signaling). Integrins are bidirectional signaling molecules, as both signaling directions take place along an allosteric pathway.[15] The two TM helices of the integrin heterodimer are pivotal in signaling events as linkers between the extracellular and intracellular domains. Hence, a variety of groups have pursued different strategies to understand the role of the TM domains in signaling.[16–23]

It was postulated that the TM helices were not merely connectors between the extra- and the intracellular space but active structures forming specific heterodimers.[24,25] However, the structural details of this TM heterodimer have remained elusive until recently. In their recent report, Lau et al. have solved the structure of the αIIbβ3 integrin heterodimer in its resting state using highly sophisticated, well-designed NMR spectroscopy experiments on the TM domains in bicelles.[7] This experimentally determined structure allows a solid, structural basis for the prior experimental biochemical results by providing a foundation for an atomistic understanding of TM integrin signaling.

The experimental structure shows a right-handed helical dimer (Figure 1). Given that the majority of soluble helix dimers form left-handed structures,[26] this unusual right-

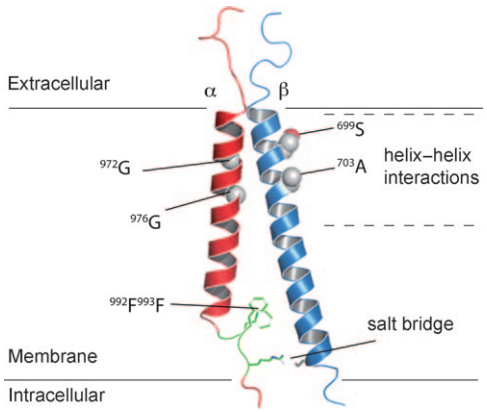

Figure 1. Overview of the αIIbβ3 integrin TM structure recently solved by Lau et al. The α subunit is shown in red, the GFFKR motif in green, and the β subunit in blue. Several important residues described in the text are highlighted.

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handed conformation and the resulting lower thermodynamic stability may play an important role in signaling. It has been demonstrated that the TM domains separate in the course of signaling.[27] Therefore, a fine-tuned energy balance favoring specific interactions versus the propensity to separate needs to be encoded in the interaction between these helices. A right-handed helical dimer is particularly suited by offering the needed structural flexibility. This propensity to separate, which complicated the structure determination of this important complex,[28] is also reflected by the low affinity of the helices for each other.[29]

The TM structure can be divided into two different interaction regions. Adjacent to the extracellular domains, the helices form canonical helix–helix interactions in tight contact. Proximal to the intracellular face of the membrane, an unusual loop comprising the highly conserved GFFKR[25,30] motif of the α subunit makes important interactions between the subunits, while the helices are already well-separated.

Near the extracellular domain, the conserved ^{972}Gxxx^{976}G motif in the α subunit, a well-known interaction motif of TM helices (x is a nonconserved amino acid),[31] is located at the interface of the dimer to allow tight interactions with the β subunit (Figure 1). A similar but less canonical motif of the β subunit (^{699}Sxxx^{703}A) points out from the interface and does not participate in the interaction. The relevance of this motif remains to be determined, but it may be important for lateral association with other TM proteins such as tetraspanins or integrins in integrin clustering, or it might be involved in transient TM conformations. It would be instructive to mutate these residues in animal models to test their significance.

Towards the intracellular interface, the C-terminal end of the α subunit contains an unusual loop (initiated by ^{991}G in the α subunit). Gly is a well-known helix breaker, given its highly flexible nature resulting from its lack of side-chain atoms. The main contact between the subunits is formed in this loop region by the ^{992}F^{993}F motif and the subsequent salt bridge (Figure 1). These interactions have previously been shown to be indispensable in keeping the integrin in its resting state.[25,30]

Integrins are subjected to force during the mechanical processes of adhesion and migration. In fact, force has been identified as a facilitator for integrin activation.[32] Interestingly, the deviation from helicity in the α subunit may be significant for integrin function: if force is acting on the α subunit tail, the nonhelical loop would facilitate activation under applied force, as less of an energetic penalty is paid for straightening a less-ordered loop than a hydrogen-bond-stabilized helix. Hence, this loop is an attractive candidate for a trigger in integrin activation by force (Figure 2). Surprisingly, no such trigger is structurally obvious in the canonical helix of the β subunit, given that the main intracellular adaptor protein talin, which connects the integrin to the cytoskeleton and is likely the force-transducing protein, binds to the β and not the α subunit. It would be interesting to identify adaptor proteins that bind to the α subunit and facilitate activation by mechanical structural changes in the loop region of the α subunit. Recent evidence suggests that, at least in the case of the integrin α4β1, paxillin might be a candidate for such a mechanism.[12]

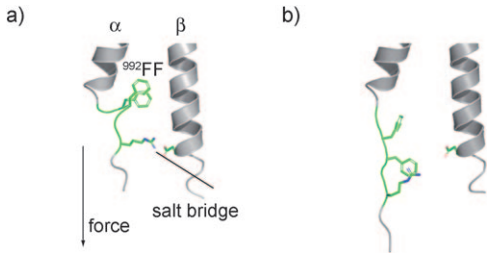

Figure 2. Potential impact of force on the α subunit. a) Experimental structure. GFFKR motif is shown in green. b) Force-induced straightening of the GFFKR motif (putative model).

