Journal of Cell Science 113, 3563-3571 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JCS1159

COMMENTARY

Integrin cytoplasmic domain-binding proteins

Shouchun Liu*, David A. Calderwood* and Mark H. Ginsberg‡

Department of Vascular Biology, VB-2, The Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla, CA 92037, USA
*These authors contributed equally to this manuscript
‡Author for correspondence (e-mail: ginsberg@scripps.edu)

Published on WWW 4 October 2000

SUMMARY

Integrins are a large family of cell surface receptors that mediate cell adhesion and influence migration, signal transduction, and gene expression. The cytoplasmic domains of integrins play a pivotal role in these integrin-mediated cellular functions. Through interaction with the cytoskeleton, signaling molecules, and other cellular proteins, integrin cytoplasmic domains transduce signals from both the outside and inside of the cell and regulate integrin-mediated biological functions. Identification and functional analyses of integrin cytoplasmic domain-binding proteins have been pursued intensively. In recent years, more cellular proteins have been reported to directly interact with integrin cytoplasmic domains and some of these interactions may play important roles in integrin-mediated biological responses. Integrin β chains, for example, interact with actin-binding proteins (e.g. talin and filamin), which form mechanical links to the cytoskeleton. These and other proteins (e.g. FAK, ILK and novel proteins such as TAP20) might also link integrins to signaling mechanisms and, in some cases (e.g. JAB1) mediate integrin-dependent gene regulation.

Key words: Integrin, Cytoplasmic domain, Binding protein, Cellular function, Signaling

INTRODUCTION

Integrin adhesion receptors are heterodimers of α and β subunits that contain a large extracellular domain responsible for ligand binding, a single transmembrane domain and a cytoplasmic domain that in most cases consists of 20-70 amino acid residues (Hynes, 1992; Sastry and Horwitz, 1993). Integrins play central roles in cell adhesion, cell migration and control of cell differentiation, proliferation and programmed cell death. They mediate signal transduction through the cell membrane in both directions: binding of ligands to integrins transmits signals into the cell and results in cytoskeletal re-organization, gene expression and cellular differentiation (outside-in signaling); on the other side, signals from within the cell can also propagate through integrins and regulate integrin ligand-binding affinity and cell adhesion (inside-out signaling; Hynes, 1992; Schwartz et al., 1995). The cytoplasmic domains of integrins play a pivotal role in these bi-directional signaling processes and intensive efforts have focused on identifying cellular proteins that can directly interact with integrin cytoplasmic domains in order to elucidate molecular mechanisms by which integrin mediate bi-directional signal transduction (Dedhar and Hannigan, 1996; Hemler, 1998; Hughes and Pfaff, 1998). Here, we focus on the most recent advances in this field.

INTEGRIN β CYTOPLASMIC DOMAIN-BINDING PROTEINS

Extensive mutational analysis has demonstrated that integrin β cytoplasmic tails play a central role in integrin functions. β_1, β_2 and β_3 integrins lacking β tails fail to localize to focal adhesions, and show reduced ligand-binding activity and impaired activation of downstream signaling molecules (Solowska et al., 1989; Hayashi et al., 1990; Marcantonio et al., 1990; O'Toole et al., 1994). Furthermore, β_{1A}, β_{1D}, β_3, β_5 and β_7 tails expressed in isolation as transmembrane chimeras localize to pre-existing focal adhesions and exhibit a dominant negative effect on the ligand-binding activity of β_1, β_3 and β_5 integrins (Akiyama et al., 1994; Chen et al., 1994; LaFlamme et al., 1992; Lukashev et al., 1994; Zent et al., 2000). Isolated β tails are also sufficient to activate downstream signaling molecules, such as FAK, and can regulate cell cycle progression and actin cytoskeleton assembly (Belkin and Retta, 1998; David et al., 1999; Tahiliani et al., 1997). β tails are thus necessary and sufficient for correct subcellular localization of integrins and for activation of signaling pathways, and regulate the affinity of integrins for their ligands.

The mechanisms by which integrin β tails function in both outside-in and inside-out signaling remain to be fully resolved. Nonetheless, these processes are probably mediated mainly through direct associations between integrin β tails and

signaling and structural proteins. A complete understanding of the molecular basis of integrin regulation will require identification of these integrin-binding proteins and characterization of their activities. At least 21 proteins are known to bind to one or more integrin β tails (Table 1). This diverse list of proteins includes actin-binding proteins, enzymes, adaptor proteins, a transcriptional co-activator and additional proteins of unknown function. As the list lengthens, the challenge becomes determination of which interactions are significant in vivo and the roles of these interactions in specific cellular activities.

Actin-binding proteins

Correct localization of integrins, and their role in cell spreading, migration and matrix assembly require connection to the actin cytoskeleton. This connection is formed by the direct or indirect association of actin-binding proteins with integrin β tails (reviewed by Calderwood et al., 2000 and Critchley, 2000). These interactions represent some of the best-characterized integrin β tail associations, and their significance has been investigated in a variety of contexts.

The first cytoplasmic protein shown to bind to integrins directly was the actin-binding protein talin (Horwitz et al., 1986). Talin colocalizes with integrins at certain sites of cell-substratum contact, and Horwitz et al., proposed that the talin-integrin interaction provides the link between integrins and the actin cytoskeleton. Subsequent experiments revealed that the β cytoplasmic tail is responsible for binding to talin (Pfaff et al., 1998; Knezevic et al., 1996; Table 1), although one report indicates that talin also binds to the α_{IIb} tail (Knezevic et al., 1996). The integrin-binding site has been localized to the head domain of talin, and overexpression of a fragment of talin containing this binding site leads to increased binding of soluble ligand (activation) by $\alpha_{IIb}\beta_3$ in CHO cells (Calderwood et al., 1999). These data, together with the observation that reduced expression of talin disrupts cell surface expression of integrins and export from the Golgi, and impairs focal adhesion formation and cell migration (Priddle et al., 1998; Martel et al., 2000), suggest that binding of talin to integrin β tails is important for a variety of integrin functions. However, proof of this hypothesis requires evidence that specific disruption of the talin-integrin interaction alters integrin-dependent functions.

To date no point mutants of talin that lack integrin-binding activity have been reported. However, fragments that lack the entire, integrin-binding, head domain cannot activate $\alpha_{IIb}\beta_3$ in CHO cells. The exact location of the talin-binding site within the β tail remains to be determined (Calderwood et al., 2000); nonetheless, point and deletion mutants that inhibit talin binding have been identified (Calderwood et al., 1999; Kaapa et al., 1999). Integrin mutants that are unable to bind talin are unable to localize to focal adhesions and fail to accumulate F-actin following integrin clustering (Reszka et al., 1992; Ylanne et al., 1995; Lewis and Schwartz, 1995). These mutants are expressed at the cell surface, although whether their trafficking from the Golgi to plasma membrane is altered has not been investigated. Furthermore, one report indicates that in v-Src-transformed cells, which exhibit reduced cell adhesion and a

