Integrins and Signal Transduction Pathways: The Road Taken

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Adhesive interactions play critical roles in directing the migration, proliferation, and differentiation of cells; aberrations in such interactions can lead to pathological disorders. These adhesive interactions, mediated by cell surface receptors that bind to ligands on adjacent cells or in the extracellular matrix, also regulate intracellular signal transduction pathways that control adhesion-induced changes in cell physiology. Though the extracellular molecular interactions involving many adhesion receptors have been well characterized, the adhesion-dependent intracellular signaling events that regulate these physiological alterations have only begun to be elucidated. This article will focus on recent advances in our understanding of intracellular signal transduction pathways regulated by the integrin family of adhesion receptors.

Integrins are the major family of cell surface receptors that mediate attachment to the extracellular matrix (ECM), and specific classes of integrins also mediate important cell-cell adhesive interactions. These integrin-mediated adhesive interactions are intimately involved in the regulation of many cellular functions, including embryonic development, tumor cell growth and metastasis, programmed cell death, hemostasis, leukocyte homing and activation, bone resorption, clot retraction, and the response of cells to mechanical stress (*1–4*). As such, they have been the focus of intense research in the past decade, and significant progress has been made in identifying their structural composition and ligand binding capabilities.

Integrins are composed of α and β transmembrane subunits selected from among 16 α and 8 β subunits that heterodimerize to produce more than 20 different receptors. Alternative splicing of the α and β subunits adds additional complexity (*5*). Though most integrins bind ligands that are components of extracellular matrices (for example, fibronectin, collagen, and vitronectin), certain integrins can also bind to soluble ligands (such as fibrinogen) or to counterreceptors [such as intracellular adhesion molecules (ICAMs)] on adjacent cells, leading to homo- or heterotypic aggregation. These ligands cross-link or cluster integrins by binding to adjacent integrin molecules on the cell surface. Both receptor clustering and ligand occupancy are critical for the activation of intracellular integrin-mediated responses (*6*). Integrin receptor engagement and clustering leads to the formation of focal adhesions where integrins link to intracellular cytoskeletal complexes and bundles of actin filaments (*7, 8*). These protein assemblies play important roles in modulating cell adhesion and inducing cell shape changes involved in cell spreading and locomotion.

Though much is known about the extracellular interactions between integrins and their ligands, significantly less is known about these intracellular biochemical pathways that integrins regulate and the cellular functions that are thereby controlled. To understand how integrins regulate these events, it is critical to identify and characterize the intracellular signaling pathways activated by integrin-ligand interactions. This article will review what is currently known about integrin-mediated signaling, focusing on the key components of integrin signaling complexes and the pathways that integrins regulate. In addition, the events that coordinate signals induced by integrins and other receptors will be examined.

Integrin Cytoplasmic Domains and the Cytoskeleton

The short cytoplasmic domains of the α and β integrin subunits do not have any intrinsic enzymatic activity and appear to function by coupling with cytoplasmic proteins that nucleate the formation of large protein complexes containing both cytoskeletal and catalytic signaling proteins (*9*). The α cytoplasmic domains contain highly divergent amino acid sequences, whereas the β subunits show partial sequence conservation. Analyses of chimeric integrin receptors or mutant α and β subunits have indicated that the β cytoplasmic domains are necessary and sufficient to target integrins to focal adhesions in a ligand-independent manner, whereas the α cytoplasmic domains regulate the specificty of the ligand-dependent interactions (*9, 10*).

Integrins link ECM proteins on the extracellular face of the cell membrane to cytoskeletal proteins and actin filaments on the cytoplasmic face. A model, based on results from in vivo localization and in vitro binding studies of integrin-cytoskeletal protein complexes that are detected in cells cultured on ECM proteins (*7–9*), is shown in Fig. 1A. However, because the precise molecular interactions involved in these large protein assemblies are difficult to dissect without disturbing the complex, it may not accurately reflect the true binary interactions in vivo.

Actin-binding proteins that co-localize with integrins in focal adhesions include α-actinin, talin, vinculin, and tensin (Table 1). Talin and α-actinin bind to integrin cytoplasmic domains in vitro; specifically, α-actinin binds to two distinct regions within the β_1 subunit (*11*). Binding sites for α-actinin have also been shown to contribute to β_1 focal contact localization (*12*). Thus, actin filaments may link to integrins through talin, α-actinin, vinculin, which binds to both talin and α-actinin, or tensin, which binds to vinculin, or through a combination of these interactions (*13, 14*). These assemblies of structural proteins are believed to play important roles in stabilizing cell adhesion and regulating cell shape, morphology, and mobility. They may also serve as a framework for the association of signaling proteins that regulate signal transduction pathways leading to integrin-induced changes in cell behavior. However, further studies will be required to determine the physiologically important interactions between integrin receptors and cytoplasmic proteins.

Integrin-Dependent Signaling Pathways

The signaling pathways activated by integrins have been identified through the analysis of biochemical events that are triggered by integrin engagement, and by the identification of proteins that associate with focal adhesion complexes. Because many of the signaling proteins regulated by integrins are also involved in signal transduction pathways activated by receptors for growth factors, information on the functions of such proteins in these signaling pathways has aided our understanding of their potential role in integrin-mediated signaling.

