

Molecular interactions in cell adhesion complexes

Kenneth M Yamada* and Benjamin Geiger†

Cell adhesions consist of multimolecular protein complexes of transmembrane adhesion receptors anchoring intracellular cytoskeletal structural proteins and signal transduction molecules. Recent advances reveal that components of cell adhesion complexes display multiple interactions and functions, which cooperate to mediate both cell adhesion and signaling. Cell–matrix and cell–cell adhesions can serve as both recipients and generators of signaling information, using hierarchical and synergistic molecular interactions regulated by aggregation, conformational changes, phosphorylation, and tension.

Addresses

*Craniofacial Developmental Biology and Regeneration Branch, National Institute of Dental Research, National Institutes of Health, Building 30, Room 421, 30 Convent Drive, MSC 4370, Bethesda, MD 20892-4370, USA; e-mail: ky4w@nih.gov

†Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel; e-mail: ligeiger@weizmann.weizmann.ac.il

Current Opinion in Cell Biology 1997, 9:76–85

Electronic identifier: 0955-0674-009-00076

© Current Biology Ltd ISSN 0955-0674

Abbreviations

APC	adenomatous polyposis coli
EGF	epidermal growth factor
FAK	focal adhesion kinase
MAP	mitogen-activated protein
PIP ₂	phosphatidylinositol 4,5-bisphosphate
SH	Src homology

Introduction

Cell adhesion links cells with other cells or with the extracellular matrix, while establishing intracellular structural linkages with cytoskeletal proteins which become organized into large supramolecular complexes. A number of reviews on cell adhesion complexes and their components has been published in the past few years, including [1*–7*]. In this brief review, we examine recent progress in characterizing cell adhesion complexes and their activation of intracellular signaling pathways, which regulate cell morphology, migration, gene expression, growth, and differentiation. We focus primarily on new findings and concepts concerning the possible molecular interactions that take place in these complexes, which affect both signaling events and assembly of adhesions. We apologize for omitting other studies because of space constraints.

Complex intermolecular interactions in adhesion complexes

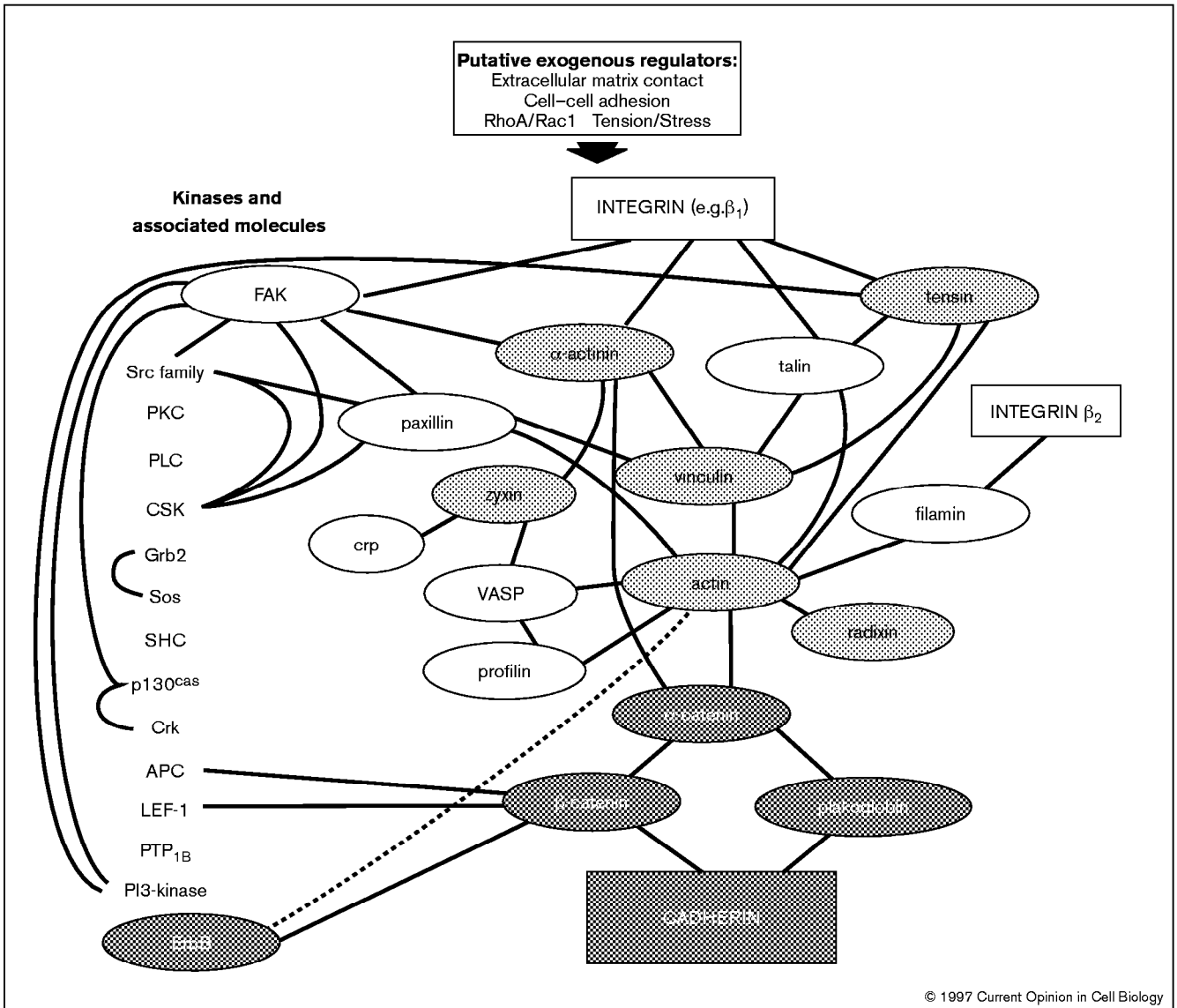
The extraordinary complexity of molecular interactions within adhesion complexes has become apparent in the

past few years. Figure 1 illustrates this complexity: it depicts known protein–protein binding interactions in such complexes, whether in cell–extracellular-matrix adhesive structures, in cell–cell adhesions, or in both types of complex. The number of these interactions described in the literature is growing nearly exponentially, so that many of these components appear to participate in complex three-dimensional webs or networks of potentially interacting proteins. For example, some proteins such as FAK (focal adhesion kinase) and vinculin bind to a number of other proteins, and may serve as linking or docking proteins, besides fulfilling other functions. As there are so many possible interactions, at least some of which are inducible, this complex network of molecules is likely to be dynamic and heterogeneous in molecular composition, though the actual *in vivo* existence of some interactions and their regulation still remain to be established. A number of well known structures display linkages to cytoskeletal proteins in anchoring functions that include desmosomes, tight junctions, adherens junctions, focal adhesions, and adhesions to extracellular matrix fibrils. Recent studies indicate, however, that these complexes also play important roles in intracellular signal transduction [1*–9*].

