Integrin activation

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Summary

The ability of cells to regulate dynamically their adhesion to one another and to the extracellular matrix (ECM) that surrounds them is essential in multicellular organisms. The integrin family of transmembrane adhesion receptors mediates both cell-cell and cell-ECM adhesion. One important, rapid and reversible mechanism for regulating adhesion is by increasing the affinity of integrin receptors for their extracellular ligands (integrin activation). This is controlled by intracellular signals that, through their action on integrin cytoplasmic domains, induce conformational changes in integrin extracellular domains that result in increased affinity for ligand. Recent studies have shed light on the final intracellular steps in this process and have revealed a vital role for the cytoskeletal protein talin.

Key words: Integrin, Talin, Cytoplasmic tails

Introduction

Integrins are heterodimeric adhesion receptors formed by the non-covalent association of α and β subunits. Each subunit is a type I transmembrane glycoprotein that has relatively large extracellular domains and, with the exception of the β4 subunit, a short cytoplasmic tail (Hynes, 2002). Integrins are present in all metazoans, and the number of integrins in the genome generally increases with the complexity of the organism (Hynes, 2002; Bokel and Brown, 2002), which is consistent with the central role of integrins in adhesion, migration and tissue organization. Mammals contain 18 α and 8 β subunits that combine to produce at least 24 different heterodimers, each of which can bind to a specific repertoire of cell-surface, ECM or soluble protein ligands.

Cell-cell and cell-substratum adhesion is mediated by the binding of integrin extracellular domains to diverse protein ligands; however, cellular control of these adhesive interactions and their translation into dynamic cellular responses, such as cell spreading or migration, requires the integrin cytoplasmic tails. These short tails bind to intracellular ligands that connect the receptors to signalling pathways and cytoskeletal networks (Critchley, 2000; Calderwood et al., 2000; Liu et al., 2000; Brakebusch and Fassler, 2003; Giancotti and Ruoslahti, 1999; Geiger et al., 2001). Hence, by binding both extracellular and intracellular ligands, integrins provide a transmembrane link for the bidirectional transmission of mechanical force and biochemical signals across the plasma membrane.

One important mechanism by which cells regulate integrin function is through tight spatial and temporal control of integrin affinity for extracellular ligands. This is achieved by rapid, reversible changes in the conformation of the extracellular domains of the integrin heterodimer, so-called integrin activation (Sims et al., 1991; Woodside et al., 2001). It has recently been noted that researchers in the integrin field use this term differently from those studying most other receptors and that improved clarity of terminology will be required to understand the complexities of integrin regulation (Humphries et al., 2003; Carman and Springer, 2003). The term activation was initially applied to integrins in early studies of the platelet GPIIb/IIIa complex (integrin αIIbβ3), where an activation-dependent change in the conformation and/or microenvironment of the complex caused activation of the fibrinogen receptor function of GPIIb/IIIa (Coller, 1985; Shattil et al., 1985; Isenberg et al., 1987). In this sense, integrin activation refers to the changes required to enhance ligand-binding activity (the primary effector function of adhesion receptors), whereas activation of signalling receptors generally refers to the changes induced by ligand binding that enhance signal transduction (the primary effector function of signalling receptors). The finding that integrins also play important roles as signalling receptors (Schwartz and Ginsberg, 2002) serves to emphasise the importance of providing clear definitions of terms. Recently, integrin priming was proposed as a term to denote integrin affinity regulation, whereas integrin activation would be defined as the process of ligand-induced propagation of intracellular signals (Humphries et al., 2003). However, priming has also been proposed to define the affinity- or valency-based regulatory events that serve to enhance ligand-binding efficiency (Carman and Springer, 2003). It is therefore essential that investigators operationally define ‘priming’ or ‘activation’ for their specific experimental system. Here, in keeping with general usage and historical precedent, I will apply the term integrin activation to describe the increase in monomeric affinity that is coupled to alterations in integrin conformation.

Despite significant recent advances, the exact nature of the conformational changes leading to integrin activation remains controversial (reviewed by Hynes, 2002; Liddington and Ginsberg, 2002; Shimaoka et al., 2002; Xiong et al., 2003a; Humphries et al., 2003). Additional affinity-independent mechanisms, such as integrin clustering, lateral diffusion of receptors, interactions with and reorganization of the cytoskeleton, and changes in integrin expression patterns also contribute to the control of integrin-mediated adhesion (reviewed by Laudanna et al., 2002; van Kooyk and Figdor, 2000; Hogg et al., 2002). Affinity-dependent and -independent

mechanisms are not mutually exclusive and can act in concert; indeed, integrin activation and clustering may be mechanistically linked such that activation, in combination with ligand occupancy, also stimulates clustering (Isenberg et al., 1987; Erb et al., 1997; Li et al., 2003).

The importance of tight regulation of integrin activation is evident during haemostasis, where activation of platelet integrin αIIbβ3 is a pivotal event in thrombus formation (Shattil et al., 1998), and during leukocyte trafficking, where β1 and β2 integrins become activated (Laudanna et al., 2002; Hogg et al., 2002). However, the importance of integrin activation is not limited to blood cells. Integrin activation is essential for normal development because it controls cell adhesion, migration and assembly of an extracellular matrix (Wu et al., 1995; Huttenlocher et al., 1996; Palecek et al., 1997). Integrin activation is also involved in angiogenesis (Byzova et al., 2000) and tumour cell metastasis (Felding-Habermann et al., 2001), and deregulated integrin activation disrupts embryonic development (Martin-Bermudo et al., 1998), and impairs cardiac function (Keller et al., 2001) and the immune response (McDowall et al., 2003). Thus, cellular control of integrin activation plays important roles in health and disease throughout development and during the course of adult life. This review will focus on the roles of integrin cytoplasmic tails and the importance of their interactions with talin for regulating integrin activation.

Integrin cytoplasmic tails

The membrane-proximal regions of the short α and β integrin cytoplasmic tails are well conserved (Fig. 1) and play crucial roles in integrin activation. Deletion of the entire α subunit cytoplasmic tail, or of only the conserved membrane-proximal GFFKR sequence, constitutively activates integrins, whereas deletions that retain the GFFKR sequence do not (O'Toole et al., 1991; Ylanne et al., 1993; O'Toole et al., 1994; Lu and Springer, 1997). Likewise, deletion of the membrane-proximal region of the β tail activates integrins, whereas deletions that are more C-terminal block activation (Hughes et al., 1995; Lu et al., 2001; Crowe et al., 1994). Mutagenesis studies suggest that a salt bridge between residues R995 of αIIb and D723 of β3 stabilizes the association of the membrane-proximal regions and so maintains αIIbβ3 in a low-affinity state (Hughes et al., 1996). Furthermore, forced association of the cytoplasmic regions of αLβ2 or αMβ2 blocks activation, and preventing their association activates integrins (Lu et al., 2001; Kim et al., 2003). Indeed, lipid-modified peptides corresponding to the membrane-proximal regions of the αIIb or α2 tails activate αIIbβ3 or α2β1 when introduced into platelets, possibly by disrupting the interaction between integrin α and β tails (Stephens et al., 1998; Wang et al., 2003). More recently, fluorescence resonance energy transfer (FRET) analysis of cyan fluorescent protein-fused and yellow fluorescent protein-fused αL and β2 has provided in vivo evidence for α-β tail associations (Kim et al., 2003). In the resting state, the α and β tails were sufficiently close together to undergo FRET; however, either stimulation with agonists, leading to integrin activation, or introduction of activating membrane-proximal α subunit mutations led to a reduction in FRET, which is indicative of conformational rearrangements of the cytoplasmic domains. Biochemical evidence for a low-affinity interaction between α and β tails also exists (Muir et al., 1994; Haas and Plow, 1997; Laplantine et al., 2000; Ginsberg et al., 2001), and activating mutations in the α subunit membrane-proximal region disrupt the interaction (Vallar et al., 1999).

The high-affinity state generated by deletion of the

A

α1	LALW **KI**GFFKRPLKKKMEK
α2	AILW **KL**GFFKRKYEKMTKNPDEIDETTELSS
α3A	LLLW **KC**GFFKRARTRALYEAKRQKAEMKSQPSETERLTDDY
α4	YVMW **KA**GFFKRQYKSILQEENRRDSWSYINSKSNDD
α5	YILY **KL**GFFKRSLPYGTAMEKAQLKPPATSDA
α6A	FILW **KC**GFFKRNKKDHYDATYHKAEIHAQPSDKERLTSDA
α7	LLLW **KM**GFFKRAKHPEATVPQYHAVKIPREDRQQFKEEKTGTILRNNWGSPRREGPDAHPILAADGHPELGPDGHPGPGTA
α8	LALW **KC**GFFDRARPPQEDMTDREQLTNDKTPEA
α9	VLLW **KM**GFFRRRYKEIIEAEKNRKENEDSWDWVQKNQ
α10	FCLW **KL**GFFAHKKIPEEEKREEKLEQ
α11	LALW **KL**GFFRSARRRREPGLDPTPKVLE
αV	FVMY RM**GFFKR**VRPPQEEQEREQLQPHENGEGNSET
αL	IVLY **KV**GFFKRNLKEKMEAGRGVPNGIPAEDSEQLASGQEAGDPGCLKPLHEKDSESGGGKD
αM	AALY **KL**GFFKRQYKDMMSEGGPPGAEPQ
αX	AVLY **KV**GFFKRQYKEMMEEANGQIAPENGTQTPSPPSEK
αD	ATLY **KL**GFFKRHYKEMLEDKPEDTATFSGDDFSCVAPNVPLS
αIIb	LAMW **KV**GFFKRNRPPLEEDDEEGE
αE	VILF **KC**GFFKRKYQQLNLESIRKAQLKSENLLEEEN
αPS1	YVLW **KV**GFFKRIRPTDPTLSGNLEKMNEEKPFLAPSKNTHHVF
αPAT2	LLLW RC**GFFKR**NRPPTEHAELRADRQPNAQYADSQSRYTSQDQYNQGRHGQML