Table 1

Binding partner	Integrin tail	Detection	Reference
Actin-binding protein			
Talin	$\beta_{1A},\beta_{1D},\beta_2,\beta_3$	COIP, PEP, EQ, INT, SLS	Horwitz et al., 1986; Knezevic et al., 1996; Pfaff et al., 1998; Goldmann, 2000
Filamin	$\beta_{1A},\beta_2,\beta_3,\beta_7$	COIP, PEP, 2HYB, SLS	Pavalko et al.,1989; Loo et al., 1998; Pfaff et al., 1998; Goldmann, 2000
α-actinin	β_{1A},β_2	PEP, INT, COIP, SLS	Otey et al., 1990; Pavalko et al., 1991; Cattelino et al., 1999
F-actin	α_2	PEP	Kieffer et al., 1995
Myosin	β_3	PEP, COIP	Jenkins et al., 1998; Sajid et al., 2000
Skelemin	β_1,β_3	2HYB, PEP	Reddy et al., 1998
Signaling protein			
ILK	β_1,β_3	2HYB, COIP	Hannigan et al., 1996
FAK	β_1,β_2,β_3	PEP, COIP	Schaller et al., 1995; Chen et al., 2000
Cytohesin-1	β_2	2HYB, COIP, PEP	Kolanus et al., 1996
Cytohesin-3	β_2	2HYB	Hmama et al., 1999
Other protein			
Paxillin	β_1,β_3,α_4	PEP, COIP	Schaller et al., 1995; Chen et al., 2000; Liu et al., 1999
Grb2	β_3	PEP	Law et al., 1996
Shc	β_3	PEP	Law et al., 1996
β3-endonexin	β_3	2HYB, INT, PEP	Shattil et al., 1995; Eigenthaler et al., 1997
TAP-20	β_5	PEP	Tang et al., 1999
CIB	α_{IIb}	2HYB, PEP, COIP	Naik et al., 1997; Shock et al., 1999; Valler et al., 199
Calreticulin	α	PEP, COIP	Rojiani et al., 1991; Leung-Hagesteijn et al., 1994; Coppolino et al., 1995
Caveolin-1	α	COIP	Wary et al., 1998
Rack1	β_1,β_2,β_5	2HYB, PEP, COIP	Liliental et al., 1998
WAIT-1	β_7	2HYB, PEP	Rietzler et al., 1998
JAB1	β_2	2HYB, PEP, COIP	Bianchi et al., 1998
Melusin	$\beta_{1A},\beta_{1B},\beta_{1D}$	2HYB, INT	Brancaccio et al., 1999
MIBP	β_{1A},β_{1D}	2HYB, PEP, COIP	Li et al., 1999
ICAP-1	β_{1A}	2HYB, PEP, INT	Chang et al., 1997; Zhang and Hemler, 1999
CD98	β_{1A},β_3	PEP	Zent et al., 2000
DRAL/FHL2	$\alpha_{3A},\alpha_{3B},\alpha_{7A},\beta$	2HYB, PEP	Wixler et al., 2000

COIP--Coimmunoprecipitation; PEP--Synthetic/recombinant peptide studies; 2HYB--Yeast two-hybrid screen; INT--Binding to purified integrins; SLS--Static light scattering; EQ--Equilibrium gel filtration.

disorganized cytoskeleton, the NPXY motif (this motif is highly conserved among different integrin β subunit cytoplasmic domains and may play important roles in integrin-mediated signal transduction) in the β_1 tail is phosphorylated on tyrosine, and talin binding is inhibited (Tapley et al., 1989). These observations strongly support the hypothesis that binding of talin to integrin β tails plays a role in integrin-mediated processes by connecting integrins to the actin cytoskeleton. However, note that the integrin mutants tested might also exhibit impaired interactions with other integrin-binding proteins whose function is not appreciated. For this reason reconstitution experiments in talin-null cells, using wild-type and mutated talin, should provide an additional test of the role of integrin-talin interactions.

The binding of integrins to two other families of actin-binding proteins, α-actinin and filamin, has also been well characterized (Table 1; Calderwood et al., 2000; Critchley, 2000). Static light scattering experiments, which can measure equilibrium binding constants between purified proteins, indicate that both filamin and α-actinin bind less tightly to integrin $\alpha_{IIb}\beta_3$ than does talin (Goldmann, 2000). Like talin, α-actinin colocalizes with integrins in focal adhesions, and α-actinin targets to focal adhesions in microinjected cells and in a cell-free system, apparently by interaction with β cytoplasmic tails (Otey et al., 1990; Pavalko and Burridge, 1991; Cattelino et al., 1999). α-actinin is also localized along stress fibers. Expression of isolated integrin-binding fragments of α-actinin disrupts stress fibers, focal adhesions and shear-induced mechanical signaling in fibroblasts and osteoblasts (Ezzell et al., 1997; Pavalko and Burridge, 1991; Pavalko et al., 1998). The α-actinin-binding site has been localized to the membrane-proximal half of the β_1 and β_2 integrin tails (Fig. 1; Otey et al., 1990), and mutations in this region alter formation of focal adhesions and stress fibers. However, truncated integrin mutants that retain α-actinin binding but cannot bind to talin still exhibit a disrupted phenotype (Retta et al., 1998; Lewis and Schwartz, 1995), which indicates that these two β tail-binding proteins have separate functions.

Filamin localizes to the cortical actin cytoskeleton and along the length of stress fibers, but is also found in some focal adhesions (Pavalko et al., 1989). Its recruitment to β_1-containing focal adhesions is stimulated by mechanical stress and leads to F-actin recruitment (Glogauer et al., 1998). In addition to providing a mechanical link between integrins and the cytoskeleton, filamin also acts as an adapter protein for a number of signaling proteins (e.g. RalA) that can regulate cytoskeletal dynamics (Ohta et al., 1999). Loss of filamin-1 expression in cultured melanocytes causes impaired migration, altered morphology and defective expression of cell surface molecules (Cunningham et al., 1992; Meyer et al., 1998), and human neurons lacking filamin-1 fail to migrate in vitro, which causes the disease periventricular heterotopia (Fox et al., 1998). The filamin binding site lies in the C-terminal portion of the β_{1A} tail, and point mutations in this region disrupt filamin-binding (Loo et al., 1998). These mutations also lead to impaired localization of integrins to focal adhesions. Thus, both α-actinin and filamin can bind to integrin β tails, colocalize with integrins, and are required for integrin-mediated processes. However, detailed analysis of which processes require direct β-tail binding awaits identification of subtle mutations that modulate integrin-filamin or integrin–α-actinin interactions.

Additional cytoskeletal proteins have been identified as integrin-β-tail-binding proteins; however, their significance is not yet clear. Jenkins et al. used ligand blotting to demonstrate association of platelet myosin with a peptide corresponding to the last 23 residues of the β_3 tail (Jenkins et al., 1998). The interaction was dependent on phosphorylation of both tyrosine residues in this sequence and was inhibited by a loss of function mutation in this region. Following platelet aggregation, phosphorylation of both tyrosines in the β_3 tail can be detected, which suggests that myosin is recruited to contribute to clot retraction. CHO cells expressing mutants that have Tyr→Phe mutations in the β_3 tail are less efficient at clot retraction, and these mutations are associated with a mild bleeding phenotype in mice (Jenkins et al., 1998; Law et al., 1999). In addition, Sajid et al. recently showed that non-muscle myosin heavy chain A coimmunoprecipitates with $\alpha_V\beta_3$ integrins following thrombospondin stimulation of smooth muscle cells (Sajid et al., 2000). It will be of interest to determine whether this stimulation leads to β_3 tail tyrosine phosphorylation, which could account for myosin recruitment.

Binding of skelemin, a cytoskeletal M-band protein, to β_1 and β_3 but not β_2 tails was also reported recently (Reddy et al., 1998). Skelemin has an unique N-terminal region followed by Ig superfamily C2 and fibronectin (FN) type II motifs. Reddy et al., localized the skelemin-binding site to the membrane proximal ten residues of β_1. Under some conditions, skelemin colocalizes with $\alpha_{IIb}\beta_3$ expressed in CHO cells, and microinjection of the integrin-binding domain of skelemin causes myoblasts to round up (Reddy et al., 1998), conceivably by disrupting integrin-cytoskeleton interactions. Skelemin is encoded by an alternatively spliced version of the myomesin gene (Steiner et al., 1999), and the integrin-binding sequence is also present in myomesin, which suggests that this protein also binds β tails. Although skelemin expression is restricted to muscle cells, antibodies against skelemin crossreact with a protein of similar size from non-muscle cells, which raises the possibility that integrin binding to a skelemin-like protein is important in many cell types.