Signaling from cell–substrate adhesion complexes

Cell–substrate adhesions, which include focal adhesions, are generally based on integrin-type receptors, whereas adherens junctions contain cadherins which mediate cell–cell adhesion. There is compelling indirect evidence for signaling originating from both cell–cell and cell–substrate adhesions, and both contain abundant populations of signaling molecules (see below). However, the molecular properties of the signaling systems associated with cell–matrix adhesions are better characterized—the study of integrin-dependent signaling has grown rapidly from a field publishing only a dozen papers in 1990 to one generating more than 150 papers over the past year. As reviewed recently [2*–4*,8*,9*], many intracellular signaling pathways are activated by cell adhesion to specific extracellular molecules. These signal transduction events range from protein tyrosine phosphorylations and mitogen-activated protein (MAP) kinase activation to Ca⁺⁺ influx, pH alterations, and inositol lipid turnover. The past year has expanded our understanding of the breadth of these signaling responses, as well as suggesting potential mechanisms of growth regulation, prevention of apoptosis, and cooperative interactions between growth factor mediated and integrin-mediated signaling pathways.

Figure 1



Schematic diagram of protein-protein interactions between cell adhesion receptors, cytoskeletal proteins, and signal transduction molecules. Lines indicate reported binding interactions between pairs of molecules, usually based on *in vitro* assays. Dotted line indicates a possible indirect interaction. Open ovals indicate intracellular molecules associated with integrin-type receptors; dark gray ovals indicate molecules associated with cadherins; and light gray ovals indicate molecules associated with both types of adhesion system. Several proposed exogenous regulators of these binding interactions are listed at the top. The kinases and associated molecules shown at the left are involved in adhesion complexes, but their specific binding interactions with other components of cell adhesions are not yet clear. PKC, protein kinase C; PLC, phospholipase C; CSK, carboxy-terminal Src kinase; LEF-1, lymphoid enhancer factor-1; PTP_{1B}, protein tyrosine phosphatase 1_B; PI3-kinase, phosphatidylinositol 3'-kinase; VASP, vasodilator-stimulated phosphoprotein.

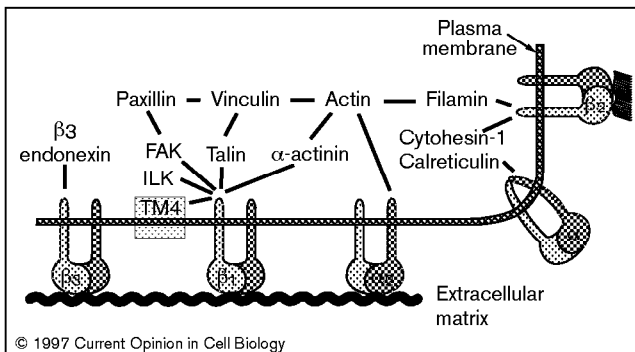
Mechanisms that link cytoskeletal and signaling molecules to integrins

The exact mechanisms by which integrins organize the cytoskeleton and induce signaling are still not known in detail, but Figure 2 shows a current view of such integrin connections. The numbers of potential connections and the fact that some can be regulated by phosphorylation or by the state of integrin aggregation or occupancy (see below) suggests the likelihood of variable compositions of adhesion complex. Direct binding interactions

with integrin β_1 subunits have been reported for talin, α -actinin, and FAK (reviewed in [8*,9*]), and filamin binds to integrin β_2 cytoplasmic domains [10]. Binding of calreticulin to integrin α subunits, and even of actin to integrin α_2 cytoplasmic domain peptides, has been reported [11]. The ability of integrins to interact with the cytoskeleton appears to be masked or suppressed in native, unoccupied integrins, which are thought to be induced to interact with cytoskeletal molecules after binding of ligand; this state can be mimicked by

constitutive exposure of the β integrin cytoplasmic tail in the form of an isolated β tail in a molecular chimera or an integrin with a missing α tail [12–16]. An early event in this process can be observed visually by tracking tiny beads coated with integrin ligands that respond to integrin occupancy by rapidly attaching to dynamic cytoskeletal networks via integrin cytoplasmic domains [17].

Figure 2



Schematic summary of a variety of integrin molecular connections. The cytoplasmic domains of integrin-type receptors have been reported to bind to a number of different proteins, which in turn bind to other molecules (see also Fig. 1). The binding of certain β integrin subunits to some cytoplasmic proteins appears to be regulated by the binding of the integrin to its ligand, for example, an extracellular matrix molecule (shown at bottom) or a molecule on an adjacent cell (shown at top right). ILK, integrin-linked kinase; TM4, tetraspan-family protein; α , α_2 , β_1 , β_2 , β_3 , integrin subunits.

Novel interaction mechanisms were described recently that involve regulation of protein binding to integrin cytoplasmic domains by phosphorylation of an integrin after ligand binding or after an intracellular signaling event. Ligand-induced tyrosine phosphorylation of the integrin β_4 cytoplasmic domain creates tyrosine-based activation motif (TAM) sequences analogous to those used by T- and B-cell receptors; these sequences mediate the binding of $\alpha_6\beta_4$ integrin to cytoskeletal elements in hemidesmosomes. Other tyrosine phosphorylation sites result in binding of the adapter/signaling molecules Shc and Grb2 by interactions using Src homology (SH)2 and SH3 domains; Shc and Grb2 provide a link to the Ras signaling pathway [18]. Phosphorylation of the integrin β_3 cytoplasmic domain after thrombin-induced platelet aggregation is also remarkably robust, permitting *in vitro* binding of the same signaling molecules, Shc and Grb2 [19], which could also link this integrin to the Ras pathway.

Hierarchies of signaling and cytoskeletal molecules

Recent studies have used beads coated with integrin ligands and anti-integrin antibodies to experimentally dissect the earliest steps in adhesion complex formation with the extracellular matrix [20,21*–24*]. A hierarchy of accumulation of molecules could be established, based on whether an integrin was clustered, occupied with a ligand,

or both [21*]. For example, FAK and tensin accumulate on the cytoplasmic side of the plasma membrane after simple clustering of β_1 integrins, accompanied by a number of signaling molecules, including Src family kinases, Ras, Raf, and MAP kinases, in a process that also required cellular tyrosine phosphorylation [21*,23*]. On the other hand, a number of cytoskeletal molecules, including α -actinin, talin, and vinculin, require both integrin clustering and integrin occupancy by a ligand to accumulate in adhesion complexes—the advantage of this dual requirement might be to ensure that the formation of long-term, cytoskeleton-stabilized adhesion complexes would occur only if there were stable attachment of an integrin to a ligand, and would not occur if unstable binding of a soluble fragment of an extracellular matrix protein to integrin, or integrin clustering followed by cell migration away from the ligand, took place. The accumulation of F-actin and paxillin also required cellular tyrosine phosphorylation, providing another level of regulation to this hierarchical system [21*].

