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SIGNAL TRANSDUCTION BY CELL ADHESION RECEPTORS AND THE CYTOSKELETON: Functions of Integrins, Cadherins, Selectins, and Immunoglobulin-Superfamily Members

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■ **Abstract** Cellular interactions with the extracellular matrix and with neighboring cells profoundly influence a variety of signaling events including those involved in mitogenesis, survival, and differentiation. Recent advances have provided insights into mechanisms underlying the ability of integrins, cadherins, selectins, and other cell adhesion molecules to regulate signal transduction cascades. These mechanisms often involve the ability of cell adhesion molecules to initiate the formation of organized structures or scaffolds that permit the efficient flow of information in signaling pathways.

OVERVIEW

The role of adhesion molecules in the generation of tissue architecture has long been an important theme of cell biological research. Over the last decade, however, it has become apparent that adhesion molecules play far more than a structural role and indeed are critically involved in multiple signal transduction processes. Thus, aspects of cell adhesion research now overlap with one of the key themes of pharmacological research, namely that of understanding the mechanistic bases of signaling cascades. This review addresses selected aspects of the recent literature in this rapidly evolving field, concentrating on themes that the author views as being particularly novel or critically important to the future development of this area of research. Although a young field, there is already a vast literature dealing with various aspects of cell adhesion and signaling. Much of the early work on adhesion molecules and signaling involved the integrin family of cell adhesion receptors; however, there are now numerous examples implicating other adhesion receptor families, including cadherins, selectins, syndecans, and the immunoglobulin superfamily of cell adhesion molecules (Ig CAMs), in aspects of signal transduction.

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For a reprise of some of the key early work in this area, the reader is referred to several previous reviews (1–8).

This review provides an overview of some exciting emerging trends in the adhesion/signaling area. More importantly it deals with the mechanistic basis of cooperation between cell adhesion receptors and conventional signaling receptors, such as receptor tyrosine kinases. There is a growing awareness that such cooperation involves the formation of scaffolds that efficiently organize the intracellular components of signaling cascades. Furthermore, it seems likely that elements of the actin-containing cytoskeleton are essential in the formation of such scaffolds; however, the molecular details have yet to be elucidated, and this will be a key issue for future progress.

This review deals primarily with events that occur in normal or malignant epithelial cells, and in fibroblasts, endothelial cells, and other mesenchymal cell types. There is a vast literature on cell adhesion and signaling in the immune system, focusing on the T-cell receptor, cognate MHC molecules, and accessory cell surface receptors, including members of the integrin and Ig CAM families (9, 10). However, because of the complex and specialized nature of immune system signaling, I do not deal with this topic here. Cell adhesion receptors also play a critical role in the development of the nervous system (11). In addition to integrins and cadherins, a variety of important receptor and ligand families are involved in neural function, including the eph tyrosine kinase-ephrin group (12), the netrins and their receptors (13), the semaphorins and their plexin receptors (14), and several others. Once again, the biological complexity of neural development precludes detailed consideration of these additional adhesion receptor families in the present context. Cell adhesion also affects events distal to the initial signaling pathways, including cell cycle traverse and programmed cell death. However, I do not deal with these topics here because of space limitations. Several excellent recent reviews on the relationships between adhesion molecules and cell cycle (1, 15) or apoptosis (16, 17) are available.

ADHESION RECEPTOR FAMILIES

The structures and functions of the major families of cell adhesion receptors have been reviewed at length elsewhere (2, 7, 18). Here I briefly mention some key features that are relevant to my discussion of the signaling properties of these molecules. (Figure 1.)

Integrins

Members of the integrin family of cell-surface glycoproteins primarily act as receptors for extracellular matrix (ECM) proteins such as fibronectin, laminin, or collagen. Cell-ECM adhesion sites supported by integrins are complex, specialized structures termed focal contacts or focal adhesions (19). Integrins are

