Integrin activation takes shape

R.C. Liddington[1] and M.H. Ginsberg[2]

[1]Program in Cell Adhesion, The Burnham Institute, La Jolla, CA 92037
[2]Division of Vascular Biology, Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037

Integrins are cell surface adhesion receptors that are essential for the development and function of multicellular animals. Here we summarize recent findings on the regulation of integrin affinity for ligand (activation), one mechanism by which cells modulate integrin function. The focus is on the structural basis of integrin activation, the role of the cytoplasmic domain in integrin affinity regulation, and potential mechanisms by which activation signals are propagated from integrin cytoplasmic domains to the extracellular ligand-binding domain.

Introduction

Integrin adhesion receptors mediate the attachment of cells to each other and to their surrounding extracellular matrix and participate in diverse events such as hemostasis, immunity, inflammation, and in pathological processes such as atherosclerosis (Schwartz et al., 1995). Integrins are noncovalent heterodimers of type I transmembrane protein subunits termed α and β. Each subunit has a large (>700 residue) NH_2-terminal extracellular domain. A single membrane-spanning domain links this extracellular domain to a generally short (13–70 residue) cytoplasmic domain. Integrin-mediated adhesion is rapidly and precisely regulated, a process that is often a central aspect of integrin function and depends on the integrin cytoplasmic domain interactions intracellular proteins (Liu et al., 2000). Cells can regulate integrin-mediated adhesion by changing integrin affinity for ligand (activation) (Schwartz et al., 1995). Rapid changes in affinity have been widely documented among integrins; however, affinity-independent mechanisms, including changes in cell shape, cooperative interactions promoted by integrin clustering, and changes in the diffusion of integrins in the plane of the membrane, are also important. The focus of this review is integrin affinity regulation (activation) and the structural mechanisms by which these changes in affinity occur.

Address correspondence to Robert C. Liddington, Program in Cell Adhesion, The Burnham Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: (858) 646-3136. Fax: (858) 646-3136.
E-mail: rlidding@burnham.org; or Mark H. Ginsberg, Div. of Vascular Biology, Dept. of Cell Biology, The Scripps Research Institute, VB-2, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: (858) 784-7124. Fax: (858) 784-7343. E-mail: ginsberg@scripps.edu

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The inside story of activation

Cytoplasmic interactions between integrin α and β subunits may regulate the affinity of the integrin extracellular domain by an allosteric mechanism (Williams et al., 1994; Hughes et al., 1996). This mechanism implies transmission of long range conformational rearrangements through the membrane-spanning region (Du et al., 1993). Mutagenesis of the membrane proximal regions of integrin cytoplasmic domains (which are highly conserved within α and β subunits) suggested that integrins are maintained in a default low affinity state, in part, by charge interactions between the α and β subunits (for integrin αIIbβ3, αIIb Arg^{995}, and β3 Asp^{723} [Hughes et al., 1996]). Indeed, the interaction between integrin α and β tails can regulate activation (Lu et al., 2001). In nuclear magnetic resonance spectroscopy (NMR)* analysis, when tethered to a lipid vesicle the integrin αIIb cytoplasmic domain adopts a closed conformation with its COOH-terminal acidic region proximal to the juxtamembrane region (Vinogradova et al., 2000) while it is unfolded in solution. In the predicted structure, αIIb Arg^{995} is surface exposed and thus could interact with the β3 Asp^{723} to mediate a proposed salt bridge (Hughes et al., 1996). Certain mutations, which activate integrin αIIbβ3, open the closed conformation of the αIIb cytoplasmic domain (Vinogradova et al., 2000). Interestingly, NMR studies with recombinant "miniintegrins" in liposomes and aqueous solution (Li et al., 2001; Ulmer et al., 2001) have not confirmed interactions of the integrin αIIb and β3 cytoplasmic domains. In contrast, other studies using smaller fragments of the cytoplasmic domains suggested such interactions (Weljie et al., 2002). These disparities may owe to subtle differences in experimental conditions used. However, the interaction of these cytoplasmic domains with each other may require some third factor such as a membrane component or cytoplasmic protein. Indeed, the interaction between these tails was readily demonstrated in the presence of antibodies directed against combinatorial epitopes formed by their interaction (Ginsberg et al., 2001). However, it is clear that the changes in the structure and/or interactions of the α and β tails is an important determinant of integrin activation (O'Toole et al., 1994; Hughes et al., 1996).

*Abbreviations used in this paper: ERM, ezrin, radixin, and moesin; FERM, band four-point-one, ezrin, radixin, moesin homology; MIDAS, metal ion-dependent adhesion site; NMR, nuclear magnetic resonance spectroscopy; PTB, phosphotyrosine binding.

