MINIREVIEW

Role of Adhesion Molecule Cytoplasmic Domains in Mediating Interactions with the Cytoskeleton (43709)

FREDRICK M. PAVALKO*,[1] AND CAROL A. OTEY†

Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, Indiana 46202 and Department of Anatomy and Cell Biology,† University of Virginia School of Medicine, Charlottesville, Virginia 22908*

Abstract. **The past ten years have seen significant progress in cell biology research aimed at understanding how cytoskeletal filaments interact with the plasma membrane. Considerable evidence suggests that both actin microfilaments and intermediate filaments attach to the membrane via the cytoplasmic domains of various membrane proteins including adhesion molecules. Interactions between the cytoskeleton and adhesion molecules appear to be essential for a variety of cellular functions, including cell-cell and cell-extracellular matrix (ECM) interactions, cell motility, receptor-ligand interactions, and receptor internalization. Recently, many of the detailed molecular mechanisms which mediate the associations between actin filaments and adhesion molecules have been identified. Among adhesion molecules that support the attachment of cytoskeletal filaments to their cytoplasmic domains are members of the integrin and cadherin families, the intracellular adhesion molecule-1 (ICAM-1, an immunoglobulin family member), and the glycoprotein Ib/IX complex in platelets. A general conclusion emerging from these studies is that physical associations between cytoskeletal filaments and transmembrane glycoproteins do not occur directly between the filaments and the cytoplasmic tails of adhesion molecules. Instead, these interactions appear to be indirect and involve a complex ensemble of intermediary linker proteins. The severe effects of cytoplasmic domain deletion and mutagenesis on adhesion-dependent functions support the view that receptor cytoplasmic domains play a vital role in regulating receptor function and in mediating communication across the membrane. Transfection studies with mutant and chimeric adhesion molecules, along with protein-binding studies, are clarifying the mechanisms which physically link the cytoskeleton to transmembrane proteins, regulate cytoskeletal organization, mediate signaling across the cell membrane, and regulate the ligand specificity and binding affinity of surface receptors.** [P.S.E.B.M. 1994, Vol 205]

[1] To whom requests for reprints should be addressed at Department of Physiology and Biophysics, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46202–5120.

P.S.E.B.M. 1994, Vol 205: 282–293

0037-9727/94/2054–0282$10.50/0

Regulation of cytoskeletal filament assembly and maintenance of cytoskeletal organization is crucial to many aspects of cellular function. The integrity of cytoskeletal filaments has been shown to affect fundamental processes in cells such as shape change, adhesion, locomotion, mitosis, cytokinesis, phagocytosis and maintenance of cell polarity and contractility. Current research strongly suggests that signaling via surface receptors is involved in regulating

the state of actin polymerization, the organization and distribution of actin filaments, and cell motility. Our understanding of many details about how the cytoskeleton interacts with the plasma membrane is lacking despite the fact that actin filaments must be anchored to the cytoplasmic face of the membrane in order to produce the force in cells necessary for performing many of these cytoskeleton-dependent functions. Similarly, intermediate filaments must be tightly connected to desmosomes in the cells of tissues which form permeability and occluding barriers throughout the body. Interactions between cytoskeletal filaments and the plasma membrane occur at discrete domains of the membrane. Sites of cytoskeletal filament anchorage are usually sites of adhesion between cells, or between the cell and the ECM. These attachment sites are found in both isolated cells and in tissues. Several classes of anchoring junctions have been distinguished and have been studied to better understand how cytoskeletal interactions with adhesion molecules are regulated. This review attempts to summarize many of the recent advances in this important area of cell biology and to present our current understanding of how cytoskeletal-adhesion molecule interactions are formed, maintained, and regulated. Two popular models for studying the interactions of actin filaments and intermediate filaments with the membrane are integrin-containing focal adhesions and cadherin-mediated cell junctions. Other recent reviews have dealt with additional related aspects of research on cytoskeletal-plasma membrane interactions (1–3).

Focal Adhesions

The actin filaments of cells grown in culture interact with the cytoplasmic face of the plasma membrane at discrete sites of tight attachment between the ventral plasma membrane and the substrate. These sites have been referred to as focal adhesions, focal contacts or adhesion plaques (4). Focal adhesions have been a valuable model for studying cytoskeleton-membrane interactions because they can be visualized easily in cell culture and they share many similar morphological and biochemical features with other sites of actin-membrane interaction throughout the body. Focal adhesions of cells grown in tissue culture are separated from the glass or plastic substrates on which the cells are grown by a distance of only 10–15 nm and contain ECM matrix proteins on the outside which are recognized by specific receptors in the plasma membrane. Table I lists some of the sites *in vivo* which morphologically and biochemically resemble focal adhesions of cells grown in tissue culture.

Integrins are a large family of cell surface receptors found in focal adhesions. The intense interest in the role of integrins as transmembrane links between the inside and outside of cells derives, in part, from the fact that integrins are expressed by nearly all cells, are involved in both cell-ECM and cell-cell interactions, and serve as sites of transmembrane communication (3–7). Several cytoskeleton-associated proteins are concentrated in focal adhesions and function as links between actin filaments and the cytoplasmic domains of integrins. Integrins in focal adhesions are involved in a wide range of cellular processes in addition to adhesion including gene activation and regulation of intracellular pH and Ca^{2+} levels (8).

Table I. Sites of Interaction Between Cytoskeletal Filaments and the Membrane

Site of cytoskeleton-membrane interaction	Type
Focal adhesions (focal contact, adhesion plaque)	Cell-ECM
Activated platelets	Cell-Cell and Cell-ECM
Myotendenous junctions	muscle-tendon
Neuromuscular junctions	nerve-muscle
Smooth muscle dense plaques	cell-cell (smooth muscle)
Intercalated disks	cell-cell (cardiac muscle)
Z-lines	cell-cell (striated muscle)
Epithelial zonula adherens (adhesion belt)	cell-cell (epithelia)
Epithelial hemi-desmosomes	epithelial-basal lamina
Desmosomes (intermediate filaments)	cell-cell (many cell types)
Neurite growth cones	nerve-ECM

Recent efforts have also focused attention on the role of integrin-mediated phosphorylation of cytoskeleton-associated proteins in the organization of actin microfilaments at focal adhesions. There is growing evidence that proteins such as $pp125^{FAK}$ (FAK = focal adhesion kinase), a protein tyrosine kinase localized to focal adhesions, as well as other kinases, play a role in intracellular signaling events and the organization of the cytoskeleton (9). For example, phosphorylation of proteins such as tension and paxillin may affect the physical attachment of the actin cytoskeleton at the membrane. Table II lists many of the proteins that have been identified in focal adhesions at the cytoplasmic face of the membrane. This list includes only those proteins which do not appear to possess enzymatic activity, and distinguishes those that have been shown to be modified by phosphorylation.