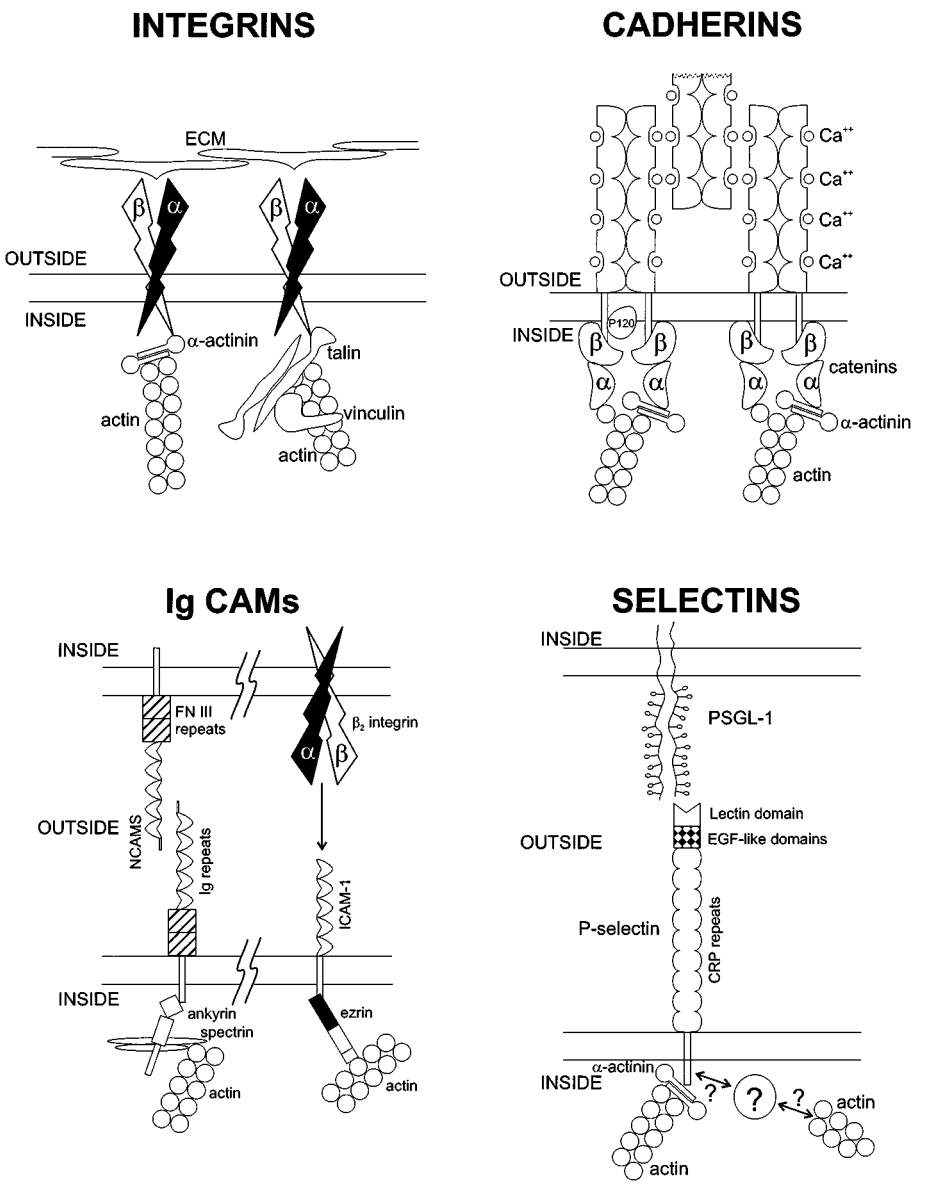


Figure 1 Cell adhesion receptors and associated cytoskeletal components. Integrins, cadherins, selectins, and Ig CAM cell adhesion receptors are depicted in association with their typical extracellular ligands and bound to the proteins that link them to the actin cytoskeleton.

heterodimers having an α and a β subunit; each subunit has a large extracellular domain, a single membrane spanning region, and a short cytoplasmic domain (except for the β4 subunit) (2, 7). The integrin receptor family of vertebrates includes at least 18 distinct α subunits and 8 β subunits. Integrins undergo dynamic changes during the ligand binding process, including relative movements of subunits and conformational changes within domains (20, 21). Integrins can exist in various affinity states for their ligands; these affinity states can be regulated either by extracellular factors (e.g., divalent cations) or by complex intracellular processes that involve the R-Ras and Rap1 small GTPases (22–24). The cytoplasmic domain of an integrin is the key nexus of interaction with intracellular structures and signaling cascades. Both the α and β subunit cytoplasmic domains make important contributions to various aspects of overall integrin function, including cytoskeletal organization, cell motility, signal transduction and regulation of integrin affinity. A large number of cytoskeletal, adaptor, and signaling proteins can interact with integrin cytoplasmic domains and may play a role in integrin-mediated functions. Many of these are listed in Table 1 (available as Supplemental Material: follow the Supplemental Material link on the Annual Reviews homepage at http://www.annualreviews.org/).

Cadherins

The cadherins comprise a family of transmembrane proteins that share an extracellular domain consisting of repeats of an approximately 100-amino acid cadherin-specific module (21, 25, 26). Members of the classic cadherin subfamily, including N, P, R, B, and E cadherins as well as about ten other members (27), contain five such modules and are primarily calcium-dependent homotypic cell-cell adhesion molecules. Classic cadherins localize in specialized sites of cell-to-cell adhesion that are termed adherence junctions where cadherins can establish linkages with the actin-containing cytoskeleton. Another important subfamily of cadherins is a group of desmosome-associated cadherins that form intracellular linkages to intermediate filaments rather than actin filaments (2). Finally, the proto-cadherins comprise a complex gene subfamily that seems particularly important in the development of the nervous system (28). Cadherins on one cell surface form a series of rigid dimers that present several of the cadherin repeats to equivalent dimers on the opposing cells (29); lateral motion of these complexes allows the cell junction site to zip up in order to form a stable adhesion (21, 30). The distal cytoplasmic domains of cadherins are bound by a group of intracellular proteins known as catenins. Beta-catenin binds directly to the cadherin cytoplasmic domain; subsequently α-catenin binds to β-catenin and serves to link the complex to the actin cytoskeleton by direct interaction with actin and by binding α-actinin, an actin-bundling protein (31). Another important part of the complex is p120-catenin, which binds to the membrane proximal domain of the cadherin and which has been implicated in signaling to Rho GTPases (32).