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How is the activation signal transmitted? How does regulatory information contained within the cytoplasmic domains of integrins propagate through the transmembrane domain to the ligand-binding site? Integrin transmembrane domains are typically thought to begin after an extracellular proline residue. The boundary between the transmembrane domain and the cytoplasmic domain is less clear (Williams et al., 1994). Most human integrin α and β subunits contain a conserved, positively charged amino acid (Arg or Lys) positioned ~23 hydrophobic amino acid residues COOH-terminal of the predicted start of the transmembrane domain. This conserved K/R residue is generally (with the exception of β4 and β8) immediately followed by a stretch of four to six hydrophobic residues, resulting in the K/R residue being flanked by hydrophobic regions. For this reason, it was proposed previously to be a weak "stop transfer" signal, demarcating the beginning of integrin α and β cytoplasmic domains (Williams et al., 1994). In an earlier review, we proposed four alternative movements (involving the conserved K/R residue) of the transmembrane domains relative to each other to explain proposed allosteric rearrangements in integrins (Williams et al., 1994). New evidence favors one of those models, the "piston" model. Elegant glycosylation mapping studies (Armulik et al., 1999) suggest that the hydrophobic region just COOH-terminal to the conserved K/R is membrane-embedded in integrin subunits α2, α5, β1, and β2. These same studies demonstrated that decreasing the hydrophobicity of this region likely shortens the transmembrane domain by extending these residues out of the membrane. This mobility of the transmembrane domain is likely facilitated by the fact that the minimal length of hydrophobic residues required to span the plasma membrane is ~20, and most of the integrin subunits contain a 27 residue hydrophobic stretch (Williams et al., 1994) (inclusive of the conserved K/R). Thus, the length or orientation of the integrin transmembrane domains could vary depending on the nature of this conserved hydrophobic region. Several "activating" mutations disrupt this hydrophobic region (O'Toole et al., 1994; Hughes et al., 1996) and could result in a bias toward the shorter transmembrane domain. Changes in the length or orientation of the integrin transmembrane domain could also occur during physiological integrin activation. Binding of the cytoplasmic proteins such as talin to integrin β cytoplasmic domains activates integrins (Calderwood et al., 1999), and the binding site of the β2 integrin regulatory protein, cytohesin-1, is in this conserved hydrophobic region of integrin β2 (Nagel et al., 1998). Thus, the binding of proteins to integrin cytoplasmic domains may change the border of the integrin transmembrane and cytoplasmic domain in a piston-like motion. Such changes could lead to the proposed conformational rearrangements in the extracellular domains.

What is the intracellular activation signal? The central role of integrin cytoplasmic domains in regulating activation suggested that the regulated interaction of cytoplasmic proteins with the integrin tails was a central control point in the process (O'Toole et al., 1994). Two candidates for the proximal regulators are cytohesin-1 and talin. The former is reported to bind to the β2 cytoplasmic domain and regulate β2 integrin–mediated adhesion in response to activation of phosphatidylinositol 3-kinase (Nagel et al., 1998). Furthermore, cytohesin-1 is a guanine nucleotide exchanger for the ARF GTPase, and both its exchange activity and capacity to bind to the integrin cytoplasmic domain are required for its effects on β2 integrins. However, cytohesin-1 is β2 integrin specific. and integrin activation appears to be a general property of these receptors. Thus, talin, which binds to most integrin β cytoplasmic domains (Calderwood et al., 1999), may represent a more general activator.

Talin is an actin-binding protein that links integrins to the actin cytoskeleton (Horwitz et al., 1986) and colocalizes with clustered integrins. Furthermore, genetic and cell biological analyses show that talin is involved in integrin clustering into focal adhesions (Priddle et al., 1998). Talin consists of an NH_2-terminal ~47-kD globular head domain (talin-H) and an ~190-kD, COOH-terminal rod (talin-R) domain (Rees et al., 1990). It is an elongated (60 nm), flexible protein that binds to the cytoplasmic domains of integrins β1A, β1D (but not β1B and β1C), β2, and β3 (Liu et al., 2000). The talin head domain contains a band four-point-one, ezrin, radixin, moesin homology (FERM) domain and expression of talin fragments containing the head domain or of the FERM domain alone (Calderwood et al., 1999, 2002) activates integrins. The talin FERM domain is similar to those present in ezrin, radixin, and moesin (ERM) proteins (Pearson et al., 2000), which mediate the binding of the ERM proteins to the cytoplasmic domains of certain transmembrane receptors (Bretscher et al., 2000). In ERM proteins, the membrane protein-binding site in the FERM domains are masked in the intact molecule by interactions with the COOH-terminal tail domain (Pearson et al., 2000). The binding activity of the FERM domain can be unmasked by phosphorylation or the interaction of ERM proteins with polyphosphoinositides (Bretscher et al., 2000). Similarly, talin's integrin-binding site is masked in the intact molecule and can be uncovered by proteolytic cleavage (Yan et al., 2001) or binding to polyphosphoinositides (Martel et al., 2001). Furthermore, talin can be phosphorylated; however, the effects of these phosphorylation events on integrin binding have not been reported. Thus, talin is an integrin-activating protein that can manifest regulated interactions with integrin β cytoplasmic domains.