B

β1A	LLIW **KL**LMII**HDRRE**FAKFEKEKMNAK**W**DTGE**NPI**YKSAVTTVV--------NPKYEGK
β2	LVIW **KA**LIHLS**DLRE**YRRFEKEKLKSQ**W**NND-**NPL**FKSATTTVM--------NPKFAES
β3	LLIW **KL**LITI**HDRKE**FAKFEEERARAK**W**DTAN**NPL**YKEATSTFT--------NITYRGT
β5	LAIW **KL**LVTI**HDRRE**FAKFQSERSRAR**Y**EMAS**NPL**YRKPISTHTVDFTFNKFNKSYNGTVD
β6	LCIW **KL**LVSF**HDRKE**VAKFEAERSKAK**W**QTGT**NPL**YRGSTSTFK--------NVTYKHREKQKVDLSTDC
β7	VLAY R**LS**VEI**Y**DRRE**Y**SRFEKEQQQLN**W**KQDS**NPL**YKSAITTTI--------NPRFQEADSPTL
βPS	LLLW **KL**LTTI**HDRRE**FARFEKERMNAK**W**DTGE**NPI**YKQATSTFK--------NPMYAGK
βPAT3	LLLW **KL**LTVL**HDRSE**YATFNNERLMAK**W**DTNE**NPI**YKQATTTFK--------NPVYAGKAN

Fig. 1. Alignment of integrin cytoplasmic tails. The amino acid sequences of human α tails and *Drosophila* αPS1 and *Caenorhabditis elegans* αPAT2 (A), and human β and *Drosophila* βPS and *C. elegans* βPAT3 (B) were manually aligned. The divergent human β4 and β8 and alternative splice variants of α3, α6, β1 and β3 were omitted. The interface between the transmembrane and cytoplasmic regions is generally assumed to lie between the conserved W/Y and K residues, shown in bold. The conserved membrane-proximal α subunit GFFKR and β subunit LLxxxHDREE are shown in red. The conserved β tail residues involved in talin binding are indicated; the first, β turn-forming, NPxY motif is shown in blue and the conserved tryptophan is shown in pink.

membrane-proximal region of either the α or β tail is independent of cell type and metabolic energy, whereas cell-type-dependent and energy-dependent activation requires additional, more C-terminal, sequences (O'Toole et al., 1994; Hughes et al., 1995). Deletion of β tail sequences outside the membrane-proximal region blocks activation, and specific β tail regions important for activation have been identified (Hibbs et al., 1991a; Hibbs et al., 1991b; Chen et al., 1994a; O'Toole et al., 1994; O'Toole et al., 1995; Hughes et al., 1995; Wang et al., 1997). Tyrosine to alanine mutations in one of these sites, the first conserved NPxY motif, strongly inhibit integrin activation and induce structural changes at the NPxY site and within the membrane-proximal region (Ulmer et al., 2001). Mutations in the NPxY motif also perturb the binding of numerous cytoskeletal and signalling proteins to integrin β tails (Liu et al., 2000), and αIIbβ3 activation by the β tail-binding protein talin requires an intact NPxY sequence (Calderwood et al., 2002; Calderwood et al., 1999). Thus, membrane-distal portions of the β tail might control integrin activation through interactions with regulatory proteins and direct effects on the conformation of membrane-proximal regions. Isolated β1A, β1D, β3 or β5 tails, but not α tails nor β tails containing membrane-distal inactivating mutations, inhibit integrin activation in a dose-dependent manner, presumably by competing for limiting amounts of intracellular activators (Chen et al., 1994b; Fenczik et al., 1997; Mastrangelo et al., 1999; Zent et al., 2000; Bodeau et al., 2001).

The α tail contributes to the cell-type specificity of integrin activation (O'Toole et al., 1994), and deletion of α subunit residues after GFFKR inhibits cell-type-specific and agonist-induced α2β1-, α4β1- and αLβ2-mediated adhesion (Kawaguchi and Hemler, 1993; Kassner and Hemler, 1993; Weber et al., 1997; Tohyama et al., 2003). If an additional 5-7 amino acids is included after the GFFKR sequence then this is prevented. However, with the notable exception of Rap1-induced αLβ2-mediated adhesion in BAF/3 cells, the sequence of these additional residues is unimportant (Kassner et al., 1994; Tohyama et al., 2003). In the case of αIIbβ3, a portion of the αIIb tail immediately C-terminal to the conserved GFFKR may interact with the β3 tail to inhibit formation of an active conformation (Ginsberg et al., 2001). In the presence of this sequence, the β3 tail forms epitopes present in native inactive αIIbβ3 and in β3 tails containing an inactivating mutation. Furthermore, palmitoylated peptides spanning this region of αIIb suppress αIIbβ3 activation in platelets (Ginsberg et al., 2001) and a P988A, P999A mutation within this region alters the conformation of the α tail (Vinogradova et al., 2000), prevents formation of the combinatorial epitope (Ginsberg et al., 2001) and activates αIIbβ3 (Leisner et al., 1999).

The membrane-proximal regions of the α and β tails thus play crucial roles in integrin activation, probably by interacting with one another to stabilize an inactive conformation. The more distal regions of the β tails regulate activation through interactions with signalling proteins that might disrupt the membrane-proximal interaction, whereas membrane-distal α sequences regulate β tail conformation and association with activator proteins in a cell-type-specific manner.

Structure of integrin tails

Nuclear magnetic resonance (NMR) studies of peptide constructs representing sequences from the membrane-proximal regions of the αIIb and β3 tails, the entire cytoplasmic tails, or the cytoplasmic tails fused to the transmembrane sequences, either free in solution, fused to soluble coiled-coil regions, tethered to membranes or embedded in micelles, have shed further light on interactions involving integrin tails (Vinogradova et al., 2000; Vinogradova et al., 2002; Ulmer et al., 2001; Ulmer et al., 2003; Li et al., 2001; Li et al., 2002; Weljie et al., 2002). Although the membrane-proximal regions of both β3 and αIIb generally tend to form α helices, and evidence exists for a turn at the β3 subunit NPLY, there are significant differences between the various structures obtained. This probably reflects the different constructs and conditions used, and the dynamic flexible nature of the integrin tails. Crystallography reveals that, when bound to the phosphotyrosine binding (PTB)-like domain of talin, the β3 tail NPLY motif forms a turn and the preceding seven residues form a β strand that augments the β sandwich present in talin (Garcia-Alvarez et al., 2003). Thus, integrin tails might rely on interaction with intracellular factors to stabilize their structure. Nonetheless, despite the flexibility of the tails, point mutations can disrupt their conformation and inhibit their interactions with intracellular proteins (Vinogradova et al., 2000; Ulmer et al., 2001; Ulmer et al., 2003).

Two NMR studies detected no interaction between the αIIb and β3 tails (Ulmer et al., 2001; Li et al., 2001), whereas one study detected a weak membrane-proximal interaction (Vinogradova et al., 2002) and, using truncated tails, another study observed two distinct αβ tail complexes, each significantly different from that seen by Vinogradova et al. (Weljie et al., 2002). Isolated tail complexes may be difficult to observe because of the low-affinity interaction between tails (Vallar et al., 1999). α and β subunit extracellular domains form a complex that, in intact integrins, limits the relative mobility of the tails and might facilitate complex formation; nonetheless, tethering α and β tails together did not reveal an interaction (Ulmer et al., 2001). The only αβ interaction observed using full-length tails was evident from small chemical shifts and was absent in the presence of micelles (Vinogradova et al., 2002), which raises questions about its biological significance. However, the complex was disrupted by activating membrane-proximal α subunit mutations and by the integrin activator talin. Activating, but not non-activating, talin fragments perturb the membrane-proximal residues of β3 tails and tethering the αIIb tail adjacent to the β3 tail inhibits this effect (Ulmer et al., 2003). Thus, although integrin cytoplasmic tails may be largely unstructured in solution and their modes of interaction remain controversial, structural studies provide some support for a membrane-proximal interaction between αIIb and β3 tails and for regulation of this by the integrin-activator talin.

Talin is a critical integrin-activating protein

Recent data indicate that talin, a major cytoskeletal actin-binding protein that binds to integrin, tails and colocalizes with activated integrins (Critchley, 2000), plays a crucial role in integrin activation (Tadokoro et al., 2003). Talin, like integrin, is required for normal development in mice (Monkley et al., 2000), *Drosophila* (Brown et al., 2002) and *Caenorhabditis elegans* (Cram et al., 2003). In *Drosophila* and *C. elegans*, talin

deficiency generates phenotypes similar to those produced by integrin deficiency, indicating that talin is required for normal integrin function in vivo. However, talin regulates integrins in several ways (Calderwood and Ginsberg, 2003), and the role of defective integrin activation in the phenotypes of talin-deficient animals has not been assessed.

Talin is an antiparallel homodimer of two ~270 kDa subunits (Critchley, 2000). Each subunit consists of an N-terminal ~50 kDa globular head and an ~220 kDa C-terminal rod (Rees et al., 1990). Talin binds strongly to β1A, β1D, β2, β3 and β5 integrin tails and weakly to the β7 integrin tail (Horwitz et al., 1986; Knezevic et al., 1996; Pfaff et al., 1998; Sampath et al., 1998; Calderwood et al., 1999; Calderwood et al., 2001; Calderwood et al., 2003). The major integrin-binding site lies within the talin head (Calderwood et al., 1999; Patil et al., 1999; Yan et al., 2001) and, when over-expressed, talin fragments containing this binding site activate β3 integrins (Calderwood et al., 1999; Calderwood et al., 2002).