Ig CAM Superfamily

Ig CAMs comprise a very diverse group of adhesive receptors. Members of this family are defined by the presence of one or more copies of the Ig fold, a compact structure with two cysteine residues separated by 55–75 amino acids arranged as two antiparallel beta sheets (33). Typically Ig CAMs have a large amino-terminal extracellular domain containing the Ig folds, a single transmembrane helical segment, and a cytoplasmic tail (7). Members of the Ig CAM family function in a wide variety of cell types and are involved in many different biological processes.

One of the most important contexts for Ig CAMs is the developing nervous system, where many different members of this superfamily are involved in axon guidance and in the establishment and maintenance of neural connections (11). The classic example of a neural Ig superfamily adhesion receptor is NCAM, which contains five Ig folds in its extracellular portion (34). NCAM functions as a homotypic, calcium-independent cell-cell adhesion receptor; however, the precise mechanism of NCAM-mediated cell-cell interaction remains controversial. Several other neural cell adhesion molecules belong to the Ig superfamily, including L1, neural glial cell adhesion molecule (Ng CAM), TAG1, contactin, and *Drosophila* fasciclin II (34, 35). Another group of Ig CAMs important in neural development are the netrin receptors, such as deleted in colon carcinoma (DCC), that interact with laminin-like netrins in the extracellular matrix and provide specific guidance cues to migrating axons (13). Yet another group of key Ig CAM receptors involved in neural development are the dozen or so members of the Eph subfamily of transmembrane tyrosine kinases that bind their cognate ligands (ephrins) on neighboring cells (12). Thus Ig CAMs can be involved in either homotypic (NCAM) or heterotypic (DCC, Eph kinases) adhesive interactions.

There is relatively little known about the interactions of Ig CAMs with cytoplasmic proteins. One possibility is a linkage between L1 and actin mediated by ankyrin (34). Members of the intercellular cell adhesion molecule (ICAM) subfamily of Ig CAMs clearly interact with ezrin in a PIP2-enhanced fashion; ezrin is a member of the ezrin/radixin/moesin family of proteins that serve to directly link certain membrane receptors to the actin cytoskeleton (36). However, it seems likely that additional unknown cytoskeletal interactions exist that contribute to the functions of Ig CAMs.

Selectins

The L-, E-, and P-selectin molecules comprise a small family of lectin-like adhesion receptors (37). Selectin structure includes an amino-terminal domain that is homologous to calcium-dependent animal lectins, followed by an epidermal growth factor (EGF)-type domain, two to nine complement regulatory protein repeats, a transmembrane helical segment, and a short cytoplasmic tail. Selectins mediate heterotypic cell-cell interactions through calcium-dependent recognition of sialylated glycans. A major physiological role for selectins involves leukocyte adherence to endothelial cells and platelets during inflammatory processes (38),

but other functions of selectins have been reported (39). There is tight regulation of the expression and function of selectins so that they come into play only at the appropriate time, such as when leukocytes need to stick to the vessel wall during normal immune system cellular trafficking or during inflammation. Physiological ligands for selectins include sialyl-Lewis^X saccharides in the context of macromolecular scaffolds (40). The best documented high-affinity counterreceptor for a selectin is PSGL-1, a mucin-like transmembrane glycoprotein found on leukocytes and lymphoid cells (41). Little is known about selectin association with the cytoskeleton. One report has indicated an interaction between the cytoplasmic domain of L-selectin and α-actinin (42); however, additional cytoskeletal interactions may also exist.

DIRECT SIGNALING BY INTEGRINS

A concept that may initially be surprising is that integrins can directly activate intracellular signaling processes. Integrin subunits (except for β4) have very abbreviated cytoplasmic domains and were thus initially thought not to be capable of signaling. However, it is now abundantly clear that integrin engagement with ligand, accompanied by integrin clustering, can recruit a number of structural and signaling components and lead to activation of important signal transduction cascades, especially pathways leading to activation of mitogen-activated protein (MAP) kinases. The abundant literature on direct integrin signaling has been comprehensively reviewed (1, 7). Here I sketch out some of the basic ideas and discuss some intriguing recent findings.

Focal Adhesion Kinase

One of the earliest insights into the possibility of integrin signaling was the observation that integrin-mediated adhesion and/or clustering led to enhanced tyrosine phosphorylation (43). It quickly became apparent that adhesion was activating a nonreceptor tyrosine kinase now known as focal adhesion kinase (FAK) (44, 45). This protein is comprised of a central kinase domain flanked by large amino-terminal and carboxy-terminal extensions. A region of the c terminus known as the focal adhesion targeting sequence is responsible for recruiting FAK to its eponymous sites within the cell, the integrin-rich adhesion structures known as focal adhesions or focal contacts. FAK is capable of binding to a number of other signaling and structural proteins including c-Src, PI-3-kinase, GRAF (a RhoGAP), paxillin, talin, and p130 Cas. Tyrosine phosphorylation and activation of FAK accompanies integrin-mediated adhesion, and dephosphorylation promptly occurs when cells are detached (7, 46, 47). The precise mechanisms regulating these events remain largely undefined. There is little evidence for a direct association of FAK with integrins; indeed, at least in some cell types, FAK phosphorylation seems to be a relatively late event that is downstream of actin filament assembly (48).