Signaling from adherens junctions

It is now clear that another major adhesion and signaling system exists, in addition to cell–matrix adhesions, that is based on adherens-type cell–cell junctions, which are also potent signaling complexes [4*,6*,7*]. Key elements in these adherens-based interactions appear to be cadherins and their intracellular partner β -catenin, which is a junctional plaque protein that interacts directly with cadherins and indirectly with the cytoskeleton via α -catenin (Fig. 1). Although β -catenin can undergo tyrosine phosphorylation and can associate with a transmembrane protein tyrosine phosphatase that can dephosphorylate it *in vitro* [25], the function of phosphorylation in β -catenin adhesive functions still remains to be established definitively. β -catenin also appears to be involved in additional interactions, however: in growth factor activated cells, it binds to members of the ErbB family of receptor tyrosine kinases, including the epidermal growth factor (EGF) receptor [26,27], and then localizes them, together with additional components of their signaling pathway, to the adherens junction area. It is still unclear whether this localization is important for activation of the ErbB signaling pathway. Moreover, it is clear that β -catenin and its homolog in *Drosophila*, Armadillo, are part of the *wnt/wingless* signaling pathway [6*,28]. This pathway contains β -catenin as a central element, and involves the tumor suppressor adenomatous polyposis coli (APC), p120, plakoglobin, and plakophilin. However, the pool of cytoplasmic and nuclear β -catenin molecules involved in Wnt signaling may be distinct from the pool involved in cadherin-mediated junctional adhesion [29].

It has been recently shown that β -catenin and plakoglobin can complex with a transcription factor, the lymphoid enhancer factor-1 (LEF-1), and bind upstream to the E-cadherin gene [30,31*]. The translocation of β -catenin into the nucleus, driven by LEF-1, leads to the induction

of the dorsal mesoderm in *Xenopus*. The Arm-repeating peptide motifs in β -catenin have been implicated in its binding to LEF-1, APC, cadherins, and EGF receptors (reviewed in [28]). Thus, β -catenin has been implicated in a variety of interactions ranging from binding ErbB receptors to regulating embryonic axis formation. These findings raise the possibility that components of the junctional plaque have complex extrajunctional roles.

To add further interest and complexity, β -catenin and its relative, plakoglobin, also bind to the actin-bundling protein fascin [32]. Fascin is involved in the formation of F-actin bundles, particularly in cellular sites involved in motility such as filopodia and lamellipodia, but also in actin stress fibers. Fascin and β -catenin colocalize at the dynamic leading edges of cells, as well as at cell–cell junctions. This interaction is independent of cadherins, however, and in fact the cadherin cytoplasmic domain and fascin appear to compete for the same site on β -catenin [32]. This additional direct link to a cytoskeletal regulatory molecule further enhances the rapidly expanding role of β -catenin as a centrally located regulatory molecule that can interact with components of the cadherin system, with APC, with fascin, and with transcription factors.

Multiple functions and cross-talk between the various components of cell–cell and cell–substrate adhesions

It has become increasingly clear that the distinction between ‘structural proteins’ (which provide the mechanical linkage between the cell exterior and the cytoskeleton) and ‘signaling molecules’ (which are involved in the generation and transduction of long-range signals affecting nuclear events and cell metabolism) is rather artificial. Instead, another view is that adhesion complexes containing cytoskeletal and plaque proteins are sites of clustering or aggregation of the components of many signaling pathways. Clustering could both concentrate reactants and lead to activation of pathways, and this process could be facilitated by multifunctional enzymes such as FAK, which may play a mechanical role in binding a number of signaling and cytoskeletal molecules, in parallel with its kinase activity. Figure 3 depicts the remarkably wide-ranging interactions of FAK, whose binding and kinase activities can be modulated by phosphorylation, although the full range of phosphorylation-mediated regulation of FAK remains to be established (e.g. see [33]). In addition, some cells express the FAK homolog Pyk2 (cell adhesion kinase β or CAK β), which has been implicated in signaling involving MAP kinase and ion channel regulation [34*].

Roles of tyrosine phosphorylation

The precise role of tyrosine kinases in adhesion complex formation still remains unclear. A key mechanism involves the binding of SH2 domains present in many signaling molecules and some cytoskeletal molecules to protein tyrosine phosphorylation sites [35]. This mechanism

provides a readily regulated mechanism for binding of one protein to another, regulated by a host of protein tyrosine kinases and reversed by growing numbers of known tyrosine phosphatases; the balance between these enzyme activities, which can often be cell- and protein-specific, probably regulates many important pathways. It is known that inhibition of tyrosine kinases with inhibitors such as herbimycin will inhibit focal contact formation [36], and that striking time-dependent changes can be induced by enhancing tyrosine phosphorylation by inhibiting phosphatases with orthovanadate or by stimulating phosphorylation via the EGF receptor [4*].

Another approach to studying the roles of tyrosine phosphorylation is to mutate the genes encoding tyrosine kinases. Mutant mice that lacked genes for one of the Src family members Src, Fyn, or Yes all formed normal focal adhesions and stress fibers [37*], although kinetic effects were reported [38*], and all mutants phosphorylated FAK normally, although there were effects on phosphorylation of the putative docking molecule p130^{cas} [37*]. A mouse with a mutation in the gene encoding FAK did not display disrupted formation of focal adhesions either, and in fact the focal adhesions were enlarged [39*]; however, it was not clear that these mice did not express a truncated version of FAK. Even if *src* gene function is not essential for focal adhesion formation, enhanced expression of the amino-terminal portion of Src, which lacks the kinase domain, somehow enhances phosphotyrosine staining as well as the overall size of focal adhesions [40]. Gene-knockout experiments and overexpression experiments must both be interpreted with some caution, however, as ablation of one gene may lead to compensation by another gene during development, and nonphysiologically high concentrations of a particular protein or a particular domain of a protein may lead to effects that can be either instructive or misleading. A combination of approaches will probably be the safest route to understanding these unusually complex regulatory interactions.

Although tyrosine phosphorylation has been studied intensively, including its appearance at low stoichiometry on a variety of cytoskeletal proteins, other types of phosphorylation are also likely to play significant roles in the formation of adhesion complexes. For example, protein kinase C has been implicated in the formation of focal adhesions [41]. Striking serine phosphorylation of paxillin was observed after adhesion of macrophages to vitronectin via the integrin $\alpha_v\beta_5$ in a process that was independent of FAK and apparently mediated by protein kinase C [42]. In fact, in some cells, this same $\alpha_v\beta_5$ integrin has only limited function until protein kinase C is activated: activation permits this integrin to mediate interactions with a variety of cytoskeletal proteins including α -actinin and vinculin (but not talin) and to stimulate the phosphorylation of FAK in cells expressing this kinase [43].