FERM and phosphotyrosine-binding domain–integrin interactions; a structural paradigm for diversity in integrin signaling. Based on the crystal structures of the FERM domains from moesin, radixin, and band 4.1, the talin FERM domain was predicted to contain three subdomains, F1, F2, and F3. The isolated 96 amino acid F3 subdomain activates integrins (Calderwood et al., 2002). The F3 domain was predicted to be a sandwich of two orthogonal antiparallel β sheets followed by an α helix resembling the phosphotyrosine-binding (PTB) domain. The interaction of integrin β cytoplasmic domains with talin resembles the binding of a PTB domain to a peptide ligand in several ways. PTB-binding sequences, including NPxY/F motifs, form β turns when bound to a PTB domain (Forman-Kay and Pawson, 1999). Talin-binding integrin β tails contain such NPxY/F motifs, and the integrin β3 NPxY motif has the propensity to form a β turn (Ulmer et al., 2001). Mutations of the NPxY motif abolish the transient β turn and block both talin binding (Pfaff et al., 1998) and integrin activation (O'Toole et al.,

1995). Additional PTB–ligand interactions are provided by the amino acid sequence NH_2-terminal to the β turn of the recognition motif (Forman-Kay and Pawson, 1999). Consistent with such an interaction, minimal talin-binding peptides contain the conserved first NPxY motif of integrin β1A along with the preceding five amino acids (Horwitz et al., 1986), providing additional similarities between the peptide-binding properties of talin and authentic PTB domains. Hence, the integrin β tail–talin interaction bears many of the characteristics of PTB–ligand interactions, and the ability of the PTB-like talin F3 subdomain to activate integrins may require a stable β turn at the conserved NPxY/F motif.

The NPxY-dependent integrin binding to the PTB-like FERM subdomain of talin suggests general paradigms for the control of cellular response to integrin-dependent cell adhesion. There are a large number of FERM and PTB domain–containing proteins; thus, the remarkable conservation of the NPxY/F motif in integrin β subunits suggests the possibility that other FERM and PTB domains might also bind integrins. Furthermore, PTB domain binding to peptide ligands may be favored or disfavored by phosphorylated tyrosines in the context of NPxY motifs (Forman-Kay and Pawson, 1999). In the case of integrin–PTB interactions, the binding of talin F3 subdomain (Calderwood et al., 2002) and ICAP-1α (Chang et al., 2002) to integrin β tails is phosphorylation independent, whereas the binding of Shc to the β3 tail is favored by tyrosine phosphorylation (Cowan et al., 2000). These several general principles of integrin–cytoplasmic protein interaction are suggested. Specifically, integrin β cytoplasmic domains may utilize their conserved NPxY/F motifs to bind to cytoplasmic proteins that contain PTB (or PTB-like) modules. Furthermore, by analogy with the masking of the integrin-binding site in talin, posttranslational modifications and interactions of the PTB domain–containing proteins could regulate their binding to the integrin tails. Conversely, tyrosine phosphorylation of the β3 tail switches its PTB-binding preference from talin to Shc, a shift likely to promote cell migration (Calderwood et al., 2002). Thus, phosphorylation-regulated changes in PTB domain binding specificity may represent molecular toggle switches that designate biological responses to integrin-dependent adhesion. Finally, as with talin and ICAP1-α, it is likely that there will be integrin-specific PTB domain interactions. The matrix of multiple integrin β tails and multiple PTB domain proteins and the biochemical regulation of their interactions provide a rich alphabet for encoding signals from integrins.

Activation and the integrin extracellular domain

Ligand-binding sites in integrins. At least three regions of integrins have been implicated in ligand binding. Within all α subunits, there exist seven repeated modules which were predicted to fold into seven four-stranded β sheets arranged in a torus around an axis of pseudosymmetry to form a β propeller (Springer, 1997). The existence of this structure has been confirmed in the crystal structure of αvβ3 (Xiong et al., 2001), and its upper surface comprises a ligand-binding domain (Xiong et al., 2002). Second, at least eight integrin α subunits contain a ~200 residue I (or A) domain that participates in ligand binding. The I domains contain a DXSXS motif that participates in coordinating bound cation (Lee et al., 1995). Two other coordination positions are filled by oxygenated residues, a threonine and aspartic acid near the DXSXS motif in the folded protein, but >50 residues distant in the primary sequence. Thus, these residues form the novel metal ion-dependent adhesion site (MIDAS) (Lee et al., 1995). Several solved I domain structures indicate that the overall structure is that of a dinucleotide-binding fold with a central parallel β sheet surrounded by α helices. The MIDAS motif lies at the COOH-terminal end of the β sheet. Residues involved in ligand binding to the I domain cluster about the MIDAS face, with a glutamic acid from the ligand completing the coordination sphere of the metal ion (Emsley et al., 2000). Third, β subunits contain a highly conserved DXSXS motif involved in ligand binding that was predicted to be part of an I-like domain (Lee et al., 1995). The existence of this I-like domain has been confirmed in the structure of integrin αvβ3 (Xiong et al., 2001). Integrin activation is likely to involve alteration of the secondary and tertiary structures of one or more of these three modules. In addition, it seems likely that alterations of the quaternary structure of the integrin leading to changes in the physical relationships or accessibility of these modules can regulate ligand-binding affinity (see below).