Cell signaling proteins

In addition to providing a link to the actin cytoskeleton, integrin-β-tail-binding proteins also regulate outside-in and inside-out signaling. The absence of any detectable enzymatic activity in integrin cytoplasmic tails suggests that integrin-mediated signaling requires direct binding of signaling proteins, such as non-receptor kinases, or of adaptor proteins that can recruit these molecules.

Two kinases, focal adhesion kinase (FAK) and integrin linked kinase (ILK), have been reported to bind to integrin β tails (Table 1). FAK colocalizes with integrins at cell-substratum contact sites, and its phosphorylation state and tyrosine kinase activity are regulated by binding of cells to the ECM (reviewed by Schlaepfer and Hunter, 1998). FAK phosphorylation can also be induced by clustering of isolated integrin β tails. FAK has been implicated as a central player in integrin-mediated signaling (Schlaepfer and Hunter, 1998). However, what, if any, role FAK binding to integrin β tails plays in the process is not clear. Schaller et al. demonstrated that FAK binds to synthetic peptides corresponding to the

membrane-proximal region of the β_1, β_2 and β_3 tails, and that mutations in this region inhibit FAK binding to a full-length β_1 tail peptide (Schaller et al., 1995). FAK has also been reported to co-immunoprecipitate with β_1 integrins from co-cultures of Schwann cells and neurons (Chen et al., 2000). Under these conditions paxillin is also co-precipitated with both FAK and β_1 integrins, which raises the possibility that FAK is linked indirectly to β_1 integrins. FAK binding to talin (Borowsky and Hynes, 1998) provides yet another mechanism by which FAK might be indirectly linked to integrin. The integrin-binding site in FAK was localized to the region N-terminal to the kinase domain; however, this region is not required for localization of FAK to focal adhesions. Instead, a region C-terminal to the kinase domain, the focal-adhesion-targeting domain, is necessary and sufficient for localization to focal adhesions. Furthermore, the reported FAK-binding region within β_3 tail (Schaller et al., 1995) is neither required nor sufficient for FAK activation following clustering of isolated β_3 tails (Tahiliani et al., 1997).

ILK is the subject of several recent reviews (Dedhar, 2000; Dedhar et al., 1999; Wu, 1999) and was initially identified in a yeast two-hybrid screen for β_1-tail-binding protein. It localizes to focal adhesions in a manner dependent on both the C-terminal domain, which contains the integrin-binding site, and the most N-terminal ankyrin repeat (Wu, 1999). This repeat is responsible for binding the LIM domain protein PINCH. PINCH in turn binds to other adaptor proteins, which could regulate additional signaling pathways or actin polymerization (Calderwood et al., 2000). Null mutations in the gene that encodes *C. elegans* ILK, *pat-4*, have a phenotype similar to that of integrin- or PINCH-null mutants, providing additional evidence for a functional link between these molecules. However, although ILK has been implicated in regulation of cell adhesion, fibronectin (FN) matrix assembly, anchorage-dependent cell growth, and cell cycle progression, whether direct integrin-ILK binding is required for any of these processes awaits studies using mutants in which integrin binding is selectively disrupted.

Another class of signaling molecule capable of binding to integrin β tails is the cytohesins. Cytohesins have guanine nucleotide exchange activity for the ARF family of small GTPases (Ogasawara et al., 2000). Cytohesin-1 and subsequently, cytohesin-3 were identified as β_2-tail-binding proteins in yeast two-hybrid screens, and the integrin-binding site was localized to the Sec7 domain of the molecule (Kolanus et al., 1996; Korthauer et al., 2000). Cytohesin-1 also coprecipitated with β_2, but not β_1, integrins from Jurkat cell lysate, and recombinant cytohesin-1 bound to peptides corresponding to the β_2 tail. Overexpression of cytohesin-1 or cytohesin-3, but not cytohesin-2, increased β_2-integrin-mediated adhesion of unstimulated Jurkat cells, and antisense cytohesin-1 oligonucleotides reduced LPS-induced adhesion of THP1 cells to β_2 integrin ligands (Korthauer et al., 2000; Hmama et al., 1999).

Recent data from Geiger et al. (2000) have identified membrane-proximal residues in β_2 that are required for cytohesin-1 binding. Mutation of these residues inhibits $\alpha_L\beta_2$-mediated cell adhesion, possibly by preventing cytohesin-1 binding. However, although overexpression of cytohesin-1 induces expression of an activation epitope on $\alpha_L\beta_2$, binding of soluble ligand is unaffected. Unfortunately, whether cytohesin-1 could induce this epitope on the mutated integrin was not tested. Geiger et al. (2000) did show that ARF-GEF activity was required for the effects on cell adhesion and spreading, but not for induction of the activation epitope, suggesting that a major role for cytohesin lies in regulation of cell shape, which may in turn affect the adhesive properties of the cell. Some controversy remains as to the localization of cytohesins within the cell (i.e. whether they reside in the cytoplasm, the plasma membrane or Golgi membranes). This probably reflects differences in cell type and cytohesin isoforms present, along with a signal-dependent regulation of cytohesin localization (Lee and Pohajdak, 2000; Korthauer et al., 2000; Venkateswarlu et al., 1999). The PH domain of cytohesins is capable of binding phosphatidylinositol phosphate, and this might regulate cytohesin localization and GAP activity, and thereby explain the effect of phosphatidylinositol phosphate levels on β_2-integrin-mediated adhesion (Hmama et al., 1999).

The actin-binding proteins and enzymes discussed above probably play additional roles as adaptor proteins (Calderwood et al., 2000), but three previously characterized adaptor proteins, paxillin, SHC and GRB2, also bind to peptides corresponding to integrin β tails (Schaller et al., 1995; Law et al., 1996). Paxillin also binds strongly to the α_4 tail (Liu et al., 1999) and is discussed in detail below. Binding of GRB2 and SHC required phosphorylation of the β_3 tail peptide, a process dependent on platelet aggregation (Law et al., 1996). Further experiments will be necessary to determine whether β_3 associates with GRB2 and SHC in vivo.

Several other proteins that have no clear enzymatic activity have been reported to bind β tails (Table 1). Most of these have been identified in yeast two-hybrid screens. The first β-tail-binding protein identified in this manner was β_3-endonexin. β_3-endonexin binds specifically to β_3, but not β_1 or β_2, tails through both membrane proximal and distal motifs (Shattil et al., 1995; Eigenthaler et al., 1997). When expressed in CHO cells, β_3-endonexin increased the ligand-binding activity of $\alpha_{IIb}\beta_3$ (Kashiwagi et al., 1997). β_3 mutants incapable of binding β_3-endonexin are insensitive to β_3-endonexin transfection, which suggests that binding of β_3-endonexin to the β_3 tail is important for integrin regulation. β_3-endonexin can also bind to cyclin A and inhibit the cyclin-A–Cdk2 kinase activity (Ohtoshi et al., 2000), which raises the possibility that it is involved in integrin-mediated regulation of cell cycle progression.