Cadherin-Mediated Cell Junctions

Another class of cytoskeleton-associated junctions contain members of the family of transmembrane glycoprotein cell adhesion molecules called cadherins (10). Interactions between the cytoskeleton and cadherins occur in the zonula adherens and desmosomes of epithelial cells (11). Actin microfilaments are associated with the adherens-type junctions involving E-cadherin. Intermediate filaments are associated with desmosomes and involve other members of the transmembrane cadherin family of surface proteins, includ-

Table II. Nonenzymatic Proteins at the Cytoplasmic Face of Focal Adhesions

Protein	Function at focal adhesion	Phosphorylated
Actin	Structural	—
α-Actinin	Actin-integrin linker	—
Talin	Actin-integrin linker	+
Vinculin	Binds to talin, α-actinin, tensin, and paxillin	+
Zyxin	Binds to α-actinin	?
Paxillin	Binds to vinculin	+
Tensin	Binds to actin and vinculin	+
Dystrophin	Links actin to a transmembrane glycoprotein which binds laminin (found only in focal adhesions of cultured skeletal muscle cells)	?
Fimbrin	Bundles actin filament in microvilli	?

ing desmogleins and desmocollins. Each of the latter two members of the cadherin family are actually represented by multiple subfamily members, including desmogleins 1, 2, and 3 and desmocollins 1, 2, and 3. To further complicate the desmocollin story, each of these three desmocollins exist in two alternatively spliced forms (1a, 1b, 2a, 2b, 3a, and 3b).

Two common features of different types of junctional sites are involvement of multiple linker proteins and a requirement for the cytoplasmic domains of adhesion molecules to mediate attachments with cytoskeletal filaments. The cytoplasmic domains of E- and N-cadherin which anchor actin filaments interact with a complex of cytoplasmic proteins, including the catenins, radixin, and perhaps vinculin and α-actinin. The desmogleins and desmocollins, which are attachment sites for keratin, desmin, and vimentin intermediate filaments, also contain an enrichment of the desmosomal plaque proteins plakoglobin and desmoplakin I which may function to link intermediate filaments.

Other Types of Cytoskeletal-Membrane Interactions

In platelets, one of the major mechanisms of attachment of the membrane skeleton to the plasma membrane occurs through the linkage of actin filaments to the membrane glycoprotein Ib/IX complex (GP Ib/IX) (12). This nonintegrin receptor appears to link to the actin cytoskeleton via a high molecular weight actin-binding protein (ABP) in platelets. A 19 amino acid ABP binding region within the α-chain of GPIb has been identified (12). In the cellular slime mold, Dictyostelium, the primary mechanism for attaching actin filaments to the plasma membrane is via the protein ponticulin (2). A member of the immunoglobulin family of cell surface receptors, the intracellular adhesion molecule-1 (ICAM-1) has also been shown to interact with the cytoskeleton (13). In striated muscle, the cytoplasmic protein dystrophin is thought to form a link between actin and a transmembrane glycoprotein complex which also interacts with the extracellular matrix protein laminin (14).

We will focus on recent progress towards understanding the molecular interactions necessary for establishing and maintaining interactions between the cytoskeleton and receptors in the plasma membrane. One conclusion from studies that examine cytoskeleton-plasma membrane interactions is that the cytoplasmic domains of membrane receptors are absolutely necessary for proper receptor positioning and function in the membrane.

Cytoskeletal Interactions with Integrins

Integrins are expressed on almost all types of cells and many cells express more than one type of integrin. Integrins are α/β heterodimers and, to date, 8 β subunits and 15 α subunits have been identified that interact as non-covalently associated pairs. Not all of the over 100 possible pair combinations of these 8 β and 15 α subunits appear to exist in cells. In fact, only 20–25 distinct α/β heterodimers have been identified (4). Some heterodimers recognize only a single ligand while other receptors are more promiscuous. For example, α_5/β_1, recognizes only fibronectin while α_{IIb}/β_3 binds to fibronectin, fibrinogen, von Willebrand factor, vitronectin, and thrombospondin. Considerable evidence suggests that the short cytoplasmic domains of many integrin subunits can interact with the cytoskeleton. Experiments using both purified proteins (*in vitro*) and living cells (*in vivo*) have been used to examine mechanisms of interaction between actin filaments and integrins.

Recently, splice variants of several integrin subunits have been identified. These include α_3, α_6, α_7, β_1, and β_3. These variants are produced by alternative mRNA processing, and they are identical to their more classic counterparts, except for differences in the cytoplasmic domain. Two variant forms of the β_1 subunit have been described, so that the classic β_1 subunit has been renamed β_{1A}, and the variants are called β_{1B} and β_{1C}. These variants are found as minor forms in cells that also express β_{1A}. As shown in Table III, the cytoplasmic tail of the β_{1B} subunit is shorter by 21 amino acids than that of the classic β_{1A}, and it has 12 new amino acids in place of the last 21 amino acids of β_{1A} (15). β_{1B} behaves similarly to β_{1A} in terms of its association with α subunits and its binding to fibronectin, but unlike the β_{1A} form, β_{1B} does not localize to focal adhesions. This suggests that the C-terminal end of the β_{1A} cytoplasmic domain may play a major role in focal adhesion localization. The β_{1C} variant (originally called β_{1S}) has a cytoplasmic tail that is longer by 27

Table III. Cytoplasmic Domain Sequences of β_{1A}, β_{1B}, and β_{1C} Integrins

β_{1A}	KLLMIIHDRREFAKFEKEKMNAKWDTGENPIYKSAVTTVVNPKYEGK
β_{1B}	KLLMIIHDRREFAKFEKEKMNAKWDTVSKYKTSKKQSGL
β_{1C}	KLLMIIHDRREFAKFEKEKMNAKWDTSLSVAQPGVQWCDISSLQPLTSRFQQFSCLSLPSTWDYRVKILFIRVP

The conserved region is underlined.

amino acids than that of the classic β_{1A} (16). It is not yet known how these β_1 variants interact with the cytoskeleton, but the presence of multiple forms of β_1 integrin in the same cell raises the interesting possibility that these splice variants are filling specialized roles within the cell.

The diversity of integrins and the fact that many cells express multiple integrins suggest that different α/β combinations might transduce different signals to the interior of the cell. The α subunits are interesting in this regard because they possess highly divergent cytoplasmic domains, whereas the cytoplasmic domains of the β subunits are more highly conserved. Additional variety is provided by the alternatively spliced α subunit variants, which in some cases are developmentally regulated (17). However, the roles of the α subunit cytoplasmic domain in integrin mediated functions such as cell adhesion and spreading, cell migration, and cell signaling are not yet clearly understood.

Little is known about the binding of cytoskeletal proteins to the α subunits of integrin. It is thought that the integrin α subunits might bind to some of the same proteins that bind to the β subunit, and that the α-chain might help to stabilize these interactions. Binding studies utilizing synthetic peptides to represent the cytoplasmic tails of α_3, α_4, and α_5 have shown that talin binds to both α_4, and α_5, but not to α_3. Interestingly, of these three α subunits, α-actinin binds only to the cytoplasmic domain of α_4 (Simon *et al.*, in preparation). It may be that one of the roles of the α subunit cytoplasmic domain is to regulate the interactions of the β subunit with linkage proteins such as α-actinin and talin.

Recently, transfection studies have been performed to investigate the role of the α_5 subunit in the functioning of the α_5/β_1 fibronectin receptor (18). Using truncated or full-length forms of the α_5 subunit, these authors demonstrated that the region of the α_5 cytoplasmic tail that is adjacent to the membrane is important in cytoskeletal organization and cell motility, but the α_5 cytoplasmic domain is not required for integrin-mediated tyrosine phosphorylation. Additional studies will be needed to determine if this is a common feature of all integrin α subunits.