Rearrangements in the tertiary structure of the I domain and activation. The crystal structure of an integrin I domain–ligand complex demonstrates that conformational changes are induced by ligand binding to the α subunit I domain (Emsley et al., 2000). Such conformational changes are likely to underlie affinity regulation. The structure of the α2 I domain in complex with a triple helical fragment of collagen shows that ligand binding alters the conformation of the I domain in a dramatic way; in particular, a subtle alteration in metal coordination at the MIDAS motif, which allows the formation of a strong bond between the Mg^{2+} ion and a glutamic acid from the collagen, is linked to a 10 Å shift of the COOH-terminal helix toward the lower surface of the domain (Emsley et al., 2000). In the absence of the conformational switch, collagen could still bind to the I domain, but it could not form a strong bond to the metal ion. This immediately suggests that ligand-binding affinity is regulated by the contacts between the lower surface of the domain and the body of the integrin that restrain the conformational switch (see below). Structure-based mutagenesis has confirmed, using "gain of function" studies, that these conformational changes occur in the context of whole integrin and underlie affinity regulation (Shimaoka et al., 2000). The structural changes in the α2 collagen complex are essentially identical to those seen earlier in two crystal forms of the αM I domain (Lee et al., 1995). Therefore, it seems very likely that there are just two conformations for all α subunit I domains ("open" and "closed"). For example, NMR data are consistent with a similar conformational change in the αL-I domain (Legge et al., 2000). The next major challenge is to understand the link between these tertiary changes in the I domain and quaternary changes in the whole integrin.

Quaternary rearrangements in structure: lessons from the structure of the αvβ3 integrin extracellular domain. Early electron microscopic and immunochemical studies suggested that integrin activation was associated with large

changes in the overall structure of the protein (Du et al., 1993). Furthermore, activation appears to change the accessibility of the ligand-binding pocket, suggesting that an important role for changing quaternary structure in the activation process (Beer et al., 1992). The recent determination of the crystal structure of the complete extracellular portion of integrin αvβ3 (Xiong et al., 2001, 2002) provides a major advance to the field and provides strong indications that such quaternary rearrangements could underlie activation. The αv subunit lacks an α-I domain, so the ligand-binding head is comprised of the β subunit I–like domain (featuring a MIDAS motif very similar to the α subunit MIDAS motif) sitting on top of the α subunit β propeller, very similar to the arrangement seen in the G protein heterodimer (Bohm et al., 1997). Now that we know the atomic details of the association, however, we are in a much stronger position to propose quaternary rearrangements that could lead to activation.

The crystal structure shows that the α subunit propeller and β-I–like domain engage in an intimate embrace. With respect to the long axis of the integrin (as deduced from EM images), the propeller axis is tilted by nearly 90°, with its axis pointing into the side the β-I domain, which inserts an arginine side chain from a 3_{10} helix into the cavity along the propeller axis. The β-MIDAS motif is exposed at the top of the head, apparently available for binding ligands, whereas the ligand-binding epitopes on the propeller are at least partly obscured by the β-I domain as predicted. Within the published structures, we propose that the β-I domain is in a conformation that resembles the closed or inactive conformation. First, the principal marker of the closed conformation is the position of the COOH-terminal helix (α7) and its connecting loop with respect to the central β sheet. In the αvβ3 crystal structure, this loop and helix are at the same height as found in the closed conformation of the α-I domain. Second, the arrangement of the MIDAS loops is much more similar to that of the closed conformation. The 3_{10} helix that inserts into the propeller axis is just a few residues upstream of the MIDAS loop 3 (Fig. 1); it is analogous to the "switch II" region in G proteins, which is similarly linked to changes in metal ion coordination, suggesting how the packing of the integrin β-I domain against the propeller could regulate its conformation and affinity. Third, three mutations that disrupt activation of integrin αIIbβ3 (Baker et al., 1997) map to residues on two strands (Fig. 1, B) adjacent to MIDAS loop 3. These strands could thus link conformational changes in the metal-binding loop to a large shift of the COOH-terminal helix. Note that in the scheme presented in Fig. 1, it is the β-I domain that moves while the α7 helix remains fixed; however, the relative motion is the same. We suggest that the effects of these mutations on activation provide strong circumstantial evidence that the integrin β-I domain undergoes comparable conformational changes to those seen in α-I domain.

In further support of this notion, the recent structure of an RGD peptide ligand bound to crystals of αVβ3 reveals conformational changes within the β-I domain that are in the direction of those observed in the α-I domain but appear to be limited by the constraints of the quaternary organization (Xiong et al., 2002). MIDAS loop 1 shifts by ~2 Å toward MIDAS loop 2, just as it does in α-I, allowing a metal ion to coordinate sidechains from both loops simultaneously. The top of the COOH-terminal helix appears to be squeezed out by this motion, but the large motion of the analogous helix observed in α-I is prevented in β-I by contacts with the immunoglobulin-like hybrid domain (Xiong et al., 2002). Similarly, MIDAS loop 3 is shifted little in the liganded β-I domain, and its movement is presumably frustrated by its link to the loop that locks into the propeller.