Interestingly, a novel 175-residue protein, TAP20, shares 55% amino acid sequence similarity with β_3-endonexin over the first 110 residues and appears to bind to and regulate β_5 integrins (Tang et al., 1999). TAP20 expression in rat capillary endothelial cells is induced by protein kinase Cθ (PKCθ), which regulates $\alpha_V\beta_5$ mediated endothelial cell migration. TAP20 overexpression in cultured endothelial cell lines reduces $\alpha_V\beta_5$-mediated adhesion and increases $\alpha_V\beta_5$-mediated migration on vitronectin. Recombinant TAP20 binds to β_5, but not β_1, β_3 or α_V, tails. Thus, two related proteins that bind to and regulate β_3 or β_5 integrins have been identified. It remains to be seen whether TAP20 regulates the affinity state of β_5 integrins, as β_3-endonexin does for β_3 integrins, although it is noteworthy that β_3-endonexin leads to activation of β_3 integrins whereas TAP20 appears to downregulate β_5 function.

The WD repeat proteins Rack1 (receptor for activated

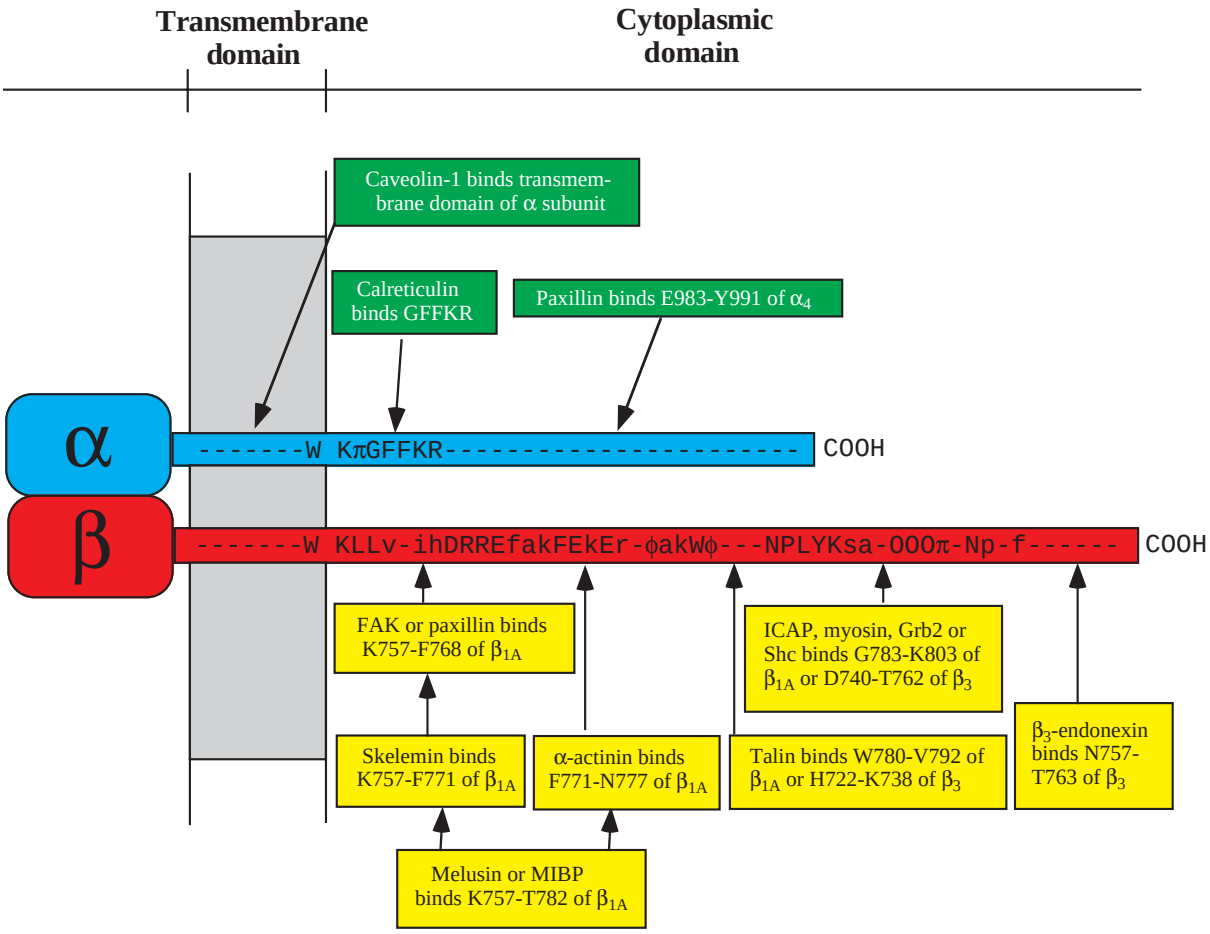

Fig. 1. Binding sites on integrin α and β subunits for integrin cytoplasmic domain binding proteins. The reported interacting sites on integrin tails for integrin-tail-binding proteins are indicated by boxes and arrows. Conserved amino acid sequences among integrin α and β cytoplasmic domains are illustrated: uppercase letters correspond to near-invariant residues; lowercase letters correspond to residues conserved in at least three subunits; O represents conserved hydroxylated residues; π and ϕ represent conserved apolar and polar residues, respectively; and dashes represent unconserved residues and/or gaps.

protein kinase C) and WAIT-1 (WD protein associated with integrin tails) have also been identified in yeast two-hybrid screens for β-tail-binding proteins (Liliental and Chang, 1998; Zhang and Hemler, 1999). Rack1 is composed of seven WD repeats, and the integrin-binding site is localized to repeats 5-7. The binding site within the integrin β_1, β_2 and β_5 tails lies in the membrane proximal region. Interaction of full-length Rack1 with integrins requires stimulation of the cell with phorbol esters, which indicates that the Rack1-integrin interaction is regulated. The significance of the interaction between Rack-1 and integrins has not been well defined and the specificity of this interaction has been questioned because Rack1 also interacts with α_4 and α_V tails (Zhang and Hemler, 1999). The other β-tail-binding WD repeat protein, WAIT-1, also binds to both α and β tails, specifically β_7, α_4 and α_E, but not β_2, β_1 or α_L (Rietzler et al., 1998). WAIT-1 can therefore bind to both tails of the $\alpha_4\beta_7$ and $\alpha_E\beta_7$ integrins. However, whereas β_7 expression is localized to leukocytes, *WAIT-1* mRNA appears to be expressed in a variety of cell types, which suggests that WAIT-1 has additional functions besides binding to β_7 integrins. Like Rack1, WAIT-1 interacts with a membrane-proximal region of the β subunit. No functional data suggesting a role for their interaction with β tails have been provided.

Similarly, the muscle-specific proteins Melusin and MIBP (muscle-specific β_1-integrin-binding protein) both bind to membrane-proximal regions of the β_1 tail. However, the functional significance of this interaction has not been addressed (Brancaccio et al., 1999; Li et al., 1999). Binding of Melusin to the β_1 tail appears to be regulated by calcium, which is consistent with the identification of potential calcium-binding motif at its C terminus. Melusin also contains putative binding sites for SH2 and SH3 domains at its N terminus, which raises the possibility that it functions as an adaptor protein to recruit additional signaling proteins to the β tail.

The most recently identified integrin-binding protein, JAB1 (Jun-activation-domain-binding protein), binds to the β_2 but not to the α_L tail in a yeast two-hybrid screen and in studies using recombinant proteins (Bianchi et al., 2000). JAB1 is found both in the nucleus and in the cytoplasm, and its shuttling between the two might be related to its transcriptional co-activator activity. Bianchi et al., observed some colocalization of JAB1 with $\alpha_L\beta_2$ integrins at the cell membrane, and following integrin engagement the nuclear

pool of JAB1 increased. However, whether direct β_2-JAB1 interaction is involved in integrin regulation of gene expression or other JAB1-mediated effects awaits further study.