However, certain mutations in the integrin β subunit cytoplasmic tail can have a strong effect on FAK activation, suggesting specific, if indirect, linkages (49). FAK clearly plays important roles in the regulation of cell motility and in the control of apoptosis. Immediately below I consider the potential role of FAK in integrin-mediated activation of MAP kinase pathways.

Direct Activation of MAP Kinase Cascades by Integrins

An intriguing and somewhat controversial aspect of integrin signaling concerns activation of the Erk and c-Jun kinase (Jnk) MAP kinase pathways. Several quite different mechanisms have been proposed to account for this phenomenon (Figure 2). One concept is that FAK acts as a surrogate for a receptor tyrosine kinase in activating the canonical tyrosine kinase-Ras-Erk cascade. Thus, upon integrin engagement with ECM proteins, FAK is recruited to focal contacts and is autophosphorylated at Y397. This provides a recognition site for the c-Src SH2 domain and recruits Src, which then phosphorylates FAK at additional sites. One of these, Y925, provides a binding site for the SH2 domain of the adaptor protein Grb-2. Recruitment of Grb-2, along with its partner Sos, an exchange factor for Ras, sets the stage for activation of Ras followed by activation of the downstream kinase cascade comprised of Raf-1, Mek, and Erk (7, 50). There is substantial evidence in support of this mechanism, largely derived from studies in which various activated or dominant-negative versions of FAK or Src were overexpressed. However, there are also studies that indicate that integrin-dependent Erk activation can take place independently of FAK activation (51, 52).

Another very interesting model is that of integrin-mediated activation of the Erk cascade involving the transmembrane protein caveolin-1, the Src-family kinase Fyn, and the adaptor protein Shc, but not FAK. In this model, a subset of integrin α subunits are able to activate Fyn, thus causing tyrosine phosphorylation of Shc and subsequent recruitment of the Grb-2/Sos complex. This then triggers Ras and the downstream kinase cascade leading to Erk activation (1, 53). While most integrins can bind caveolin, only certain integrin heterodimers can activate Fyn; the basis for this difference is currently unclear. Interestingly, depletion of caveolin using antisense technology disrupts integrin-mediated adhesion and signaling (54).

In both of the above models Ras plays a key role in propagation of the signal from integrins to Erk. However, there is also evidence for Ras-independent mechanisms. For example, integrin-dependent adhesion can activate a mutant form of Raf-1 that is defective in its ability to bind Ras (55). One possibility is that a protein kinase C isoform plays a role in integrin-dependent activation of the Erk cascade (55, 56).

The existence of several somewhat contradictory models for integrin-mediated Erk activation, each supported by seemingly strong data, may be a reflection of the fact that signaling processes are often highly cell-context dependent. One interesting possibility is that the relative roles of the FAK-dependent and Shc-dependent pathways may reflect the levels of Raf-1 and B-Raf in various cell types. In addition to the postulated FAK/Grb-2/Sos/Ras connection, FAK binds