Protein phosphorylation is one of the earliest events detected in response to integrin stimulation. Studies in platelets provided the first evidence that integrin receptors can

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regulate agonist-induced tyrosine phosphorylation (*15*). Subsequently, tyrosine phosphorylation has been shown to be a common and perhaps ubiquitous response to integrin engagement in many cell types including fibroblasts, carcinoma cells, and leukocytes (*2*, *5*, *16*). The ability of tyrosine kinase inhibitors to inhibit the formation of focal adhesions suggests a role for tyrosine phosphorylation in the signaling pathways linked to integrin receptors (*17*). Serine-threonine kinase families such as protein kinase C (PKC) and mitogen-activated protein (MAP) kinases are also activated upon integrin stimulation, and inhibitors of PKC block cell attachment and spreading in certain cell systems (*18*– *21*). Integrin engagement can also induce an increase in the intracellular calcium concentration, a key regulator of intracellular signaling via multiple integrins (*2*, *22*, *23*). Numerous phospholipid metabolites generated in response to integrin engagement also function in integrin-mediated signal transduction, in part by activating PKC (*24*). Elevation of intracellular pH, as a result of the activation of a proton antiporter, accompanies cell spreading and appears to be regulated by several different integrins (*3*). Integrins also regulate changes in gene expression which are critical for developmental and proliferative responses (*25*, *26*). Lastly, integrins appear to affect cell survival by regulating programmed cell death, a response that is also dependent on tyrosine phosphorylation (*3*). The pathways involved in regulating many of these biochemical events as well as several important integrin-dependent protein-protein interactions that have recently been identified will be the focus of this section.

Several proteins that associate with integrin–nucleated protein complexes contain modular domains, termed Src homology 2 (SH2) and 3 (SH3), that specifically mediate protein-protein coupling. SH2 domains bind to proteins through interactions with specific peptide motifs containing phosphotyrosine, whereas SH3 domains bind to short proline-rich peptide motifs on their protein targets (*27*, *28*). These domains play critical roles in intermolecular interactions that couple proteins associated with membrane receptor complexes.

Tyrosine kinases, phosphatases, and their cytoskeletal substrates. Several protein tyrosine kinases have been implicated in integrin signaling events by virtue of their integrin-dependent activation or their localization to focal contacts (Table 2). The focal adhesion kinase (FAK) appears to play a central role in integrin-mediated signal transduction. This kinase is tyrosine-phosphorylated, and its tyrosine kinase activity enhanced, upon integrin engagement (*29*). Integrin-induced phosphorylation of FAK requires the cytoplasmic domain of the β integrin subunit, and clustering of chimeric integrin receptors expressing several β cytoplasmic domains is sufficient to induce FAK phosphorylation (*29*, *30*). Thus, information contained within the β cytoplasmic domain is necessary (and perhaps sufficient) for FAK activation. However, calcium transients and PKC activity may be required as costimulatory events for FAK phosphorylation (*18*, *31*, *32*).

Table 1. Structural components of focal adhesions. PI-3K, phosphatidylinositol-3 kinase; SH2, Src homology 2.

Molecule	Characteristics
Integrins	
β_1	Integrin receptors containing the β_1 subunit and any 1 of 10 α subunits; ECM ligands include fibronectin, laminin, collagen, and vitronectin.
β_2	Integrin receptors containing the β_2 subunit; found in leukocytes; mainly involved in cell-cell adhesion; ligands include ICAMs and fibrinogen.
β_3	Integrin receptors containing the β_3 subunit and any one of two α subunits; ligands include fibrinogen and vitronectin.
Cytoskeletal proteins	
Actin	Structural component of microfilaments.
α-Actinin	Homodimer that binds to and cross-links actin filaments; binds to integrin cytoplasmic face, vinculin, and PI-3K.
Talin	Binds to integrin cytoplasmic face; binds to vinculin and phospholipids.
Vinculin	Binds to α-actinin, talin, paxillin, tensin, actin filaments, and phospholipids.
Paxillin	Tyrosine-phosphorylated; binds to vinculin.
Tensin	Tyrosine-phosphorylated; contains an SH2 domain; binds to vinculin and actin filaments.

The targeting of FAK to focal adhesions appears to involve multiple binding interactions (Fig. 1B). A COOH-proximal focal adhesion targeting (FAT) sequence is necessary and sufficient to localize FAK or a FAT-containing chimeric protein to focal adhesions (*33*), whereas NH_2-proximal sequences of FAK can bind to synthetic peptides derived from several β cytoplasmic domains (*34*). The cytoskeletal protein paxillin also associates with FAK through a COOH-terminal sequence of FAK distinct from the FAT domain (*35*). It appears likely that FAK utilizes several of these domains in its in vivo localization to focal adhesions. Finally, FAK tyrosine phosphorylation sites can serve as binding sites to couple FAK with cellular proteins that contain SH2 domains (*21*, *28*). Through these linkages, FAK is capable of integrating multiple signals triggered by integrins.