RNAi knockdown of talin expression has revealed that talin is essential for β1 and β3 integrin activation in a variety of cell types (Tadokoro et al., 2003). Notably, in megakaryocytes (the precursors of platelets), talin knockdown prevents αIIbβ3 activation following stimulation with physiological agonists. Talin mediates cellular-energy-dependent integrin activation by binding to integrin β tails, and disruption of this interaction, by mutations in either talin or the β3 tail, inhibits activation (Tadokoro et al., 2003). The requirement for talin cannot be bypassed by physiological agonists or by expression of other putative integrin-activating proteins (Tadokoro et al., 2003). Thus, its binding to integrin β tails is an essential final step in integrin activation.

The talin head possesses a FERM (4.1, ezrin, radixin, moesin) domain (Rees et al., 1990; Calderwood et al., 2002). FERM domains have three subdomains – F1, F2 and F3 – and often mediate interactions with the cytoplasmic tails of transmembrane proteins (Pearson et al., 2000). The talin F2 and F3 subdomains bind specifically to integrin β3 tails; however, F3 binds with a fourfold higher affinity (K_d = 120 nM) than F2 (Calderwood et al., 2002). Furthermore, expression of talin F3, but not F2 or other high-affinity β tail-binding proteins, activates αIIbβ3 (Calderwood et al., 2002). Thus, the major integrin-binding and activating fragment of talin lies within the 96-residue F3 subdomain.

X-ray crystallography of a talin fragment spanning the F2 and F3 subdomains revealed that, as in other FERM domains, F2 is largely α-helical and resembles the acyl-CoA-binding protein, whereas F3 is a sandwich of two orthogonal antiparallel β-sheets followed by an α-helix (Garcia-Alvarez et al., 2003). The F3 fold is very similar to that of PTB domains, which often recognize peptide ligands containing β turns formed by NPxY motifs (Schlessinger and Lemmon, 2003). NPxY motifs are highly conserved in integrin β tails (Fig. 1), and mutations that disrupt this motif perturb β turn formation, inhibit talin binding and interfere with integrin activation (Ulmer et al., 2001; Pfaff et al., 1998; Calderwood et al., 2002; O'Toole et al., 1995). The crystal structure of talin F3-engaging residues 739-750 of the β3 tail reveals that residues 740-742 (DTA) form a β strand that augments the β sheet of F3, and residues 744-747 (NPLY) form a reverse turn with Y747 pointing into an acidic and hydrophobic pocket (Garcia-Alvarez et al., 2003) (Fig. 2). Both the turn and augmentation of the β strand are classical features of PTB domain-ligand interactions (Schlessinger and Lemmon, 2003). A distinctive feature of the β3-F3 complex is a pocket occupied by the side chain of a conserved β tail tryptophan residue (W739). The talin-β3 interaction is disrupted and integrin activation inhibited by mutation of either this tryptophan, or of residues involved in β turn formation, or of talin residues that contact the β tail (Garcia-Alvarez et al., 2003; Tadokoro et al., 2003) (Fig. 2). The key sites mediating talin binding are well conserved in integrin β tails (Fig. 1), suggesting that the talin-β3 structure represents a general integrin-activation complex.

Talin binding also affects the membrane-proximal regions of the β3 tail and this is probably required for transmission of the activation signal (Vinogradova et al., 2002; Ulmer et al., 2003). The crystallized talin-β3 complex does not include the membrane-proximal β3 sequence, but NMR analysis reveals that NPxY-mediated talin binding induces spectral perturbations in the membrane-proximal residues (Vinogradova et al., 2002; Ulmer et al., 2003; Garcia-Alvarez et al., 2003). This might be owing to direct interactions with the membrane-proximal region of the tail (Patil et al., 1999) or to indirect conformational changes induced following formation of the complex. Notably, only integrin-activating talin fragments affect the membrane-proximal regions of β3, and tethering the αIIb tail parallel to the β3 tail inhibits this effect (Ulmer et al., 2003). Furthermore, deletion of the α subunit membrane-proximal regions generates integrins that remain activated in the absence of talin or metabolic energy (O'Toole et al., 1994; Tadokoro et al., 2003). Finally, the talin head domain prevents detection of the membrane-proximal interaction between αIIb and β3 tail peptides by NMR (Vinogradova et al., 2002) and, more recently, has been shown to reduce the FRET between fluorphore-tagged α and β integrin in living cells (Kim et al., 2003). Binding of the talin PTB domain to the integrin NPxY motif might thus perturb β tail membrane-proximal residues and disrupt an inhibitory α-β tail interaction.

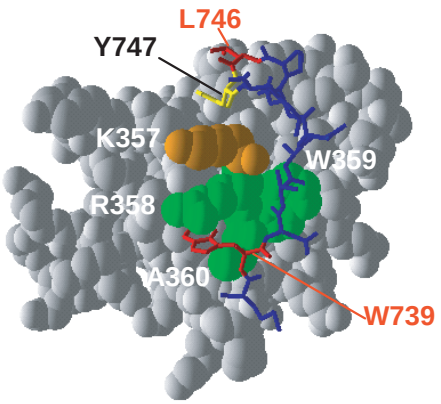

Fig. 2. Structure of a β3 tail-talin F3 complex. The structure of the β3 tail residues 738-748 (shown as sticks) bound to the talin PTB-like domain (PDB: 1MK7). Mutations at talin residues R358, W359 or A360 (shown in green) inhibit β3 tail binding, whereas mutation of K357 (shown in orange) did not. Integrin residues L746 and W739, which selectively inhibit talin binding and integrin activation when mutated to alanine, are shown in red; Y747, which inhibits talin, filamin and Syk binding when mutated to alanine, is shown in yellow. This figure was first published as Supporting Online Material at *Science* online (Tadokoro et al., 2003).

Other regulators of integrin activation

At least two other β tail-binding proteins, β3-endonexin (Kashiwagi et al., 1997) and cytohesin (Kolanus et al., 1996), and one α tail-binding protein, calcium- and integrin-binding protein (CIB) (Tsuboi, 2002), might also directly activate integrins.

β3-endonexin binds specifically to β3, but not β1 or β2, tails through both membrane-proximal and -distal motifs (Shattil et al., 1995; Eigenthaler et al., 1997) and GFP-β3-endonexin activates αIIbβ3 in CHO cells (Kashiwagi et al., 1997). However, in the absence of talin, this activation is very weak (Tadokoro et al., 2003). Therefore, β3-endonexin may cooperate with talin during αIIbβ3 activation in platelets.

Cytohesins-1 and -3 bind β2 integrin tails and have guanine nucleotide exchange factor (GEF) activity for the ARF family of small GTPases (Kolanus et al., 1996; Korthauer et al., 2000; Ogasawara et al., 2000). Antisense cytohesin-1 oligonucleotides reduce β2 integrin-mediated cell adhesion (Hmama et al., 1999), whereas over-expression of cytohesin-1 or -3 increases adhesion (Kolanus et al., 1996; Korthauer et al., 2000). However, cytohesin-1 over-expression induces appearance of an activation epitope on αLβ2 but binding of soluble ligand is unaffected (Geiger et al., 2000); therefore, cytohesins might increase cell adhesion through affinity-independent processes, such as integrin clustering, rather than integrin activation.

One report indicates that CIB activates αIIbβ3 by binding to the αIIb tail (Tsuboi, 2002); however, others implicate CIB in post-receptor occupancy events not activation (Vallar et al., 1999; Haataja et al., 2002; Naik and Naik, 2003). The C-terminal domains of CIB bind specifically to the membrane-proximal residues of the αIIb tail and a portion of the predicted transmembrane region in a divalent-cation-dependent fashion (Naik et al., 1997; Vallar et al., 1999; Shock et al., 1999; Barry et al., 2002), and this might account for any effects of CIB on integrin activation.

These putative activators are each selective for only one integrin tail and thus probably represent specialist pathways that collaborate with talin-mediated activation in specific cells. The type II transmembrane protein CD98 heavy chain (CD98hc) – an amino acid transporter – is another class of β tail-binding protein implicated in integrin activation (Fenczik et al., 1997; Zent et al., 2000; Kolesnikova et al., 2001). Its over-expression does not activate integrins but can reverse suppression of integrin activation mediated by over-expression of free β tails (Fenczik et al., 1997). This effect is independent of its role in amino acid transport and is dependent on its binding to the suppressive β tail (Fenczik et al., 2001; Zent et al., 2000). However, CD98hc is unable to reverse integrin suppression mediated by RNAi knockdown of talin (Tadokoro et al., 2003). Clustering cell-surface CD98 activates pathways of integrin signalling and stimulates cell adhesion, possibly in a Rap1-dependent manner (Fenczik et al., 1997; Rintoul et al., 2002; Suga et al., 2001). Thus, CD98 is important for integrin function but is probably not directly involved in integrin activation.

Finally, the importance of the talin PTB domain-NPxY interaction in integrin activation, along with the observation that many PTB domains bind integrin β tails (Calderwood et al., 2003), suggests that other integrin-binding PTB or FERM domain-containing proteins could activate integrins. Those tested to date, Numb, Dok-1 and kindlerin (Calderwood et al., 2002; Kloeker et al., 2003), do not activate αIIbβ3, and (as discussed below) some PTB domain proteins might antagonize talin binding to integrins and so inhibit activation. The role of other PTB-like domains during integrin activation requires further study.