The formation of a complex between the integrin β tail and talin has been shown to be the common final step in integrin activation.[33] During complex formation, talin displaces the α subunit, thus inducing TM separation and integrin activation.[34,35] The new structure of the TM domains in combination with recently solved structures of the integrin–talin complex now enables a structural test of this hypothesis. Surprisingly, talin can bind to the integrin β subunit without structural constraints imposed by the α subunit (Figure 3).

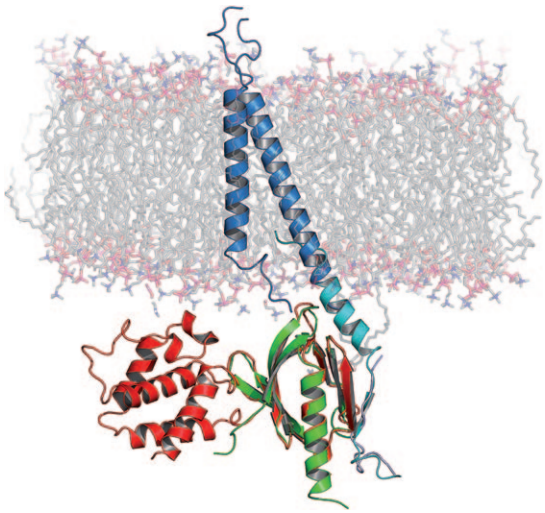

Figure 3. Concatenation of TM structure (blue) and two integrin–talin complexes (red and green). Background: approximate position of the membrane.

Hence, a possible mechanism of a purely sterical displacement of the α subunit needs to be refined. It must be remembered, however, that the intracellular tail of the α subunit is longer than the portion that was resolved by NMR spectroscopy.

In the past, models of the TM domains have been generated by a variety of approaches.[19,24,36–42] The publication of the structure of the TM domains allows the predictive power of TM structure versus computer modeling to be tested. We published in 2002 a first computational model of the core of the TM domains of the integrin αIIbβ3 calculated in the absence of experimental data on the TM domains.[37] Despite this lack of experimental support at the time of

calculation, the predicted conformation and the experimental structure are identical with a root-mean-square deviation (RMSD) of less than 1 Å across the Cα atoms over 34 residues (Figure 4). Later computations by the groups of

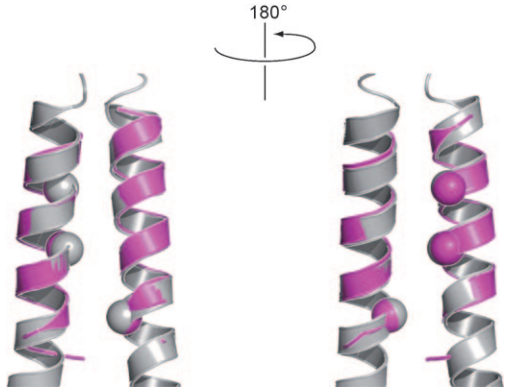

Figure 4. Superposition of experimental (gray) and predicted (magenta) TM domain conformation. Two Gly residues on the α chain and one Gly on the β chain are shown as spheres to demonstrate rotational orientation for a front and rear view. The predicted conformation was superimposed on Model 1 of the NMR ensemble.

DeGrado,[39] Torres,[41] and most recently Springer,[43] obtained with different methods, led to very similar models. The Springer group even correctly predicted the unusual C-terminal loop of the α subunit using a large number of experimental restraints together with an ab initio structure-prediction tool. This close agreement between experiment and computation impressively underlines the power of computational approaches for these kinds of systems.

Obtaining the structure of integrin TM domains provides the foundations for a structural understanding of TM integrin signaling. However, open questions remain. These include the impact of force on the TM domains, the possible existence of structural intermediates between complexed and uncomplexed subunits, and the mechanism of TM activation through talin binding. Other important issues are the possibility of formation of homooligomers and their role in formation of focal adhesion. The molecular nature of cell migration along concentration gradients as well as the spatiotemporal demands of chemotactic proteins in cooperation with integrin ligands also require further study.[44,45] Furthermore, the details of transducing information of ligand binding from the headgroup to the transmembrane region or vice versa are still not well-understood. The now available structure of the transmembrane complex is a tremendous step forward in understanding the function of these important molecules. Future computational and experimental efforts will address the questions on the basis of this structure.

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