Members of the Src family of tyrosine kinases, which contain an SH3 and SH2 domain, have also been implicated in integrin signaling events. Activated Src associates with integrin-dependent cytoskeletal complexes in platelets and fibroblasts (*15*, *21*). This localization appears to be dependent on the Src SH3 domain (*36*), possibly through interaction with proline-rich sequences within paxillin (*37*). The localization of Src family kinases to focal adhesions may also be mediated by their SH2 domains which bind to the FAK autophosphorylation site (*38*). Therefore, it appears that Src SH2 and SH3 domains, restricted by intramolecular interactions in unactivated Src, are capable of interacting with components of focal adhesions upon kinase activation. Furthermore, in cells lacking the kinase Csk that phosphorylates the COOH-terminal tyrosine of c-Src, Src is activated and localized to focal adhesions (*36*). This suggests that Csk may regulate both kinase activation and localization of Src. The Csk kinase also associates with FAK and paxillin through its SH2 domain (*39*). As with FAK, Src family kinases and Csk appear to have multiple sites of attachment to components of focal adhesions.

Syk, a tyrosine kinase with two SH2 domains NH_2-terminal to the catalytic domain, also associates with integrin-dependent cytoskeletal structures and is activated in platelets by the engagement of several integrins (*40*). The mechanism whereby Syk associates with the cytoskeleton is currently under investigation and is presumed to occur through an SH2-phosphotyrosine interaction. Expression of Syk is restricted predominantly to cells of hematopoietic origin, so its involvement in integrin signaling would also be restricted to these cell types.

How does integrin engagement regulate tyrosine kinase activity? Many tyrosine kinases are activated by receptor clustering (*41*, *42*). By extrapolation, ligand-induced integrin clustering may activate tyrosine kinases that are coupled to integrin receptors, either directly or indirectly. No direct association between integrins and tyrosine kinases has been shown in cell lysates, although FAK can interact in vitro with peptides from several β subunit cytoplasmic domains (*34*). Indirectly, many tyrosine kinases associate with integrins through interactions with cytoskeletal complexes induced by the cross-linking of integrins. Kinase activation may be mediated by such