Figure 3

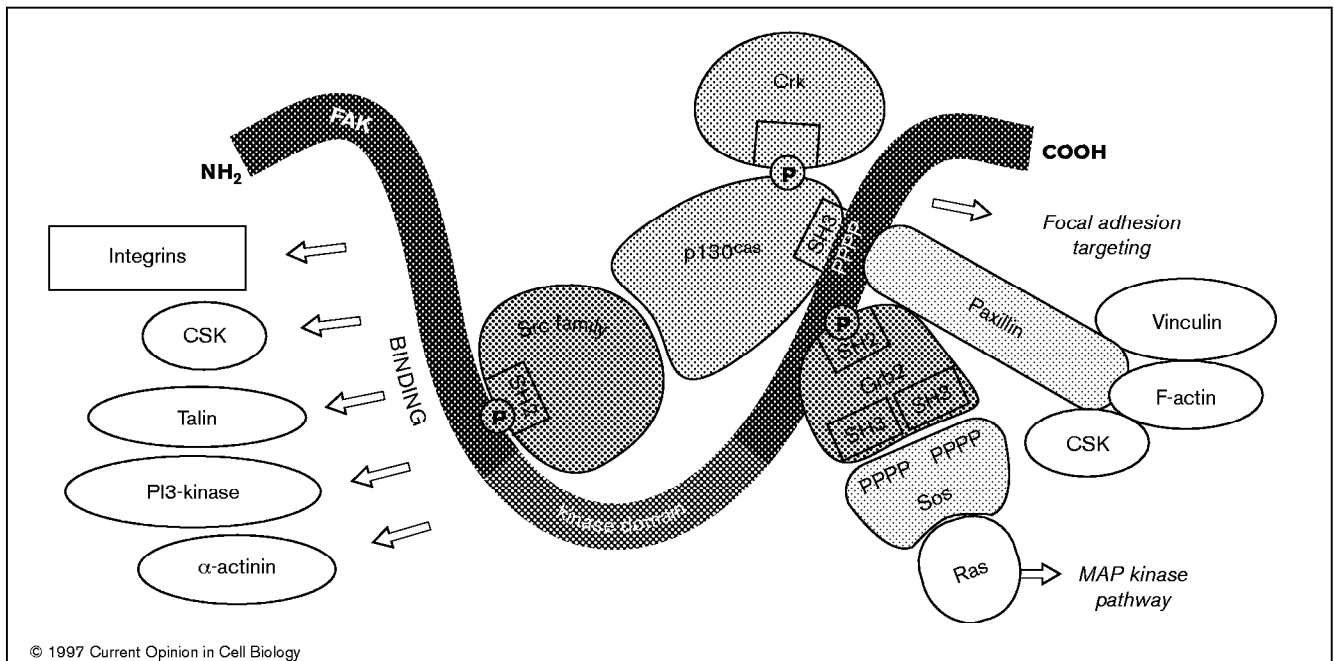


Diagram of putative molecular interactions of FAK. FAK is a multifunctional tyrosine kinase that binds to, or is bound by, a remarkable number of cytoskeletal and signaling molecules, including components that may link it to the MAP kinase pathway (although this link is not yet established directly). Src family kinases bind to one phosphotyrosine site (P) of FAK and mediate the phosphorylation of a second site required for binding of Grb2. A site that remains to be defined can target the binding of FAK to focal adhesions. NH₂, amino terminus; COOH, carboxyl terminus; CSK, carboxy-terminal Src kinase; PI3-kinase, phosphatidylinositol 3'-kinase.

In addition to changes in phosphorylation, functionally important changes in integrin functions can result from alternative splicing. Previous studies indicated that signaling or cell adhesion was inhibited by certain types of alternative splicing of β_1 and β_3 integrins [44,45]. Recent studies indicate a role for the β_{1c} splice form in the regulation of cell growth, although the mechanisms remain to be determined [46,47].

Adhesion complexes as both recipients and generators of signaling information

The studies discussed above suggest that adhesion complexes can be regulated by extracellular and intracellular inputs. For example, the phosphorylation state of specific components or integrin aggregation and occupancy can modulate cytoskeletal interactions involved in adhesion. Conversely, the accumulation of signal transduction enzymes and substrates in close proximity appears to lead to FAK phosphorylation and activation of signaling cascades, including the extracellular signal regulated kinase (ERK) and the Jun amino-terminal kinase (JNK) pathways of MAP kinase signaling (e.g. see [21*,48]). Signaling probably promotes further aggregation of phosphorylated proteins; signaling/adhesion complexes therefore receive and emit signals, which might be self-reinforced. The role of Ras as an intermediary between integrins, FAK (or some other early activating molecule), and the downstream members of the MAP

kinase signaling pathways is presently controversial, with evidence both for [2*] and against [49] such a role. One explanation may be that dominant-negative Ras inhibitors are not particularly potent, and in fact other systems such as protein kinase C activation may also be involved in MAP kinase activation. Roles of integrin-associated molecules such as IRS-1 (insulin receptor substrate-1) and FAK in growth regulation and suppression of apoptosis appear to be linked to integrin interactions with the extracellular matrix [50–52], though the mechanisms remain to be elucidated. Another puzzle associated with integrin-mediated signaling involves the duration of the signals. Although integrin-induced MAP kinase signals can be moderately prolonged [53*], they are nevertheless transient (less than several hours) in comparison with cell growth stimulation on extracellular matrix substrates over spans of days. Conversely, cell–cell adhesion appears to restrain cell proliferation, but the mechanisms also remain obscure. Such regulation of cellular functions and the regulatory cross-talk between cell–matrix and cell–cell adhesion/signaling complexes should become fruitful areas of future research.

Rho and integrins

The dual functions of adhesion sites as both source and target of signaling become particularly complex when considering the interactions of Rho with integrin function. The Rho family of GTPases may have both upstream

and downstream functions in relation to integrin signaling complexes. As reviewed by Tapon and Hall (this issue, pp 86–92), activated members of the Rho family of small GTPases play important upstream regulatory roles in inducing focal adhesion formation in 3T3 cells, and inhibition of Rho function by serum starvation inhibits integrin clustering and focal adhesion formation [54*]. Nevertheless, in addition to Rho, integrin stimulation by cell adhesion to the matrix was also needed to form focal adhesions [54*]. On the other hand, inhibition of Rho by C3 transferase increased integrin $\alpha_5\beta_1$ mediated adhesion in a monocyte cell line [55], so findings in one cell type cannot necessarily be generalized to all cells. In terms of potential effects of integrins on Rho, integrin clustering can alter Rho localization: Rho was found to accumulate at early integrin complexes induced by ligand-coated beads *in vitro* [21*], and Rho was also localized weakly along linear strands of clustered integrins in native extracellular matrix complexes [20].

Shear stress and tension

Other potentially important regulators of adhesion complexes and their interaction with the cytoskeleton are shear stress and tension. Normal physiology involves a variety of physical forces on cells, from the effects of shear due to the flow of blood in the circulation over and around cells, to tension due to muscle contraction, to intracellular forces acting on individual adhesions with the extracellular matrix or with other cells. Recent work is beginning to address the effects and possible mechanisms of stress, whether acute or cyclic. Examples of effects include a rapid stiffening response to twisting shear as integrins interact with the cytoskeleton [4*] and changes in integrin $\alpha_v\beta_3$ and vinculin localization in endothelial cells undergoing shear stress [56].