Control of talin-integrin interactions

Integrin activation is dynamically regulated and a major new challenge will be to understand how cellular signalling pathways control binding of talin to integrin tails and so integrin activation. Several potential mechanisms exist, including integrin phosphorylation (Tapley et al., 1989), talin proteolysis (Yan et al., 2001), phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] binding (Martel et al., 2001) and competition between integrin tail-binding proteins (Bouvard et al., 2003) (Fig. 3), but their relative significance, their effect on integrin activation and details of their in vivo roles remain to be determined.

Although PTB domains were initially characterized as domains that bind to phosphorylated tyrosines, their binding is often independent of phosphotyrosine (Schlessinger and Lemmon, 2003), and tyrosine phosphorylation of the β tail NPxY motif inhibits talin binding (Tapley et al., 1989). The side chain of β3 Y747 occupies an uncharged pocket in talin F3, and this lack of positively charged residues is consistent with recognition of non-phosphorylated tyrosine and disruption of integrin binding by phosphorylation (Garcia-Alvarez et al., 2003). Tyrosine phosphorylation of the NPxY motifs in integrin β tails by Src-family kinases reduces cell adhesion (Datta et al., 2002), leads to integrin displacement from focal adhesions (Johansson et al., 1994) and is important in cell migration (Sakai et al., 1998), hemostasis (Law et al., 1999) and transformation (Datta et al., 2001; Sakai et al., 2001). These effects are consistent with disruption of integrin-talin interactions and are reversed by expression of non-phosphorylatable integrins (Datta et al., 2002; Sakai et al., 2001). Tails containing non-phosphorylatable NPxF motifs retain talin-binding activity and can be activated by physiological stimuli (Xi et al., 2003; Law et al., 1999; Kaapa et al., 1999; Sampath et al., 1998). Integrin phosphorylation, by Src-family kinases or other kinases, may therefore be an important negative regulator of integrin activation. Src-family kinases may also phosphorylate talin (Pasquale et al., 1986). However, despite numerous reports of tyrosine, serine or threonine phosphorylation of talin (e.g. Turner et al., 1989; Beckerle, 1990), the effects of these modifications remain unclear.

Talin head has a sixfold higher affinity than intact talin for β3, which suggests that the β3 tail-binding site is masked in intact talin (Yan et al., 2001). In ERM proteins, interactions between the FERM domain and the C-terminal portion of the molecule also mask ligand-binding sites (Pearson et al., 2000; Hamada et al., 2003). The protease calpain provides an in vivo mechanism for the separation of talin N- and C-terminal domains to unmask the integrin-binding site, and calpain cleavage increases talin binding to integrins in vitro (Yan et al., 2001). Calpain is implicated in αVβ3 activation on vascular cells (Byzova et al., 2000; Byzova and Plow, 1998) and activation of platelets leads to calpain-mediated talin cleavage and αIIbβ3 activation (Hayashi et al., 1999; Schoenwaelder

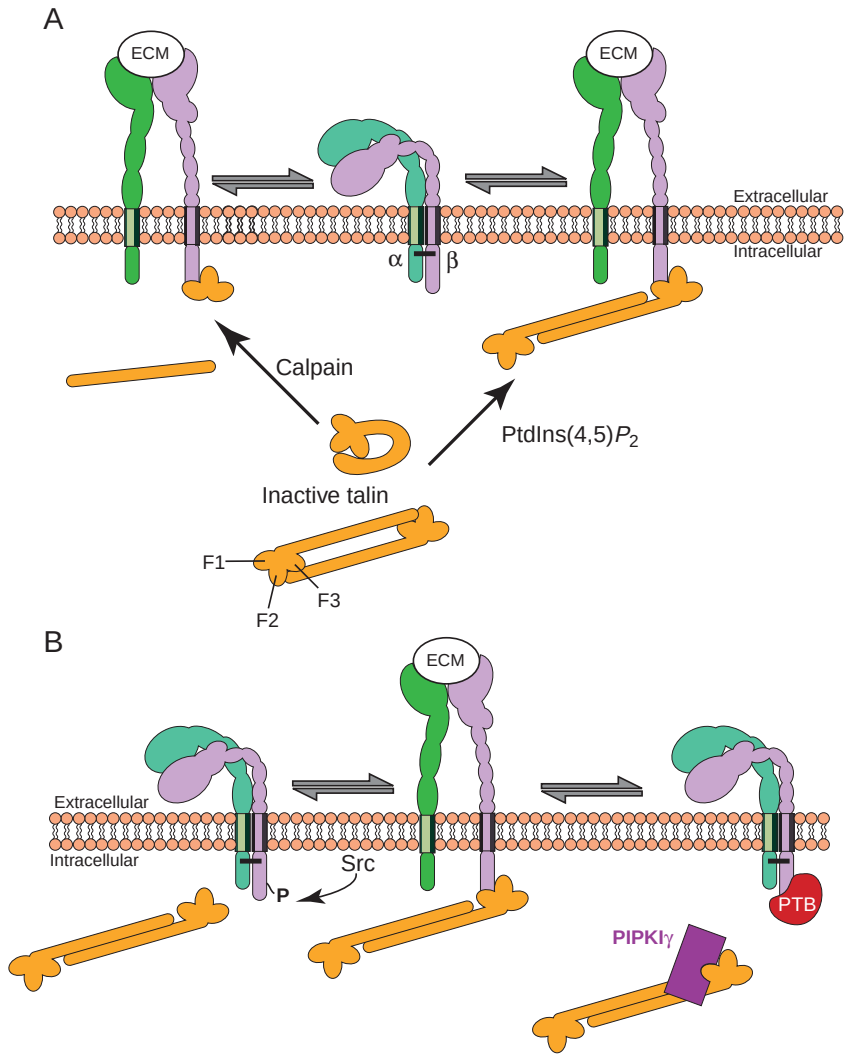

Fig. 3. Potential mechanisms regulating talin-mediated integrin activation. Talin binding to integrin β tails induces conformational changes in the extracellular domain, increasing their affinity for ligands (the nature of the conformational changes remains controversial and the model shown represents only one of several possibilities). Mechanisms that regulate talin binding may therefore control integrin activation. The putative salt bridge stabilizing the interaction between membrane-proximal regions of the α and β tails in the inactive conformation is illustrated as a black bar. The three-lobed FERM domain within the talin head is indicated. (A) Stimulation of talin binding. Two hypothetical models of inactive talin are shown, where regions of the rod mask the β tail-binding site in the F3 subdomain. Calpain cleavage or PtdIns(4,5)P_2 binding unmasks the binding site, potentially activating integrins. (B) Inhibition of talin binding. Src-mediated tyrosine phosphorylation (P) of integrin NPxY motifs, and competition with other β tail-binding proteins (e.g. PTB domain proteins), or other talin-binding proteins (e.g. PIPKIγ-90), may prevent integrin-talin interactions, so inhibiting integrin activation. Hence, dynamic interplay between the stimulatory and inhibitory pathways might determine the integrin activation state.

et al., 2000), suggesting that calpain could regulate talin-mediated αIIbβ3 activation. However, calpain also plays roles downstream of integrin activation (Fox et al., 1993; Schoenwaelder et al., 2000), and it will be important to determine whether talin cleavage is sufficient to activate integrins. Calpain also cleaves integrin β tails (Pfaff et al., 1999), albeit more slowly than talin (Xi et al., 2003); hence, calpain might induce integrin activation by cleaving talin and subsequently downregulating activation by cleaving the β tail.

Binding of PtdIns(4,5)P_2 to talin induces a conformational change that unmasks the tail-binding site within the talin FERM domain and enhances its association with integrin β1 tails (Martel et al., 2001). Notably, talin binds to and activates one splice variant of the PtdIns(4,5)P_2-producing enzyme: phosphatidylinositol phosphate kinase type Iγ-90 (PIPKIγ-90) (Ling et al., 2002; Di Paolo et al., 2002). Therefore, talin can stimulate PtdIns(4,5)P_2 production that in turn enhances talin-integrin interactions, which suggests that PIPKIγ-90 may positively regulate integrin activation. However, PIPKIγ-90 and integrin β tails compete for overlapping binding sites on the talin F3 subdomain (Barsukov et al., 2003) and so, under some conditions, PIPKIγ-90 might inhibit integrin activation by displacing talin from β tails.

In addition to the talin F3 subdomain, many other PTB domains can bind integrin NPxY motifs (Calderwood et al., 2003), raising the possibility that they might compete with talin. One such protein, the integrin cytoplasmic domain-associated protein-1α (ICAP-1α), binds to integrin β1A through a PTB domain-NPxY interaction (Chang et al., 2002) and inhibits β1A-talin association (Bouvard et al., 2003). It remains to be determined whether, by preventing talin binding to integrin β1 tails, ICAP-1α inhibits integrin activation. However, calcium/calmodulin-dependent protein kinase II, a kinase implicated in the control of β1 activation, phosphorylates ICAP-1α and this phosphorylation appears to regulate integrin-mediated processes (Bouvard et al., 1998; Bouvard and Block, 1998). ICAP-1α binds to the second NPxY motif in β1 and it is likely that tyrosine phosphorylation at this site inhibits binding (Chang et al., 2002). Talin interacts with the first β tail NPxY motif, and differential phosphorylation of the β tail tyrosines may therefore provide a means to control ICAP-1α or talin binding independently. It will be important to determine the roles of ICAP-1α and the other integrin-binding PTB or FERM domains as positive or negative regulators of integrin activation. Furthermore, the role of integrin tyrosine phosphorylation in determining PTB-

domain binding specificity and the consequences of this on integrin activation require investigation.