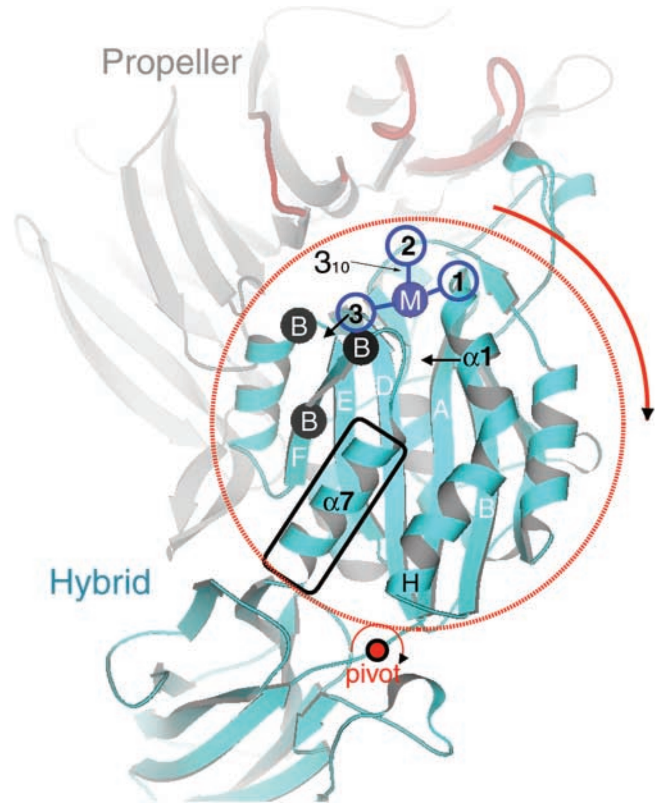

Figure 1. **Speculative model of the initial tertiary and quaternary changes during occupancy of activated integrins that lack an α-I domain.** Ligand binding to the MIDAS motif of the β-I domain (M) triggers conformational changes in the three MIDAS loops (numbered 1–3) that cause a shift of helix α1 and MIDAS loop 3 toward the βF-α7 loop (black arrows) and a loosening of the contacts between the β-I domain and the propeller. These movements cause steric clashes that are relieved by a rotation of the β-I domain around the indicated pivot point (red circle) at the hybrid–β-I domain boundary, separating the β-I from the propeller. Helix α7 remains in position, thus freeing itself from its contacts with MIDAS loop 3 and helix α1. That is, there is a net shift of helix α7 with respect to the β-I domain as observed in the α-I domain. Since occupancy activates integrins (Du et al., 1993), a reversal of the order of these events could account for integrin activation. In support of this idea, three mutations (B) shown previously to block activation (Baker et al., 1997) define a path between the movement of MIDAS loop 3 and helix α7; an activation epitope maps to the base of α1 (labeled H) at the β-I–hybrid interface. Red highlighted loops on the propeller map the location of chimeras that switch ligand recognition specificities between integrin αv and α5 subunits.

What kinds of quaternary motion are possible? The interface between the β-I and the β hybrid domains is principally polar and could be disrupted with only small energetic consequences. For example, a hinge motion somewhere around E108-D109 (Fig. 1, pivot point) at the NH_2 terminus of

β-I–like domain could provide the necessary pull on the COOH-terminal helix and lift the I-like domain clear of the β propeller (Fig. 1). Thus, tertiary rearrangements within the I-like domain of the β subunit could lead to activation as suggested by the finding that (Mould et al., 2002) the epitope for an activating antibody maps to the base of helix α1 of the β-I domain (Fig. 1, H). Although the authors interpreted this as evidence for a conformational change distinct from that of the α-I domain, another interpretation is that the antibody is recognizing the motion of the β-I domain with respect to the β-hybrid and propeller domain. A piston-like quaternary shift of the β subunit could create such a motion.

This model for activation proposed above extends the analogy with the G proteins, where the I-like domain would separate completely from the propeller during activation. The structure would then resemble the EM images presented by Hantgan et al. (1999) of the activated state with the head pieces separated. It could also allow for the ∼30 Å separation of the distinct propeller and I domain ligand-binding epitopes as determined by Mould et al. (1997) for the binding of fibronectin to α5β1. We also note that it may be necessary for the propeller to reorient such that its ligand-binding surface becomes coplanar with that of the β-I domain; this would require a hinge motion in the α subunit, either at the propeller-thigh interface or the "genu" as defined by Arnaout and colleagues (Xiong et al., 2001).

Activation of integrins containing an α-I domain. In those integrins containing an α subunit I domain, it is likely that the domain sits on top of the propeller, with its COOH-terminal helix binding to and/or blocking the β-MIDAS motif. Those interactions with the β-I domain could lock the α-I domain in the closed conformation or alternatively, as proposed recently (Alonso et al., 2002), in an open conformation. Thus, mutations to surface-exposed residues on the COOH-terminal helix of the αL-I domain and the neighboring loop at the base of strand βF lead to activation of integrin αLβ2, possibly by disrupting the interactions between the α- and β-I domains (Lupher et al., 2001). Similar activation occurs with mutation of the buried Ile^{235} to Ala, which disrupts the packing of the COOH-terminal helix against the body of the α-I domain (Xiong et al., 2000). This intimate link between conformational changes in the α- and β-I domains suggests that in some cases the β-I domain could regulate the α-I domain rather than serving as a primary ligand-binding site. This would explain the apparent paradox that the α-I domain is usually the primary ligand-binding site, yet mutations in the β-I domain can often block ligand binding (Bajt et al., 1995). It is also noteworthy that several mutations to the outside of helix α7 and the COOH-terminal connector of α-I domains cause inactivation of the integrin (Lupher et al., 2001). One interpretation of these data is that bonds between the α-I and β-I domains in the active state of the integrin stabilize the open state of the α-I domain. Indeed, Arnaout's group (Alonso et al., 2002) has proposed that a conserved Glu just COOH-terminal to the integrin I domain α7 helix is part of a ligand for the integrin β-I domain. They suggest that the engagement of this internal ligand leads to downward displacement of the α7 helix, switching the α subunit I domain to its open conformation. This proposed "ligand relay" mechanism would provide a cogent explanation for bidirectional conformational rearrangements in the signaling reactions of integrins that posses α-I domains.