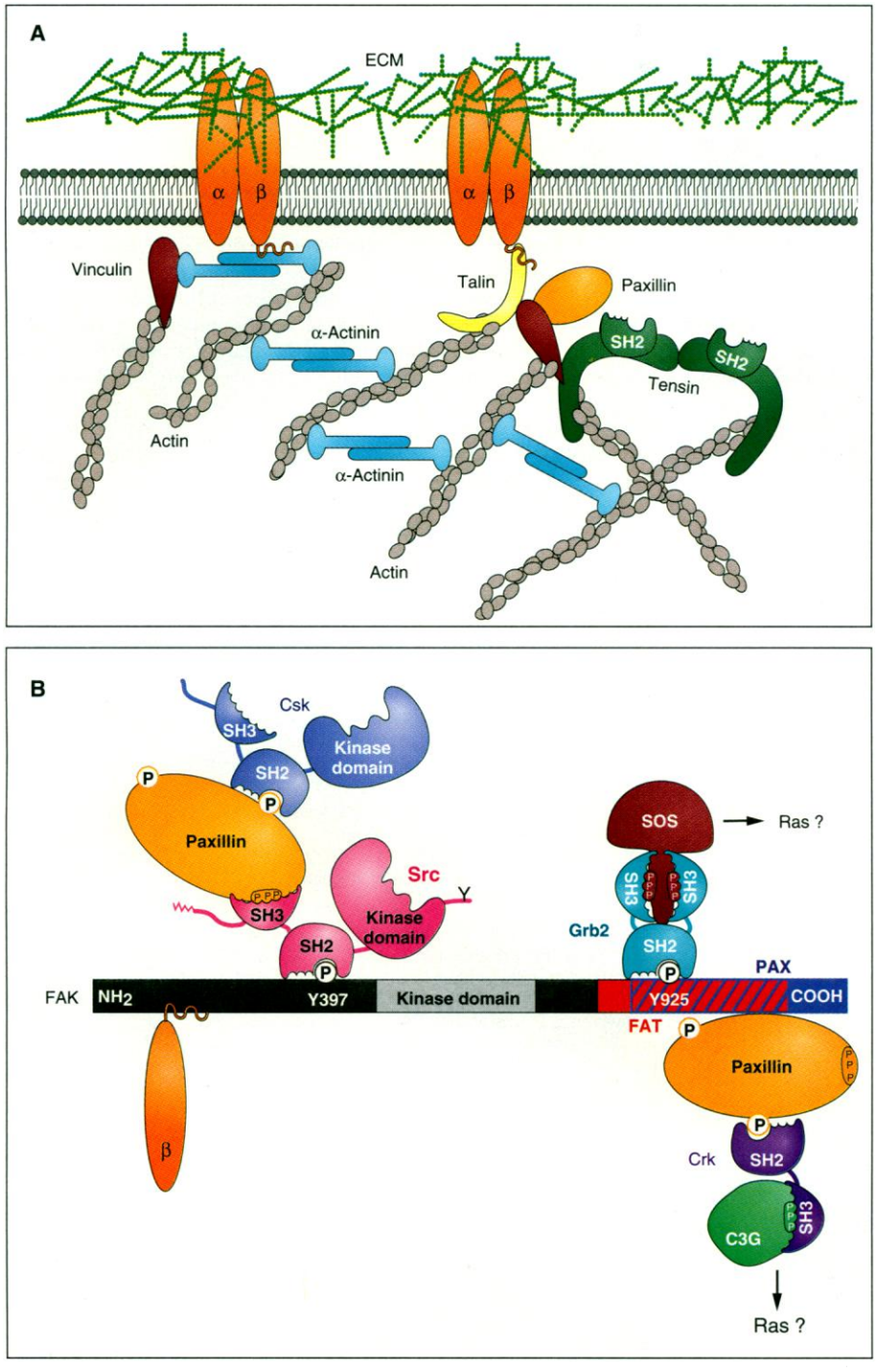

Fig. 1. The focal adhesion. Arrangements of cytoskeletal proteins that localize to focal adhesions in vivo and bind to integrins in vitro are shown in (**A**). Transmembrane integrins bound to the extracellular matrix (ECM) also bind within the cell to a complex of cytoskeletal proteins that include α-actinin, vinculin, talin, paxillin, and tensin and link them to actin filaments. These integrin-cytoskeletal assemblies also form the foundation for the construction of signaling complexes that include the focal adhesion kinase (FAK). Signaling molecules that associate with FAK in vivo or in vitro as described in the text are shown in (**B**). Src homology 2 (SH2) domains bound to phosphotyrosine-containing peptides (P) and SH3 domains bound to proline-rich peptide motifs (PPP) allow the formation of supramolecular complexes of signaling proteins. These proteins include the tyrosine kinases Src and Csk, the adapter proteins Grb2 and Crk, and the guanine nucleotide exchange factors SOS and C3G. PI-3K (not shown) also associates with FAK, though the binding site has not been established. FAK also associates with the cytoplasmic tail of the β integrin subunit, though FAK appears to bind to focal adhesions through a COOH-terminal FAT (focal adhesion targeting) domain. The FAT domain partially overlaps with the region of FAK bound by the cytoskeletal protein paxillin (PAX) and includes a tyrosine at position 925 (Y925) that, when phosphorylated, can bind to the SH2 domain of Grb2. One model for integrin signaling suggests that integrin clustering activates FAK, inducing its phosphorylation on tyrosine (Y397) and subsequent binding to the Src SH2 domain. Src may then phosphorylate Y925 and thereby localize Grb2-SOS complexes (and Ras activation) to focal adhesions (*21*).

clustering. This hypothesis is attractive because inhibitors of cytoskeletal assembly also inhibit the activation of a number of tyrosine kinases (*4*, *29*). Binding of autophosphorylated (activated) FAK to the SH2 domain of Src may activate Src through disruption of the COOH-terminal phosphotyrosine-SH2 interaction. Protein tyrosine phosphatases (PTPs) may also participate in the activation of Src family kinases by dephosphorylating the negative regulatory COOH-terminal phosphotyrosine. To date, two PTPs have been identified as components of integrin-mediated signaling: CD45, a transmembrane PTP that is necessary for the activation of Src family kinases in lymphocytes and is involved in integrin-induced tyrosine phosphorylation in neutrophils (*16*); and the cytosolic PTP1B that is activated after aggregation of $\alpha_{IIb}\beta_3$ in platelets (*43*). It is likely that additional PTPs involved in integrin signaling remain to be identified.

The identification of the cytoskeletal proteins paxillin and tensin as substrates for tyrosine kinases suggests a possible mechanism for the assembly and regulation of integrin-mediated signaling complexes and pathways (Fig. 1). The focal adhesion protein paxillin binds to vinculin and is tyrosine-phosphorylated in adherent cells, presumably by one of the tyrosine kinases (FAK, Csk, or Src) with which it associates (*7*, *44*). Tensin, which is tyrosine-phosphorylated in adherent cells and localized to focal adhesions (where it is implicated as an anchor for actin filaments), is capable of forming signaling complexes with tyrosine-phosphorylated proteins via its SH2 domain (*14*).

SH2-SH3 adapter proteins. Though many signaling proteins that contain SH2 and SH3 domains carry additional catalytic domains, several proteins (known as adapter proteins) are composed exclusively of SH2 and SH3 domains (*27*). Several of these proteins have recently been implicated in the protein-protein interactions detected in integrin-mediated signaling. One such protein, growth factor receptor–bound protein 2 (Grb2), links activated receptor tyrosine kinases to an activator of the Ras pathway, mSOS1, a guanine nucleotide exchange factor (GNEF) that functions by converting inactive Ras·GDP to active Ras·GTP (*41*) (GDP is guanosine diphosphate and GTP is

guanosine triphosphate). In cells adhering to fibronectin, Grb2 and mSOS1 associate with FAK in an integrin-dependent manner. In vitro the SH2 domain of Grb2 binds to a phosphotyrosine sequence within the COOH-terminus of FAK (*21*), suggesting a role for Grb2 and mSOS1 in linking integrin-mediated FAK activation with activation of a Ras signal transduction pathway (see Fig. 1B).

The adapter protein Crk, which contains both SH2 and SH3 domains, may also regulate integrin-mediated Ras signaling. The Crk protein binds to C3G, a putative GNEF for Ras, through the SH3 domain of Crk (*45*). The Crk protein also associates with tyrosine-phosphorylated paxillin, which binds in vitro to the SH2 domain of Crk (*46*). Thus, Grb2 and Crk could potentially link integrins and the cytoskeleton to the Ras signaling pathway through interactions with FAK, mSOS1, paxillin, and C3G.