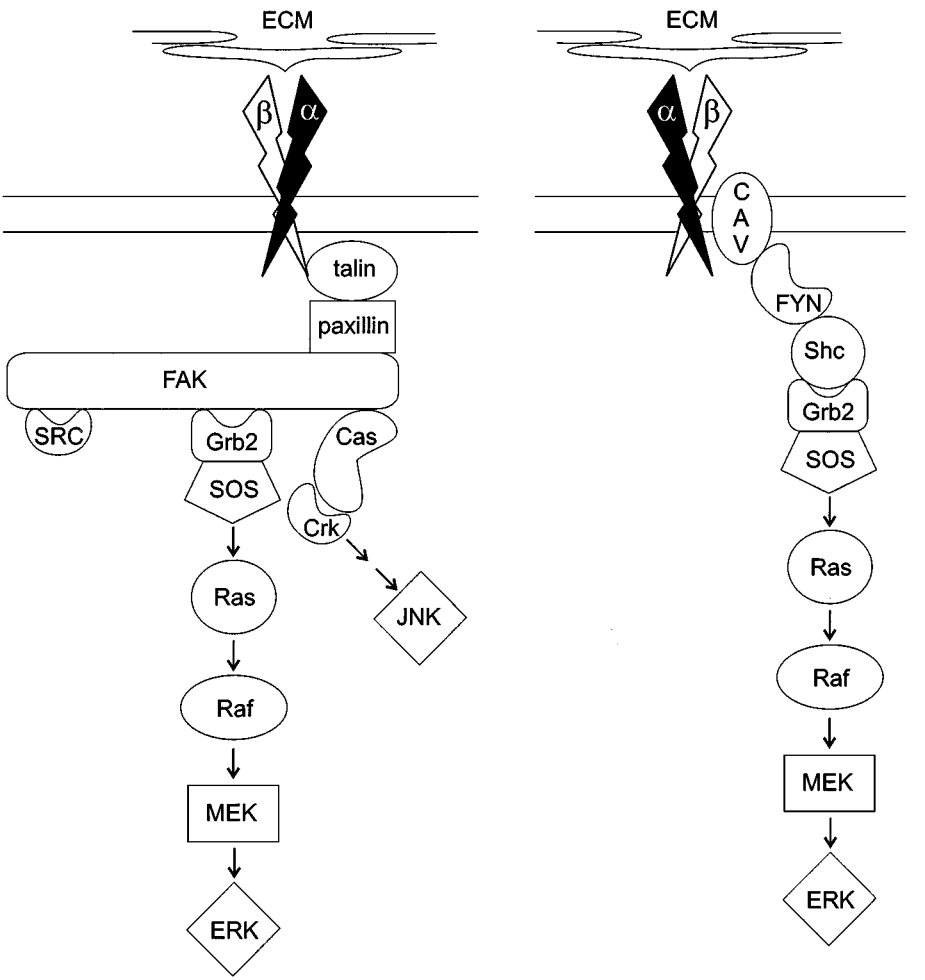


Figure 2 Direct signaling by integrins. Two models are shown depicting how integrins may directly stimulate the Erk/MAPK cascade. In the model on the left integrin engagement causes recruitment and activation of FAK and its autophosphorylation. This creates a binding site for the Src tyrosine kinase, which then further phosphorylates FAK. This allows the Grb2 SOS complex to bind, thus triggering Ras activation and subsequent activation of Raf, Mek, and Erk. A second pathway leads from p130 Cas and Crk to c-Jun kinase activation. In the model on the right certain integrins (but not all) associate with Fyn and Shc via caveolin. The phosphorylation of Shc by the Fyn tyrosine kinase allows recruitment of Grb2-SOS and activation of the cascade.

p130Cas, which can be phosphorylated by FAK and Src, thus creating binding sites for the CrkII-C3G adaptor protein-exchange factor complex (8). C3G can activate the Rap1 GTPase, which, in most instances, is antagonistic to Ras signaling (57). However, Rap1 can activate B-Raf, which in turn can activate Mek and Erk. Thus, depending on the ratio of Raf isoforms, integrin signaling to Erk might be predominantly through Shc (when Raf-1 is high) or through FAK (when B-Raf is high) (58). Recent evidence consistent with this indicates that the C3G-CrkII complex can play a negative role in anchorage regulation of Erk activation, but that this can be reversed by overexpression of B-Raf (59). The Raf-1 vs. B-Raf divergence seems to be an example of the role of cell context in determining the precise details of integrin-signaling pathways.

Integrin engagement has also been reported to directly activate other arms of the MAP kinase pathway including c-Jun kinase (Jnk) and p38. The integrin-mediated activation of Jnk is dependent on FAK activation and may play a role in cell cycle traverse (60) and/or in cell survival (61). Engagement of the $\alpha 2\beta 1$ integrin has been reported to selectively activate p38α, as part of upregulation of collagen gene expression (62).

The physiological significance of direct integrin activation of MAP kinase cascades remains obscure. Integrin-mediated Erk activation is not sufficient to push cells into cycle; additional signals provided by growth factors are still required. One interesting possibility is that integrin modulation of the Erk pathway provides a mechanism for localized control of contractility, because Erk can activate myosin light chain kinase (63).

Integrin Effects on Rho GTPases

Recent research has indicated a link between direct signaling through integrins and activation of the Rho-related branch of the Ras GTPase superfamily, particularly Rho, Rac, and CDC42 (Figure 3). In addition, it is clear that several Ras superfamily members influence the activation state of integrins, as was recently reviewed elsewhere (24). Thus there is an intricate bi-directional pattern of communication between integrins, small GTPases, and the actin cytoskeleton.

Members of the Rho-family of small GTPases influence many key cellular processes but are particularly important in regulation of the actin cytoskeleton (4, 64). RhoA promotes the formation and maintenance of stress fibers, whereas Rac and CDC 42 regulate cortical actin structures such as lamellipodia and filopodia. Some of the mechanisms linking Rho GTPases to control of actin filament assembly have been clarified recently. Thus, both the Rho-stimulated kinase ROCK and the Rac-stimulated kinase p21 activated kinase (PAK) can phosphorylate and activate LIM-kinase, which then phosphorylates and inactivates cofilin, an actin depolymerizing protein, thus promoting actin filament assembly (65–67). While the LIM-kinase/cofilin connection is quite important, there are also other linkages between Rac, CDC42, and actin organization, including those mediated through WASP and WAVE adaptor proteins and the Arp 2,3 complex in forming