Concerning the assembly of adhesion complexes and signaling, two recent papers underscore the importance of tension and contractility. In one, pharmacological disruption of microtubules alone could induce the rapid assembly of focal adhesions and actin microfilament bundles, tyrosine phosphorylation, and even stimulation of DNA synthesis [57*]. These effects required concurrent interaction with the extracellular matrix, tyrosine phosphorylation, and cell contractility, yet occurred in serum-starved cells where Rho is apparently suppressed, suggesting the existence either of a Rho-independent activation of an integrin-dependent signaling cascade via cell contractility that can induce matrix adhesions and DNA synthesis, or of contraction-dependent activation of Rho without internal signals [57*]. The mechanism linking tension to these effects is still a mystery. A complementary paper shows that effects of Rho on actin microfilament bundle formation are linked to myosin light chain (MLC) phosphorylation, and lead to contractility, resulting in aggregation of integrins into focal adhesions and increased tyrosine phosphorylation [58]. An underlying mechanism may be direct activation of MLC kinase by Rho (possibly

due to Rho-kinase activity), leading to increased tension (see Tapon and Hall, this issue, pp 86–92).

Synergism between integrin adhesion complexes and growth factors or cytokines

Additional examples of integrin–growth-factor synergy and information about mechanisms of synergy have recently emerged. Chondrocyte integrins were reported to induce or enhance synthesis of four matrix metalloproteinases, including stromelysin, and they reportedly synergized with IL-1 in this process [59]. In myoblast differentiation, different integrin α subunits appeared to mediate different synergistic interactions with growth factor stimulation; for example, the choice between growth or differentiation depended on the ratio of $\alpha_5:\alpha_6$ integrin cytoplasmic tail signals and whether the environment provided transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), or other growth factors [60]. These and a number of previously reported examples of growth-factor–integrin synergy in growth and differentiation need mechanistic explanations. One potential mechanism has been described on the basis of linked changes in enzyme activity and levels of a phosphoinositol substrate to generate a synergistic response via increases in second messengers (reviewed in [8*]). A novel alternative mechanism has been proposed on the basis of recent observations of integrin-mediated concentrations of growth factor receptors at adhesion/signaling complexes [23*,61], synergistic enhancement of growth factor receptor tyrosine phosphorylation if EGF, PDGF, or basic fibroblast growth factor is present, and markedly augmented MAP kinase activation [61]. These effects require both aggregation and occupancy of integrins, and they are transient, which could provide a mechanism for brief bursts of integrin–growth-factor stimulation of this key regulatory system for growth and differentiation.

Non-integrin signaling and cytoskeletal interactions

In addition to integrins, an integrin ligand and an alternative adhesion receptor have also been recently implicated in the formation of signaling or cytoskeletal adhesion complexes. Intercellular interaction of the leukocyte integrin LFA-1 (lymphocyte function associated antigen-1) and its ligand (or counter-receptor) ICAM-1 (intercellular adhesion molecule-1) was required for tyrosine phosphorylation of p130^{cas} in a B-cell line, and clustering of either the integrin or its ligand alone could not induce this signal [62]. The phosphorylated p130^{cas} becomes a docking protein for the SH2 domains of the regulatory adapter proteins CrkII and CrkL. Clustering of E-selectin also induces cytoskeletal protein accumulation associated with the E-selectin cytoplasmic domain [63]. Although a number of similar cytoskeletal proteins, such as α -actinin, vinculin, and paxillin, accumulate as with β_1 integrin clustering and occupancy, the protein talin does not [63]. Another study reports that the L-selectin cytoplasmic domain binds directly to α -actinin in association with

vinculin and possibly talin [64]. This interaction requires the carboxyl terminus of the tail, which is necessary for leukocyte adhesion and rolling *in vivo*, although L-selectin molecules lacking this site were still able to localize normally to microvilli; this interaction therefore appears to be important for biological function even though other mechanisms can mediate L-selectin localization. Selectins seem to differ in their capacity or need to interact with the cytoskeleton, as neither E-selectin nor P-selectin cytoplasmic domains bound to α -actinin *in vitro*, and truncation of either cytoplasmic domain did not inhibit their function in leukocyte adhesion [65]. Selectins also appear to play important roles in 'inside-out' integrin signaling, as binding of selectins to their glycoconjugate ligands leads to intracellular effects that produce an activation of integrins in a stepwise process that leads from selectin-mediated cell rolling of leukocytes on the endothelium to strong, stable adhesion to the endothelium mediated by the activation of integrins [66].

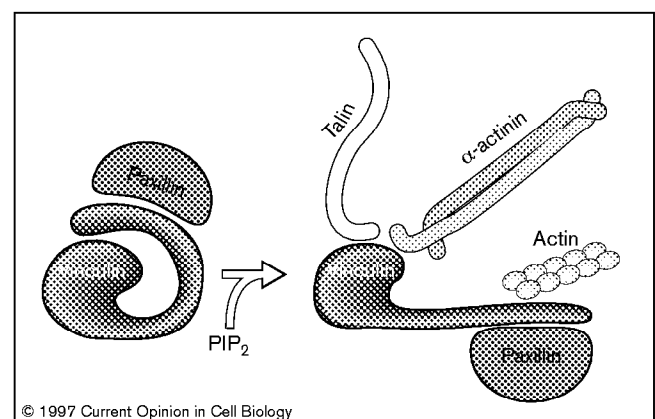
Regulation of components of cell adhesion complexes by aggregation and by conformational alterations

Signal transduction is basically a complex series of protein interactions, induced by and inducing post-translational modifications such as phosphorylation, and triggered by external stimuli. The functions of the components of adhesion/signaling complexes may be regulated in a major fashion by two potentially interrelated physical properties: their state of aggregation and their conformation. Aggregation to form dimers or larger complexes is well known as a mechanism of activation of receptors such as tyrosine kinase receptors for growth factors, and, as reviewed above, integrins also respond to aggregation. This property may be a central element of the response to cell adhesion. Physical aggregation by itself can either induce signaling via phosphorylation of neighboring molecules, or increase susceptibility to *trans*phosphorylation. The primary role of adhesion itself for triggering signaling should not be underestimated: by the binding of receptors to localized sites, it concentrates them together and can thereby trigger a massive molecular cascade in which many other molecules are triggered to accumulate and, ultimately, to signal. In general, molecules that were isolated are suddenly forced to 'talk' to one another by aggregation via integrins and then via SH2, SH3 and other domains. Accordingly, the phenomenon of 'cross-talk' between adhesive and signaling pathways will probably become increasingly common. The actual first phosphorylation signal(s) involved in integrin- and cadherin-mediated signaling is not yet known with certainty. Obvious candidates are *trans*phosphorylation of aggregated FAK molecules, activation of FAK and other molecules by a Src family kinase, or a combination of these events.