The Ras–MAP kinase pathway. The association of Grb2 and mSOS1 with FAK suggests that integrins may regulate Ras. This possibility is supported by studies in T lymphoblastoid cells showing that Ras is activated after engagement of the collagen receptor (*47*). In addition, one of the downstream targets of the Ras pathway, MAP kinase, has been shown to be activated in 3T3 cells adherent to fibronectin (*20*,•*21*). This activation requires an intact cytoskeleton, suggesting that the integrin-dependent cytoskeletal complexes are instrumental in the activation of the MAP kinase pathway (Fig. 2A). Furthermore, because growth factors also stimulate the Ras–MAP kinase pathway, it is likely that integrins and growth factor receptors may synergize to enhance Ras–MAP kinase activation.

What are the functional consequences of MAP kinase activation by integrins? Because MAP kinase can phosphorylate and activate transcription factors (*48*), it appears that MAP kinase may be involved in the integrin regulation of gene expression (see below). In addition, MAP kinase phosphorylates and activates cytoplasmic phospholipase A_2 ($cPLA_2$) which hydrolyzes glycerophospholipids to yield (most often) arachidonic acid and lysophospholipid (*49*). Integrin clustering also induces the release of arachidonic acid, an event that occurs concomitant with the phosphorylation of $cPLA_2$ but precedes cell spreading (*50*, *51*). Furthermore, inhibition of $cPLA_2$ prevents cell spreading, an effect that is overcome by the addition of exogenous arachidonic acid (*51*), suggesting that $cPLA_2$ may play a role in integrin-mediated cell spreading. Studies with inhibitors of leukotriene production have shown that arachidonic acid–induced leukotriene production is essential for actin polymerization (*52*), and lipoxygenase inhibitors block HeLa cell spreading on collagen (*51*). Taken together, these results loosely outline a pathway involving MAP kinase and $cPLA_2$ that may regulate cytoskeletal changes necessary for cell spreading (Fig. 2A).

Phospholipid kinases, phospholipases, and protein kinase C. Phosphatidylinositol (PI)-3 kinase (PI-3K), which phosphorylates PI(4)phosphate (PIP) or PI(4,5)bisphosphate (PIP_2) to generate $PI(3,4)P_2$ or $PI(3,4,5)P_3$, respectively, has been shown to associate with integrin-regulated cytoskeletal complexes in platelets (*53*) and to coprecipitate with FAK in cells spread on fibronectin (*54*). Preliminary studies suggest that the SH2 domain of the p85 subunit of PI-3K may be important for its association with tyrosine-phosphorylated FAK (*54*). The role of PI-3K in integrin-induced changes in cell behavior is not understood; however, the evidence that inhibition of PI-3K blocks growth factor–induced actin rearrangements suggests a possible involvement in integrin-regulated cytoskeletal rearrangements (*55*).

PIP-5 kinase (PIP-5K), which phosphorylates PIP to generate PIP_2, has also been implicated in integrin signaling processes. The adhesion of mouse fibroblasts to fibronectin induces an increase in the production of PIP_2, and conversely, the dissociation

Table 2. Components of integrin-mediated signaling pathways.

Molecule	Characteristics
Kinases	
FAK	Tyrosine kinase that localizes to focal adhesions through a COOH-terminal FAT domain; activated by β_1, β_2, and β_3 integrin cross-linking; associates with Src, Csk, Grb2, PI-3K, paxillin, and β_1 integrin peptide.
Src	Tyrosine kinase containing SH2 and SH3 domains; associates with integrin-dependent cytoskeletal complexes, FAK, and paxillin.
Fgr	Src family tyrosine kinase containing SH2 and SH3 domains; activated by tumor necrosis factor in a β_2 integrin–dependent manner.
Csk	Tyrosine kinase containing SH2 and SH3 domains; suppresses Src family kinase activity; associates with FAK and paxillin.
Syk	Tyrosine kinase in hematopoietic cells containing two SH2 domains; activated by β_1 and β_3 integrin cross-linking.
PKC	Serine-threonine kinase that localizes to focal adhesions; activated by phospholipid metabolites and calcium.
MAP kinase	Serine-threonine kinase activated by mitogens or integrin cross-linking; phosphorylated on tyrosine and threonine.
SH2-SH3 signaling	
Crk	SH2-SH3-SH3–containing adapter protein that associates with paxillin (through its SH2 domain) and C3G (through its COOH-terminal SH3 domain).
Grb2	SH2-SH3-SH2–containing adapter protein that links receptors to Ras activation; associates with FAK (through its NH_2-terminal SH2 domain) and mSOS1 (through its SH3 domain).
PI-3K	Lipid kinase specific for phosphatidyl inositol on the D3 position; integrin-dependent association with tyrosine kinases (FAK and Src) and the cytoskeleton (α-actinin); activated in vitro by Rho.
PLC	Phospholipase that hydrolyzes inositol phospholipids such as PIP_2 into diacylglycerol and IP_3; γ isoform contains two SH2 and an SH3 domain and is tyrosine-phosphorylated after engagement of integrin and growth factor receptors.
Small molecular weight GTPases	
Ras	Small molecular weight GTPase that hydrolyzes GTP into GDP; activated upon integrin engagement in T cells.
Rho	Small molecular weight GTPase that hydrolyzes GTP into GDP; essential for serum-induced formation of focal adhesions and stress fibers.
mSOS1	Guanine nucleotide exchange factor that is the mammalian homolog of the *Drosophila son of sevenless* (*SOS*) gene; converts inactive Ras·GDP to active Ras·GTP; binds to the SH3 domains of Grb2.
C3G	Guanine nucleotide exchange factor that binds to the Crk-SH3.
RasGAP	GTPase-activating protein (GAP) for Ras that contains two SH2 and an SH3 domain in its NH_2-terminal; binds to p190, a putative RhoGAP.
Phospholipid mediators	
PIP-5K	Lipid kinase specific to PI(4)phosphate, yielding PI(4,5)bisphosphate; activated in vitro by Rho.
$cPLA_2$	Catalyzes the hydrolysis of glycerophospholipid at the sn-2 position, yielding arachidonic acid and lysophospholipid; phosphorylated and activated by MAP kinase in a calcium-dependent manner.
Arachidonic acid	Precursor for eicosanoids that is released from adherent cells; essential for the spreading of HeLa cells on collagen.
5-Lipoxygenase	Oxygenates arachidonic acid into leukotrienes; essential for the spreading of HeLa cells on collagen.