Talin and α-Actinin as Direct Links to Integrin Cytoplasmic Domains

Experiments designed to unravel the complex sets of interactions which may be involved in linking the cytoskeleton to integral membrane proteins can be broadly grouped into two categories. One of these categories includes those experiments which use purified proteins and peptides to examine the ability of isolated proteins to associate *in vitro*. The second category are those experiments designed to determine if interactions which appear to occur *in vitro* actually occur *in vivo*. First, we will summarize the data which suggest that two cytoskeletal proteins, talin and α-actinin, can interact directly with integrin cytoplasmic domains *in vitro*.

Talin is one of several cytoskeleton-associated proteins that is concentrated in focal adhesions (19). Using a gel filtration assay designed to detect low affinity interactions between proteins *in vitro*, purified talin was shown to bind integrins that had been isolated from membranes (20). The recent demonstration that talin can bind to actin filaments suggests that talin can function as a direct link between actin and the membrane (21, 22). Another focal adhesion protein, vinculin, can also bind both talin and α-actinin, suggesting that this protein may participate in linking actin filaments to integrins as part of a multiprotein chain between integrins and actin (4, 6, 7). The interaction between talin and integrin is, at least *in vitro*, of relatively low affinity (20). These data suggest that actin can link to integrins through either talin alone or a multiprotein chain via talin, vinculin, and α-actinin. The relatively low affinity of these protein-protein interactions could be compensated for by the high concentration of each of these proteins in focal adhesions. It is also possible that the phosphorylation state of talin (which is not controlled in binding assays *in vitro*) may affect its affinity for other components in focal adhesions.

An interaction of higher affinity than occurs between talin and integrin has been demonstrated between the cytoplasmic domain of the integrin β_1, β_2 and β_3 subunits and the protein α-actinin using cytoplasmic domain peptide affinity chromatography and solid phase binding assays (23, 24). The binding site for integrin is contained within the rod domain of α-actinin and is preserved in a proteolytic fragment of α-actinin which is distinct from the actin-binding domains (23). The actin-binding region also contains a binding domain for the proteins zyxin (25). The binding domains of α-actinin are diagrammed in Figure 1. A series of peptide binding studies has mapped two noncontiguous α-actinin binding regions with the cytoplasmic do-

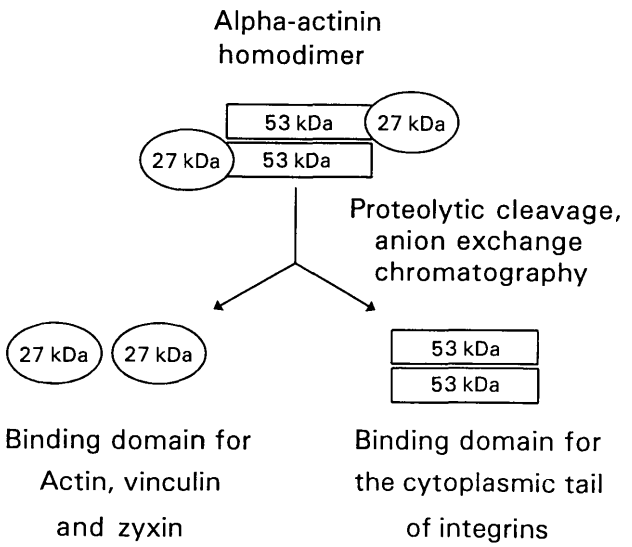
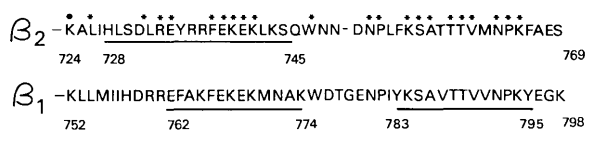


Figure 1. α-Actinin contains distinct binding domains. α-Actinin is a homodimer comprised of two identical subunits that interact in an antiparallel manner. Each end of the molecule contains a globular head domain that binds to F-actin. The central portion of the molecule is a rigid rod-like structure. Proteolysis of α-actinin yields two fragments of 27 and 53 kDa corresponding to the globular head and rod portion, respectively. The binding domain for integrin is contained in the rod portion of the molecule. The actin-binding head region also contains binding sites for both vinculin and zyxin.

main of the integrin β_1 subunit. These two regions may represent two distinct binding sites, or they may contribute to a single binding site when the cytoplasmic domain of the β subunit is folded (26). Figure 2 shows the putative α-actinin binding domains within the integrin β_1 and β_2 cytoplasmic domains. Despite the important role of integrin β subunits in directly linking actin filaments, the role of the α subunits should not be overlooked. Ongoing studies may reveal important structural or regulatory functions for the integrin α subunits in mediating attachments to the cytoskeleton.

It is possible that α-actinin may play a role in signal transduction at the focal adhesions. α-Actinin binds to zyxin, which is a low-abundance protein that colocalizes with α-actinin in focal adhesions, at the ends of stress fibers and in cell-cell junctions (25). Sequence analysis has revealed that zyxin contains three



*Represents amino acids that are identical in the beta-1 and beta-2 cytoplasmic domains

Underlined regions are sequences involved in binding to alpha-actinin

Figure 2. Amino acid sequences of the human integrin β_1 and β_2 subunit cytoplasmic domains. The underlined regions bind to α-actinin *in vitro* binding assays and the * mark residues that are identical in two cytoplasmic domains. Note that within the two regions which bind α-actinin there is a high degree of sequence conservation.

tandem LIM domains (27). Zyxin binds to another protein called the cysteine-rich protein or CRP, which also contains LIM domains (27). LIM domains are potential zinc-binding motifs and have been identified in a number of proteins that are thought to be involved in cell differentiation and gene regulation. Thus, the interactions between α-actinin, zyxin, and cCRP are of great interest to workers in these fields who want to understand how signals from the ECM affect behavior such as cellular differentiation and growth.

Evidence for Cytoskeletal-Membrane Attachments *in Vivo*

The identification of interactions between purified proteins provides important clues about the mechanisms which may attach actin filaments to the membrane in living cells. Confirmation that these interactions are relevant *in vivo* is more difficult. Several cytoplasmic proteins are found at focal adhesions. Two of these proteins, talin and α-actinin, have been shown to bind directly to the cytoplasmic domain of the β_1 integrin subunit *in vitro* and may form direct links between actin filaments and integrins in focal adhesions. Immunofluorescence and immunoelectron microscopy has been used as the primary tool for identifying proteins that are present in focal adhesions. In the case of α-actinin, it has frequently been suggested that this protein may be located some distance farther from the membrane than are other proteins such as talin or vinculin (28, 29). Immunofluorescence using antibodies against α-actinin reveals staining along stress fibers consistent with α-actinin's functions as an F-actin cross-linker, but staining of α-actinin in focal adhesions is usually very weak. This has raised the question of whether or not α-actinin is actually located near the plasma membrane where it would be needed to directly link actin to integrins in focal adhesions. When α-actinin is fluorescently labeled and microinjected into cells, however, it incorporates prominently in focal adhesions, suggesting that an antibody accessibility problem in focal adhesions is responsible for weak immunofluorescence and immunoelectron microscopy labeling (30, 31). This is most likely due to stearic hindrance of an immunodominant epitope on α-actinin by other proteins in focal adhesions.