A final mechanism of regulation of adhesion complex formation and function involves conformational changes

and changes in steric accessibility. The theme of altered protein conformation runs throughout all the steps in adhesive interaction and response. Integrins are 'activated' by cytoplasmic signals that produce changes in the conformation of their extracellular domain to augment their binding to a wide variety of ligands (reviewed in [8]). Conversely, the response of an integrin to binding of a ligand results in a transmembrane conformational change or a change in the interactions of integrin α and β tails, leading to the ability of the integrin β cytoplasmic domain to bind to the cytoskeleton; this process can mediate integrin movement and help to form an adhesion site [3,17]. Src family kinases appear to have cryptic kinase domains that are exposed by changes in tyrosine phosphorylation [67]. A potentially important regulatory mechanism in focal adhesions is depicted in Figure 4. As adhesion proceeds, phosphatidylinositol 4,5-bisphosphate (PIP_2) is thought to produce a conformational change in vinculin, which unfolds to expose binding sites for talin and actin, thereby helping to assemble an adhesion complex [68,69]. These and other conformational changes contribute to the remarkable ability of a cell to assemble adhesion and signaling complexes rapidly from existing molecules, in turn unleashing a cascade of important signaling and cytoskeletal changes.

Figure 4



Conformational opening of vinculin to permit protein binding. The signaling molecule PIP_2 can regulate vinculin interactions with actin, α -actinin and talin. In contrast, interactions with paxillin appear to occur with or without this regulatory lipid. Other regulated conformational changes (not shown) probably play important roles in adhesion complex formation and function.

Conclusions

Recent advances in the study of cell adhesion complexes have established the following concepts:

1. Cell adhesion complexes contain many cytoskeletal and signal transduction molecules that bind to a number of other molecules, which are thereby likely to form elaborate three-dimensional networks of varying compositions.

2. Compelling indirect evidence exists for signaling originating from both cell–cell and cell–substrate adhesions.
3. These adhesion complexes can serve as both recipients and generators of signaling information.
4. As exemplified by FAK, there are likely to be multiple functions of, and extensive cross-talk between, components of adhesion complexes. Each component may serve both structural and signaling functions.
5. Adhesion/signaling complexes containing a number of molecules are associated with integrin cytoplasmic domains. These interactions can involve α or β integrin tails, and some β cytoplasmic domains can be modified by tyrosine phosphorylation.
6. A hierarchy of signaling and cytoskeletal molecules binding to integrins has been identified, beginning with FAK and tensin; the types of molecules that bind depend upon the ligand-occupancy and aggregation state of the integrins, as well as on cellular tyrosine phosphorylation.
7. Other important synergistic interactions exist between integrin and growth-factor signaling pathways, one mechanism of which involves integrin-mediated growth factor receptor aggregation, which is associated with enhanced phosphorylation and MAP kinase signaling.
8. Another system of signaling from adherens junctions has been identified that centers on the cytoplasmic molecule β -catenin, which can interact with cadherins and with a number of regulatory molecules.
9. Basic physical mechanisms that underlie the regulation of adhesion complex formation and function include aggregation of individual signaling molecules and conformational changes that lead to altered accessibility of key molecular binding sites.
10. Tyrosine phosphorylation and other protein phosphorylation events probably also play important roles in cell adhesion complex formation and regulation, but the details at the level of roles of specific kinases remain confusing.
11. Tension, contractility, and shear stress can contribute to the regulation and function of cell adhesions.

Researchers in this field have thus made impressively rapid progress in defining and understanding many aspects of cell adhesion and signaling complexes. An exciting research opportunity for the near future will be to establish the actual detailed molecular mechanisms that link adhesion receptors to the organization and function of these complexes in cell movement and signaling. An equally exciting challenge will be to elucidate their regulation and coordination via cross-talk between the

many different pathways of intermolecular interaction, complex formation, and signal transduction.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Jockusch BM, Bubeck P, Giehl K, Kroemker M, Moschner J, Rothkegel M, Rudiger M, Schuler K, Stanke G, Winkler J: **The molecular architecture of focal adhesions.** *Annu Rev Cell Dev Biol* 1995, **11**:379–416.
 - A comprehensive review describing the many components of focal adhesions, their interactions, and their many potential functions.
 2. Clark EA, Brugge JS: **Integrins and signal transduction pathways: the road taken.** *Science* 1995, **268**:233–239.
 - This broad review covers many key aspects of integrin signaling and its relationship to more classical signaling pathways.
 3. Yamada KM, Miyamoto S: **Integrin transmembrane signaling and cytoskeletal control.** *Curr Opin Cell Biol* 1995, **7**:681–689.
 - A review focusing on recent advances in integrin-induced signaling and the regulation of localization of a variety of cytoskeletal proteins to form adhesion complexes.
 4. Geiger B, Yehuda-Levenberg S, Bershadsky AD: **Molecular interactions in the submembrane plaque of cell–cell and cell–matrix adhesions.** *Acta Anat* 1995, **154**:46–62.
 - This review identifies the components, and compares the compositions and functions, of adhesion complexes in cell–cell versus cell–matrix adhesions.
 5. Gilmore AP, Burridge K: **Molecular mechanisms for focal adhesion assembly through regulation of protein–protein interactions.** *Structure* 1996, **4**:647–651.
 - A brief review of recent advances that discusses the potential mechanisms of integrin-mediated regulation of focal adhesion assembly.
 6. Gumbiner BM: **Cell adhesion: the molecular basis of tissue architecture and morphogenesis.** *Cell* 1996, **84**:345–357.
 - This unusually wide-ranging review captures the essence of the many advances in studies of cell adhesion and its roles in morphogenesis and tissue structure.
 7. Kirkpatrick C, Peifer M: **Not just glue: cell–cell junctions as cellular signaling centers.** *Curr Opin Genet Dev* 1995, **5**:56–65.
 - An interesting review focusing on potential roles of cell–cell adhesive junctions as sites of generation of signaling.
 8. Schwartz MA, Schaller MD, Ginsberg MH: **Integrins – emerging paradigms of signal-transduction.** *Annu Rev Cell Dev Biol* 1995, **11**:549–599.
 - A comprehensive, thought-provoking review and analysis of recent findings and concepts in the area of integrin-mediated signal transduction.
 9. Parsons JT: **Integrin-mediated signaling – regulation by protein-tyrosine kinases and small GTP-binding proteins.** *Curr Opin Cell Biol* 1996, **8**:146–152.
 - This review provides insights into the regulation of integrin signaling by two important types of protein, namely, tyrosine kinases and molecules such as Rho.
 10. Sharma CP, Ezzell RM, Arnaout MA: **Direct interaction of filamin (ABP-280) with the beta 2-integrin subunit CD18.** *J Immunol* 1995, **154**:3461–3470.
 11. Leung-Hagesteijn CY, Milankov K, Michalak M, Wilkins J, Dedhar S: **Cell attachment to extracellular matrix substrates is inhibited upon downregulation of expression of calreticulin, an intracellular integrin alpha-subunit-binding protein.** *J Cell Sci* 1994, **107**:589–600.
 12. Laflamme SE, Thomas LA, Yamada SS, Yamada KM: **Single subunit chimeric integrins as mimics and inhibitors of endogenous integrin functions in receptor localization, cell spreading and migration, and matrix assembly.** *J Cell Biol* 1994, **126**:1287–1298.
 13. Laflamme SE, Akiyama SK, Yamada KM: **Regulation of fibronectin receptor distribution.** *J Cell Biol* 1992, **117**:437–447.
 14. Geiger B, Salomon D, Takeichi M, Hynes RO: **A chimeric N-cadherin/beta 1-integrin receptor which localizes to both cell–cell and cell–matrix adhesions.** *J Cell Sci* 1992, **103**:943–951.