ICAP-1 (integrin-cytoplasmic-domain-associated protein 1) binds specifically to the membrane-distal 13 residues of the β_{1A} tail but not other β tails (Chang et al., 1997; Zhang and Hemler, 1999; Fig. 1). Point mutations within the C-terminal NPXY motif or between the two NPXY motifs inhibit ICAP-1 binding to the β_{1A} tail. ICAP-1 is rich in serine residues, contains consensus phosphorylation sites for PKC, cAMP- or cGMP-dependent kinases and calcium/calmodulin-dependent protein kinase II (CaMKII), and is phosphorylated in response to cell adhesion to FN. ICAP-1-overexpressing COS cells exhibit increased β_1-mediated migration on FN and in CHO cells expressing chimeric β_1/β_5 integrins migration correlates with the ICAP-1 binding ability of the chimera. In addition, point mutations within the putative ICAP-1 CaMKII-phosphorylation site that mimic phosphorylation inhibit CHO cell spreading on FN, whereas mutations that prevent phosphorylation stimulate cell spreading to levels seen in cells that express wild-type ICAP-1 in the presence of a CaMKII inhibitor (Bouvard and Block, 1998). Thus, ICAP-1 binding to β tails might regulate cell spreading and migration in a manner dependent on CaMKII phosphorylation of ICAP-1. Further experiments are required to determine whether CaMKII does indeed phosphorylate ICAP-1, whether this alters its binding to the β_1 tail and whether this phosphorylation is regulated by cell adhesion.

Finally, we have recently shown that CD98 can bind to recombinant models of β_{1A} and β_3, but not β_{1D} or β_7, tails (Zent et al., 2000). CD98 is a type II transmembrane protein first discovered as a T-cell activation antigen and is involved in regulation of amino acid transport, cell fusion and integrin activation. CD98 was identified in an expression cloning screen for proteins that reverse the dominant suppression of integrin activation by isolated β_{1A} tails (Fenczik et al., 1997). The binding of CD98 to different β tails correlates with its ability to reverse the suppression, which suggests that CD98-integrin binding is important for regulation of integrin activation (Zent et al., 2000). Antibody crosslinking of CD98 within the cell membrane stimulates β_1-integrin-dependent cell adhesion of small lung cancer cells, and, in combination with anti-CD3 antibodies, antibodies to CD98 cause proliferation of peripheral blood T lymphocytes (Fenczik et al., 1997; Warren et al., 2000). This co-stimulatory activity is inhibited in the presence of an anti-β_1-integrin antibody. The ongoing characterization of regions within CD98 responsible for integrin β tail binding should facilitate investigation of which other CD98-mediated functions require β tail binding.

INTEGRIN α CYTOPLASMIC DOMAIN BINDING PROTEINS

In contrast to β subunits, different α subunit cytoplasmic domains share little sequence similarity, except for the membrane proximal KXGFFKR sequence (Fig. 1); this suggests that each tail plays a unique role in integrin function. However, each α subunit is highly conserved among different species, which indicates that the α cytoplasmic domains are important for integrin functions (Hynes, 1992; Sastry and Horwitz, 1993). Indeed, different α cytoplasmic domains differentially regulate integrin-mediated biological responses. A classical example of this is the α_4 integrins. α_4 integrins regulate cell migration, cytoskeletal organization and gene expression differently from other integrin α subunits. α_4 integrins increase cell migration and oppose cell spreading and focal adhesion formation (Chan et al., 1992; Hemler et al., 1992; Kassner et al., 1995). These unusual biological properties depend on the α_4 cytoplasmic domain. Indeed, when joined to other integrin α subunits, the α_4 tail markedly enhances cell migration and opposes cell spreading and focal adhesion formation (Kassner et al., 1995; Liu et al., 1999).

Parise and colleagues reported that R-Ras, a Ras-related GTPase, promotes migration of cells expressing integrin chimeras containing the α_2, but not the α_5, cytoplasmic domain (Keely et al., 1999). Furthermore, $\alpha_2\beta_1$-mediated migration is inhibited by the expression of excess α_2- but not α_5-cytoplasmic-domain-containing chimeras, which suggests that there are limiting factors that bind the α_2 tail (Keely et al., 1999). Weber et al. have shown that two β_2 integrins expressed on lymphocytes, $\alpha_M\beta_2$ (Mac-1) and $\alpha_L\beta_2$ (LFA-1), display different kinetics of integrin activation upon chemokine stimulation. This difference appears to reside within the cytoplasmic domains of α_M and α_L, since exchange of the cytoplasmic tail conferred the α tail-specific integrin activation (Weber et al., 1999). Therefore, α_M and α_L tails transduce distinct pathways for integrin activation possibly through interaction with different cellular components.

Sastry et al. reported that integrin-mediated cell cycle withdrawal and onset of terminal differentiation are controlled differently by the α_5 and α_{6A} cytoplasmic domains (Sastry et al., 1999). However, both of the α tails did not appear to initiate these signals but instead to regulate β_1 signaling (Sastry et al., 1999). In another study, Shaw et al. reported that the α_{6A} tail induced integrin-dependent paxillin phosphorylation more effectively than did the α_{6B} tail (Shaw et al., 1995). These data suggest that each α cytoplasmic tail uniquely regulates integrin functions either by directly initiating signaling events, by modulating β subunit signaling or by regulating β tail ligand binding (e.g. the α_4 tail partially inhibited filamin binding to the β_{1A} tail; Liu et al., 1999). Extensive efforts have focused on identifying cellular proteins that can directly associate with α cytoplasmic domains. However, thus far, most of the key α tail partners remain elusive.

Kieffer et al. have reported that F-actin binds directly to the α_2 cytoplasmic domain and removal of five amino acid residues from the C terminus of the tail disrupt the binding; this suggests that this region is responsible for F-actin binding (Kieffer et al., 1995). This F-actin–α_2 association might play a role in the focal adhesion localization of α_2 integrins and the enhanced collagen gel contraction by the α_2 tail (Chan et al., 1992; Kassner et al., 1995). However, the molecular mechanism and effect of this interaction on α_2-integrin-mediated biological properties have not been reported.

Calreticulin, a luminal endoplasmic reticulum calcium-binding protein directly interacts with the KXGFFKR motif, the highly conserved membrane-proximal sequence of α cytoplasmic domains (Fig. 1; Rojiani et al., 1991). Calreticulin can directly bind to the synthetic KLGFFKR peptide, co-precipitates with different α integrins and co-localizes with integrins (Leung-Hagesteijn et al., 1994; Coppolino et al.,

1995). Calreticulin-deficient cells have a severe defect in integrin-mediated cell adhesion; however, this defect can be rescued by expression of calreticulin. Furthermore, transient elevation of intracellular calcium concentration initiated by integrin-mediated adhesion is also absent in calreticulin-deficient cells (Coppolino et al., 1997). Thus, the α-tail–calreticulin interaction might modulate integrin-mediated cell adhesion and signal transduction, although the mechanism by which calreticulin gains access to the cytoplasm is unclear.

CIB (calcium- and integrin-binding protein) is another calcium-binding protein that specifically binds to α_{IIb}, but not α_V, α_2, α_5, β_1 or β_3, tails in the yeast two-hybrid system (Naik et al., 1997). CIB also interacts with intact $\alpha_{IIb}\beta_3$ integrin in an enzyme-linked immunosorbent assay and can be coimmunoprecipitated with intact $\alpha_{IIb}\beta_3$ upon Mn^{2+} activation (Shock et al., 1999; Vallar et al., 1999). Thus, it is suggested that CIB is a candidate for a regulator of integrin $\alpha_{IIb}\beta_3$ and might be involved in $\alpha_{IIb}\beta_3$ post-receptor-occupancy events (Nail et al., 1997; Vallar et al., 1999).