Many cell signalling pathways regulate integrin activation, including those involving the Ras family of GTPases (Kinbara et al., 2003); however, their final effectors have not been identified. The inability of R-Ras to activate integrins in talin-deficient cells (Tadokoro et al., 2003) suggests that talin might be the final step between these pathways and the integrin cytoplasmic tails.

Transmission of intracellular rearrangements to the extracellular domain

Integrin activation must involve transmission of conformational rearrangements from the cytoplasmic domains through the transmembrane and membrane-proximal extracellular domains to the membrane-distal ligand-binding site. Very little is known about the role of the transmembrane domains in this process, but conformational changes in the membrane-proximal extracellular domains have been documented, and the folding/unfolding of domains linking this region to the ligand-binding domains might transmit the activation signal (Du et al., 1993; Beglova et al., 2002). As in the case of the intracellular membrane-proximal regions, modification of the extracellular membrane-proximal regions to inhibit subunit separation impairs activation, whereas enhancing separation promotes activation (Takagi et al., 2001; Takagi et al., 2002; Xiong et al., 2003b). Several non-mutually exclusive models have been proposed to explain transmission of the activation signal (Williams et al., 1994; Woodside et al., 2001; Liddington and Ginsberg, 2002; Hynes, 2002; Takagi et al., 2002; Li et al., 2003). These all involve some change in orientation of the subunits relative to one another and to the membrane, and include pistoning, twisting, separation and hinging. All could potentially be regulated by altering the association of the transmembrane regions or their membrane-proximal flanking sequences. However, despite recent experimental support for the pistoning model (Armulik et al., 1999; Liddington and Ginsberg, 2002) and the separation model (Kim et al., 2003), there is currently insufficient data to distinguish clearly between the various activation models.

Conclusions and perspectives

Recent studies have significantly deepened our understanding of the molecular basis of integrin activation. X-ray crystallography has revealed ligand-induced and activating conformational changes in isolated extracellular domains (Emsley et al., 2000; Shimaoka et al., 2003). In addition, crystal structures of the αvβ3 extracellular domains (Xiong et al., 2001; Xiong et al., 2002), along with lower-resolution X-ray scattering (Mould et al., 2003) and electron microscopy (Takagi et al., 2002; Takagi et al., 2003; Adair and Yeager, 2002), have provided a basis for modelling extracellular rearrangements during integrin activation. However, additional structures of active and inactive integrins along with integrin-ligand co-crystals will be required to elucidate fully these conformational changes. The importance of integrin cytoplasmic tails and, in particular, their membrane-proximal regions and of talin-integrin β tail interactions is now well established, and structural data on the tails are becoming available. Resolving the structure of the whole β tail, or αβ heterodimer, when bound to activating or non-activating talin fragments might reveal how talin alters membrane-proximal regions to propagate activation signals. Investigation of the structure, orientation and interactions of integrin transmembrane regions and the consequences of associations with specific lipid micro-domains will also need to be extended. The pathways that regulate integrin activation and the mechanisms by which they act (e.g. through control of integrin-talin interactions or through the potential positive or negative effects of other tail-binding proteins) all need further explanation. Finally, improved techniques for visualizing in vivo integrin activation in real time will be required if we are to understand fully not only how this important process works but also where and when.

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References

Adair, B. D. and Yeager, M. (2002). Three-dimensional model of the human platelet integrin αIIbβ3 based on electron cryomicroscopy and X-ray crystallography. *Proc. Natl. Acad. Sci. USA* **99**, 14059-14064.

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

Barry, W. T., Boudignon-Proudhon, C., Shock, D. D., McFadden, A., Weiss, J. M., Sondek, J. and Parise, L. V. (2002). Molecular basis of CIB binding to the integrin αIIb cytoplasmic domain. *J. Biol. Chem.* **277**, 28877-28883.

Barsukov, I. L., Prescot, A., Bate, N., Patel, B. C., Floyd, D. N., Bhanji, N., Bagshaw, C. R., Letinic, K., Di Paolo, G., De Camilli, P. et al. (2003). PIP kinase type 1γ and β1-integrin cytoplasmic domain bind to the same region in the talin FERM domain. *J. Biol. Chem.* **278**, 31202-31209

Beckerle, M. C. (1990). The adhesion plaque protein, talin, is phosphorylated in vivo in chicken embryo fibroblasts exposed to a tumor-promoting phorbol ester. *Cell Regul.* **1**, 227-236.

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

Bodeau, A. L., Berrier, A. L., Mastrangelo, A. M., Martinez, R. and LaFlamme, S. E. (2001). A functional comparison of mutations in integrin β cytoplasmic domains: effects on the regulation of tyrosine phosphorylation, cell spreading, cell attachment and β1 integrin conformation. *J. Cell Sci.* **114**, 2795-2807.

Bokel, C. and Brown, N. H. (2002). Integrins in development: moving on, responding to, and sticking to the extracellular matrix. *Dev. Cell* **3**, 311-321.

Bouvard, D. and Block, M. R. (1998). Calcium/calmodulin-dependent protein kinase II controls integrin α5β1-mediated cell adhesion through the integrin cytoplasmic domain associated protein-1α. *Biochem. Biophys. Res. Commun.* **252**, 46-50.

Bouvard, D., Molla, A. and Block, M. R. (1998). Calcium/calmodulin-dependent protein kinase II controls α5β1 integrin-mediated inside-out signaling. *J. Cell Sci.* **111**, 657-665.

Bouvard, D., Vignoud, L., Dupe-Manet, S., Abed, N., Fournier, H. N., Vincent-Monegat, C., Retta, S. F., Fassler, R. and Block, M. R. (2003). Disruption of focal adhesions by integrin cytoplasmic domain-associated protein-1α. *J. Biol. Chem.* **278**, 6567-6574.

Brakebusch, C. and Fassler, R. (2003). The integrin-actin connection, an eternal love affair. *EMBO J.* **22**, 2324-2333.

Brown, N. H., Gregory, S. L., Rickoll, W. L., Fessler, L. I., Prout, M., White, R. A. and Fristrom, J. W. (2002). Talin is essential for integrin function in *Drosophila*. *Dev. Cell* **3**, 569-579.

Byzova, T. V. and Plow, E. F. (1998). Activation of αVβ3 on vascular cells controls recognition of prothrombin. *J. Cell Biol.* **143**, 2081-2092.

Byzova, T. V., Goldman, C. K., Pampori, N., Thomas, K. A., Bett, A., Shattil, S. J. and Plow, E. F. (2000). A mechanism for modulation of cellular responses to VEGF: activation of the integrins. *Mol. Cell* **6**, 851-860.

Calderwood, D. A. and Ginsberg, M. H. (2003). Talin forges the links between integrins and actin. *Nat. Cell Biol.* **5**, 694-697.

Calderwood, D. A., Zent, R., Grant, R., Rees, D. J., Hynes, R. O. and

Ginsberg, M. H. (1999). The Talin head domain binds to integrin β subunit cytoplasmic tails and regulates integrin activation. *J. Biol. Chem.* **274**, 28071-28074.

Calderwood, D. A., Shattil, S. J. and Ginsberg, M. H. (2000). Integrins and actin filaments: reciprocal regulation of cell adhesion and signaling. *J. Biol. Chem.* **275**, 22607-22610.

Calderwood, D. A., Huttenlocher, A., Kiosses, W. B., Rose, D. M., Woodside, D. G., Schwartz, M. A. and Ginsberg, M. H. (2001). Increased filamin binding to β-integrin cytoplasmic domains inhibits cell migration. *Nat. Cell Biol.* **3**, 1060-1068.

Calderwood, D. A., Yan, B., de Pereda, J. M., Alvarez, B. G., Fujioka, Y., Liddington, R. C. and Ginsberg, M. H. (2002). The phosphotyrosine binding-like domain of talin activates integrins. *J. Biol. Chem.* **277**, 21749-21758.

Calderwood, D. A., Fujioka, Y., de Pereda, J. M., Garcia-Alvarez, B., Nakamoto, T., Margolis, B., McGlade, C. J., Liddington, R. C. and Ginsberg, M. H. (2003). Integrin β cytoplasmic domain interactions with phosphotyrosine- binding domains: a structural prototype for diversity in integrin signaling. *Proc. Natl. Acad. Sci. USA* **100**, 2272-2277.

Carman, C. V. and Springer, T. A. (2003). Integrin avidity regulation: are changes in affinity and conformation underemphasized? *Curr. Opin. Cell Biol.* **15**, 547-556.

Chang, D. D., Hoang, B. Q., Liu, J. and Springer, T. A. (2002). Molecular basis for interaction between ICAP-1α PTB domain and β1 integrin. *J. Biol. Chem.* **277**, 8140-8145.

Chen, Y.-P., O'Toole, T. E., Ylanne, J., Rosa, J.-P. and Ginsberg, M. H. (1994a). A point mutation in the integrin β3 cytoplasmic domain (S752-P) impairs bidirectional signaling through αIIbβ3 (platelet glycoprotein IIb-IIIa). *Blood* **84**, 1857-1865.

Chen, Y.-P., O'Toole, T. E., Shipley, T., Forsyth, J., LaFlamme, S. E., Yamada, K. M., Shattil, S. J. and Ginsberg, M. H. (1994b). 'Inside-out' signal transduction inhibited by isolated integrin cytoplasmic domains. *J. Biol. Chem.* **269**, 18307-18310.

Coller, B. S. (1985). A new murine monoclonal antibody reports an activation-dependent change in the conformation and/or microenvironment of the platelet GPIIb/IIIa complex. *J. Clin. Invest.* **76**, 101-110.

