

# Integrin Structure, Activation, and Interactions

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Integrins are large, membrane-spanning, heterodimeric proteins that are essential for a metazoan existence. All members of the integrin family adopt a shape that resembles a large “head” on two “legs,” with the head containing the sites for ligand binding and subunit association. Most of the receptor dimer is extracellular, but both subunits traverse the plasma membrane and terminate in short cytoplasmic domains. These domains initiate the assembly of large signaling complexes and thereby bridge the extracellular matrix to the intracellular cytoskeleton. To allow cells to sample and respond to a dynamic pericellular environment, integrins have evolved a highly responsive receptor activation mechanism that is regulated primarily by changes in tertiary and quaternary structure. This review summarizes recent progress in the structural and molecular functional studies of this important class of adhesion receptor.

The name “integrin” was suggested for an integral membrane protein complex first characterized in 1986 (Tamkun et al. 1986). The name was devised because the protein identified linked the extracellular matrix to the cytoskeleton (early developments in this field have been well described [Hynes 2004]). In the 25 years since that first characterization, a vast amount of work has been performed, with consequent increased understanding. The essential role of integrins in tissue organization and cell development, their signal transduction mechanisms (from outside to in and inside to out!), and their potential as therapeutic targets is now established. In this article, we provide an overview of the structure of integrins, the conformational changes that determine activation state, and the mechanisms of ligand binding.

## INTEGRIN STRUCTURE

### Overall Structure

Integrins are heterodimers of non-covalently associated  $\alpha$  and  $\beta$  subunits. In vertebrates, there are 18  $\alpha$  and 8  $\beta$  subunits that can assemble into 24 different receptors with different binding properties and different tissue distribution (Hynes 2002; Barczyk et al. 2010). The  $\alpha$  and  $\beta$  subunits are constructed from several domains with flexible linkers between them. Each subunit has a single membrane-spanning helix and, usually, a short unstructured cytoplasmic tail. The size varies but typically the  $\alpha$ - and  $\beta$ -subunits contain around 1000 and 750 amino acids, respectively. Numerous reviews on integrin structure and function have been published (Arnaout et al. 2007; Luo et al.



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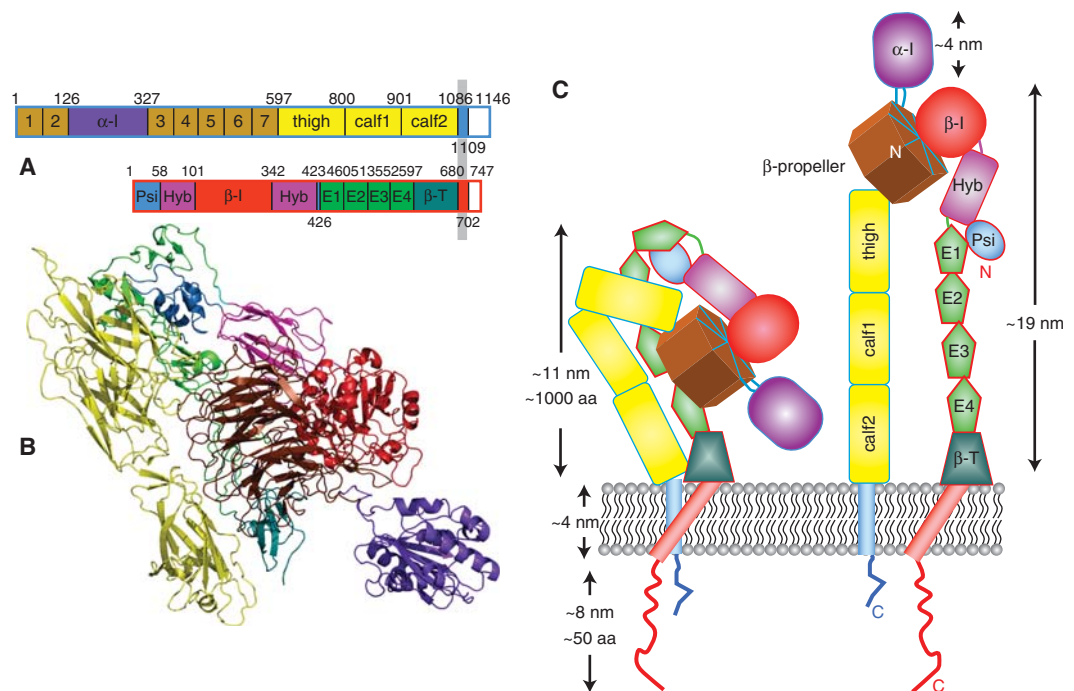
2007; Askari et al. 2009; Bennett et al. 2009) so here we concentrate mainly on the implications of recent structural work that includes studies of intact ectodomains, membrane spanning regions, cytoplasmic tails and their ligands.

### The Ectodomains

The breakthrough crystal structure of  $\alpha V\beta 3$  (Xiong et al. 2001) started a deluge of structural information about integrin ectodomains. Structures of  $\alpha V\beta 3$ , with and without ligand (Xiong et al. 2001, 2002),  $\alpha IIb\beta 3$  (Zhu et al. 2008), and  $\alpha x\beta 2$  (Xie et al. 2009) are all now available. These crystal structures are all in a similar overall “bent” conformation that would place the ligand binding site near the membrane surface. The overall topology and structure of integrin ectodomains is illustrated in Figure 1 for the case of  $\alpha x\beta 2$  (Xie et al. 2009), which has an inserted  $\alpha$ -I domain.