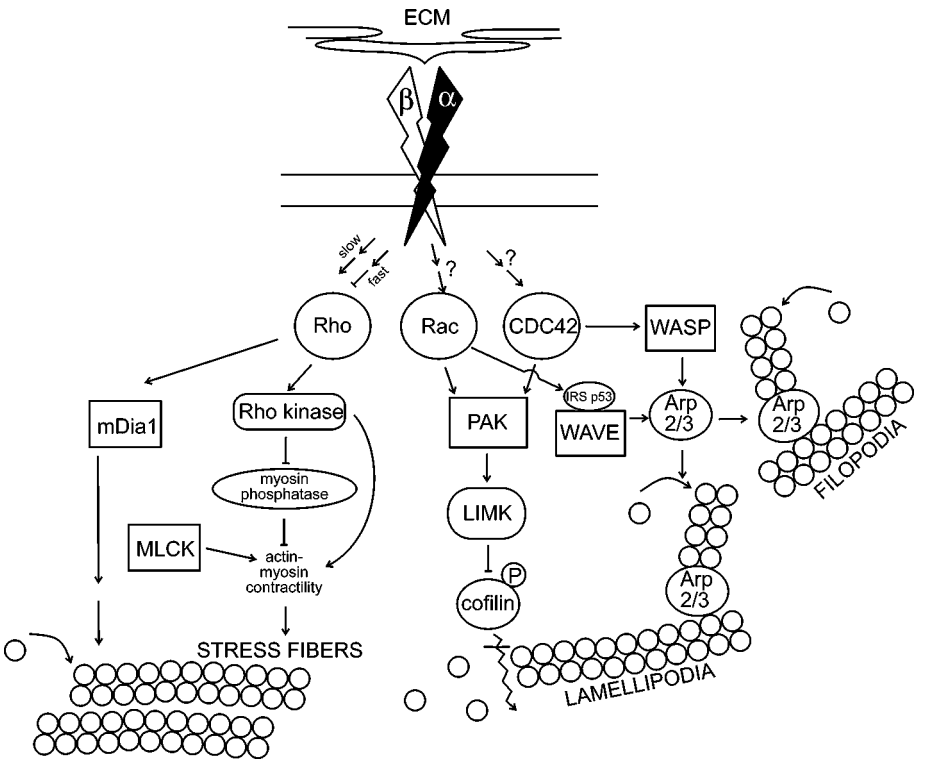


Figure 3 Integrin signaling to the cytoskeleton via Rho GTPases. Integrins can modulate the activity of Rho, Rac, and CDC42, although the precise mechanisms remain largely obscure. CDC42 binds WASP to activate the Arp2/3 complex and trigger filopodia formation. Rac binds to IRSp53 and thence to WAVE to activate Arp2/3 and cause lamellipodia formation. Rho activates Rho kinase to activate myosin via direct phosphorylation of the light chain and by inhibition of myosin light chain phosphatase; Rho also interacts with mDia1 to modulate actin filament formation. The joint action of mDia1 and Rho kinase leads to stress fiber formation. Activation of PAK by Rac results in LIM kinase activation and inhibition of its target, the actin-severing protein cofilin, thus promoting actin filament growth.

actin networks (68, 69), and those mediated through mDia1 in forming stress fibers (70).

Studies have shown that integrin engagement activates several Rho-family GTPases (71–75). Activated Rac and Cdc42 can couple to a variety of downstream effectors, and this coupling process is also influenced by integrins. Thus in a recent study it was found that constitutively activated Rac is not sufficient to activate PAK in nonanchored cells, whereas in adherent cells, Rac translocates to the membrane and PAK is activated (76). These results indicate that integrin engagement with the ECM enables membrane association between Rac and PAK, thus allowing

kinase activation. Another effector downstream of a Rho GTPase that is activated by integrin engagement is activated Cdc42-associated kinase-2 (ACK-2). ACK-2 binds specifically to both Cdc42 and β1 integrins and is activated by β1 integrin ligation (77).

The mechanism(s) of integrin-mediated modulation of Rho-family GTPases have yet to be worked out in detail; presumably exchange factors and/or GTPase-activating proteins are important. The most information is available for RhoA, which undergoes a complex response, first dipping in activity and then displaying increased activity as integrins are engaged. The initial dip in RhoA activity has been linked to either FAK activation (78) or Src activity, leading to tyrosine phosphorylation and activation of p190 RhoGAP (79). The initial dip in RhoA activity may serve to decrease local actinomyosin contractility and thus promote cell spreading on the ECM at initial sites of cell adhesion (79). In any case, it is clear that integrin modulation of Rho GTPases likely plays a significant role in cytoskeletal organization and cell motility. Activation or deactivation of the appropriate Rho-family GTPase in the vicinity of an integrin-mediated adhesive contact may be a key aspect for localized control of actin filament assembly and contractility.

INTEGRIN MODULATION OF SIGNAL TRANSDUCTION CASCADES

One of the most biologically significant aspects of integrin function concerns the ability of integrins and their associated cytoskeletal components to regulate or modulate signaling cascades initiated by other receptors. Thus, integrin-mediated cell anchorage has been observed to have profound effects, in terms of amplitude and/or duration, on signaling processes associated with receptor tyrosine kinases, G protein-coupled receptors, and cytokine receptors. This coupling between integrins and more "conventional" signaling receptors allows cells to integrate positional information concerning cell-cell or cell matrix contacts, with information about the availability of growth or differentiation factors.

Integrin and Cytoskeletal Modulation of the RTK/Ras/MAPK Cascade

Over the last few years there has been a substantial amount of work exploring the multiple connections between integrins, cytoskeletal components, and the canonical receptor tyrosine kinase (RTK)/Ras/MAP kinase cascade. It has become apparent that integrin-mediated cell anchorage, and the accompanying formation of cytoskeletal complexes, can regulate the RTK/Ras/MAPK pathway in three distinct ways. The first is at the level of activation of the RTK. The second is at the level of coupling between upstream and downstream events in the pathway. The third concerns the transmission of the signal between the cytoplasm and nucleus (Figure 4).