15. Briesewitz R, Kern A, Marcantonio EE: **Ligand-dependent and -independent integrin focal contact localization: the role of the alpha chain cytoplasmic domain.** *Mol Biol Cell* 1993, 4:593-604.
16. Ylanne J, Huuskonen J, O'Toole TE, Ginsberg MH, Virtanen I, Gahmberg CG: **Mutation of the cytoplasmic domain of the integrin beta 3 subunit. Differential effects on cell spreading, recruitment to adhesion plaques, endocytosis, and phagocytosis.** *J Biol Chem* 1995, 270:9550-9557.
17. Felsenfeld DP, Choquet D, Sheetz MP: **Ligand binding regulates the directed movement of beta1 integrins on fibroblasts.** *Nature* 1996, 383:438-440.
18. Mainiero F, Pepe A, Wary KK, Spinardi L, Mohammadi M, Schlessinger J, Giancotti FG: **Signal transduction by the alpha 6 beta 4 integrin: distinct beta 4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes.** *EMBO J* 1995, 14:4470-4481.
19. Law DA, Nannizzi-Alaimo L, Phillips DR: **Outside-in integrin signal transduction. Alpha IIb beta 3-(GP IIb IIIa) tyrosine phosphorylation induced by platelet aggregation.** *J Biol Chem* 1996, 271:10811-10815.
20. Burbelo PD, Miyamoto S, Utani A, Brill S, Yamada KM, Hall A, Yamada Y: **p190-B, a new member of the Rho GAP family, and Rho are induced to cluster after integrin cross-linking.** *J Biol Chem* 1995, 270:30919-30926.
21. Miyamoto S, Teramoto H, Coso OA, Gutkind JS, Burbelo PD, Akiyama SK, Yamada KM: **Integrin function: molecular hierarchies of cytoskeletal and signaling molecules.** *J Cell Biol* 1995, 131:791-805.
- This study identified over 20 signal transduction molecules associated with integrin-triggered adhesion complexes and established a hierarchy of regulation of assembly.
22. Miyamoto S, Akiyama SK, Yamada KM: **Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function.** *Science* 1995, 267:883-885.
- Demonstration that integrins respond in different ways to occupancy, aggregation, or a combination of these two signals in inducing aggregation of intracellular molecules.
23. Plopper GE, McNamee HP, Dike LE, Bojanowski K, Ingber DE: **Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex.** *Mol Biol Cell* 1995, 6:1349-1365.
- Direct demonstration of the accumulation of a variety of important signaling molecules in focal adhesion complexes, including proteins involved in growth factor receptor signaling.
24. Lewis JM, Schwartz MA: **Mapping *in vivo* associations of cytoplasmic proteins with integrin beta 1 cytoplasmic domain mutants.** *Mol Biol Cell* 1995, 6:151-160.
- Parallel study to [21*-23*], examining integrin sequence requirements for association of cytoplasmic proteins with integrin tails.
25. Kypka RM, Su H, Reichardt LF: **Association between a transmembrane protein tyrosine phosphatase and the cadherin-catenin complex.** *J Cell Biol* 1996, 134:1519-1529.
26. Shibamoto S, Hayakawa M, Takeuchi K, Hori T, Oku N, Miyazawa K, Kitamura N, Takeichi M, Ito F: **Tyrosine phosphorylation of beta-catenin and plakoglobin enhanced by hepatocyte growth factor and epidermal growth factor in human carcinoma cells.** *Cell Adhes Commun* 1994, 1:295-305.
27. Hoschuetzky H, Aberle H, Kemler R: **Beta-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor.** *J Cell Biol* 1994, 127:1375-1380.
28. Miller JR, Moon RT: **Signal transduction through beta-catenin and specification of cell fate during embryogenesis.** *Genes Dev* 1996, 10:2527-2539.
29. Sanson B, White P, Vincent JP: **Uncoupling cadherin-based adhesion from wingless signalling in *Drosophila*.** *Nature* 1996, 383:627-630.
30. Molenaar M, Van De Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destree O, Clevers H: **XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos.** *Cell* 1996, 86:391-399.
31. Behrens J, Von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W: **Functional interaction of beta-catenin with the transcription factor LEF-1.** *Nature* 1996, 382:638-642.
- Describes the important linkage between beta-catenin and the transcription factor LEF-1 (lymphoid enhancer factor-1), including evidence that these proteins together form a complex with DNA that affects DNA bending. The authors provide a mechanism for signal transduction from cell adhesion complexes, or complexes with Wnt, to the nucleus.
32. Tao YS, Edwards RA, Tubb B, Wang S, Bryan J, McCreagh PD: **Beta-catenin associates with the actin-bundling protein fascin in a noncadherin complex.** *J Cell Biol* 1996, 134:1271-1281.
33. Schlaepfer DD, Hunter T: **Evidence for *in vivo* phosphorylation of the Grb2 SH2-domain binding site on focal adhesion kinase by Src-family protein-tyrosine kinases.** *Mol Cell Biol* 1996, 16:5623-5633.
34. Lev S, Moreno H, Martinez R, Canoll P, Peles E, Musacchio JM, Plowman GD, Rudy B, Schlessinger J: **Protein tyrosine kinase PYK2 involved in Ca(2+)-induced regulation of ion channel and MAP kinase functions.** *Nature* 1995, 376:737-745.
- Evidence for the role of a FAK homolog in modulation of ion channel function and activation of the MAP kinase pathway.
35. Pawson T: **Protein modules and signalling networks.** *Nature* 1995, 373:573-580.
36. Burrridge K, Turner CE, Romer LH: **Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly.** *J Cell Biol* 1992, 119:893-903.
37. Bockholt SM, Burrridge K: **An examination of focal adhesion formation and tyrosine phosphorylation in fibroblasts isolated from *src-*, *fyn-*, and *yes-* mice.** *Cell Adhes Commun* 1995, 3:91-100.
- Provides evidence that gene knockouts of each of three Src kinase family members do not affect mature focal adhesions or FAK phosphorylation, indicating that understanding the roles of these kinases will probably be difficult.
38. Kaplan KB, Swedlow JR, Morgan DO, Varmus HE: **c-Src enhances the spreading of *src-/-* fibroblasts on fibronectin by a kinase-independent mechanism.** *Genes Dev* 1995, 9:1505-1517.
- Provides kinetic evidence for a role for c-Src in early fibroblast spreading, with roles for its kinase activity, subcellular localization, and a kinase-independent function in fibroblast spreading.
39. Illic D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T: **Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice.** *Nature* 1995, 377:539-544.
- FAK deficiency results in embryonic lethality, but, surprisingly, the authors find that FAK-deficient cells have an increased number of focal adhesions, with reduced cell motility *in vitro*. They suggest that FAK may be involved in turnover of focal adhesions during cell migration.
40. Kaplan KB, Bibbins KB, Swedlow JR, Arnaud M, Morgan DO, Varmus HE: **Association of the amino-terminal half of c-Src with focal adhesions alters their properties and is regulated by phosphorylation of tyrosine 527.** *EMBO J* 1994, 13:4745-4756.
41. Woods A, Couchman JR: **Protein kinase C involvement in focal adhesion formation.** *J Cell Sci* 1992, 101:277-290.
42. De Nichilo MO, Yamada KM: **Integrin alpha5 beta5-dependent serine phosphorylation of paxillin in cultured human macrophages adherent to vitronectin.** *J Biol Chem* 1996, 271:11016-11022.
43. Lewis JM, Cheresch DA, Schwartz MA: **Protein kinase C regulates alpha v beta 5-dependent cytoskeletal associations and focal adhesion kinase phosphorylation.** *J Cell Biol* 1996, 134:1323-1332.
44. Balzac F, Retta SF, Albin A, Melchiorri A, Koteliansky VE, Geuna M, Silengo L, Tarone G: **Expression of beta 1B integrin isoform in CHO cells results in a dominant negative effect on cell adhesion and motility.** *J Cell Biol* 1994, 127:557-565.
45. Akiyama SK, Yamada SS, Yamada KM, Laflamme SE: **Transmembrane signal transduction by integrin cytoplasmic domains expressed in single-subunit chimeras.** *J Biol Chem* 1994, 269:15961-15964.
46. Fornaro M, Zheng DQ, Languino LR: **The novel structural motif Gln795-Gln802 in the integrin beta 1C cytoplasmic domain regulates cell proliferation.** *J Biol Chem* 1995, 270:24666-24669.
47. Meredith J Jr, Takada Y, Fornaro M, Languino LR, Schwartz MA: **Inhibition of cell cycle progression by the alternatively spliced integrin beta 1C.** *Science* 1995, 269:1570-1572.