Current knowledge of ectodomains has also been enhanced by structures of various integrin fragments including isolated  $\alpha$ -I domains (Lee et al. 1995a) and  $\beta 2$ -leg fragments (Beglova et al. 2002; Xiao et al. 2004; Shi et al. 2005).

Because of observed flexibility in studies by electron microscopy (EM) and the existence of conformationally sensitive antibody recognition sites (Humphries 2004), there is a general acceptance that conformations other than the bent one are possible and are functionally relevant (see below). The structural studies of intact ectodomains (Xiong et al. 2001; Xie et al. 2009; Zhu et al. 2009) all postulated that an upright structure of the sort illustrated in Figure 1C could exist as well as the bent structure. There has, however, been controversy about whether this large change between bent and upright structures—the “switchblade” model (Luo et al. 2007)—has to take place or if more conservative changes around the bent structure can explain the data—the “deadbolt” model (Arnaout et al. 2005).



**Figure 1.** Integrin structure. (A) Domain structure of  $\alpha x\beta 2$  (Xie et al. 2009); (B) structure of  $\alpha x\beta 2$  using same color code as A (drawn with PyMOL [DeLano Scientific] using PDB coordinates 3K6S); (C) cartoon representation of bent and upright conformations showing approximate dimensions.



### The Structure of $\alpha$ Subunit Ectodomains

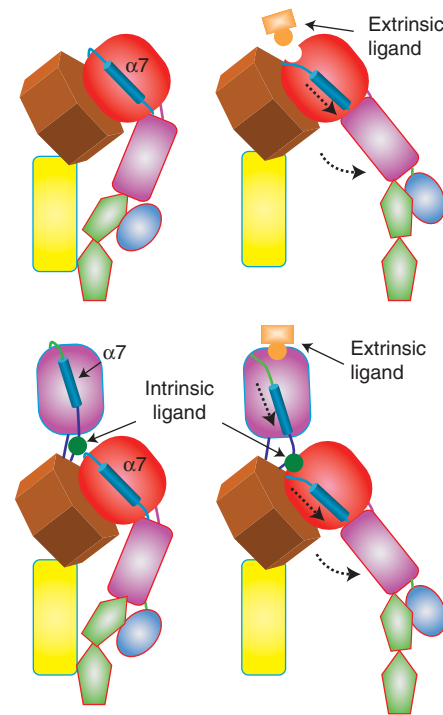
The  $\alpha$ -chain consists of four or five extracellular domains: a seven-bladed  $\beta$ -propeller, a *thigh*, and two *calf* domains. Nine of the 18 integrin  $\alpha$  chains have an  $\alpha$ -I domain of around 200 amino-acids, inserted between blades 2 and 3 of the  $\beta$ -propeller (Larson et al. 1989). The I domain, a copy of which also appears in the  $\beta$ -chain, has five  $\beta$ -sheets surrounded by seven  $\alpha$  helices; it is similar to von Willebrand A domains. The last three or four blades of the  $\beta$ -propeller contain domains that bind  $\text{Ca}^{2+}$  on the lower side of the blades facing away from the ligand-binding surface.  $\text{Ca}^{2+}$  binding to these sites has been shown to influence ligand binding (Oxvig and Springer 1998; Humphries et al. 2003).

The thigh and calf domains have similar, immunoglobulin-like,  $\beta$ -sandwich folds (Xiong et al. 2001). They have 140–170 residues with more  $\beta$ -strands than typical Ig-like domains ( $\sim 100$  residues). There are two main regions of interdomain flexibility. One is the linker between the  $\beta$ -propeller and the thigh; the other is the “genu” or knee at the bend between the thigh and the calf-1 domain. The  $\alpha$ -subunit genu is located close to the similar bend in the  $\beta$  subunit, thereby allowing extension by a hinging at the knees. The  $\alpha$ -I domain in  $\alpha\beta 2$  is inserted in the  $\beta$ -propeller domain with flexible linkers (Fig. 1C). Unlike the other four  $\alpha$ -leg domains, which have relatively rigid structures, I domains show conformational changes within the domain that are important for regulating binding affinity (see below and Fig. 2).

### The Structure of $\beta$ Subunit Ectodomains

The  $\beta$ -leg has seven domains with flexible and complex interconnections. A  $\beta$ -I domain is inserted in a hybrid domain, which is, in turn, inserted in a plexin-semaphorin-integrin (PSI) domain. These domains are followed by four cysteine-rich *epidermal growth factor* (EGF) modules and a  $\beta$ -tail domain. The hybrid domain in the upper  $\beta$ -leg has a  $\beta$ -sandwich construction. The  $\beta$ -I domain, which is homologous to the  $\alpha$ -I domain, is inserted into the

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**Figure 2.** An illustration of the movement of  $\alpha 7$  helix in the I domains and the swing-out of the hybrid domain (the domains are defined in Fig. 1). The *top* pair corresponds to the closed and open conformations of an integrin without an inserted  $\alpha$ -I domain whereas the *lower* pair represents the situation when there is an  $\alpha$ -I domain present. The intrinsic ligand is a glutamate (E310 in  $\alpha$ L).

hybrid domain. The small PSI domain, with an  $\alpha/\beta$  fold, is also split into two portions (Xiao et al. 2004; Xiong et al. 2004) connected, in  $\beta 3$ , by a long-range Cys-13 to Cys-435 disulfide bond.