α-Actinin-Integrin Interaction *in Vivo*

Evidence that α-actinin plays a role in linking actin stress fibers to the membrane at focal adhesions in living cells comes from at least two types of experimental approaches. First, microinjection studies using the isolated integrin binding domain of α-actinin that had been identified *in vitro* have shown that this fragment of α-actinin associates with focal adhesion in fibroblasts and epithelial cells (31). This was determined

by microinjecting high concentrations of the fluorescently-labeled 53 kDa rod domain of α-actinin into living cells, which resulted in the rapid colocalization of the integrin-binding α-actinin fragment with integrin in focal adhesions. Interestingly, localization of the α-actinin rod domain in focal adhesions was quickly followed by a loss of endogenous α-actinin from these sites and the detachment of actin stress fibers from the membrane. This result argues that the cell's endogenous, intact, α-actinin molecules are necessary for the attachment of actin filaments to focal adhesions *in vivo,* and endogenous α-actinin can be competitively displaced from the focal adhesion by α-actinin fragments.

The importance of the integrin cytoplasmic domains to normal integrin function and cytoskeletal organization is indicated by studies in which mutated integrins with modified cytoplasmic domains have been transfected into cells, resulting in abnormal integrin localization, altered cytoskeletal interactions, and reduced ligand binding activity (32–34). Furthermore, mutant and chimeric integrins with normal β_1 integrin cytoplasmic domains but nonfunctional extracellular ligand binding regions have been shown to localize properly to focal adhesions and to support the attachment of actin filaments (35). Thus, the β subunit cytoplasmic domain appears to be crucial, and perhaps sufficient, to direct integrins to focal adhesions and to attach actin filaments. This idea is supported by other recent studies in which the α subunit has been shown to be unnecessary for focal adhesion formation and normal cell spreading (36). The α subunit does, however, appear to be necessary for regulating the ligand binding specificity of certain integrins (36).

Another line of evidence indicates that α-actinin interacts with integrins in living cells and mediates the attachment of actin filaments to integrins via the β subunit cytoplasmic domain. This evidence arises from recent studies using human neutrophils which indicate that an interaction between α-actinin and the integrin β_2 subunit is induced upon activation of neutrophils with chemotactic peptides (24). Mac-1, LFA-1, and p150,95 are members of a unique integrin subfamily that are present only on leukocytes. These three integrins share a common β subunit (β_2 or CD18) while each has a unique α subunit. These leukocyte integrins are interesting because they possess the property of rapidly changing from a low avidity to a high avidity state upon activation by chemotactic peptides as well as a variety of other agents. Cytoplasmic domain deletion experiments with LFA-1 suggest that the cytoplasmic domain of the β_2 subunit is necessary for proper integrin function and imply that interactions with cytoskeletal components are necessary (37). α-Actinin interacts with a region of the β_2 cytoplasmic domain that overlaps with one of the α-actinin binding sites in the β_1 subunit (24, 26). Evidence that this interaction may be physiologically relevant was obtained by demonstrating that α-actinin coimmunoprecipitates with the integrin β_2 subunit from neutrophils activated with the chemotactic peptide FMLP, but does not coprecipitate with the β_2 subunit from unactivated neutrophils (24). The association between α-actinin and integrin was transient, peaking 5–10 min after activation of the neutrophils and decreasing to near resting levels by 20 min. No association between either talin or vinculin could be detected in these experiments. Cytoskeletal association with integrins may be necessary for neutrophil locomotion and it is interesting that this time course of α-actinin-integrin interaction is very similar to the time course of neutrophil transit across the endothelium at sites of inflammation (38). The observation that α-actinin remains associated with β_2 integrins in coprecipitation experiments that involved extensive washes in the presence of nonionic detergents suggests that the binding affinity between these two proteins is high. Using the same rationale, the possibility that weak interaction occurs between talin and the integrin β_2 subunit cannot be ruled out. The activation-dependent association of α-actinin with the integrin β_2 subunit is compatible with a direct role for cytoskeletal interactions in regulating the increased avidity of β_2 integrins and the enhanced adhesiveness of neutrophils following activation.

In lymphocytes, activation via the T cell receptor may induce a similar association of the β_2 integrin LFA-1 with the actin cytoskeleton involving α-actinin (39). In this study, activation of the lymphocytes was achieved by treating the cells with antibodies against CD3, a component of the T cell receptor (TCR) complex. Although this study did not determine which subunit of LFA-1 was involved in this interaction, it was shown to be dependent on protein kinase C (PKC). Inhibitors of PKC also blocked the transient hyperphosphorylation of the LFA-1 α subunit suggesting that this subunit may be involved in regulating the association of LFA-1 with actin filaments. Taken together with the microinjection studies using the integrin-binding domain of α-actinin described above, these coimmunoprecipitation studies strongly suggest that α-actinin serves a physiologically relevant role linking actin filaments to integrins *in vivo* in a variety of cell types and may be involved in the activation-dependent adhesion of leukocytes.

Talin Function *in Vivo*

Direct evidence that talin plays a role in linking the actin cytoskeleton to focal adhesions in fibroblasts comes from studies in which antibodies against talin were microinjected into fibroblasts that were in the process of spreading onto a substrate (40). These an-

tibodies were able to inhibit cell spreading and the formation of normal focal adhesions, presumably by interfering with talin's function. Interestingly, antibodies against talin failed to disrupt established focal adhesions in well spread cells suggesting that talin's role in focal adhesion formation and actin-membrane attachment may be most critical during the initial formation of new adhesions and less important in maintaining established adhesions. In contrast, as mentioned above, disruption of α-actinin's function by microinjection of the integrin-binding fragment of α-actinin was able to disrupt established adhesions. Together, these results support a model in which talin may be critical to the initiation of focal adhesion formation while α-actinin is required for maintaining and perhaps strengthening mature focal adhesions (41).

Talin has also been shown to bind to membrane phospholipids (42) and to nucleate the polymerization of actin filaments at the plasma membrane (43). It may function by binding to G-actin and facilitating the formation of actin nuclei which leads to actin filament elongation. Consistent with a role in actin filament nucleation, talin has been shown to be concentrated at sites in motile cells called F-actin ribs which appear to be precursors for the formation of actin stress fibers in ruffling membranes (44). This is also consistent with the idea that talin may be involved in the early stages of new focal adhesion formation and filament attachment, while α-actinin may be recruited to focal adhesions somewhat latter to strengthen the interactions of actin filaments with the membrane.

Indirect evidence has also suggested that talin is involved in mediating changes in the interaction of actin filaments with the membrane. In platelets, activation leads to clot formation and a dramatic redistribution of talin from a random distribution throughout the cell to nearer the membrane (45). Ultrastructural studies using high-resolution electron microscopy has consistently localized talin almost exclusively to sites where actin filaments attach to the membrane. There is also evidence demonstrating that increased talin phosphorylation correlates with changes in cytoskeletal organization. In epithelial cells, treatment with phorbol esters leads to actin filament disassembly and a loss of focal adhesions which correlates with increased phosphorylation of talin (46). In canine trachealis smooth muscle, stimulation with acetylcholine to induce contraction of the tissue results in rapid increase in talin phosphorylation (47). Such modification of talin may be involved in regulating changes in actin filament interactions with the smooth muscle membrane at dense plaques that are necessary for the production of force during contraction.