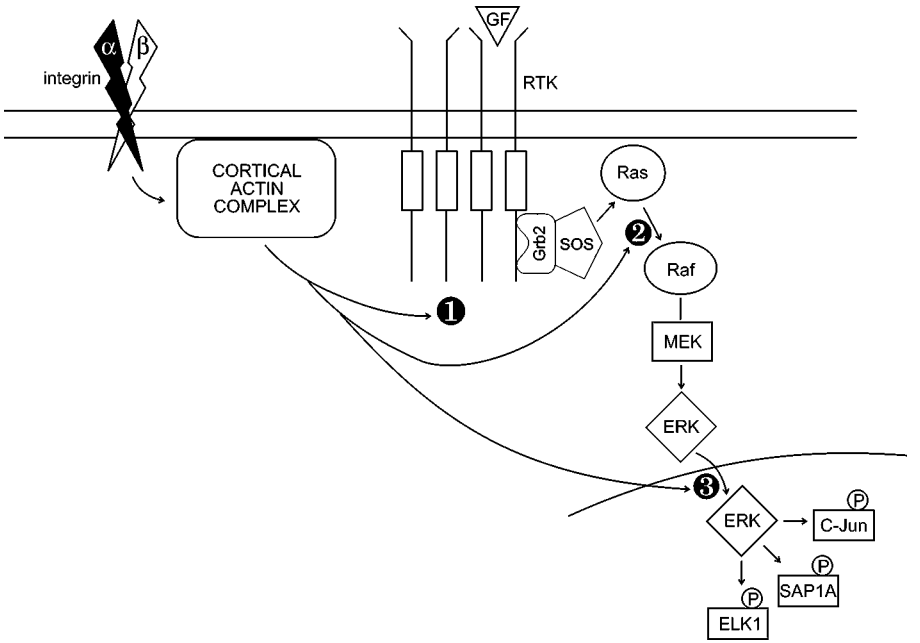


Figure 4 Integrin-cytoskeletal complexes regulate signaling in the RTK/Ras/MAPK pathway. The integrin-dependent cytoskeletal complex can influence signaling downstream of receptor tyrosine kinases at several points: (*a*) integrin-cytoskeletal complexes can enhance the activation of the RTKs themselves, (*b*) the linkage between upstream and downstream events can be affected, probably at the level of Ras to Raf coupling, and (*c*) traffic of signaling components to the nucleus can be affected.

A role for integrins in efficient activation of RTKs has been demonstrated in many cell systems. Early work (80) showed that integrin aggregation and ligand occupancy were required for triggering tyrosine phosphorylation of epidermal growth factor (EGF), platelet derived growth factor (PDGF), and fibroblast growth factor (FGF) receptors. The enhanced RTK autophosphorylation was then reflected in increased Erk/MAPK activity. There are at least two examples of integrin activation of RTKs that take place in the absence of growth factors, one involving PDGFβ receptor (81) and the other EGFR (82). In the case of EGFR, a physical association with β1 integrins may lead to EGFR clustering and autoactivation. A more common situation is that both integrin engagement and the presence of growth factor are required to promote efficient RTK activation. Direct association between RTKs and integrins have been observed for the cases of αvβ3 integrins and PDGFβR, insulin receptor, and vascular endothelial cell growth factor R2 (83, 84). Recently there have been a large number of reports of apparent signaling synergisms between integrins and pathways activated through growth factor receptors. Table 2 (available as Supplemental Material: follow the Supplemental

Material link on the Annual Reviews homepage at http://www.annualreviews.org/) lists a number of these examples. In most cases relatively little is known concerning the mechanistic basis of the observed synergisms; in some cases they may occur at levels other than RTK activation.

Some insights are beginning to emerge concerning the underlying molecular mechanisms for RTK/integrin collaboration. It seems obvious that formation of direct or indirect complexes between the RTKs and the integrins could lead to enhanced opportunities for RTK dimerization and cross-phosphorylation. Recently evidence has emerged indicating that integrin-associated cytoskeletal components may play a key role in these putative complexes. For example, an association between the *Neu* RTK and actin has been observed in mammary carcinoma cells (85). A very compelling recent report has proposed a coupling between integrins and RTKs via FAK (86). FAK can link indirectly to integrins via its carboxy domain, which can bind paxillin and talin; in turn, paxillin binds the $\alpha 4$ subunit, whereas talin can bind various integrin β subunit cytoplasmic tails (see Table 1). The amino terminal domain of FAK, whose function has long been debated, seems to be able to associate with activated EGFR or PDGFR, although the interaction may be indirect. This is a very satisfying model that could account for some of the reported integrin/RTK/MAPK cascade synergisms (87).