Unusual EGF module boundaries were first proposed in the  $\alpha\text{V}\beta 3$  structure (Xiong et al. 2004), but recent crystal structures suggest that each EGF module has an even number of eight cysteines, bonded in a C1-C5, C2-C4, C3-C6, and C7-C8 pattern except for EGF1, which lacks the C2-C4 disulfide. The  $\alpha\text{IIb}\beta 3$  structure shows that all 56 cysteines in the integrin  $\beta 3$  subunit are disulfide bonded (Zhu et al. 2008).

The  $\beta$ -tail domain has an  $\alpha + \beta$  fold (Xiong et al. 2001). The weak electron density of this

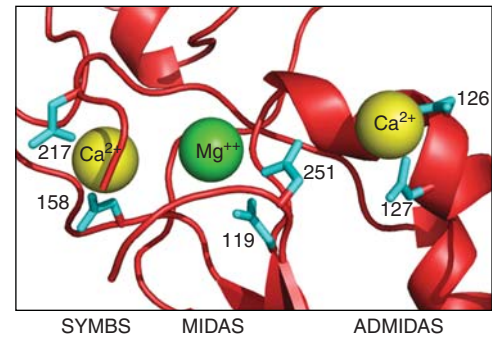
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domain observed in the  $\alpha$ I $\beta$ 3 crystal structure was taken to suggest a flexible connection to other regions of the  $\beta$ -leg by a mobile “ankle” (Zhu et al. 2008). A contact between the CD loop of the  $\beta$ -tail domain and the  $\alpha$ 7 helix of the  $\beta$ -I domain has been proposed to inhibit integrin activation—the “deadbolt” model (Arnaout et al. 2005). This contact is, however, small and no such contact is observed in the  $\alpha$ I $\beta$ 3 or  $\alpha$ X $\beta$ 2 structures.

In general, the  $\beta$ -leg seems to be more flexible than the  $\alpha$ -leg. Evidence for this comes from the ten different structures of  $\alpha$ X $\beta$ 2 observed in three different crystal lattices (Xie et al. 2009). The EGF domain region is relatively plastic, especially between EGF1 and EGF2, the  $\beta$  knee, and at the PSI/hybrid and hybrid/I-EGF1 junctions. There is evidence for important conformational changes occurring in the  $\beta$ -I/hybrid region. A transition from a “closed” to an “open” conformation of the  $\beta$ -I domain has been observed when the  $\beta$ -I  $\alpha$ 7-helix moves toward the hybrid domain (Xiao et al. 2004). The connecting rodlike motion of the  $\alpha$ 7-helix causes the hybrid domain to swing-out by  $\sim 60^\circ$  (see Fig. 2).

### Cation Binding Sites

As described below, ligand binding in  $\alpha$ -I less integrins takes place at the largest interface between the two subunits (the  $\beta$ -propeller/ $\beta$ -I domain interface); binding is dependent on the cations  $Mg^{++}$ ,  $Ca^{2+}$ , and  $Mn^{++}$ . From a structure of an  $\alpha$ -I domain it was suggested that integrin ligand binding involves a  $Mg^{++}$  ion, a “metal-ion-dependent adhesion site” (MIDAS) (Lee et al. 1995b); a crystal form with bound  $Mn^{++}$  also showed considerable movement of the  $\alpha$ 7-helix on activation (Lee et al. 1995a). In the recent  $\alpha$ I $\beta$ 3 structure (Zhu et al. 2008) strong electron densities were ascribed to cations at three sites formed by loops in the  $\beta$ -I domain (Fig. 3);  $Mg^{++}$  was assigned to the central MIDAS site with  $Ca^{2+}$  at the two flanking sites (ADMIDAS) binds an inhibitory  $Ca^{2+}$  ion; binding of  $Mn^{++}$  here results in a structural change that gives an active integrin (Humphries



**Figure 3.** Three  $\beta$ -I domain metal-binding sites in  $\alpha$ X $\beta$ 2 (Zhu et al. 2008); aspartate ligands to the metal ions are shown in cyan (from PDB:3FCS); figure drawn using PyMOL (DeLano Scientific).

et al. 2003). The second  $Ca^{2+}$ -binding site has been called the synergistic metal ion binding site (SyMBS) (Zhu et al. 2008). Mutational studies show that the SyMBS site is responsible for  $Ca^{2+}$  synergy (Chen et al. 2003; Mould et al. 2003a).

As mentioned above, the  $\beta$ -I domain has distinct closed and open conformational states, involving movement of the  $\alpha$ 7 helix, in  $\alpha$ -I-less integrins (Xiao et al. 2004; Luo et al. 2007). Similar conformational changes are seen in  $\alpha$ -I-domains. When integrin ligands, such as Arg-Gly-Asp, bind the open state, the MIDAS  $Mg^{++}$  ion coordinates the Asp side chain of the ligand. In  $\alpha$ -I integrins it has been suggested that the  $\beta$ -I MIDAS may bind an intrinsic ligand, an invariant Glu, Glu-318 in  $\alpha$ X (Xie et al. 2009). Support for this model comes from Glu mutations that abolish integrin activation (Huth et al. 2000; Alonso et al. 2002); the observed flexibility of the  $\alpha$ X  $\alpha$ -I domain would also facilitate such interdomain interactions (Xie et al. 2009).