Cram, E., Clark, S. and Schwarzbauer, J. E. (2003). Talin loss-of-function uncovers roles in cell contractility and migration in *C. elegans*. *J. Cell Sci.* **116**, 3871-3878.

Critchley, D. R. (2000). Focal adhesions – the cytoskeletal connection. *Curr. Opin. Cell Biol.* **12**, 133-139.

Crowe, D. T., Chiu, H., Fong, S. and Weissman, I. L. (1994). Regulation of the avidity of integrin $\alpha_4\beta_7$ by the β_7 cytoplasmic domain. *J. Biol. Chem.* **269**, 14411-14418.

Datta, A., Shi, Q. and Boettiger, D. E. (2001). Transformation of chicken embryo fibroblasts by v-src uncouples β1 integrin-mediated outside-in but not inside-out signaling. *Mol. Cell Biol.* **21**, 7295-7306.

Datta, A., Huber, F. and Boettiger, D. (2002). Phosphorylation of β3 integrin controls ligand binding strength. *J. Biol. Chem.* **277**, 3943-3949.

Di Paolo, G., Pellegrini, L., Letinic, K., Cestra, G., Zoncu, R., Voronov, S., Chang, S., Guo, J., Wenk, M. R. and De Camilli, P. (2002). Recruitment and regulation of phosphatidylinositol phosphate kinase type 1 γ by the FERM domain of talin. *Nature* **420**, 85-89.

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

Eigenthaler, M., Hofferer, L., Shattil, S. J. and Ginsberg, M. H. (1997). A conserved sequence motif in the integrin β_3 cytoplasmic domain is required for its specific interaction with β_3-endonexin. *J. Biol. Chem.* **272**, 7693-7698.

Emsley, J., Knight, C. G., Farndale, R. W., Barnes, M. J. and Liddington, R. C. (2000). Structural basis of collagen recognition by integrin α2β1. *Cell* **101**, 47-56.

Erb, E. M., Tangemann, K., Bohrmann, B., Muller, B. and Engel, J. (1997). Integrin αIIbβ3 reconstituted into lipid bilayers is nonclustered in its activated state but clusters after fibrinogen binding. *Biochemistry* **36**, 7395-7402.

Felding-Habermann, B., O'Toole, T. E., Smith, J. W., Fransvea, E., Ruggeri, Z. M., Ginsberg, M. H., Hughes, P. E., Pampori, N., Shattil, S. J., Saven, A. et al. (2001). Integrin activation controls metastasis in human breast cancer. *Proc. Natl. Acad. Sci. USA* **98**, 1853-1858.

Fenczik, C. A., Sethi, T., Ramos, J. W., Hughes, P. E. and Ginsberg, M. H. (1997). Complementation of dominant suppression implicates CD98 in integrin activation. *Nature* **390**, 81-85.

Fenczik, C. A., Zent, R., Dellos, M., Calderwood, D. A., Satriano, J., Kelly, C. and Ginsberg, M. H. (2001). Distinct domains of CD98hc regulate integrins and amino acid transport. *J. Biol. Chem.* **276**, 8746-8752.

Fox, J. E. B., Taylor, R. G., Taffarel, M., Boyles, J. K. and Goll, D. E. (1993). Evidence that activation of platelet calpain is induced as a consequence of binding of adhesive ligand to the integrin, glycoprotein IIb-IIIa. *J. Cell Biol.* **120**, 1501-1507.

Garcia-Alvarez, B., de Pereda, J. M., Calderwood, D. A., Ulmer, T. S., Critchley, D. R., Campbell, I. D., Ginsberg, M. H. and Liddington, R. C. (2003). Structural determinants of integrin recognition by talin. *Mol. Cell* **11**, 49-58.

Geiger, B., Bershadsky, A., Pankov, R. and Yamada, K. M. (2001). Transmembrane crosstalk between the extracellular matrix–cytoskeleton crosstalk. *Nat. Rev. Mol. Cell Biol.* **2**, 793-805.

Geiger, C., Nagel, W., Boehm, T., van Kooyk, Y., Figdor, C. G., Kremmer, E., Hogg, N., Zeitlmann, L., Dierks, H., Weber, K. S. et al. (2000). Cytohesin-1 regulates β-2 integrin-mediated adhesion through both ARF-GEF function and interaction with LFA-1. *EMBO J.* **19**, 2525-2536.

Giancotti, F. G. and Ruoslahti, E. (1999). Integrin signaling. *Science* **285**, 1028-1032.

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

Haas, T. A. and Plow, E. F. (1997). Development of a structural model for the cytoplasmic domain of an integrin. *Protein Eng.* **10**, 1395-1405.

Haataja, L., Kaartinen, V., Groffen, J. and Heisterkamp, N. (2002). The small GTPase Rac3 interacts with the integrin-binding protein CIB and promotes integrin αIIbβ3-mediated adhesion and spreading. *J. Biol. Chem.* **277**, 8321-8328.

Hamada, K., Shimizu, T., Yonemura, S., Tsukita, S., Tsukita, S. and Hakoshima, T. (2003). Structural basis of adhesion-molecule recognition by ERM proteins revealed by the crystal structure of the radixin-ICAM-2 complex. *EMBO J.* **22**, 502-514.

Hayashi, M., Suzuki, H., Kawashima, S., Saido, T. C. and Inomata, M. (1999). The behavior of calpain-generated N- and C-terminal fragments of talin in integrin-mediated signaling pathways. *Arch. Biochem. Biophys.* **371**, 133-141.

Hibbs, M. L., Jakes, S., Stacker, S. A., Wallace, R. W. and Springer, T. A. (1991a). The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 β subunit: sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site. *J. Exp. Med.* **174**, 1227-1238.

Hibbs, M. L., Xu, H., Stacker, S. A. and Springer, T. A. (1991b). Regulation of adhesion to ICAM-1 by the cytoplasmic domain of LFA-1 integrin beta subunit. *Science* **251**, 1611-1613.

Hmama, Z., Knutson, K. L., Herrera-Velit, P., Nandan, D. and Reiner, N. E. (1999). Monocyte adherence induced by lipopolysaccharide involves CD14, LFA-1, and cytohesin-1. Regulation by Rho and phosphatidylinositol 3-kinase. *J. Biol. Chem.* **274**, 1050-1057.

Hogg, N., Henderson, R., Leitinger, B., McDowall, A., Porter, J. and Stanley, P. (2002). Mechanisms contributing to the activity of integrins on leukocytes. *Immunol. Rev.* **186**, 164-171.

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

Hughes, P. E., O'Toole, T. E., Ylanne, J., Shattil, S. J. and Ginsberg, M. H. (1995). The conserved membrane-proximal region of an integrin cytoplasmic domain specifies ligand binding affinity. *J. Biol. Chem.* **270**, 12411-12417.

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

Humphries, M. J., McEwan, P. A., Barton, S. J., Buckley, P. A., Bella, J. and Paul Mould, A. (2003). Integrin structure: heady advances in ligand binding, but activation still makes the knees wobble. *TIBS* **28**, 313-320.

Huttenlocher, A., Ginsberg, M. H. and Horwitz, A. F. (1996). Modulation of cell migration by integrin-mediated cytoskeletal linkages and ligand-binding affinity. *J. Cell Biol.* **134**, 1551-1562.

Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673-687.

Isenberg, W. M., McEver, R. P., Phillips, D. R., Shuman, M. A. and Bainton, D. F. (1987). The platelet fibrinogen receptor: An immunogold-surface replica study of agonist-induced ligand binding and receptor clustering. *J. Cell Biol.* **104**, 1655-1663.

Johansson, M. W., Larsson, E., Luning, B., Pasquale, E. B. and Ruoslahti,

E. (1994). Altered localization and cytoplasmic domain-binding properties of tyrosine-phosphorylated β_1 integrin. *J. Cell Biol.* **126**, 1299-1309.

Kaapa, A., Peter, K. and Ylanne, J. (1999). Effects of mutations in the cytoplasmic domain of integrin β1 to talin binding and cell spreading. *Exp. Cell Res.* **250**, 524-534.

Kashiwagi, H., Schwartz, M. A., Eigenthaler, M., Davis, K. A., Ginsberg, M. H. and Shattil, S. J. (1997). Affinity modulation of platelet integrin αIIbβ3 by β3- endonexin, a selective binding partner of the β3 integrin cytoplasmic tail. *J. Cell Biol.* **137**, 1433-1443.

Kassner, P. D. and Hemler, M. E. (1993). Interchangeable α chain cytoplasmic domains play a positive role in control of cell adhesion mediated by VLA-4, a β_1 integrin. *J. Exp. Med.* **178**, 649-660.

Kassner, P. D., Kawaguchi, S. and Hemler, M. E. (1994). Minimum α chain cytoplasmic tail sequence needed to support integrin-mediated adhesion. *J. Biol. Chem.* **269**, 19859-19867.

Kawaguchi, S. and Hemler, M. E. (1993). Role of the α subunit cytoplasmic domain in regulation of adhesive activity mediated by the integrin VLA-2. *J. Biol. Chem.* **268**, 16279-16285.

Keller, R. S., Shai, S. Y., Babbitt, C. J., Pham, C. G., Solaro, R. J., Valencik, M. L., Loftus, J. C. and Ross, R. S. (2001). Disruption of integrin function in the murine myocardium leads to perinatal lethality, fibrosis, and abnormal cardiac performance. *Am. J. Pathol.* **158**, 1079-1090.

Kim, M., Carman, C. V. and Springer, T. A. (2003). Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. *Science* **301**, 1720-1725.