Integrin bending and activation. The crystal structure of αvβ3 revealed a surprising quaternary structure. The "stalks" of both subunits were severely bent bringing the "head region," including the propeller and thigh domain in the α subunit, and the I domain and hybrid domain of the β subunit into close proximity to the presumed membrane interface (Xiong et al., 2001). This structure does not conform to consensus view of the integrin structure in membranes or in solution as derived from rotary shadowed images (Parise and Phillips, 1985; Du et al., 1993) and biophysical measurements (Hantgan et al., 1999). Although some of these bends may reflect an underlying flexibility, they are the sites of major crystal contacts within the crystal lattice, suggesting that the bends are induced by crystallization. Furthermore, some of the stalk domains are poorly ordered, which is suggestive of a strained conformation. However, it is noteworthy that the COOH termini of the two chains are found in close proximity, since prior mutational studies have established that the separation of these COOH termini leads to activation of integrin α5β1 (Takagi et al., 2001). Blacklow, Springer, and colleagues have shown that the epitopes for two activating antibodies map to the outside of the β thigh domain, but are obscured by the β hybrid domain in the crystal structure, leading them to suggest that the bent conformation represents the inactive integrin. They further propose that a "switch-blade" opening of the integrin is associated with activation (Beglova et al., 2002). Clearly, evidence from EM studies is required to support this model.

In summary, both the tertiary structure of the β-I–like domain and its mode of association with the propeller (as described above) and the interaction of the COOH termini suggest that the αVβ3 crystal structure is not in the high affinity (active) state. Indeed, if the active or open state does resemble the G protein, then it is unlikely that it could be crystallized in the absence of a bivalent ligand that engaged both the β-I domain and propeller. However, the soluble αVβ3 crystallized by Arnaout and colleagues had been shown previously to bind ligand in the presence of Mn^{++} and to therefore be active in solution (Mehta et al., 1998). Clearly, a precise definition of the activation state of the solved αVβ3 structure will be essential in understanding the activation process.

Concluding remarks

Several mechanisms can modulate cell adhesion. Among them is the regulation of integrin affinity for ligand. Affinity regulation has been observed for many integrins and has important functional consequences in development, angiogenesis, hemostasis, wound repair, inflammation, and immunity. Importantly, affinity changes can have their major impact on integrin functions other than adhesion, such as cell migration or assembly of a fibronectin matrix. Integrin cytoplasmic domains control integrin activation. The mechanism by which structural information contained within these cytoplasmic domains is propagated through integrin

transmembrane domains to influence ligand binding continues to be an area of intense interest. Understanding the mechanisms of integrin activation will provide insight into a signal transduction event of broad biological significance.

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References

Alonso, J.L., M. Essafi, J.P. Xiong, T. Stehle, and M.A. Arnaout. 2002. Does the integrin alphaa domain act as a ligand for its betaA domain? *Curr. Biol.* 12: R340–R342.

Armulik, A., I. Nilsson, G. von Heijne, and S. Johansson. 1999. Determination of the border between the transmembrane and cytoplasmic domains of human integrin subunits. *J. Biol. Chem.* 274:37030–37034.

Bajt, M.L., T. Goodman, and S.L. McGuire. 1995. β2 (CD18) is involved in ligand recognition by I domain integrins, LFA-1 (αLβ2 CD11a/CD18) and MAC-1 (αmβ2 CD11b/CD18). *J. Biol. Chem.* 270:94–98.

Baker, E.K., E.C. Tozer, M. Pfaff, S.J. Shattil, J.C. Loftus, and M.H. Ginsberg. 1997. A genetic analysis of integrin function: Glanzmann thrombasthenia *in vitro. Proc. Natl. Acad. Sci. USA.* 94:1973–1978.

Beer, J.H., K.T. Springer, and B.S. Coller. 1992. Immobilized Arg-Gly-Asp (RGD) peptides of varying lengths as structural probes of the platelet glycoprotein IIb/IIIa receptor. *Blood.* 79:117–128.

Beglova, N., S.C. Blacklow, J. Takagi, and T.A. Springer. 2002. Cysteine-rich module structure reveals a fulcrum for integrin rearrangement upon activation. *Nat. Struct. Biol.* 9:282–287.

Bohm, A., R. Gaudet, and P.B. Sigler. 1997. Structural aspects of heterotrimeric G-protein signaling. *Curr. Opin. Biotechnol.* 8:480–487.

Bretscher, A., D. Chambers, R. Nguyen, and D. Reczek. 2000. ERM-Merlin and EBP50 protein families in plasma membrane organization and function. *Annu. Rev. Cell Dev. Biol.* 16:113–143.

Calderwood, D.A., R. Zent, R. Grant, D.J.G. Rees, R.O. Hynes, and M.H. Ginsberg. 1999. The talin head domain binds to integrin beta subunit cytoplasmic tails and regulates integrin activation. *J. Biol. Chem.* 274:28071–28074.