### The Membrane Spanning Helices

The current view is that association of integrin  $\alpha$  and  $\beta$  transmembrane (TM) segments, results in an inactive resting receptor (Wegener and Campbell 2008). Evidence for this includes experiments using EM (Adair and Yeager 2002), disulfide cross-linking (Luo et al. 2004), activating mutations (Partridge et al. 2005), and FRET





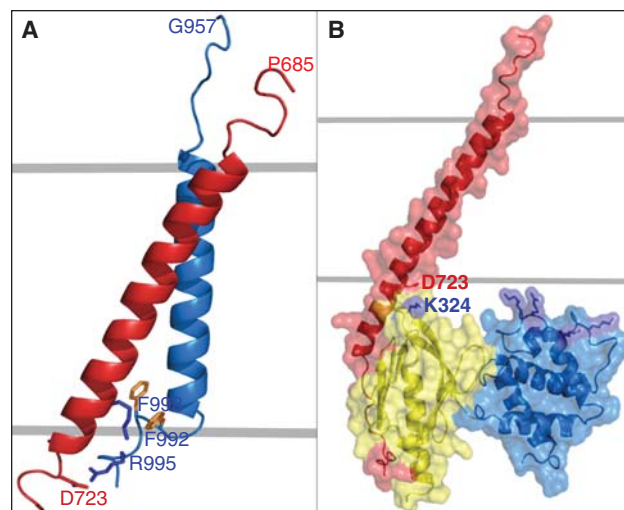
of labeled cytoplasmic tails (Kim et al. 2003). Recent studies have given new insight into the structure of the resting state. The structures of  $\beta 3$  and  $\alpha \text{IIb}$  TM segments in phospholipid bicelle model membranes have been solved by NMR separately (Lau et al. 2008a,b) and in complex (Lau et al. 2009). A similar structure was obtained for the TM region in intact  $\alpha \text{IIb}\beta 3$  using disulfide-based distance restraints combined with protein modeling (Zhu et al. 2009). A recent structure of a complex in organic solvents has been described (Yang et al. 2009) but the relevance of a system without a phospholipid/water interface is questionable. A bacterial reporter system has been used to define the sequence motif required for TM helix-helix interactions in  $\beta 1$  and  $\beta 3$  integrin subfamilies (Berger et al. 2010). Several modeling studies of the TM regions have also been published (Gottschalk 2005; Metcalf et al. 2009; Wang and Luo 2010). [Note that whereas most evidence supports the heterodimeric form, there is also evidence that homomeric TM oligomers can form in vitro (Li et al. 2003; Parthasarathy

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et al. 2008); nevertheless, the evidence for homodimeric forms mainly comes from experiments or simulations performed without the ectodomains, whose presence would be expected to favor the heterodimeric form.]

The NMR structure of the  $\alpha \text{IIb}\beta 3$  TM complex is shown in Figure 4. The  $\alpha \text{IIb}$  helix is perpendicular to the membrane whereas the  $\beta 3$  helix is tilted. There are glycines at the helix-helix interface in the membrane and an unusual  $\alpha \text{IIb}$  backbone reversal that packs a consecutive pair of Phe residues against the  $\beta 3$  TM helix, promoting electrostatic interactions between  $\alpha \text{IIb}(\text{D723})$  and  $\beta 3(\text{R995})$ . The two TM segments have essentially the same structure when studied separately suggesting that the topological features of the TM segments will remain unchanged in the separated, active state.

The ectodomain-TM linkers seem to be flexible (Lau et al. 2009; Zhu et al. 2009) and are thus unlikely to constrain the orientation between the ectodomain and the membrane. Although  $\alpha \text{X}\beta 2$  is bent in a similar way to



**Figure 4.** (A) NMR structure of the complex between the  $\alpha \text{IIb}$  (blue) and  $\beta 3$  (red) TM domains (PDB: 2K9J). The approximate position of the membrane glycerol backbone is shown by gray lines. (B) The talin F2 (blue)/F3 (yellow) domain pair in complex with a  $\beta$  integrin tail (red). The salt bridge that forms between K324 on F3 and D723 in the tail is shown; some key Lys and Arg residues are indicated in blue near the putative membrane interface with the F2 domain. B was constructed from a composite of coordinates of the talin/ $\beta$  complex (PDB: 3G9W; [Anthis et al. 2009]) and the membrane complex (PDB:2K9J [Lau et al. 2009]). Images made using PyMOL (DeLano Scientific).

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integrins without an  $\alpha$ -I domain, the terminal domains of the  $\alpha$ -legs and  $\beta$ -legs, calf-2, and  $\beta$ -tail domains, are oriented differently (Xie et al. 2009). These observations are all consistent with the flexible transmembrane domain separation model of activation rather than stiff pistons or levers. The TM complex is also likely to be stabilized by the resting ectodomain.