of cells from the extracellular matrix (ECM) causes a rapid loss of cellular PIP_2 (*56*, *57*). The enhanced amounts of PIP_2 induced by integrin engagement could be important for the polymerization of actin, which is induced upon integrin engagement, because PIP_2 can regulate actin-binding proteins such as profilin (*58*, *59*). In addition, because PIP_2 also serves as the preferred substrate for phospholipase C (PLC), the production of PIP_2 by ECM-adherent cells is critical to provide substrate for this enzyme. Integrin regulation of PIP_2 production can thus serve an important function in regulating other agonist pathways that are dependent on PIP_2 hydrolysis by PLC (see below).

Recently, PLC was shown to be activated by collagen and antibodies to β_2 integrins through a pathway that is dependent on tyrosine phosphorylation (*60*, *61*). The generation of the important second messengers diacylglycerol (DAG) and inositol trisphosphate (IP_3) upon PLC activation enhances the activity of multiple forms of PKC (*19*), some of which localize to focal adhesions in serum-stimulated 3T3 cells (*62*). Integrin-mediated PKC activation precedes cell spreading, and activators of PKC enhance cell spreading and tyrosine phosphorylation of FAK (*18*, *51*, *63*). PKC inhibitors prevent cell spreading and enhance tyrosine phosphorylation of FAK under certain experimental conditions (*18*) but not under others (*51*, *63*), though focal contacts are not observed when PKC is inhibited under any of these conditions. In addition, PKC inhibition prevents integrin-mediated cellular alkalinization (*3*). Taken together, these results suggest that integrin-mediated cell adhesion activates PKC, enhancing adhesion through the formation of focal contacts (Fig 2B). PKC may, under certain conditions, also be necessary for cell spreading and FAK phosphorylation (*18*, *32*). Finally, PKC may also regulate actin-membrane interactions through phosphorylation of the PKC-specific substrate MARCKS, a phosphoprotein that localizes to focal contact-like sites (*59*).

Intracellular calcium regulation. Though integrin engagement induces an increase in the concentration of intracellular calcium, the exact response appears to be specific to the integrin, ligand, and cell type (*2*, *22*, *23*). For example, pancreatic acinar cells spread on collagen show an increase in the intracellular calcium concentration (*60*), whereas collagen-adherent endothelial cells do not (*64*). Furthermore, endothelial cells spread on vitronectin show a rise in calcium and are dependent on this event for cell migration, whereas endothelial cells migrate on collagen in the absence of a calcium response (*64*). Though the mechanisms regulating integrin-dependent increases in intracellular calcium have not been elucidated, in some instances they appear to involve either PLC-mediated, IP_3-induced calcium mobilization from the endoplasmic reticulum or the transport of extracellular calcium through plasma membrane channels (*22*, *65*).

Gene expression. Integrins regulate gene expression in several cell types through activation of tyrosine kinase pathways (*25*, *66*), perhaps involving the MAP kinase pathway as discussed above. The complexity of the regulation of these signaling pathways is highlighted in studies on fibroblasts in which the $\alpha_5\beta_1$ integrin receptor enhances the expression of specific metalloproteinase genes, whereas $\alpha_4\beta_1$ can counter the effect (*67*). Elucidation of the specific transcription factors responsible for integrin-regulated gene expression may help in uncovering the mechanisms involved in this event.