Giancotti's group has reported that caveolin-1, a transmembrane adaptor, can interact with some integrins through the transmembrane domain of the α subunit (Wary et al., 1998). They report that the association of caveolin-1-α subunit can physically and functionally link integrins to the tyrosine kinase, Fyn (or Yes), recruit the adaptor protein Shc and subsequently Grb2 and Sos and thereby regulate Ras-ERK signaling and cell cycle progression (Wary et al., 1998).

DRAL/FHL2, a LIM-only protein, has recently been reported to bind α_{3A}, α_{3B} and α_{7A}, as well as several β integrin cytoplasmic domains (Wixler et al., 2000). The amino acid residues C-terminal of the conserved GFFKR sequence among α subunits are crucial for DRAL/FHL2 binding. DRAL/FHL2 is also localized to focal adhesion complexes; thus, it is suggested that DRAL/FHL2 is involved in integrin-mediated signal transduction (Wixler et al., 2000).

We have recently reported that paxillin, an intracellular adaptor protein, binds directly and tightly to the α_4 tail but not other α tails (Liu et al., 1999). Direct association between paxillin and the α_4 tail was also confirmed by immunoprecipitation with intact α_4 or $\alpha_{IIb}\alpha_4$ chimeric integrins. Binding of paxillin to the α_4 tail markedly enhanced the rates of $\alpha_{IIb}\beta_3$-dependent tyrosine phosphorylation of FAK and cell migration. It also reduced cell spreading, focal adhesion and stress fiber formation. We have identified a nine-residue (E983-Y991) region conserved among the α_4 cytoplasmic domains that is sufficient for paxillin binding (Liu and Ginsberg, 2000). A point mutation within this region (Y991A) reversed the α_4-tail-specific effects (Liu et al., 1999; Liu and Ginsberg, 2000). Furthermore, $\alpha_4\beta_1$-dependent adhesion to VCAM-1 led to spreading of paxillin-null cells and reconstitution of paxillin inhibited spreading (Liu et al., 1999). Paxillin directly interacts with several intracellular signaling and adaptor molecules (Turner, 1998) and most paxillin-interacting proteins, such as FAK, Src, PTP-PEST, vinculin, Crk, p95PKL as well as PIX and PAK (Turner, 1998; Turner et al., 1999), have been implicated in regulation of cell migration, cytoskeletal organization and gene expression. Thus, the direct association of paxillin with the α_4 tail might facilitate the rapid recruitment and activation of these signaling molecules and therefore account for some of the unusual biological properties of α_4 integrins.

CONCLUSIONS AND FUTURE DIRECTIONS

The list of reported integrin cytoplasmic domain binding proteins is relatively long and still expanding. Most of the interactions have been identified in vitro. This raises two major questions: which of these proteins interact with integrin cytoplasmic domains in vivo and are these interactions important for integrin functions? Addressing these concerns will be a major challenge in future studies. In evaluating these associations, several considerations apply. (1) Do mutations that prevent the interactions affect integrin-dependent functions? (2) Does absence of the integrin-binding protein affect integrin-dependent functions? (3) Does the protein associate with endogenous integrins when both proteins are present at physiological levels? (4) Is the protein in physical proximity to integrins in intact cells? (5) Ultimately the significance of the association should be validated in vivo; thus, what, if any, are the phenotypes of organisms bearing mutations in binding proteins that disrupt its interactions with integrins and are these phenotypes related to integrin functions?

The large number of integrin cytoplasmic domain binding partners suggests that cellular regulation of the interactions might be important. To date, little is known about post-translational modifications, such as phosphorylation or proteolytic cleavage of integrin tails and their binding partners and changes in their subcellular localization are likely to play roles in regulating integrin-dependent biological responses.

This work was supported in part by grants from the National Institutes of Health (to M.H.G.) and a Scientist Development Grant from American Heart Association (to S.L.). D.A.C. is the recipient of a fellowship from the Susan G. Komer Breast Cancer Foundation.

REFERENCES

Akiyama, S. K., Yamada, S. S., Yamada, K. M. and LaFlamme, S. E. (1994). Transmembrane signal transduction by integrin cytoplasmic domains expressed in single-subunit chimeras. *J. Biol. Chem.* **269**, 15961-15964.

Belkin, A. M. and Retta, S. F. (1998). β1D integrin inhibits cell cycle progression in normal myoblasts and fibroblasts. *J. Biol. Chem.* **273**, 15234-15240.

Bianchi, E., Denti, S., Granata, A., Bossi, G., Geginat, J., Villa, A., Rogge, L. and Pardi, R. (2000). Integrin LFA-1 interacts with the transcriptional co-activator JAB1 to modulate AP-1 activity. *Nature* **404**, 617-621.

Borowsky, M. L. and Hynes, R. O. (1998). Layilin, a novel talin-binding transmembrane protein homologous with C-type lectins, is localized in membrane ruffles. *J. Cell Biol.* **143**, 429-442.

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.

Brancaccio, M., Guazzone, S., Menini, N., Sibona, E., Hirsch, E., De Andrea, M., Rocchi, M., Altruda, F., Tarone, G. and Silengo, L. (1999). Melusin is a new muscle-specific interactor for β1 integrin cytoplasmic domain. *J. Biol. Chem.* **274**, 29282-29288.

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.* (in press).

Cattelino, A., Albertinazzi, C., Bossi, M., Critchley, D. R. and de Curtis, I. (1999). A cell-free system to study regulation of focal adhesions and of the connected actin cytoskeleton. *Mol. Biol. Cell* **10**, 391.

Chan, B. M. C., Kassner, P. D., Schiro, J. A., Byers, H. R., Kupper, T. S. and Hemler, M. E. (1992). Distinct cellular functions mediated by different VLA integrin α subunit cytoplasmic domains. *Cell* **68**, 1051-1060.

Chang, D. D., Wong, C., Smith, H. and Liu, J. (1997). ICAP-1, a novel β1 integrin cytoplasmic domain-associated protein, binds to a conserved and functionally important NPXY sequence motif of β_1 integrin. *J. Cell Biol.* **138**, 1149-1157.

Chen, L. M., Bailay, D. and Fernandez-Valle, C. (2000). Association of β1 integrin with focal adhesion kinase and paxillin in differentiating schwann cells. *J. Neurosci.* **20**, 3776-3784.

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

Coppolino, M., Leung-Hagesteijn, C., Dedhar, S. and Wilkins, J. (1995). Inducible interaction of integrin $\alpha_2\beta_1$ with calreticulin. *J. Biol. Chem.* **270**, 23132-23138.

Coppolino, M. G., Woodside, M. J., Demaurex, N., Grinstein, S., St-Arnaud, R. and Dedhar, S. (1997). Calreticulin is essential for integrin-mediated calcium signalling and cell adhesion. *Nature* **386**, 843-847.

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

Cunningham, C. C., Gorlin, J. B., Kwiatkowski, D. J., Hartwig, J. H., Janmey, P. A., Byers, H. R. and Stossel, T. P. (1992). Actin-binding protein requirement for cortical stability and efficient locomotion. *Science* **255**, 325-327.

David, F. S., Zage, P. E. and Marcantonio, E. E. (1999). Integrins interact with focal adhesions through multiple distinct pathways. *J. Cell Physiol.* **181**, 74-82.

Dedhar, S. and Hannigan, G. E. (1996). Integrin cytoplasmic interactions and bidirectional transmembrane signalling. *Curr. Opin. Cell Biol.* **8**, 657-669.