Taken together, current data in the field of actin filament/integrin interactions suggests that cells may use a number of mechanisms to regulate the attachment of actin filaments to the cytoplasmic domains of integrins. At lest three mechanisms for linking F-actin to integrins are suggested: (i) via talin which can bind both integrin and F-actin; (ii) via α-actinin which can bind both integrin and F-actin; and (iii) through a more complex multiprotein chain involving talin binding to integrin, vinculin binding to talin, and α-actinin binding to both vinculin and F-actin.

Cytoskeletal Interactions with Nonintegrin Adhesion Molecules

A dramatic reorganization of the actin cytoskeleton appears to be crucial to the function of activated platelets *in vivo*. A primary mechanism for attachment of actin filaments in platelets is the heterotrimeric membrane complex, GP Ib/IX (12). This nonintegrin receptor binds to von Willebrand factor and mediates the adhesion of platelets to injured blood vessels. One of the first demonstrations of a direct linkage between actin filaments and the plasma membrane came with the finding that platelet ABP mediates actin membrane attachment by linking actin filaments to the cytoplasmic domain of the α-chain of GPIb. Actin filaments in platelets may also associate with the integrin glycoprotein IIb/IIIa via the cytoplasmic domain of GPIIIa, which is the integrin β_3 subunit (48).

Since the identification of an association between the actin cytoskeleton and integrins via α-actinin, another nonintegrin receptor has been found to associate with the cytoskeleton via its cytoplasmic domains through a mechanism which involves α-actinin. The intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin (Ig) family, is present on endothelial cells, serves as a ligand for LFA-1 and Mac-1, and is also a receptor for rhinoviruses. ICAM-1 has been shown to associate with the actin-cytoskeleton via α-actinin, and this association influences the distribution of ICAM-1 on the surface of cells (13). Several other Ig family members have been known for some time to redistribute or form "caps" on the surfaces of cells in an energy-dependent fashion when cross-linked by divalent antibodies. Capping has been clearly shown to require a functional actin cytoskeleton, but the proteins which mediate these interactions have not always been carefully investigated. Association of the actin cytoskeleton with the CD44 molecule on macrophages has also been shown recently to be regulated by phosphorylation of the CD44 cytoplasmic domain (49). Molecular details of how this phosphorylation affects actin-CD44 interaction may yield important clues as to how other types of receptors link to actin filaments.

Mechanical Force Transduction via Integrins

Integrins are logical candidate molecules for the transduction of force across cell membranes because

they mediate physical attachments between the extracellular matrix outside the cell and the cytoskeleton inside the cell. Wrinkles formed by fibroblasts grown on flexible rubber substrates, for example, can be easily seen in the light microscope (50). Formation of these wrinkles requires force generation by the cells that must be transduced across the membrane. Generation and transmission of this force in cardiomyocytes has been shown to be dependent on the actin cytoskeleton (51). Nearly seven years have elapsed, however, between the identification of integrins as physical links between the extra- and intracellular environments and the recent demonstration that integrins can actually function as mechanoreceptors (52). This important study used magnetic beads, coated with a small peptide (GRGDS) that binds to integrins, to apply a rotating force directly to integrins using a weak magnetic field. The result is to twist the integrin molecules in the membrane (and the underlying cytoskeleton to which the integrins attach) without affecting the gross morphology of the cell. Using this novel approach, in combination with specific drugs which inhibit cytoskeletal function, it was demonstrated that cytoskeletal interaction with integrins are responsible for causing a stiffening of the receptors that could be conveniently measured by quantifying the resistance of the integrins to twisting in the magnetic field. This study has led to the idea of a "tensegrity" model in which the cytoskeleton forms a complex of rigid cables and elastic fibers which respond by stiffening when stress is applied to the receptor.

Cytoskeletal Interactions with Cadherins

A second category of adhesive junctions, distinct from those involving integrins, are cell-cell adhesions that require calcium and are mediated by the family of adhesion molecules called cadherins (10). Within this category, two types of filament anchorage at the cytoplasmic face of the membrane can be distinguished. One involves the attachment of actin filaments at sites such as the zonula adherens of epithelial cells. These sites contain the transmembrane glycoprotein E-cadherin and a number of cytoplasmic "plaque" proteins which may attach actin filaments to cadherins. Transfection of cells with mutated cadherin cDNA which code for a molecule lacking portions of the cytoplasmic domain demonstrate that these regions are necessary for cytoskeletal-cadherin associations (53). Specifically, the carboxyl terminal 70 amino acids have been shown to be necessary to attach actin filaments. Both the colocalization of cytoskeletal proteins with cadherins and the demonstration that nonionic detergents fail to remove cadherins from adhesive junctions support the existence of a physical association of cadherins with the cytoskeleton. Several cytoplasmic proteins have been identified that colocalize with cadherins or exist as a complex of proteins that coimmunoprecipitate with E-cadherin including α-, β-, and τ-catenins, α-actinin, vinculin and radixin (54). Whether these latter three proteins bind directly to cadherin cytoplasmic domains is uncertain. It is thought that the cytoplasmic domains of cadherins and the catenins are required for desmosome assembly and cytoskeletal filament anchorage.

A second type of cadherin-containing adhesive junctions provides attachment sites for intermediate filaments composed of cytokeratins, desmin, or vimentin. These sites, called desmosomes, contain members of a complex subfamily of transmembrane cadherins called desmogleins and desmocollins (55). At least three distinct desmogleins and three desmocollins have been identified, and alternatively spliced forms of these proteins have been demonstrated. Desmosomal plaques also contain the proteins desmoplakin and plakoglobin at the cytoplasmic face of the membrane as well as several other cell-type-specific proteins. Several studies using chimeric molecules transfected into cells lacking endogenous cadherins have shown that the cytoplasmic tails of E-cadherin and of desmosomal cadherins, particularly the highly conserved carboxyl-terminal domains, contain sufficient information to direct the recruitment of plaque-associated proteins and of microfilaments or intermediate filaments which insert at the plaques (56). The specific molecular interactions which link cytoskeletal filaments to cadherins have not been determined. With regard to this question, it is noteworthy that α-catenin shows homology to the focal adhesion protein vinculin, which appears to be involved in linking actin filaments to integrins at these sites (57).

Cadherin Cytoplasmic Domains in Development

An important role for the cytoplasmic domain of N-cadherin in Xenopus embryo development has also been demonstrated (58). In this study, mRNA encoding a deletion mutant of N-cadherin which lacked most of the extracellular domain was microinjected into the ectoderm of embryos, and this inhibited cadherin-mediated cell adhesion. This result, which demonstrated the production of a dominant negative phenotype, suggested that the cytoplasmic domain of a nonfunctional (nonadhesive) cadherin could compete with other endogenous cadherins for binding to intracellular proteins. Therefore, because the endogenous cadherins did not have a complete repertoire of intracellular proteins to interact with, their normal adhesion function was compromised. Another important finding from this study was that deletion of carboxyl terminal amino acids from the cytoplasmic domain which bind to catenins, along with the extracellular domain deletions, did not eliminate the dominant negative phenotype of the injected mRNA on embryo de-

velopment. This suggests that a sequence(s) within the N-cadherin cytoplasmic domain other than the catenin binding region is necessary for normal interactions with cytoplasmic proteins. Studies such as these provide compelling evidence that interactions between intracellular proteins and cadherin cytoplasmic domains are absolutely necessary for proper cadherin function.