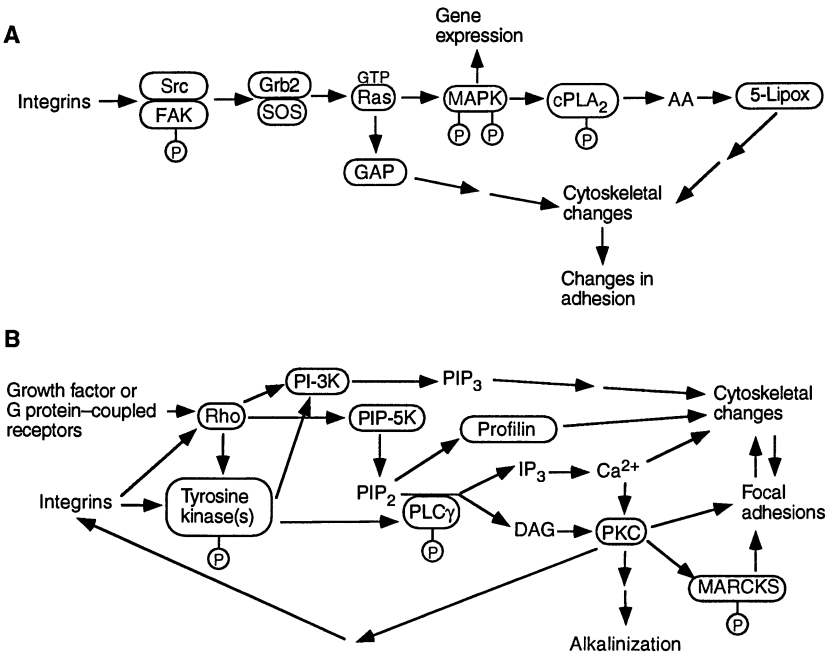

Fig. 2. (**A** and **B**) Potential integrin-mediated signaling pathways. Integrin stimulation induces the formation of focal adhesions through a complex set of signaling pathways. The pathways outlined here are hypothetical, compiled from several studies performed in a variety of cell types. In addition, several intervening steps may be represented by a single arrow. In (A), the integrin-dependent activation of the MAP kinase pathway is shown as a single linear pathway; in reality, the pathway probably involves numerous signaling pathways that converge on and diverge from the pathway shown here, and the involvement of Ras as an intermediate in the MAP kinase pathway has not been firmly established. AA, arachidonic acid; GAP, GTPase-activating protein; 5-lipox, 5-lipoxygenase; MAPK, MAP kinase; P, phosphate; MARCKS, major alanine-rich C kinase.

Signal Coordination

Integrin receptors do not function in a vacuum. Integrin signaling pathways synergize with other receptor pathways to enhance or dampen signals elicited by each receptor. Therefore, one of the most interesting challenges in this field is to identify the signaling molecules that mediate cross talk between these receptor pathways. One protein that appears to play a key role in integrating signals induced by integrins and growth factors is the small molecular weight guanosine triphosphatase (GTPase), Rho. The functions of this protein have been best defined in Swiss 3T3 cells. When these cells are deprived of serum, they lose their focal adhesions and actin stress fibers. Microinjection of activated Rho into these cells induces the rapid formation of actin stress fibers, and inactivation of Rho leads to their disassembly (*68*). Stress fiber formation is inhibited by tyrosine kinase inhibitors (*69*), and inhibition of tyrosine phosphatases induces the formation of focal adhesions (*62*), suggesting that the Rho-induced activation of one or more tyrosine kinases is required for focal adhesion and stress fiber formation (*53*, *56*). Rho-induced changes in focal adhesions and stress fibers may also involve the lipid kinases PI-3K and PIP-5K because the addition of Rho to cell extracts activates these lipid kinases (*53*, *56*).

The Ras GTPase-activating protein (RasGAP) has also been proposed as a key effector of growth factor–mediated changes in cell shape and adhesion. Expression of an NH_2-terminal fragment of RasGAP, which has two SH2 domains and an SH3 domain, correlates with the disruption of actin stress fibers, a reduction in focal contacts, and decreased adherence to fibronection (*70*). This fragment of RasGAP binds constitu-

tively to a protein (p190) that has GAP activity toward Rho. Thus, GAP may regulate adhesion-dependent cytoskeletal rearrangements by interacting with p190 and Rho (*70*). Both RasGAP and Rho may integrate these signals from integrins with those from growth factor receptors, receptors coupled to heterotrimeric GTP-binding proteins (G proteins), and cytokine receptors.

Growth factor receptor tyrosine kinases. Synergistic interactions between growth factor and integrin signaling pathways are involved in the regulation of cell proliferation, adhesion, and migration. For example, adherence to the ECM is critical for stimulation by growth factor or serum of cell proliferation in most cell types (*3*). At least one function of the ECM that is important for growth factor signaling is the activation of PIP-5K–catalyzed PIP_2 formation (*56*). This phospholipid is required as a substrate for growth factor–activated PLC and for the subsequent stimulation of PKC and calcium mobilization, events that are involved in the proliferative response (*56, 57*). In addition, stem cell factor (the Kit receptor ligand) enhances mast cell adhesion to fibronectin in a PI-3K–dependent manner (*71*), and hepatocyte growth factor stimulates the formation of focal adhesions (*72*). Furthermore, the migration of FG carcinoma cells on a vitronectin matrix requires epidermal growth factor signaling pathways that involve tyrosine kinases, PKC, and PLCγ (*73*).

There are numerous examples of growth factor–induced regulation of components of integrin signaling pathways. Platelet-derived growth factor stimulates tyrosine phosphorylation of paxillin and FAK (events that are dependent on the integrity of the cytoskeleton) and induces the association of PI-3K with FAK in adherent Swiss 3T3 cells (*74*). Insulin treatment induces an association between the $\alpha_v\beta_3$ integrin receptor and insulin receptor substrate–1 (*75*), a tyrosine-phosphorylated protein that couples with signaling molecules (including Grb2, SOS, and PI-3K) and mediates insulin signaling (*76*). This interaction may be of functional importance because there is a 2.5-fold increase in DNA synthesis when cells are plated on vitronectin, an $\alpha_v\beta_3$ ligand (*75*). These results suggest that growth factors may regulate integrin signaling complexes as part of the proliferative response.