Kinbara, K., Goldfinger, L. E., Hansen, M., Chou, F. L. and Ginsberg, M. H. (2003). Ras GTPases: integrins' friends or foes? *Nat. Rev. Mol. Cell Biol.* **4**, 767-776.

Kloeker, S., Major, M. B., Calderwood, D. A., Ginsberg, M. H., Jones, D. A. and Beckerle, M. C. (2003). The Kindler syndrome protein is regulated by TGFβ and involved in integrin-mediated adhesion. *J. Biol. Chem.* Nov 21 [Epub ahead of print].

Knezevic, I., Leisner, T. and Lam, S. (1996). Direct binding of the platelet integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) to talin. *J. Biol. Chem.* **271**, 16416-16421.

Kolanus, W., Nagel, W., Schiller, B., Zeitlmann, L., Godar, S., Stockinger, H. and Seed, B. (1996). αLβ2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1, a cytoplasmic regulatory molecule. *Cell* **86**, 233-242.

Kolesnikova, T. V., Mannion, B. A., Berditchevski, F. and Hemler, M. E. (2001). β1 integrins show specific association with CD98 protein in low density membranes. *BMC Biochem.* **2**, 10.

Korthauer, U., Nagel, W., Davis, E. M., Le Beau, M. M., Menon, R. S., Mitchell, E. O., Kozak, C. A., Kolanus, W. and Bluestone, J. A. (2000). Anergic T lymphocytes selectively express an integrin regulatory protein of the cytohesin family. *J. Immunol.* **164**, 308-318.

Laplantine, E., Vallar, L., Mann, K., Kieffer, N. and Aumailley, M. (2000). Interaction between the cytodomains of the α3 and β1 integrin subunits regulates remodelling of adhesion complexes on laminin. *J. Cell Sci.* **113**, 1167-1176.

Laudanna, C., Kim, J. Y., Constantin, G. and Butcher, E. (2002). Rapid leukocyte integrin activation by chemokines. *Immunol. Rev.* **186**, 37-46.

Law, D. A., DeGuzman, F. R., Heiser, P., Ministri-Madrid, K., Killeen, N. and Phillips, D. R. (1999). Integrin cytoplasmic tyrosine motif is required for outside-in αIIbβ3 signalling and platelet function. *Nature* **401**, 808-811.

Leisner, T. M., Wencel-Drake, J. D., Wang, W. and Lam, S. C. (1999). Bidirectional transmembrane modulation of integrin αIIbβ3 conformations. *J. Biol. Chem.* **274**, 12945-12949.

Li, R., Babu, C. R., Lear, J. D., Wand, A. J., Bennett, J. S. and DeGrado, W. F. (2001). Oligomerization of the integrin αIIbβ3: roles of the transmembrane and cytoplasmic domains. *Proc. Natl. Acad. Sci. USA* **98**, 12462-12467.

Li, R., Babu, C. R., Valentine, K., Lear, J. D., Wand, A. J., Bennett, J. S. and DeGrado, W. F. (2002). Characterization of the monomeric form of the transmembrane and cytoplasmic domains of the integrin β3 subunit by NMR spectroscopy. *Biochemistry* **41**, 15618-15624.

Li, R., Mitra, N., Gratkowski, H., Vilaire, G., Litvinov, R., Nagasami, C., Weisel, J. W., Lear, J. D., DeGrado, W. F. and Bennett, J. S. (2003). Activation of integrin αIIbβ3 by modulation of transmembrane helix associations. *Science* **300**, 795-798.

Liddington, R. C. and Ginsberg, M. H. (2002). Integrin activation takes shape. *J. Cell Biol.* **158**, 833-839.

Ling, K., Doughman, R. L., Firestone, A. J., Bunce, M. W. and Anderson, R. A. (2002). Type I γ phosphatidylinositol phosphate kinase targets and regulates focal adhesions. *Nature* **420**, 89-93.

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

Lu, C. F. and Springer, T. A. (1997). The α subunit cytoplasmic domain regulates the assembly and adhesiveness of integrin lymphocyte function-associated antigen-1. *J. Immunol.* **159**, 268-278.

Lu, C., Takagi, J. and Springer, T. A. (2001). Association of the membrane proximal regions of the α and β subunit cytoplasmic domains constrains an integrin in the inactive state. *J. Biol. Chem.* **276**, 14642-14648.

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

Martin-Bermudo, M. D., Dunin-Borkowski, O. M. and Brown, N. H. (1998). Modulation of integrin activity is vital for morphogenesis. *J. Cell Biol.* **141**, 1073-1081.

Mastrangelo, A. M., Homan, S. M., Humphries, M. J. and LaFlamme, S. E. (1999). Amino acid motifs required for isolated β cytoplasmic domains to regulate 'in trans' β1 integrin conformation and function in cell attachment. *J. Cell Sci.* **112**, 217-229.

McDowall, A., Inwald, D., Leitinger, B., Jones, A., Liesner, R., Klein, N. and Hogg, N. (2003). A novel form of integrin dysfunction involving β1, β2, and β3 integrins. *J. Clin. Invest.* **111**, 51-60.

Monkley, S. J., Zhou, X. H., Kinston, S. J., Giblett, S. M., Hemmings, L., Priddle, H., Brown, J. E., Pritchard, C. A., Critchley, D. R. and Fassler, R. (2000). Disruption of the talin gene arrests mouse development at the gastrulation stage. *Dev. Dyn.* **219**, 560-574.

Mould, A. P., Symonds, E. J., Buckley, P. A., Grossmann, G. J., McEwan, P. A., Barton, S. J., Askari, J. A., Craig, S. E., Bella, J. and Humphries, M. J. (2003). Structure of an integrin-ligand complex deduced from solution X-ray scattering and site-directed mutagenesis. *J. Biol. Chem.* **278**, 39993-39999.

Muir, T. W., Williams, M. J., Ginsberg, M. H. and Kent, S. B. H. (1994). Design and chemical synthesis of a neoprotein structural model for the cytoplasmic domain of a multisubunit cell-surface receptor: integrin αIIbβ3 (platelet GPIIb-IIIa). *Biochemistry* **33**, 7701-7708.

Naik, U. P. and Naik, M. U. (2003). Association of CIB with GPIIb/IIIa during outside-in signaling is required for platelet spreading on fibrinogen. *Blood* **102**, 1355-1362.

Naik, U. P., Patel, P. M. and Parise, L. V. (1997). Identification of a novel calcium-binding protein that interacts with the integrin αIIb cytoplasmic domain. *J. Biol. Chem.* **272**, 4651-4654.

O'Toole, T. E., Mandelman, D., Forsyth, J., Shattil, S. J., Plow, E. F. and Ginsberg, M. H. (1991). Modulation of the affinity of integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) by the cytoplasmic domain of α_{IIb}. *Science* **254**, 845-847.

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

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

Ogasawara, M., Kim, S. C., Adamik, R., Togawa, A., Ferrans, V. J., Takeda, K., Kirby, M., Moss, J. and Vaughan, M. (2000). Similarities in function and gene structure of cytohesin-4 and cytohesin-1, guanine nucleotide-exchange proteins for ADP-ribosylation factors. *J. Biol. Chem.* **275**, 3221-3230.

Palecek, S. P., Loftus, J. C., Ginsberg, M. H., Horwitz, A. F. and Lauffenburger, D. A. (1997). Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* **385**, 537-540.

Pasquale, E. B., Maher, P. A. and Singer, S. J. (1986). Talin is phosphorylated on tyrosine in chicken embryo fibroblasts transformed by Rous sarcoma virus. *Proc. Natl. Acad. Sci. USA* **83**, 5507-5511.

Patil, S., Jedsadayanmata, A., Wencel-Drake, J. J., Wang, W., Knezevic, I. and Lam, S. C. T. (1999). Identification of a talin-binding site in the integrin β3 subunit distinct from the NPLY regulatory motif of post-ligand binding functions. *J. Biol. Chem.* **274**, 28575-28583.

Pearson, M. A., Reczek, D., Bretscher, A. and Karplus, P. A. (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., Liu, S., Erle, D. J. and Ginsberg, M. H. (1998). Integrin β cytoplasmic domains differentially bind to cytoskeletal proteins. *J. Biol. Chem.* **273**, 6104-6109.

Pfaff, M., Du, X. and Ginsberg, M. H. (1999). Calpain cleavage of integrin beta cytoplasmic domains. *FEBS Lett.* **460**, 17-22.