Dedhar, S., William, B. and Hannigan, G. (1999). Integrin-linked kinase (ILK): a regulator of integrin and growth-factor signaling. *Trends Cell Biol.* **9**, 319-323.

Dedhar, S. (2000). Cell-substrate interactions and signaling through ILK. *Curr. Opin. Cell Biol.* **12**, 250-256.

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.

Ezzell, R. M., Goldmann, W. H., Wang, N., Parasharama, N. and Ingber, D. E. (1997). Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton. *Exp. Cell Res.* **231**, 14-26.

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* **370**, 81-85.

Fox, J. W., Lamperti, E. D., Eksioglu, Y. Z., Hong, S. E., Feng, Y., Graham, D. A., Scheffer, I. E., Dobyns, W. B., Hirsch, B. A., Radtke, R. A., Berkovic, S. F., Huttenlocher, P. R. and Walsh, C. A. (1998). Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* **21**, 1315-1325.

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

Glogauer, M., Arora, P., Chou, D., Janmey, P. A., Downey, G. P. and McCulloch, C. A. (1998). The role of actin-binding protein 280 in integrin-dependent mechanoprotection. *J. Biol. Chem.* **273**, 1689-1698.

Goldmann, W. H. (2000). Kinetic determination of focal adhesion protein formation. *Biochem. Biophys. Res. Commun.* **271**, 553-557.

Hayashi, Y., Haimovich, B., Reszka, A., Boettiger, D. and Horwitz, A. (1990). Expression and function of chicken integrin β1 subunit and its cytoplasmic domain mutants in mouse NIH 3T3 cells. *J. Cell Biol.* **110**, 175-184.

Hemler, M. E., Kassner, P. D. and Chan, B. M. C. (1992). Functional roles for integrin α subunit cytoplasmic domains. *Cold Spring Harbor Symp. Quant. Biol.* **57**, 213-220.

Hemler, M. E. (1998). Integrin associated proteins. *Curr. Opin. Cell Biol.* **10**, 578-585.

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.

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. and Pfaff, M. (1998). Integrin affinity modulation. *Trends Cell Biol.* **8**, 359-364.

Hynes, R. O. (1992). Integrins: Versatility, modulation, and signalling in cell adhesion. *Cell* **69**, 11-25.

Jenkins, A. L., Nannizzi-Alalmo, L., Silver, D., Sellers, J. R., Ginsberg, M. H., Law, D. A. and Phillips, D. R. (1998). Tyrosine phosphorylation of the β3 cytoplasmic domain mediates integrin-cytoskeletal interactions. *J. Biol. Chem.* **273**, 13878-13885.

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**, 534.

Kashiwagi, H., Schwartz, M. A., Eigenthaler, M., Davis, K. A., Ginsberg, M. H. and Shattil, S. J. (1997). Affinity modulation of platelet integrin $\alpha_{IIb}\beta_3$ by β_3-endonexin, a selective binding partner of the β_3 integrin cytoplasmic tail. *J. Cell Biol.* **137**, 1433-1443.

Kassner, P. D., Alon, R., Springer, T. A. and Hemler, M. (1995). Specialized functional properties of the integrin α4 cytoplasmic domain. *Mol. Biol. Cell* **6**, 661-674.

Keely, P. J., Rusyn, E. V., Cox, A. D. and Parise, L. V. (1999). R-Ras signals through specific integrin α cytoplasmic domains to promote migration and invasion of breast epithelial cells. *J. Cell Biol.* **145**, 1077-1088.

Kieffer, J. D., Plopper, G., Ingber, D. E., Hartwig, J. H. and Kupper, T. S. (1995). Direct binding of F actin to the cytoplasmic domain of the α2 integrin chain in vitro. *Biochem. Biophys. Res. Comm.* **217**, 466-474.

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.

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

LaFlamme, S. E., Akiyama, S. K. and Yamada, K. M. (1992). Regulation of fibronectin receptor distribution. *J. Cell Biol.* **117**, 437-447.

Law, D. A., Nannizzi-Alalmo, L. and Phillips, D. R. (1996). Outside-in integrin signal transduction: $\alpha_{IIb}\beta_3$-(GPIIb-IIIa) tyrosine phosphorylation induced by platelet aggregation. *J. Biol. Chem.* **271**, 10811-10815.

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 signaling and platelet function. *Nature* **401**, 808-811.

Lee, S. Y. and Pohajdak, B. (2000). N-terminal targeting of guanine nucleotide exchange factor (GEF) for ADP ribosylation factor (ARF) to the Golgi. *J. Cell Sci.* **113**, 1883-1889.

Leung-Hagesteijn, C. Y., Milankov, K., Michalak, M., Wilkins, J. and Dedhar, S. (1994). Cell attachment to extracellular matrix substrates is inhibited upon downregulation of expression of calreticulin, an intracellular integrin α-subunit-binding protein. *J. Cell Sci.* **107**, 589-600.

Lewis, J. M. and Schwartz, M. A. (1995). Mapping in vivo associations of cytoplasmic proteins with integrin β1 cytoplasmic domain mutants. *Mol. Biol. Cell* **6**, 151-160.

Li, J., Mayne, R. and Wu, C. (1999). A novel muscle-specific beta1 integrin binding protein (MIBP) that modulates myogenic differentiation. *J. Cell Biol.* **147**, 1391-1398.

Liliental, J. and Chang, D. D. (1998). Rack1, a receptor for activated protein kinase C, interacts with integrin β subunit. *J. Biol. Chem.* **273**, 2379-2383.

Liu, S., Thomas, S. M., Woodside, D. G., Rose, D. M., Kiosses, W. B., Pfaff, M. and Ginsberg, M. H. (1999). Binding of paxillin to α4 integrins modifies integrin-dependent biological responses. *Nature* **402**, 676-681.

Liu, S. and Ginsberg, M. H. (2000). Paxillin binding to a conserved sequence motif in the α4 integrin cytoplasmic domain. *J. Biol. Chem.* **275**, 22736-22742.

Loo, D. T., Kanner, S. B. and Aruffo, A. (1998). Filamin binds to the cytoplasmic domain of the β1-integrin. Identification of amino acids responsible for this interaction. *J. Biol. Chem.* **273**, 23304-23312.

Lukashev, M. E., Sheppard, D. and Pytela, R. (1994). Disruption of integrin function and induction of tyrosine phosphorylation by the autonomously expressed β1 integrin cytoplasmic domain. *J. Biol. Chem.* **269**, 18311-18314.

Marcantonio, E. E., Guan, J.-L., Trevithick, J. E. and Hynes, R. O. (1990).

Mapping of the functional determinants of the integrin β1 cytoplasmic domain by site-directed mutagenesis. *CR* **1**, 597-604.

Martel, V., Vignoud, L., Dupe, S., Frachet, P., Block, M. R. and Albiges-Rizo, C. (2000). Talin controls the exit of the integrin α5β1 from an early compartment of the secretory pathway. *J. Cell Sci.* **113**, 1951-1961.

Meyer, S. C., Sanan, D. A. and Fox, J. E. (1998). Role of actin-binding protein in insertion of adhesion receptors into the membrane. *J. Biol. Chem.* **273**, 3013-3020.

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.

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.

Ohta, Y., Suzuki, N., Nakamura, S., Hartwig, J. H. and Stossel, T. P. (1999). The small GTPase RalA targets filamin to induce filopodia. *Proc. Nat. Acad. Sci. USA* **96**, 2122-2128.

Ohtoshi, A., Maeda, T., Higashi, H., Ashizawa, S., Yamada, M. and Hatakeyama, M. (2000). Beta3-endonexin as a novel inhibitor of cyclin A-associated kinase. *Biochem. Biophys. Res. Commun.* **267**, 947-952.