### The Cytoplasmic Tails

Several NMR studies of cytoplasmic tails have been published, although there is little agreement among them. Some studies could not detect an interaction between  $\alpha$  and  $\beta$  tails, whether they were connected by a coiled coil construct (Ulmer et al. 2001) or inserted in a membrane with TM segments (Li et al. 2001). A study of isolated mixed peptides found two distinct structures of the  $\alpha\beta$  complex (Weljie et al. 2002), both significantly different from the most detailed published structure of the  $\alpha\beta$  tail complex (Vinogradova et al. 2002). The latter structure does not seem to be consistent with the recently solved structures of the TM regions (Lau et al. 2009; Zhu et al. 2009). The most obvious explanation for these discrepancies is that the tails are rather flexible, only forming transient structures in solution in the absence of a protein interaction partner and that complexes between the tails and their binding proteins are likely to provide the most significant insights into transduction events.

### Cytoplasmic Tail Ligands

Because they are extended and flexible, the cytoplasmic tails, especially  $\beta$ , can “flycast” (Shoemaker et al. 2000) and reach out to form “hub” interactions with a number of proteins (Wegener and Campbell 2008; Legate and Fassler 2009). In particular, PTB domains bind to one of the two conserved NPXY motifs in  $\beta$ -tails. Especially important for activation of integrins from inside the cell are the proteins talin and kindlin. The talin PTB domain (the head F3 subdomain) binds the first  $\beta$ -NPXY and the membrane proximal helix (Wegener and Campbell 2008) whereas kindlin binds the

second NPXY (Moser et al. 2008). It has been proposed that the F2.F3 subdomains of talin make a defined contact with the membrane surface via numerous lysine and arginine residues especially in the F2 domain (Fig. 4B; Anthis et al. 2009). Binding of F3 to the  $\beta$ -tail promotes tail dissociation by breaking the salt bridge between the  $\alpha$  and  $\beta$  tails ( $\alpha$ IIB[D723]- $\beta$ 3[R995]); a new salt bridge is formed with K324 on the F3 domain. Binding to  $\beta$  plus the F2.F3 contact with the membrane can also influence the orientation of the  $\beta$ -helix, again helping promote separation of the TM and cytoplasmic domains.

### Structural Studies of Intact Integrins

Although crystallography of ectodomains and NMR of TM domains have provided detailed information about integrin structure, other techniques have been applied to intact integrins in attempts to distinguish among current models, such as “switchblade” and “deadbolt.” In principle this should be relatively easy because the switchblade model predicts a near doubling in molecular height on activation (Fig. 1C), whereas the deadbolt model predicts a modest change. In general, however, the various studies have not been in good agreement, possibly because of multiple integrin conformations in solution.

Cryoelectron tomography of ice-embedded specimens was used to obtain three-dimensional images of full-length  $\alpha$ IIB $\beta$ 3 incorporated into small liposomes (Ye et al. 2008). No significant height change was observed between the inactive state, and the active state induced by  $Mn^{++}$ . In a FRET study,  $Mn^{++}$  activation caused an increase of 5 nm in the separation between membrane and ligand binding site, consistent with a conformational change to the upright configuration (Chigaev et al. 2003). However, FRET between fluorescently labeled Fab fragments and the  $\beta$ 3  $\beta$ -I domain indicated only small changes on platelet activation (Gupta et al. 2007). A small angle neutron scattering (SANS) study of intact  $\alpha$ IIB $\beta$ 3 in  $Ca^{2+}$ /detergent solutions found “arched” and “handgun” forms (Nogales et al. 2010) although a  $Mn^{++}$

activated form was not studied. Analytical ultracentrifugation and EM were used to investigate  $\alpha$ IIb $\beta$ 3; measurement of frictional changes under different conditions suggested considerable plasticity in the structure (Rocco et al. 2008).

EM studies of ectodomains are also not entirely consistent although, on balance, they favor the switchblade model. Single-particle reconstructions of the negatively stained  $\alpha$ v $\beta$ 3 ectodomain bound to a fibronectin (FN) fragment suggested that the bent conformation can bind its physiological ligand (Adair et al. 2005). EM of negatively stained  $\alpha$ v $\beta$ 3 ectodomain with a cleavable clasp engineered into the carboxyl terminus showed a majority of molecules in the bent conformation when inactive, and a majority in the upright conformation when active (Takagi et al. 2002). Negative stain EM of a shorter ectodomain construct of  $\alpha$ 5 $\beta$ 1 bound to FN showed similar results (Takagi et al. 2003). The recent x-ray structure papers of intact ectodomains contain EM results consistent with the switchblade model (Zhu et al. 2008; Xie et al. 2009).

A recent study of integrin activation using EM and other studies gives useful insight (Ye et al. 2010). Membrane nanodiscs were synthesized with a single lipid-embedded integrin. The majority (~90%) of the class-averaged integrin nanodisc EM images in the absence of ligand and the talin head domain had a compact structure with a height of  $11 \pm 1$  nm; i.e., corresponding to the bent conformation in Figure 1C. In the presence of talin, ~25% of unliganded integrins had an extended structure with a height of  $19 \pm 1$  nm. In contrast, at least 40% of the fibrin-bound integrins were extended. This study provides evidence that talin binding is sufficient to activate and extend membrane-embedded integrin  $\alpha$ IIb $\beta$ 3 without applied force or clustering.