G protein–coupled receptors (thrombin, bombesin, and lysophosphatidic acid). Ligands for several seven-transmembrane, G protein–linked receptors are known to enhance integrin-dependent tyrosine phosphorylation by presently unknown mechanisms (*15, 77, 78*). Mitogenic neuropeptides such as bombesin, vasopressin, and endothelin stimulate the tyrosine phosphorylation of FAK in adherent Swiss 3T3 cells (*77*). Bombesin-stimulated FAK phosphorylation requires both a functional actin cytoskeleton and an activated Rho pathway but is independent of both PKC and calcium pathways (*79*). Thrombin also stimulates the tyrosine phosphorylation of FAK in platelets through integrin-, PKC-, and calcium-dependent pathways (*32*). Finally, lysophosphatidic acid, which activates a putative G protein–coupled receptor, stimulates phosphorylation of FAK, paxillin, and MAP kinase (*80*) in adherent Swiss 3T3 cells. These results suggest that G protein–coupled receptors can enhance integrin-mediated signal transduction.

Cytokines and immune response receptors. Numerous cytokine and immune response receptors also costimulate integrin-dependent signaling pathways. Neutrophils treated with tumor necrosis factor (TNF) show an enhanced, integrin-dependent inflammatory response and tyrosine phosphorylation of paxillin (*81*). In addition, the Src family kinase Fgr is activated by TNF, but this activation does not take place in β_2 integrin-deficient neutrophils (*82*). Aggregation of immunoglobulin E receptors on the surface of mast cells spread on a fibronectin matrix enhances the calcium- and PKC-dependent tyrosine phosphorylation of FAK and paxillin and the release of histamine (*83*). Coligation of the T cell receptor and the β_2 integrin receptor LFA-1 causes a synergistic enhancement of T cell receptor proliferative responses (*84*).

Therefore, it appears that multiple receptor systems can synergize with integrins to regulate cell proliferation, motility, secretion, and other cellular events. The signaling proteins activated by these synergistic agents are common to many receptor pathways. Thus, although unique pathways may be activated by individual classes of receptors, cross talk between integrins and other receptor pathways is critically involved in the integration of signals that converge on cells in their natural environments in vivo.

Future Prospects

The disciplines of cell adhesion and signal transduction are rapidly converging. Both areas have benefited from the cross-fertilization of ideas, and this union is stimulating progress in understanding integrin-mediated signal transduction. The challenge now facing those undertaking this research is to understand the circuitry of intracellular signal transduction pathways that is established to respond to signals coming from each integrin-ligand interaction and how other receptor pathways negatively or positively regulate integrin-mediated pathways.

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Calcium Signaling in Neurons: Molecular Mechanisms and Cellular Consequences

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Neuronal activity can lead to marked increases in the concentration of cytosolic calcium, which then functions as a second messenger that mediates a wide range of cellular responses. Calcium binds to calmodulin and stimulates the activity of a variety of enzymes, including calcium-calmodulin kinases and calcium-sensitive adenylate cyclases. These enzymes transduce the calcium signal and effect short-term biological responses, such as the modification of synaptic proteins and long-lasting neuronal responses that require changes in gene expression. Recent studies of calcium signal-transduction mechanisms have revealed that, depending on the route of entry into a neuron, calcium differentially affects processes that are central to the development and plasticity of the nervous system, including activity-dependent cell survival, modulation of synaptic strength, and calcium-mediated cell death.

Ionic conductances in neurons have long been a focus of research in neurobiology because of their central role in the control of cell excitability and synaptic transmission. More than 40 years ago our understanding of the propagation of electrical signals in neurons was revolutionized by the work of Hodgkin, Huxley, Katz, and others who described how voltage-sensitive sodium and potassium conductances mediate the active propagation of action potentials along the axon (*1*). In subsequent years, study of ionic conductances during synaptic transmission led to the current view that entry of calcium ions (Ca^{2+}) into the presynaptic terminal is the trigger for neurotransmitter release. It is now widely accepted that the propagation of electrical signals between cells is mediated by the binding of neurotransmitter molecules to their receptors, which act as ligand-gated channels that regulate the influx and efflux of ions into and from the postsynaptic cell (*1*).

Although the importance of ion fluxes in fast synaptic transmission and the role of intracellular calcium in modulating the biophysical properties of membranes have been known for some time (*1*), it has become increasingly apparent during the last decade that changes in intracellular Ca^{2+} can act much more broadly to influence events such as neuronal survival (*2*, *3*), axon outgrowth (*4*), and changes in synaptic strength (*5*). Moreover, depending on the mode of Ca^{2+} entry and the cellular context, Ca^{2+} can mediate disparate biological effects. For example, Ca^{2+} influx through voltage-sensitive Ca^{2+} channels can lead to increased cell survival of embryonic neurons from the central and peripheral nervous systems (*2*). Yet Ca^{2+} influx does not always result in cell survival, and Ca^{2+} influx via the *N*-methyl D-aspartate (NMDA) subtype of glutamate receptors in postnatal neurons mediates excitotoxic cell death (*3*). Although it is not clear how Ca^{2+} could cause such dramatically different outcomes, an intriguing possibility is that the mode of Ca^{2+} entry may be a critical determinant of cell survival (*6*).

Ca^{2+} also appears to be a central mediator of adaptive responses (plasticity) in the nervous system. In recent years the function of Ca^{2+} in synaptic plasticity has been most extensively examined in a cellular model of plasticity called long-term potentiation (LTP) (*5*). LTP and long-term depression (LTD) are examples of synaptic transmission–dependent changes in synaptic efficacy, and the mechanisms that underlie this kind of synaptic plasticity are thought to represent the molecular substrates of learn-

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