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

Rintoul, R. C., Buttery, R. C., Mackinnon, A. C., Wong, W. S., Mosher, D.,

Haslett, C. and Sethi, T. (2002). Cross-linking CD98 promotes integrin-like signaling and anchorage-independent growth. *Mol. Biol. Cell* **13**, 2841-2852.
Sakai, T., Zhang, Q., Fassler, R. and Mosher, D. F. (1998). Modulation of β1A integrin functions by tyrosine residues in the β1 cytoplasmic domain. *J. Cell Biol.* **141**, 527-538.
Sakai, T., Jove, R., Fassler, R. and Mosher, D. F. (2001). Role of the cytoplasmic tyrosines of β1A integrins in transformation by v-src. *Proc. Natl. Acad. Sci. USA* **98**, 3808-3813.
Sampath, R., Gallagher, P. J. and Pavalko, F. M. (1998). Cytoskeletal interactions with the leukocyte integrin β2 cytoplasmic tail. Activation-dependent regulation of associations with talin and alpha-actinin. *J. Biol. Chem.* **273**, 33588-33594.
Schlessinger, J. and Lemmon, M. A. (2003). SH2 and PTB domains in tyrosine kinase signaling. *Sci. STKE.* **2003**, RE12.
Schoenwaelder, S. M., Yuan, Y. and Jackson, S. P. (2000). Calpain regulation of integrin $\alpha_{IIb}\beta_3$ signaling in human platelets. *Platelets* **11**, 189-198.
Schwartz, M. A. and Ginsberg, M. H. (2002). Networks and crosstalk: integrin signalling spreads. *Nat. Cell Biol.* **4**, E65-E68.
Shattil, S. J., Hoxie, J. A., Cunningham, M. and Brass, L. F. (1985). Changes in the platelet membrane glycoprotein IIb-IIIa Complex during platelet activation. *J. Biol. Chem.* **260**, 11107-11114.
Shattil, S. J., O'Toole, T. E., Eigenthaler, M., Thon, V., Williams, M. J., Babior, B. and Ginsberg, M. H. (1995). β_3 endonexin, a novel polypeptide that interacts specifically with the cytoplasmic tail of the integrin β_3 subunit. *J. Cell Biol.* **131**, 807-816.
Shattil, S. J., Kashiwagi, H. and Pampori, N. (1998). Integrin signaling: the platelet paradigm. *Blood* **91**, 2645-2657.
Shimaoka, M., Takagi, J. and Springer, T. A. (2002). Conformational regulation of integrin structure and function. *Annu. Rev. Biophys. Biomol. Struct.* **31**, 485-516.
Shimaoka, M., Xiao, T., Liu, J. H., Yang, Y., Dong, Y., Jun, C. D., McCormack, A., Zhang, R., Joachimiak, A., Takagi, J. et al. (2003). Structures of the αL I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. *Cell* **112**, 99-111.
Shock, D. D., Naik, U. P., Brittain, J. E., Alahari, S. K., Sondek, J. and Parise, L. V. (1999). Calcium-dependent properties of CIB binding to the integrin αIIb cytoplasmic domain and translocation to the platelet cytoskeleton. *Biochem. J.* **342**, 729-735.
Sims, P. J., Ginsberg, M. H., Plow, E. F. and Shattil, S. J. (1991). Effect of platelet activation on the conformation of the plasma membrane glycoprotein IIb-IIIa complex. *J. Biol. Chem.* **266**, 7345-7352.
Stephens, G., O'Luanaigh, N., Reilly, D., Harriott, P., Walker, B., Fitzgerald, D. and Moran, N. (1998). A sequence within the cytoplasmic tail of GpIIb independently activates platelet aggregation and thromboxane synthesis. *J. Biol. Chem.* **273**, 20317-20322.
Suga, K., Katagiri, K., Kinashi, T., Harazaki, M., Iizuka, T., Hattori, M. and Minato, N. (2001). CD98 induces LFA-1-mediated cell adhesion in lymphoid cells via activation of Rap1. *FEBS Lett.* **489**, 249-253.
Tadokoro, S., Shattil, S. J., Eto, K., Tai, V., Liddington, R. C., de Pereda, J. M., Ginsberg, M. H. and Calderwood, D. A. (2003). Talin binding to integrin β tails: a final common step in integrin activation. *Science* **302**, 103-106.
Takagi, J., Erickson, H. P. and Springer, T. A. (2001). C-terminal opening mimics 'inside-out' activation of integrin α5β1. *Nat. Struct. Biol.* **8**, 412-416.
Takagi, J., Petre, B. M., Walz, T. and Springer, T. A. (2002). Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. *Cell* **110**, 599-511.
Takagi, J., Strokovich, K., Springer, T. A. and Walz, T. (2003). Structure of integrin α5β1 in complex with fibronectin. *EMBO J.* **22**, 4607-4615.
Tapley, P., Horwitz, A., Buck, C. A., Duggan, K. and Rohrschneider, L. (1989). Integrins isolated from Rous sarcoma virus-transformed chicken embryo fibroblasts. *Oncogene* **4**, 325-333.
Tohyama, Y., Katagiri, K., Pardi, R., Lu, C., Springer, T. A. and Kinashi, T. (2003). The critical cytoplasmic regions of the αLβ2 integrin in Rap1-induced adhesion and migration. *Mol. Biol. Cell* **14**, 2570-2582.
Tsuboi, S. (2002). Calcium integrin-binding protein activates platelet integrin αIIbβ3. *J. Biol. Chem.* **277**, 1919-1923.
Turner, C. E., Pavalko, F. M. and Burridge, K. (1989). The role of phosphorylation and limited proteolytic cleavage of talin and vinculin in the disruption of focal adhesion integrity. *J. Biol. Chem.* **264**, 11938-11944.
Ulmer, T. S., Yaspan, B., Ginsberg, M. H. and Campbell, I. D. (2001). NMR analysis of structure and dynamics of the cytosolic tails of integrin αIIbβ3 in aqueous solution. *Biochemistry* **40**, 7498-7508.
Ulmer, T. S., Calderwood, D. A., Ginsberg, M. H. and Campbell, I. D. (2003). Domain-specific interactions of talin with the membrane-proximal region of the integrin β3 subunit. *Biochemistry* **42**, 8307-8312.
Vallar, L., Melchior, C., Plancon, S., Drobecq, H., Lippens, G., Regnault, V. and Kieffer, N. (1999). Divalent cations differentially regulate integrin αIIb cytoplasmic tail binding to β3 and to calcium- and integrin-binding protein. *J. Biol. Chem.* **274**, 17257-17266.
van Kooyk, Y. and Figdor, C. G. (2000). Avidity regulation of integrins: the driving force in leukocyte adhesion. *Curr. Opin. Cell Biol.* **12**, 542-547.
Vinogradova, O., Haas, T., Plow, E. F. and Qin, J. (2000). A structural basis for integrin activation by the cytoplasmic tail of the αIIb-subunit. *Proc. Natl. Acad. Sci. USA* **97**, 1450-1455.
Vinogradova, O., Velyvis, A., Velyviene, A., Hu, B., Haas, T. A., Plow, E. F. and Qin, J. (2002). A structural mechanism of integrin αIIbβ3 'inside-out' activation as regulated by its cytoplasmic face. *Cell* **110**, 587-597.
Wang, R., Shattil, S. J., Ambruso, D. R. and Newman, P. J. (1997). Truncation of the cytoplasmic domain of β3 in a variant form of Glanzmann thrombasthenia abrogates signaling through the integrin αIIbβ3 complex. *J. Clin. Invest.* **100**, 2393-2403.
Wang, Z., Leisner, T. M. and Parise, L. V. (2003). Platelet α2β1 integrin activation: contribution of ligand internalization and the α2 cytoplasmic domain. *Blood* **102**, 1307-1315.
Weber, C., Lu, C. F., Casasnovas, J. M. and Springer, T. A. (1997). Role of αLβ2 integrin avidity in transendothelial chemotaxis of mononuclear cells. *J. Immunol.* **159**, 3968-3975.
Weljie, A. M., Hwang, P. M. and Vogel, H. J. (2002). Solution structures of the cytoplasmic tail complex from platelet integrin αIIb- and β3-subunits. *Proc. Natl. Acad. Sci. USA* **99**, 5878-5883.
Williams, M. J., Hughes, P. E., O'Toole, T. E. and Ginsberg, M. H. (1994). The inner world of cell adhesion: integrin cytoplasmic domains. *Trends Cell Biol.* **4**, 109-112.
Woodside, D. G., Liu, S. and Ginsberg, M. H. (2001). Integrin activation. *Thromb. Haemost.* **86**, 316-323.
Wu, C., Keivens, V. M., O'Toole, T. E., McDonald, J. A. and Ginsberg, M. H. (1995). Integrin activation and cytoskeletal interaction are essential for the assembly of a fibronectin matrix. *Cell* **83**, 715-724.
Xi, X., Bodnar, R. J., Li, Z., Lam, S. C. and Du, X. (2003). Critical roles for the COOH-terminal NITY and RGT sequences of the integrin β3 cytoplasmic domain in inside-out and outside-in signaling. *J. Cell Biol.* **162**, 329-339.
Xiong, J. P., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D. L., Joachimiak, A., Goodman, S. L. and Arnaout, M. A. (2001). Crystal structure of the extracellular segment of integrin αVβ3. *Science* **294**, 339-345.
Xiong, J. P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S. L. and Arnaout, M. A. (2002). Crystal structure of the extracellular segment of integrin αVβ3 in complex with an Arg-Gly-Asp ligand. *Science* **296**, 151-155.
Xiong, J. P., Stehle, T., Goodman, S. L. and Arnaout, M. A. (2003a). New insights into the structural basis of integrin activation. *Blood* **102**, 1155-1159.
Xiong, Y. M., Chen, J. and Zhang, L. (2003b). Modulation of CD11b/CD18 adhesive activity by its extracellular, membrane-proximal regions. *J. Immunol.* **171**, 1042-1050.
Yan, B., Calderwood, D. A., Yaspan, B. and Ginsberg, M. H. (2001). Calpain cleavage promotes talin binding to the β3 integrin cytoplasmic domain. *J. Biol. Chem.* **276**, 28164-28170.
Ylanne, J., Chen, Y.-P., O'Toole, T. E., Loftus, J. C., Takada, Y. and Ginsberg, M. H. (1993). Distinct functions of integrin α and β subunit cytoplasmic domains in cell spreading and formation of focal adhesions. *J. Cell Biol.* **122**, 223-233.
Zent, R., Fenczik, C. A., Calderwood, D. A., Liu, S., Dellos, M. and Ginsberg, M. H. (2000). Class- and splice variant-specific association of CD98 with integrin β cytoplasimc domains. *J. Biol. Chem.* **275**, 5059-5064.