There is evidence that mechanical force is important for regulating integrin adhesiveness (Alon and Dustin 2007; Evans and Calderwood 2007). Talin contains both integrin and actin binding sites (Critchley 2009), and therefore the cytoskeleton could exert a lateral force on the  $\beta$  subunit. Steered molecular dynamics

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was applied to a complete ectodomain to mimic effects of cell generated tension. Evidence was found that lateral force could be transmitted through the  $\beta$  leg to the hybrid domain and promote the active form (Zhu et al. 2008).

### LIGAND BINDING

Historically, the pairing of integrins and their ligands has been uncovered either by ligand affinity chromatography or through the use of subunit-specific monoclonal antibodies (mAbs) to block ligand-mediated cell adhesion. In most cases, protein-protein binding assays have confirmed the associations established by these biochemical or cell biological approaches. A characteristic feature of most integrin receptors is their ability to bind a wide variety of ligands. Conversely, many extracellular matrix (ECM) and cell surface adhesion proteins bind to multiple integrin receptors (Humphries 2000; Plow et al. 2000; van der Flier and Sonnenberg 2001). One molecular explanation for this complexity is the evolutionary selection of common acidic peptide motifs in ECM proteins that mediate integrin binding via coordination to a divalent cation-containing binding pocket.

Integrin-ligand combinations can be clustered into four main classes, based on the nature of the molecular interaction. All five  $\alpha$ V integrins, two  $\beta$ 1 integrins ( $\alpha$ 5,  $\alpha$ 8), and  $\alpha$ IIb $\beta$ 3 recognize ligands containing an RGD tripeptide active site. Crystal structures of  $\alpha$ V $\beta$ 3 and  $\alpha$ IIb $\beta$ 3 complexed with RGD ligands have been reported and they reveal an identical atomic basis for this interaction (Xiong et al. 2002; Xiao et al. 2004). RGD binds at an interface between the  $\alpha$  and  $\beta$  subunits, with the basic residue fitting into a cleft in a  $\beta$ -propeller module in the  $\alpha$  subunit, and the acidic residue coordinating a cation bound in the  $\beta$ -I-domain. RGD-binding integrins bind to a large number of ECM and soluble vascular ligands, but with different affinities that presumably reflect the preciseness of the fit of the ligand RGD conformation with the specific  $\alpha$ , $\beta$  active site pockets. Although RGD is an essential element of the ligand binding process, macromolecular ligands can contain other binding sites, the best

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characterized of which is a synergy sequence in fibronectin that also binds the  $\alpha 5$   $\beta$ -propeller (Mould et al. 1997, 2003c).

$\alpha 4\beta 1$ ,  $\alpha 4\beta 7$ ,  $\alpha 9\beta 1$ , the four members of the  $\beta 2$  subfamily, and  $\alpha E\beta 7$  recognize related sequences in their ligands.  $\alpha 4\beta 1$ ,  $\alpha 4\beta 7$ , and  $\alpha 9\beta 1$  bind to an acidic motif, termed “LDV,” that is functionally related to RGD. Fibronectin contains the prototype LDV ligand in its type III connecting segment region, but other ligands (such as VCAM-1 and MAdCAM-1) employ related sequences. Structures of this integrin subfamily are lacking, but it is highly likely that LDV peptides bind similarly to RGD at the junction between the  $\alpha$  and  $\beta$  subunits. The  $\beta 2$  family employs a similar mode of ligand binding, but the major interaction takes place via an inserted I-domain in the  $\alpha$  subunit (Shimaoka et al. 2003). Despite this fundamental mechanistic difference, the characterized sites within ligands that bind  $\beta 2$  integrins are structurally homologous to the LDV motif (Shimaoka et al. 2003). The major difference is that  $\beta 1/\beta 7$  ligands employ an aspartate residue for cation coordination, whereas  $\beta 2$  integrins use glutamate.

Four  $\alpha$  subunits containing an  $\alpha$ -I-domain ( $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 10$ , and  $\alpha 11$ ) combine with  $\beta 1$ , and form a distinct laminin/collagen-binding subfamily. A crystal structure of a complex between the  $\alpha 2$  I-domain and a triple-helical collagenous peptide has revealed the structural basis of the interaction, with a critical glutamate within a collagenous GFOGER motif providing the key cation-coordinating residue (Emsley et al. 2000). The mechanism of laminin binding is less well understood, although a recent study has suggested that the extreme carboxyl terminus of the  $\gamma$  chain and an undefined site within  $\alpha$  subunit laminin G domains together constitute an integrin-binding site (Ido et al. 2007). Three  $\beta 1$  integrins ( $\alpha 3$ ,  $\alpha 6$ , and  $\alpha 7$ ), plus  $\alpha 6\beta 4$ , are highly selective laminin receptors. Analysis of laminin fragments indicates that these receptors and the  $\alpha$ -I-domain-containing  $\beta 1$  integrins bind to different regions of the ligands. In neither case has the active site been narrowed down to a particular sequence or residue.