Calderwood, D.A., B. Yan, J.M. de Pereda, B. Garcia-Alvarez, Y. Fujioka, R.C. Liddington, and M.H. Ginsberg. 2002. The phosphotyrosine binding (PTB)-like domain of talin activates integrins. *J. Biol. Chem.* 21749–21758.

Chang, D.D., B.Q. Hoang, J. Liu, and T.A. Springer. 2002. Molecular basis for interaction between Icap1 alpha PTB domain and beta 1 integrin. *J. Biol. Chem.* 277:8140–8145.

Cowan, K.J., D.A. Law, and D.R. Phillips. 2000. Identification of Shc as the primary protein binding to the tyrosine-phosphorylated beta 3 subunit of alpha IIbbeta 3 during outside-in integrin platelet signaling. *J. Biol. Chem.* 275: 36423–36429.

Du, X., M. Gu, J.W. Weisel, C. Nagaswami, J.S. Bennett, R.D. Bowditch, and M.H. Ginsberg. 1993. Long range propagation of conformational changes in integrin $\alpha_{IIb}\beta_3$. *J. Biol. Chem.* 268:23087–23092.

Emsley, J., C.G. Knight, R.W. Farndale, M.J. Barnes, and R.C. Liddington. 2000. Structural basis of collagen recognition by integrin alpha2beta1. *Cell.* 101: 47–56.

Forman-Kay, J.D., and T. Pawson. 1999. Diversity in protein recognition by PTB domains. *Curr. Opin. Struct. Biol.* 9:690–695.

Ginsberg, M.H., B. Yaspan, J. Forsyth, T.S. Ulmer, I.D. Campbell, and M. Slepak. 2001. A membrane-distal segment of the integrin alpha IIb cytoplasmic domain regulates integrin activation. *J. Biol. Chem.* 276:22514–22521.

Hantgan, R.R., C. Paumi, M. Rocco, and J.W. Weisel. 1999. Effects of ligand-mimetic peptides Arg-Gly-Asp-X (X = Phe, Trp, Ser) on alphaIIbbeta3 integrin conformation and oligomerization. *Biochemistry.* 38:14461–14474.

Horwitz, A.F., K. Duggan, C.A. Buck, M.C. Beckerle, and K. Burridge. 1986. Interaction of plasma membrane fibronectin receptor with talin—a transmembrane linkage. *Nature.* 320:531–533.

Hughes, P.E., F. Diaz-Gonzalez, L. Leong, C. Wu, J.A. McDonald, S.J. Shattil, and M.H. Ginsberg. 1996. Breaking the integrin hinge: a defined structural constraint regulates integrin signaling. *J. Biol. Chem.* 271:6571–6574.

Lee, J., P. Rieu, M.A. Arnaout, and R. Liddington. 1995. Crystal structure of the A domain from the *alpha* subunit of integrin CR3 (CD11b/CD18). *Cell.* 80: 631–638.

Legge, G.B., R.W. Kriwacki, J. Chung, U. Hommel, P. Ramage, D.A. Case, H.J. Dyson, and P.E. Wright. 2000. NMR solution structure of the inserted domain of human leukocyte function associated antigen-1. *J. Mol. Biol.* 295: 1251–1264.

Li, R., C.R. Babu, J.D. Lear, A.J. Wand, J.S. Bennett, and W.F. DeGrado. 2001. Oligomerization of the integrin alpha IIbbeta 3: roles of the transmembrane and cytoplasmic domains. *Proc. Natl. Acad. Sci. USA.* 98:12462–12467.

Liu, S., D.A. Calderwood, and M.H. Ginsberg. 2000. Integrin cytoplasmic domain-binding proteins. *J. Cell Sci.* 113:3563–3571.

Lu, C., J. Takagi, and T.A. Springer. 2001. Association of the membrane proximal regions of the alpha and beta subunit cytoplasmic domains constrains an integrin in the inactive state. *J. Biol. Chem.* 276:14642–14648.

Lupher, M.L., Jr., E.A. Harris, C.R. Beals, L.M. Sui, R.C. Liddington, and D.E. Staunton. 2001. Cellular activation of leukocyte function-associated antigen-1 and its affinity are regulated at the I domain allosteric site. *J. Immunol.* 167:1431–1439.

Martel, V., C. Racaud-Sultan, S. Dupe, C. Marie, F. Paulhe, A. Galmiche, M.R. Block, and C. Albiges-Rizo. 2001. Conformation, localization and integrin-binding of talin depend on its interaction with phosphoinositides. *J. Biol. Chem.* 276:21217–21227.

Mehta, R.J., B. Diefenbach, A. Brown, E. Cullen, A. Jonczyk, D. Gussow, G.A. Luckenbach, and S.L. Goodman. 1998. Transmembrane-truncated alphavbeta3 integrin retains high affinity for ligand binding: evidence for an ‘inside-out’ suppressor? *Biochem. J.* 330:861–869.

Mould, A.P., J.A. Askari, S. Aota, K.M. Yamada, A. Irie, Y. Takada, H.J. Mardon, and M.J. Humphries. 1997. Defining the topology of integrin alpha5beta1-fibronectin interactions using inhibitory anti-alpha5 and anti-beta1 monoclonal antibodies. Evidence that the synergy sequence of fibronectin is recognized by the amino-terminal repeats of the alpha5 subunit. *J. Biol. Chem.* 272:17283–17292.