Regulation of Cytoskeleton-Membrane Interactions by Tyrosine Phosphorylation in Normal Cells

A tyrosine kinase that seems to play an important role in cell adhesion has been identified recently. This protein, called pp125^{FAK} or focal adhesion kinase (FAK), has been localized to focal adhesions in cultured cells (59). Several laboratories have found that clustering of integrins causes an increase in tyrosine phosphorylation of FAK (60–62). This increased phosphorylation is rapid and transient in rounded cells that have been plated onto fibronectin, reaching a peak when the cells begin to spread and assemble adhesions. The kinase activity of FAK is enhanced by its own tyrosine phosphorylation (60), suggesting that FAK may be an important element in an integrin-mediated phosphorylation cascade.

It is thought that FAK might initiate focal adhesion assembly by phosphorylating other focal adhesion components and thus regulating their protein-protein interactions (9). Two focal adhesion proteins, paxillin (62) and tensin (63), are tyrosine phosphorylated in newly adherent cells, and both could be substrates for FAK *in vivo*. Some cells express a truncated form of FAK, called FAK-related nonkinase (FRNK), that is identical to the C-terminal domain of full-length FAK (64). FRNK has also been localized to focal adhesions, which suggests that the focal-adhesion targeting sequence of FAK is contained within this C-terminal region. An important question still to be addressed is the identification of focal adhesion proteins (possibly tensin or paxillin) that bind to the C-terminus of FAK and restrict its distribution to these adhesive sites.

While the mechanism by which FAK interacts with integrins is not well understood, there is evidence that both β_1 and β_3 integrins can mediate FAK activation. Clustering of integrins with antibodies to the β_1 subunit resulted in the phosphorylation of FAK (61), and both genetic and biochemical evidence has shown that platelet activation via integrin $\alpha_{IIB}/\beta_{IIIa}$ results in the enzymatic activation of FAK (65). It is not yet known if FAK binds to integrin directly, or if intermediary proteins are involved in activating FAK, or if both the α and β subunits of integrin are required for FAK activation.

Several actin-membrane proteins have been shown to interact with tyrosine phosphorylated proteins via regions of src homology (SH2). Tensin has been shown to contain multiple actin-binding domains and an SH2 domain suggesting a role in binding to tyrosine-phosphorylated focal adhesion proteins (66). This further supports the possibility that phosphorylation on tyrosine may be part of a signal transduction pathway, mediated through integrins, which regulates cytoskeletal-membrane interactions. Table IV lists some regulatory proteins that have been localized to focal adhesions.

Regulation of Cytoskeletal-Integrin Interactions in Transformed Cells

Transformed cells exhibit decreased adhesion and a reduced number of actin-containing stress fibers and focal adhesions. Changes in adhesion can be largely explained by decreased amounts of the extracellular matrix protein fibronectin and a loss of high-affinity fibronectin receptor (α_5/β_1 integrins) on the cell surface (67). More controversial is the question of whether the loss of actin stress fibers and of focal adhesions is directly due to transformation mechanisms or simply a result of decreased adhesion to the substrate. Enhanced tyrosine kinase activity in the focal adhesions of transformed cells mediated by the tyrosine kinase pp60$^{v\text{-}src}$ suggests that phosphorylation of actin-integrin linker proteins may be involved in altered cytoskeletal organization and the disassembly of focal adhesions. Several focal adhesion proteins, including talin, vinculin, paxillin,and integrin contain slightly elevated levels of phosphotyrosine in RSV transformed cells although the functional consequences for actin-membrane interactions are unclear. Phosphorylation of vinculin, for example, has been shown to be unrelated to changes in cytoskeletal organization (68). Another significant concern is the relatively low levels of phosphorylation of many of these proteins. Where attempts have been made to estimate the stoichiometry of phosphorylation, modest values of less than 0.1 mole phosphate/mole protein to about 0.2 moles/mole have been calculated (46, 47). An exception to this may be paxillin which, although the stoichiometry has not been determined, appears to be very heavily phosphorylated in some tissues (69). It is also possible that certain subpopulations of focal adhesion proteins are more heavily phosphorylated than others. It is thought that significant pools of talin and vinculin, for example, exist in the cytoplasm of cells that are not associated with sites of actin-membrane interaction. If

Table IV. Regulatory Proteins in Focal Adhesions

Calcium-dependent protease II (CDP II; calpain II)
Protein kinase C
pp125^{FAK} (pp41^{FRNK})
pp60$^{v\text{-}src}$
pp59^{fyn}

these pools were not phosphorylated, it is possible that the pools of protein in focal adhesion may contain much higher levels of phosphate than have been estimated.

Role of Integrin α and β Subunits

Most integrin subunit cytoplasmic domains are short, ranging from about 20 to 50 amino acids. There is considerable similarity in the amino acid sequence among the various integrin β subunits with the exception of the β_4 subunit (<1000 residues). In contrast, the α subunits are much more variable. Because different integrin α and β subunits can pair to yield such a wide variety of specificities and affinities, it is likely that much of the regulation of heterodimer localization on the cell surface is controlled by the cytoplasmic domains and that much of this fine control is determined by the α subunit cytoplasmic domains. On the other hand, many of the structural attachments to the cytoskeleton may be mediated by the β subunits. Of equal importance may be the role that integrin subunit cytoplasmic domains play in regulating the affinity of receptors for various ligands. Several recent studies support such a division of responsibility between the integrin α and β subunits (18, 36, 70, 71).

Another molecule that appears to play a role in the reorganization of the actin cytoskeleton that is mediated by phosphorylation is the MARCKS protein. MARCKS is a substrate for the Ca^{2+}-dependent serine-threonine protein kinase C (PKC) and is localized to sites of actin-membrane interaction in a phosphorylation-dependent manner (72). MARCKS can crosslink actin filaments and bind directly to phospholipids, suggesting a potentially complex regulation of actin-plasma membrane interaction *in vivo*. There has been a great deal of evidence during the past several years to suggest that occupancy of integrins by extracellular ligands can initiate a complex series of intracellular signals resulting in a number of physiological changes inside the cell. Among the events that can lead to transmembrane signaling are integrin interaction with the extracellular matrix or with counter receptors on other cells, and the cross-linking of integrin heterodimers with anti-integrin antibodies. Several important responses have been demonstrated under these conditions, including changes in cell proliferation, gene expression, and intracellular pH and intracellular Ca^{2+} levels, and increases in tyrosine phosphorylation. Because the short integrin cytoplasmic domains lack detectable enzymatic activity and are not able to mediate these changes directly, it is assumed that other intermediary proteins associated either directly or indirectly with integrins are responsible for eliciting these diverse cellular responses to integrin activation.

The clinical consequences of abnormal cytoskeletal interactions can be seen in patients with leukocyte adhesion deficiency (LAD). These individuals have leukocytes that are deficient in the expression of the integrin β_2 subunit and fail to express normal levels of integrin receptors on their surfaces (73). Neutrophils from these patients have an impaired ability to adhere to substrates, undergo chemotaxis, and phagocytize bacteria. Individuals with LAD have frequently recurring and often fatal infections. A study of actin assembly in neutrophils from LAD patients found that they were able to polymerize actin normally (74). One explanation for the impaired function of these cells may be that the actin filaments are unable to associate properly with the cytoplasmic face of the membrane in cells which lack certain integrins. This could result in impairment of cellular functions that require actin filaments such as maintenance of cell shape, adhesion, locomotion, and phagocytosis.