Otey, C. A., Pavalko, F. M. and Burridge, K. (1990). An interaction between alpha actinin and the β1 integrin subunit in vitro. *J. Cell Biol.* **111**, 721-729.

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.

Pavalko, F. M., Otey, C. A. and Burridge, K. (1989). Identification of a filamin isoform enriched at the ends of stress fibers in chicken embryo fibroblasts. *J. Cell Sci.* **94**, 109-118.

Pavalko, F. M. and Burridge, K. (1991). Disruption of the actin cytoskeleton after microinjection of proteolytic fragments of α-actinin. *J. Cell Biol.* **114**, 481-491.

Pavalko, F. M., Chen, N. X., Turner, C. H., Burr, D. B. A. S., Hsieh, Y. F., Qiu, J. and Duncan, R. L. (1998). Fluid shear-induced mechanical signaling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. *Am. J. Physiol.* **275**, 1591-1601.

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.

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

Reddy, K. B., Gascard, P., Price, M. G., Negrescu, E. V. and Fox, J. E. B. (1998). Identification of an interaction between the M-band protein skelemin and integrin subunits. Colocolization of a skelemin-like protein with β1 and β3 integrins in non-muscle cells. *J. Biol. Chem.* **273**, 35039-35047.

Reszka, A. A., Hayashi, Y. and Horwitz, A. F. (1992). Identification of amino acid sequences in the integrin β1 cytoplasmic domain implicated in cytoskeletal association. *J. Cell Biol.* **117**, 1321-1330.

Retta, S. F., Balzac, F., Ferraris, P., Belkin, A. M., Fassler, R., Humphries, M. J., De Leo, G., Silengo, L. and Tarone, G. (1998). β1-integrin cytoplasmic subdomains involved in dominant negative function. *Mol. Biol. Cell* **9**, 715-731.

Rietzler, M., Bittner, M., Kolanus, W., Schuster, A. and Holzmann, B. (1998). The human WD repeat protein WAIT-1 specifically interacts with the cytoplasmic tails of β7-integrins. *J. Biol. Chem.* **273**, 27459-27466.

Rojiani, M. V., Finlay, B. B., Gray, V. and Dedhar, S. (1991). In vitro interaction of a polypeptide homologous to human Ro/SS-A antigen (calreticulin) with a highly conserved amino acid sequence in the cytoplasmic domain of integrin α subunits. *Biochemistry* **30**, 9859-9866.

Sajid, M., Hu, Z., Lele, M. and Stouffer, G. A. (2000). Protein complexes involving αvβ3 integrins, nonmuscle myosin heavy chain-A, and focal adhesion kinase from in thrombospondin-treated smooth muscle cells. *J. Invest. Med.* **48**, 190-197.

Sastry, S. K. and Horwitz, A. F. (1993). Integrin cytoplasmic domains: mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signaling. *Curr. Opin. Cell Biol.* **5**, 819-831.

Sastry, S. K., Lakonishok, M., Wu, S., Truong, T. Q., Huttenlocher, A., Turner, C. E. and Horwitz, A. F. (1999). Quantitative changes in integrin and focal adhesion signaling regulate myoblast cell cycle withdrawal. *J. Cell Biol.* **144**, 1295-1309.

Schaller, M. D., Otey, C. A., Hildebrand, J. D. and Parsons, J. T. (1995). Focal adhesion kinase and paxillin bind to peptides mimicking β integrin cytoplasmic domains. *J. Cell Biol.* **130**, 1181-1187.

Schlaepfer, D. D. and Hunter, T. (1998). Integrin signalling and tyrosine phosphorylation: just the FAKs? *Trends Cell Biol.* **8**, 151-157.

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

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.

Shaw, L. M., Turner, C. E. and Mercurio, A. M. (1995). The α6Aβ1 and α6Bβ1 integrin variants signal differences in the tyrosine phosphorylation of paxillin and other proteins. *J. Biol. Chem.* **270**, 23648-23652.

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 alphaIIb cytoplasmic domain and translocation to the platelet cytoskeleton. *Biochem. J.* **342**, 729-735.

Solowska, J., Guan, J.-L., Marcantonio, E. E., Trevithick, J. E., Buck, C. A. and Hynes, R. O. (1989). Expression of normal and mutant avian integrin subunits in rodent cells. *J. Cell Biol.* **109**, 853-861.

Steiner, F., Weber, K. and Furst, D. O. (1999). M band proteins myomesin and skelemin are encoded by the same gene: analysis of its organization and expression. *Genomics* **56**, 78-89.

Tahiliani, P. D., Singh, L., Auer, K. L. and LaFlamme, S. E. (1997). The role of conserved amino acid motifs within the integrin b3 cytoplasmic domain in triggering focal adhesion kinase phosphorylation. *J. Biol. Chem.* **272**, 7892-7898.

Tang, S., Gao, Y. and Ware, J. A. (1999). Enhancement of endothelial cell migation and in vitro tube formation by TAP20, a novel β5 integrin-modulating, PKCo-dependent protein. *J. Cell Biol.* **147**, 1073-1084.

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.

Turner, C. E. (1998). Paxillin. *Int. J. Biochem. Cell Biol.* **30**, 955-959.

Turner, C. E., Brown, M. C., Perrota, J. A., Riedy, M. C., Nikolopoulos, S. N., McDonald, A. R., Bagrodia, S., Thomas, S. M. and Leventhal, P. S. (1999). Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF-GAP protein: a role in cytoskeletal remodeling. *J. Cell Biol.* **145**, 851-863.

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.

Venkateswarlu, K., Gunn-Moore, F., Tavare, J. M. and Cullen, P. G. (1999). EGF- and NGF-stimulated translocation of cytohesin-1 to the plasma membrane of PC12 cells requires PI3-kinase activation and a functional cytohesin-1 PH domain. *J. Cell Sci.* **112**, 1957-1965.

Warren, A. P., Patel, K., Miyamoto, Y., Wygant, J. N., Woodside, D. G. and McIntyre, B. W. (2000). Convergence between CD98 and integrin-mediated T-lymphcyte co-stimulation. *Immunology* **99**, 62-68.

Wary, K. K., Mariotti, A., Zurzolo, C. and Giancotti, F. G. (1998). A requirement for caveolin-a and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell* **94**, 625-634.

Weber, K. S., Klickstein, L. B. and Weber, C. (1999). Specific activation of leukocyte β2 integrins lymphocyte function-associated antigen-1 and Mac-1 by chemokines mediated by distinct pathways via the α subunit cytoplasmic domains. *Mol. Biol. Cell* **10**, 861-873.

Wixler, V., Geerts, D., Laplantine, E., Westhoff, D., Smyth, N., Aumailley, M., Sonnenberg, A. and Paulsson, M. (2000). The LIM-only protein DRAL/FHL2 binds to the cytoplasmic domain of several α and β integrin chains and is recruited to adhesion complexes. *J. Biol. Chem*. (in press).

Wu, C. (1999). Integrin-linked kinase and PINCH: partners in regulation of cell-extracellular matrix interaction and signal transduction. *J. Cell Sci.* **112**, 4485-4489.

Ylanne, J., Huuskonen, J., O'Toole, T. E., Ginsberg, M. H., Virtanen, I. and Gahmberg, C. G. (1995). Mutation of the Cytoplasmic Domain of the Integrin β_3 Subunit. *J. Biol. Chem.* **270**, 9550-9557.

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 cytoplasmic domains. *J. Biol. Chem.* **275**, 5059-5064.

Zhang, X. A. and Hemler, M. E. (1999). Interaction of the integrin β1 cytoplasmic domain with ICAP-1 protein. *J. Biol. Chem.* **274**, 11-19.