## ACTIVATION

For the interaction of integrins with their ligands to be meaningful for cellular function, the binding event must be able to regulate signal transduction. However, adhesion is highly dynamic, with cells continuously sampling their pericellular environment, and responding by rapidly changing their position and state of differentiation, and therefore a highly responsive receptor activation mechanism is required. As integrins lack enzymatic activity, signaling is instead induced by the assembly of signaling complexes on the cytoplasmic face of the plasma membrane. Formation of these complexes is achieved in two ways; first, by receptor clustering, which increases the avidity of molecular interactions thereby increasing the on-rate of binding of effector molecules, and second, by induction of conformational changes in receptors that creates or exposes effector binding sites. Current evidence suggests that conformational regulation is the primary mode of affinity regulation of integrins. In turn, this demands a mechanism for conveying conformational changes between the cytoplasmic tails and the ligand-binding head domain over a relatively large distance ( $\sim 20$  nm).

## Evidence for Conformational Regulation

Gross conformational changes in integrins have been monitored by a variety of techniques, and for almost all of these studies,  $\alpha IIb\beta 3$  has served as a prototype. Treatment with RGD peptides elicited alterations in sedimentation coefficient and Stokes radius (Parise et al. 1987), and receptor activation on platelets triggered changes in intramolecular FRET and cross-linking (Sims et al. 1991). mAbs have proven particularly useful probes of integrin function. Early studies reported activation-dependent changes in mAb binding to  $\alpha IIb\beta 3$  that were attributed to conformational changes (McEver and Martin 1984; Collier 1985), and these were followed by the identification of a subset of anti- $\alpha IIb\beta 3$  mAbs, the epitopes for which were induced in response to ligand binding (Frelinger et al. 1990, 1991). The acronym LIBS was coined to describe these epitopes as ligand-induced



binding sites. In most cases that have been examined, activating mAbs appear to function by increasing the affinity of ligand binding. Most LIBS mAbs have epitopes that are regulated by divalent cations, and because cations also regulate ligand binding, it appears that many cation-responsive, activating mAbs recognize naturally occurring conformers of integrins. These mAbs may therefore displace a conformational equilibrium in favor of these forms that leads to an increase in the proportion of high affinity integrin. Some other activating mAb epitopes are unaffected by either ligand or cation binding and here the most likely mechanism of action is through inducing an activated conformation in the integrin (Chen et al. 1999).

The location of LIBS epitopes has contributed significantly to our understanding of the process of receptor activation. The overwhelming majority of activating mAbs recognize the  $\beta$  subunit, and their epitopes are distributed throughout the polypeptide (Humphries 2000; Byron et al. 2009). This is suggestive of a large-scale alteration in the conformation of the whole integrin during activation. The regions recognized include the  $\beta$ -I-domain, the extreme amino terminus of the  $\beta$  subunit in the PSI domain, the hybrid domain, the  $\beta$ -subunit knee region, and distal EGF-like repeats. A few activating anti- $\alpha$  subunit mAbs have been reported, the epitopes for which are found in the  $\beta$ -propeller, the heavy-light chain border and close to the transmembrane domain, suggestive of conformational changes in these regions of the molecule (Loftus et al. 1987; Keizer et al. 1988).

### How Are Conformational Changes Coupled?

As discussed above, the various structural studies in the last 10 years have greatly stimulated functional analyses. The relevance of the observed bend in the legs of the integrin dimer has been a highly contentious issue. It has been proposed that integrins are always bent, but several lines of evidence indicate that bent integrins are inactive, and extended integrins are primed. In the original crystal structure of  $\alpha$ V $\beta$ 3, the

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integrin was bent at an angle of  $135^\circ$ . Locking integrins in this state through disulphide bond engineering abolishes ligand binding by cell surface-expressed receptors (Takagi et al. 2002). Furthermore, when the gross structure of integrins was examined by electron microscopy under conditions in which ligand binding was low, e.g., in  $\text{Ca}^{2+}$ -containing buffers or following the introduction of intersubunit covalent bond constraints, predominantly bent structures were observed (Takagi et al. 2002; Nishida et al. 2006). In bent integrins, the ligand-binding pocket may be oriented toward the plasma membrane, thereby impeding ligand engagement, but flexibility at the juxtamembrane domain could enable a “breathing” movement for the conversion of bent to extended integrin (Beglova et al. 2002; Takagi et al. 2002). In this context, a cryo-EM reconstruction of unstimulated  $\alpha$ IIb $\beta$ 3 indicated a partially extended conformation (Adair and Yeager 2002). The binding of stimulatory mAbs might then displace a conformational equilibrium, leading to activation. Similarly, breaking the interactions between the  $\alpha$  and  $\beta$  cytoplasmic tails appears to lead to a loss of the interactions among the leg regions, disruption of an interface between the head and legs, and a repositioning of the head to point away from the cell surface. Major support for this model comes from studies of soluble recombinant integrins by electron microscopy (Takagi et al. 2002) and from the large number of epitopes of stimulatory mAbs that have now been shown to lie in the knee or leg regions (Humphries et al. 2003). Exposure of these epitopes is low in the bent state of the integrin (where they are masked) but high in the extended state (Beglova et al. 2002).