Future Directions

Although it appears that the unique adhesive functions of different cell types is controlled in large part by the selective expression of a vast assortment of adhesion molecules, the repertoire of cytoskeletal and regulatory proteins among cell types is very similar. From this we must draw the obvious conclusion that many of the same cytoskeletal and regulatory proteins may interact with the cytoplasmic domains of different classes of receptor. A goal of future research will be to determine if differences in receptor cytoplasmic domain sequences affect receptor function by controlling the cytoskeletal proteins with which different classes of receptors (and variants within each group) can associate. Evidence from several laboratories suggests that protein interactions with the cytoplasmic domains of receptors affect ligand interactions and important postreceptor occupancy signaling events. It is important to distinguish experimentally those interactions between membrane receptors and cytoplasmic proteins that are truly relevant to the physiology of cells. The challenge in this field will be to continue to identify all of the players involved in creating, maintaining, and regulating cytoskeletal-membrane interactions and to better understand how cells manipulate these interactions to control cell function.

1. Bretscher A. Microfilaments and membranes. Curr Biol **5**:653–660, 1993.
2. Luna EJ, Hitt AL. Cytoskeleton-plasma membrane interactions. Science **258**:955–964,1992.
3. Sastry SK, Horwitz AF. Integrin cytoplasmic domains: Mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signaling. Curr Opin Cell Biol **5**:819–831, 1993.
4. Burridge K, Fath K, Kelly T, Nuckolls G, Turner C. Focal adhesions: Transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol **4**:487–525, 1987.

5. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell **69**:11–25, 1992.
6. Turner CE, Burridge K. Transmembrane molecular assemblies in cell-extracellular matrix interactions. Curr Opin Cell Biol **3**:849–853, 1991.
7. Burridge K, Nuckolls G, Otey C, Pavalko F, Simon K, Turner C. Actin-membrane interaction in focal adhesions. Cell Diff Dev **32**:337–342, 1990.
8. Ginsberg MH, Du X, Plow EF. Inside-out integrin signalling. Curr Opin Cell Biol **4**:766–771, 1992.
9. Schaller MD, Parsons JT. Focal adhesion kinase: An integrin-linked protein tyrosine kinase. Trends Cell Biol **3**:258–262, 1993.
10. Geiger B, Ayalon O. Cadherins. Annu Rev Cell Biol **8**:307–332, 1992.
11. Schwarz M, Duden R, Cowin P, Franke WW. Desmosomes and hemidesmosomes: Constitutive molecular components. Annu Rev Cell Biol **6**:461–491, 1990.
12. Andrews RK, Fox JEB. Identification of a region in the cytoplasmic domain of platelet membrane glycoprotein Ib-IX complex that binds to purified actin-binding protein. J Biol Chem **267**:18605–18611, 1992.
13. Carpen O, Pallai P, Staunton DE, Springer TA. Association of intercellular adhesion molecule (ICAM-1) with actin-containing cytoskeleton and α-actinin. J Cell Biol **118**:1223–1234, 1992.
14. Ervasti JM, Campbell KP. Dystrophin and the membrane skeleton. Curr Biol **5**:82–87, 1993.
15. Altruda F, Cervella P, Tarone G, Botta C, Balzac F, Stefanuto G, Silengo L. A human integrin β_1 subunit with a unique cytoplasmic domain generated by alternative mRNA processing. Gene **95**:261–266, 1990.
16. Languino LR, Ruoslahti E. An alternative form of the integrin β_1 subunit with a variant cytoplasmic domain. J Biol Chem **267**:7116–7120, 1992.
17. Tamura RN, Cooper HM, Collo G, Quaranta V. Cell type–specific integrin variants with alternative α-chain cytoplasmic domains. Proc Nat Acad Sci **88**:10183–10187, 1991.
18. Bauer JS, Varner J, Schreiner C, Kornberg L, Nicholas R, Juliano RL. Functional role of the cytoplasmic domain of the integrin α_5 subunit. J Cell Biol **122**:209–221, 1993.
19. Burridge K, Connell L. A new protein of adhesion plaques and ruffling membranes. J Cell Biol **97**:359–367, 1983.
20. Horwitz A, Duggan K, Buck C, Beckerle MC, Burridge K. Interaction of plasma membrane fibronectin receptor with talin—A transmembrane linkage. Nature (London) **320**:531–533, 1986.
21. Muguruma M, Matsumura S, Fukazawa T. Direct interactions between talin and actin. Biochem Biophys Res Comm **171**:1217–1223, 1990.
22. Goldman WH, Isenber G. Kinetic determination of talin-actin binding. Biochem Biophys Res Comm **178**:718–723, 1991.
23. Otey CA, Pavalko FM, Burridge K. An interaction between α-actinin and the β_1 integrin subunit *in vitro*. J Cell Biol **111**:721–729.
24. Pavalko FM, LaRoche SM. Activation of human neutrophils induces an interaction between the integrin β_2-subunit (CD18) and the actin binding protein α-actinin. J Immunol **151**:3795–3807, 1993.
25. Crawford A, Michelsen JW, Beckerle MC. An interaction between zyxin and α-actinin. J Cell Biol **116**:1381–1393, 1992.
26. Otey CA, Vasquez GB, Burridge K, Erickson BW. Mapping of the α-Actinin binding site with the β_1 integrin cytoplasmic domain. J Biol Chem **268**:21193–21197, 1993.
27. Sadler I, Crawford AW, Michelsen JW, Beckerle MC. Zyxin and cCRP: Two interactive LIM domain proteins associated with the cytoskeleton. J Cell Biol **119**:1573–1587, 1992.
28. Chen WT, Singer SJ. Immunoelectron microscopic studies of the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. J Cell Biol **95**:205–222, 1982.
29. Samuelsson SJ, Luther PW, Pumplin DW, Bloch RJ. Structures linking microfilament bundles to the membrane at focal contacts. J Cell Biol **122**:485–496, 1993.
30. Feramisco JR. Microinjection of fluorescently labeled α-actinin into living fibroblasts. Proc Nat Acad Sci USA **76**:3967–3971, 1979.
31. Pavalko FM, Burridge K. Disruption of the actin cytoskeleton after microinjection of proteolytic fragments of α-actinin. J Cell Biol **114**:481–491, 1991.
32. Hayashi Y, Haimovich B, Reska A, Boettiger D, Horwitz A. Expression and function of chicken integrin beta 1 subunit and its cytoplasmic domain mutants in mouse NIH 3T3 cells. J Cell Biol **110**:175–184, 1990.
33. Solowska J, Guan JL, Marcantonio EE, Trevithick JE, Buck CA, Hynes RO. Expression of normal and mutant avian integrin subunits in rodent cells. J Cell Biol **109**:853–861, 1989.
34. Reszka AA, Hayashi Y, Horwitz AF. Identification of amino acid sequences in the integrin β_1 cytoplasmic domain implicated in cytoskeletal association. J Cell Biol **117**:1321–1330, 1992.
35. LaFlamme SE, Akiyama SK, Yamada KM. Regulation of fibronectin receptor distribution. J Cell Biol **117**:437–447, 1992.
36. Ylanne J, Chen Y, O'Toole TE, Loftus JC, Takada Y, Ginsberg MH. Distinct functions of integrin α and β subunit cytoplasmic domains in cell spreading and formation of focal adhesions. J Cell Biol **122**:223–233, 1993.
37. Hibbs ML, Jakes S, Stacker SA, Wallace RW, Springer TA. 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, 1991.
38. Lo SK, Detmers PA, Levin SM, Wright SD. Transient adhesion of neutrophils to endothelium. J Exp Med **169**:1779–1793, 1989.
39. Pardi R, Inverardi L, Rugarli C, Bender JR. Antigen-receptor complex stimulation triggers protein kinase C-dependent CD11a/CD18-cytoskeleton association in T lymphocytes. J Cell Biol **116**:1211–1220, 1992.
40. Nuckolls GH, Romer LH, Burridge K. Microinjection of antibodies against talin inhibits the spreading and migration of fibroblasts. J Cell Sci **102**:753–762, 1992.
41. Pavalko FM, Otey CA, Simon KO, Burridge K. α-Actinin: A direct link between actin and integrins. Biochem Soc Trans **19**:1065–1069, 1991.
42. Goldmann WH, Niggli V, Kaufmann S, Isenberg G. Probing actin and liposome interaction of talin and talin-vinculin complex: A kinetic, thermodynamic, and lipid labeling study. Biochemistry **31**:7665–7671, 1992.
43. Kaufmann S, Piekenbrock T, Goldmann WH, Barmann M, Isenberg G. Talin binds to actin and promotes filament nucleation. FEBS **284**:187–191, 1991.
44. DePasquale JA, Izzard CS. Accumulation of talin in nodes at the edge of the lamellipodium and separate incorporation into adhesion plaques of focal contacts in fibroblasts. J Cell Biol **113**:1351–1359, 1991.
45. Beckerle MC, Miller DE, Bertagnolli ME, Locke SJ. Activation-dependent redistribution of the adhesion plaque protein, talin, in intact human platelets. J Cell Biol **109**:3333–3346, 1989.
46. Turner CE, Pavalko FM, Burridge K. 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, 1989.
47. Pavalko FM, Adam L, Al-Hassani MH, Gunst SJ. A rapid and transient increase in phosphorylation of the actin-membrane attachment protein talin occurs during smooth muscle contraction. Biophysical J **64**:A261, 1993.