48. Chen Q, Kinch MS, Lin TH, Burridge K, Juliano RL: **Integrin-mediated cell adhesion activates mitogen-activated protein kinases.** *J Biol Chem* 1994, **269**:26602–26605.
49. Chen Q, Lin TH, Der CJ, Juliano RL: **Integrin-mediated activation of mitogen-activated protein (MAP) or extracellular signal-related kinase kinase (MEK) and kinase is independent of Ras.** *J Biol Chem* 1996, **271**:18122–18127.
50. Vuori K, Ruoslahti E: **Association of insulin receptor substrate-1 with integrins.** *Science* 1994, **266**:1576–1578.
51. Boudreau N, Sympson CJ, Werb Z, Bissell MJ: **Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix.** *Science* 1995, **267**:891–893.
52. Frisch SM, Vuori K, Ruoslahti E, Chan-Hui PY: **Control of adhesion-dependent cell survival by focal adhesion kinase.** *J Cell Biol* 1996, **134**:793–799.
53. Zhu X, Assoian RK: **Integrin-dependent activation of MAP kinase: a link to shape-dependent cell proliferation.** *Mol Biol Cell* 1995, **6**:273–282.
- Provides a consideration of the potential role of integrin-mediated MAP kinase activation in cell shape dependent cell cycle progression.
54. Hotchin NA, Hall A: **The assembly of integrin adhesion complexes requires both extracellular matrix and intracellular rho/rac GTPases.** *J Cell Biol* 1995, **131**:1857–1865.
- Describes the demonstration that extracellular matrix alone is not sufficient to induce focal adhesion assembly and MAP kinase signal transduction, and that these processes are dependent on the Rho family of small GTPases.
55. Aepfelbacher M: **ADP-ribosylation of Rho enhances adhesion of U937 cells to fibronectin via the alpha 5 beta 1 integrin receptor.** *FEBS Lett* 1995, **363**:78–80.
56. Girard PR, Nerem RM: **Shear stress modulates endothelial cell morphology and F-actin organization through the regulation of focal adhesion-associated proteins.** *J Cell Physiol* 1995, **163**:179–193.
57. Bershadsky A, Chausovsky A, Becker E, Lyubimova A, Geiger B: **Involvement of microtubules in the control of adhesion-dependent signal transduction.** *Curr Biol* 1996, **6**:1279–1289.
- Provides evidence for major roles of microtubules and cell contractility in the regulation of focal adhesion formation and proliferation.
58. Chrzanowska-Wodnicka M, Burridge K: **Rho-stimulated contractility drives the formation of stress fibers and focal adhesions.** *J Cell Biol* 1996, **133**:1403–1415.
59. Arner EC, Tortorella MD: **Signal transduction through chondrocyte integrin receptors induces matrix metalloproteinase synthesis and synergizes with interleukin-1.** *Arthritis Rheum* 1995, **38**:1304–1314.
60. Sastry SK, Lakonishok M, Thomas DA, Muschler J, Horwitz AF: **Integrin alpha subunit ratios, cytoplasmic domains, and growth factor synergy regulate muscle proliferation and differentiation.** *J Cell Biol* 1996, **133**:169–184.
61. Miyamoto S, Teramoto H, Gutkind JS, Yamada KM: **Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors.** *J Cell Biol* 1997, in press.
62. Petruzzelli L, Takami M, Herrera R: **Adhesion through the interaction of lymphocyte function-associated antigen-1 with intracellular adhesion molecule-1 induces tyrosine phosphorylation of p130cas and its association with c-CrkII.** *J Biol Chem* 1996, **271**:7796–7801.
63. Yoshida M, Westlin WF, Wang N, Ingber DE, Rosenzweig A, Resnick N, Gimbrone MA Jr: **Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton.** *J Cell Biol* 1996, **133**:445–455.
64. Pavalko FM, Walker DM, Graham L, Goheen M, Doerschuk CM, Kansas GS: **The cytoplasmic domain of L-selectin interacts with cytoskeletal proteins via alpha-actinin: receptor positioning in microvilli does not require interaction with alpha-actinin.** *J Cell Biol* 1995, **129**:1155–1164.
65. Kansas GS, Pavalko FM: **The cytoplasmic domains of E- and P-selectin do not constitutively interact with alpha-actinin and are not essential for leukocyte adhesion.** *J Immunol* 1996, **157**:321–325.
66. Springer TA: **Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration.** *Annu Rev Physiol* 1995, **57**:827–872.
67. Liu X, Pawson T: **Biochemistry of the Src protein-tyrosine kinase: regulation by SH2 and SH3 domains.** *Recent Prog Horm Res* 1994, **49**:149–160.
68. Johnson RP, Craig SW: **An intramolecular association between the head and tail domains of vinculin modulates talin binding.** *J Biol Chem* 1994, **269**:12611–12619.
69. Gilmore AP, Burridge K: **Regulation of vinculin binding to talin and actin by phosphatidylinositol-4-5-bisphosphate.** *Nature* 1996, **381**:531–535.
- Building on the findings of [68], the authors demonstrate that the binding of vinculin to talin and actin can be regulated by a regulatory phosphoinositol lipid by dissociating the head-to-tail interaction of vinculin and unmasking binding sites.