Mould, A.P., J.A. Askari, S. Barton, A.D. Kline, P.A. McEwan, S.E. Craig, and M.J. Humphries. 2002. Integrin activation involves a conformational change in the alpha 1 helix of the beta subunit A-domain. *J. Biol. Chem.* 277:19800–19805.

Nagel, W., L. Zeitlmann, P. Schilcher, C. Geiger, J. Kolanus, and W. Kolanus. 1998. Phosphoinositide 3-OH kinase activates the beta2 integrin adhesion pathway and induces membrane recruitment of cytohesin-1. *J. Biol. Chem.* 273:14853–14861.

O’Toole, T.E., Y. Katagiri, R.J. Faull, K. Peter, R.N. Tamura, V. Quaranta, J.C. Loftus, S.J. Shattil, and M.H. Ginsberg. 1994. Integrin cytoplasmic domains mediate inside-out signal transduction. *J. Cell Biol.* 124:1047–1059.

O’Toole, T.E., J. Ylanne, and B.M. Culley. 1995. Regulation of integrin affinity states through an NPXY motif in the β subunit cytoplasmic domain. *J. Biol. Chem.* 270:8553–8558.

Parise, L.V., and D.R. Phillips. 1985. Platelet membrane glycoprotein IIb-IIIa complex incorporated into phospholipid vesicles. Preparation and morphology. *J. Biol. Chem.* 260:1750–1756.

Pearson, M.A., D. Reczek, A. Bretscher, and P.A. Karplus. 2000. Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. *Cell.* 101:259–270.

Pfaff, M., S. Liu, D.J. Erle, and M.H. Ginsberg. 1998. Integrin beta cytoplasmic domains differentially bind to cytoskeletal proteins. *J. Biol. Chem.* 273: 6104–6109.

Priddle, H., L. Hemmings, S. Monkley, A. Woods, B. Patel, D. Sutton, G.A. Dunn, D. Zicha, and D.R. Critchley. 1998. Disruption of the talin gene compromises focal adhesion assembly in undifferentiated but not differentiated embryonic stem cells. *J. Cell Biol.* 142:1121–1133.

Rees, D.J.G., S.E. Ades, S.J. Singer, and R.O. Hynes. 1990. Sequence and domain structure of talin. *Nature.* 347:685–689.

Schwartz, M.A., M.D. Schaller, and M.H. Ginsberg. 1995. Integrins: emerging paradigms of signal transduction. *Annu. Rev. Cell Dev. Biol.* 11:549–599.

Shimaoka, M., J.M. Shifman, H. Jing, J. Takagi, S.L. Mayo, and T.A. Springer. 2000. Computational design of an integrin I domain stabilized in the open high affinity conformation. *Nat. Struct. Biol.* 7:674–678.

Springer, T.A. 1997. Folding of the N-terminal, ligand-binding region of integrin alpha-subunits into a beta-propeller domain. *Proc. Natl. Acad. Sci. USA.* 94: 65–72.

Takagi, J., H.P. Erickson, and T.A. Springer. 2001. C-terminal opening mimics ‘inside-out’ activation of integrin alpha5beta1. *Nat. Struct. Biol.* 8:412–416.

Ulmer, T.S., B. Yaspan, M.H. Ginsberg, and I.D. Campbell. 2001. NMR analysis of structure and dynamics of the cytosolic tails of integrin alphaIIbbeta3 in aqueous solution. *Biochemistry.* 40:7498–7508.

Vinogradova, O., T. Haas, E.F. Plow, and J. Qin. 2000. A structural basis for integrin activation by the cytoplasmic tail of the alpha IIb-subunit. *Proc. Natl. Acad. Sci. USA.* 97:1450–1455.

Weljie, A.M., P.M. Hwang, and H.J. Vogel. 2002. Solution structures of the cytoplasmic tail complex from platelet integrin alpha IIb- and beta 3-subunits. *Proc. Natl. Acad. Sci. USA.* 99:5878–5883.

Williams, M.J., P.E. Hughes, T.E. O'Toole, and M.H. Ginsberg. 1994. The inner world of cell adhesion: integrin cytoplasmic domains. *Trends Cell Biol.* 4:109–112.

Xiong, J.P., R. Li, M. Essafi, T. Stehle, and M.A. Arnaout. 2000. An isoleucine-based allosteric switch controls affinity and shape shifting in integrin CD11b A-domain. *J. Biol. Chem.* 275:38762–38767.

Xiong, J.P., T. Stehle, B. Diefenbach, R. Zhang, R. Dunker, D.L. Scott, A. Joachimiak, S.L. Goodman, and M.A. Arnaout. 2001. Crystal structure of the extracellular segment of integrin {alpha}V{beta}3. *Science.* 294:339–345.

Xiong, J.P., T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S.L. Goodman, and M.A. Arnaout. 2002. Crystal structure of the extracellular segment of integrin alpha Vbeta3 in complex with an Arg-Gly-Asp ligand. *Science.* 296: 151–155.

Yan, B., D.A. Calderwood, B. Yaspan, and M.H. Ginsberg. 2001. Calpain cleavage promotes talin binding to the Beta3 integrin cytoplasmic domain. *J. Biol. Chem.* 276:28164–28170.