The pathway of conformational change from the interior of the cell to the ligand-binding site of the integrin is incompletely understood, but movement of the hybrid domain appears to be a central feature of the conformational changes accompanying unbending (Fig. 2). By EM, an acute angle between the hybrid domain and  $\beta$ -I was observed in the bent state, and an obtuse angle in the extended, ligand-occupied state (Takagi et al. 2002). In the

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bent conformation, any movement of the hybrid domain relative to the  $\beta$ -I domain is prevented, and therefore unbending is probably an essential prerequisite to hybrid domain motion. A central role for hybrid domain movement in affinity regulation has been established by a number of approaches. Activating mAb epitopes in the hybrid domain map close to an interface between the hybrid domain and the  $\alpha$  subunit  $\beta$ -propeller (Mould et al. 2003b). These epitopes would be masked in bent integrins, but would become exposed when the hybrid domain swings away from the propeller. Furthermore, engineering of glycosylation sites between the hybrid domain and the  $\beta$ -I-domain produces a putative wedge that leads to integrin activation (Luo et al. 2003).

Conformational changes in the head are the key determinant of ligand-binding activity, specifically, the conformation of the  $\beta$ -I domain, which, in turn, is determined by the position of the hybrid domain. Thus, a swing-out of the hybrid domain away from the  $\alpha$ -subunit pulls downward on the  $\alpha$ 7 helix of the  $\beta$ -I domain and favors the upward movement of the  $\alpha$ 1 helix (Xiao et al. 2004). The motion of these two helices shifts the  $\beta$ -I domain from a low-affinity into a high-affinity conformation by backbone movements of loops that contain cation-coordinating residues. Mutations that favor a downward shift of the  $\alpha$ 7 helix (Mould et al. 2003b; Hato et al. 2006; Cheng et al. 2007) also result in a high-affinity state.

Although current models of integrin function strongly suggest that conversion to a high-affinity receptor requires extension, there is some evidence to suggest that ligand-bound integrin can adopt a bent conformation. Crystallized  $\alpha$ V $\beta$ 3 can bind a cyclic RGD peptide in the bent conformation (Xiong et al. 2002) and electron microscopy images also show bent  $\alpha$ V $\beta$ 3 in complex with a fragment of fibronectin (Adair et al. 2005). In addition, studies that have either used FRET or competition ELISA to measure the distance between a fluorescently tagged ligand peptide and labeled cell membrane or between mAbs directed against the head piece and leg regions of  $\alpha$ IIb $\beta$ 3 on platelets have revealed partial unbending

(Calzada et al. 2002; Chigaev et al. 2003, 2007). Nevertheless, when FRET-FLIM is employed to analyze the conformation of  $\alpha$ 5 $\beta$ 1 in adherent cells, by measuring FRET between a fluorescently labeled Fab bound to  $\alpha$ 5 $\beta$ 1 and fluorescent dye intercalated into the cell membrane, it has been shown that integrins are extended in focal adhesions and bent elsewhere (Askari et al. 2010).

### Integrin Antagonists as Therapeutic Agents

The short acidic peptides that serve as ligand active sites are essentially pro-drugs, and both RGD and LDV peptides have been converted into small molecule therapeutics. RGD-based, peptidomimetic antagonists of  $\alpha$ IIb $\beta$ 3, such as eptifibatid (from 1998) and tirofiban (from 1998), are now used widely as antithrombotic agents (Hanson et al. 2004), and LDV-based compounds are in development for treatment of asthma and multiple sclerosis. In addition, mAbs that block integrin function and cell adhesion have been developed as therapeutic agents. These agents were originally assumed to compete with ligands for receptor binding, but this now appears not to be the case, with many anti-integrin mAbs having been shown to function via allosteric mechanisms. Current evidence suggests that mAbs inhibit ligand binding either by stabilizing the unoccupied state of the receptor or by preventing a conformational change necessary for ligand occupancy. In turn, the allosteric inhibition of ligand binding by anti-functional anti-integrin mAbs implies that it may be feasible to synthesize small molecule inhibitors that function in the same way. Such inhibitors could have advantages over competitive inhibitors in that a partial inhibition of function may be obtained and therefore adhesion may be more easily controlled, and they may not possess the agonistic properties of ligand mimetics, and may therefore not suffer from mechanism-related side-effects. Small molecule allosteric inhibitors that bind to  $\alpha$ -I-domains have now been reported. These molecules appear to stabilize the low affinity conformation of the  $\alpha$ -I-domain by blocking downward movement of the terminal  $\alpha$ 7-helix



and thereby preventing rearrangements at the ligand-binding pocket necessary for high affinity ligand binding (Kallen et al. 1999).

## CONCLUSION

There has been remarkable progress in our understanding of integrin structure and function in the last 10 years. The basis of much previous work on conformation, which was performed with conformationally sensitive antibodies, and ligand binding, which was largely based on mutational analyses, can now be modeled at atomic resolution. A unifying biophysical model of integrin function, which incorporates features such as catch bonds, extreme flexibility at the knees and the on- and off-rates of ligand and effector binding is therefore within reach. The process of integrin activation from inside the cell is also now quite well understood at a structural level. However, a number of major questions remain unresolved. These include outside-in signaling, which is much less well understood compared with inside-out signaling and it is unclear how similar or different the two processes are. We still do not know how an integrin allows a cell to interpret the binding of different ligands, and therefore how microenvironmental sensing is achieved at a molecular level. Looking further ahead, the process of “inactivation,” where integrins return to their resting state, is not understood, and we are just starting to develop approaches to measure force transduction at adhesion sites.

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