48. Smyth SS, Joneckis CC, Parise LV. Regulation of vascular integrins. Blood **81**:2827–2843, 1993.
49. Camp RL, Kraus TA, Pure E. Variations in the cytoskeletal interaction and post-translational modifications of the CD44 homing receptor in macrophages. J Cell Biol **115**:1283–1292, 1991.
50. Danowski BA, Harris AK. Changes in fibroblast contractility, morphology, and adhesion in response to a phorbol ester tumor promoter. Exp Cell Res **177**:47–59, 1988.
51. Danowski BA, Imanaka-Yoshida K, Sanger JM, Sanger JW. Costameres are sites of force transmission to the substratum in adult rat cardimyocytes. J Cell Biol **118**:1411–1420, 1992.
52. Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. Science **260**:1124–1127, 1993.
53. Fujimori T, Takeichi M. Disruption of epithelial cell-cell adhesion by exogenous expression of a mutated nonfunctional N-cadherin. Mol Biol Cell **4**:37–47, 1993.
54. Ozawa M, Kemler R. Molecular organization of the uvomorulin-catenin complex. J Cell Biol **116**:989–996, 1992.
55. Troyanovsky SM, Eshkind LG, Troyanovsky RB, Leube RE, Franke WW. Contributions of cytoplasmic domains of desmosomal cadherins to desmosome assembly and intermediate filament anchorage. Cell **72**:561–574, 1993.
56. Nagafuchi A, Takeichi M. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. EMBO J **7**:3679–3684, 1988.
57. Nagafuchi A, Takeichi M, Tsukita S. The 102 Kd cadherin-associated protein: Similarity to vinculin and post-translational regulation of expression. Cell **65**:849–857, 1991.
58. Kitner C. Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain. Cell **69**:225–236, 1992.
59. Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB, Parsons JT. pp125^{FAK}, a structurally distinctive protein-tyrosine kinase associated with focal adhesions. Proc Nat Acad Sci USA **89**:5192–5296, 1992.
60. Guan JL, Shalloway D. Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. Nature **358**:690–692, 1992.
61. Kornberg LJ, Earp SH, Turner CE, Prockop C, Juliano RL. Signal transduction by integrins: Increased protein tyrosine phosphorylation caused by clustering of β_1 integrins. Proc Nat Acad Sci USA **88**:8392–8396, 1991.
62. Burridge K, Turner CE, Romer LH. Tyrosine phosphorylation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: A role in cytoskeletal assembly. J Cell Biol **119**:893–904, 1992.
63. Bockholt SM, Burridge K. Cell spreading on extracellular matrix proteins induces tyrosine phosphorylation of tensin. J Biol Chem **268**:14565–14567, 1993.
64. Schaller MD, Borgman CA, Parsons JT. Autonomous expression of a noncatalytic domain of the focal adhesion-associated protein tyrosine kinase pp125^{FAK}. Mol Cell Biol **13**:785–791, 1993.
65. Lipfert L, Haimovich B, Schaller MD, Cobb BS, Parsons JT, Brugge JS. Integrin-dependent phosphorylation and activation of the protein tyrosine kinase pp125FAK in platelets. J Cell Biol **119**:905–912, 1992.
66. Davis S, Lu ML, Lo SH, Lin S, Butler JA, Druker BJ, Robers TM, An W, Chen LB. Presence of an SH2 domain in the actin binding protein tensin. Science **252**:712–715, 1991.
67. Burridge K. Substrate adhesions in normal and transformed fibroblasts: Organization and regulation of cytoskeletal, membrane, and extracellular matrix components at focal contacts. Cancer Rev **4**:18–78, 1986.
68. Rohrshneider L, Rosok MJ. Transformation parameters and pp60^{src} localization in cells infected with partial transformation mutants of Rous sarcoma virus. Mol Cell Biol **3**:731–746, 1983.
69. Turner CE. Paxillin is a major phosphotyrosine-containing protein during embryonic development. J Cell Biol **115**:201–207, 1991.
70. Chan BM, Kasner PD, Schiro JA, Byers HR, Kupper TS, Hemler ME. Distinct cellular functions mediated by different VLA integrin α subunit cytoplasmic domains. Cell **68**:1051–1060, 1992.
71. O'Toole TE, Mandelman D, Forsythe J, Shattil SJ, Plow EF, Ginsberg MH. Modulation of the affinity of integrin aIIbb3 (GPIIb/IIIa) by the cytoplasmic domain of aIIb. Science **254**:845–847, 1991.
72. Hartwig JH, Thelen M, Rosen A, Janmey PA, Nairn AC, Aderem A. MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. Nature **356**:618–622, 1992.
73. Crowley CA, Curnutte JT, Rosin RE, Andre-Schwartz J, Gallin JI, et al. An inherited abnormality of neutrophil adhesion: Its genetic transmission and its association with a missing protein. N Engl J Med **302**:1163–1170, 1980.
74. Southwick FS, Howard TH, Holbrook T, Anderson DC, Stossel TP, Arnaout MA. The relationship between CR3 deficiency and neutrophil actin assembly. Blood **73**:1973–1979, 1989.