A second locus of integrin regulation of the RTK/Ras/Erk cascade concerns the coupling between upstream and downstream elements in the pathway. Thus, my group has found that the loss of integrin-mediated cell anchorage blocks the propagation of the signal from Ras to Raf-1. In this system (3T3 cells) growth factor–mediated autophosphorylation of RTKs and subsequent GTP loading of Ras take place normally in cells in suspension. However, the activation of Raf-1 and the downstream kinases Mek and Erk are dramatically impaired (88). Thus there seems to be an anchorage-dependent step between Ras and Raf in the signaling cascade triggered by peptide mitogens. Somewhat similar results in 3T3 cells were observed by another group (89), with the exception that the locus of anchorage-dependent regulation was placed between Raf and Mek. Another report using a different strategy has also suggested that anchorage regulation lies upstream of Raf (90). Another interesting recent study has indicated that integrin-mediated adhesion of primary mouse fibroblasts can enhance RTK driven signaling activities by reducing association of RasGAP with the receptor; this may be due to recruitment of the phosphatase SHP-2 that then dephosphorylates RasGAP binding sites on the receptor (91). In contrast to the cases mentioned previously, this would place anchorage regulation above the level of Ras activation.

Anchorage regulation of Erk activation seems clearly to involve the actin cytoskeleton (92). A detailed analysis has suggested that it is cortical actin filaments rather than focal contacts and stress fibers that are important, since doses of cytochalasin D sufficient to disrupt the latter but not the former, had little effect on Erk activation by growth factors (93). Consistent with this notion, ectopic expression of active CDC42, which promotes cortical actin assembly, partially rescued Erk activation in suspended cells (93). The role of PI-3-kinase products

in modulating the activation of Ras and Erk (94) is also at least consistent with the notion of cytoskeletal involvement. Thus, there is considerable evidence that integrin-mediated cell anchorage, and subsequent actin cytoskeletal organization, can regulate upstream to downstream coupling of the Ras/Raf/Mek/Erk intracytoplasmic signaling cascade in various cells. However, the precise locus of regulation has not been consistently defined.

A third locus of anchorage-dependent regulation concerns the transmission of the signal from cytoplasm to nucleus. Clues to the existence of this aspect of regulation came from studies showing that forced activation of Erk is insufficient to drive cells into the cell cycle (90). This led to an examination of the role of integrin-mediated anchorage in the trafficking of Erk between the cytoplasm and nucleus (95). The subcellular localization of Erk and its role in growth control has lately been of considerable interest (96). The basic picture is that inactive Erk is held in the cytoplasm by virtue of its association with Mek, a protein that has a classic nuclear export signal (97, 98). Upon activation, Erk is dually phosphorylated, dissociates from Mek, and enters the nucleus, possibly as a dimer (99). Upon dephosphorylation, inactive nuclear Erk associates with Mek and is exported from the nucleus (98). To this picture one must now add a level of regulation by integrins and the actin cytoskeleton. Thus, in suspension cells, or in cells treated with cytochalasin D, the normal trafficking of Erk is disrupted. Despite activation by forced expression of active Mek or Raf, Erk fails to enter the nucleus and thus cannot phosphorylate its key immediate targets, members of the ETS family of transcription factors such as Elk1 (95). The mechanism underlying this actin-based modulation of Erk trafficking is completely undefined at this point. Somewhat similar observations have also been reported elsewhere; thus signaling triggered by macrophage-stimulating protein requires cell adhesion in order to permit Erk translocation to the nucleus (100). The identification of two intracellular loci for anchorage regulation of the RTK/Ras/Erk pathway raises many interesting questions. One concerns the possible role of kinase scaffolding proteins such as MP1 (101), RKIP (102), and KSR (103) in anchorage regulation of signaling. Hypothetically, association of such scaffolds with integrin-mediated cytoskeletal assemblies could provide a powerful means to regulate signaling activity.

Integrin and Cytoskeletal Modulation of Signaling through G Protein–Coupled Receptors

Interesting connections between integrins, the cytoskeleton, and G protein-coupled receptors (GPCRs) have emerged over the last few years. For example, several GPCR agonists including bombesin, gastrin, endothelin, and various muscarinic agents can trigger autophosphorylation of FAK (104, 105), a process that depends on the actin cytoskeleton and requires functional Rho GTPase (104). RGD peptides that interfere with integrin binding to extracellular matrix proteins block activation of FAK by muscarinic acetylcholine receptors; thus it has been

suggested that muscarinic signaling activates integrins, which then trigger the tyrosine phosphorylation of FAK (105).

Integrin-mediated cell anchorage also impacts on GPCR signaling to the Erk/MAPK pathway, with an attenuation of the signal in cells held in suspension and thus deprived of integrin-mediated anchorage (89, 106). One report has shown that treatment with RGD peptides, or with the actin depolymerizing agent cytochalasin D, can block lysophosphatidic acid receptor or thrombin receptor activation of Erk in PC12 cells (107). Recently, the role of integrin-mediated cell anchorage in the pathway leading from P2Y receptors to phospholipase Cβ and then to Erk activation has been carefully dissected. Interestingly, all upstream aspects of this signaling cascade from ligand binding to IP3 generation and calcium release were anchorage independent, but downstream events including activation of Mek and Erk were highly anchorage dependent (108). Thus a series of studies have shown that integrin-mediated anchorage modulates signaling between various GPCRs and the Erk/MAPK module, with the regulation likely occurring in the coupling between upstream and downstream events. The precise molecular mechanism underlying these events remains to be defined (Figure 5).

A very interesting series of studies has linked an integrin-associated protein (IAP, CD47) with heterotrimeric G proteins. IAP is a multispanning membrane protein that is a member of the Ig superfamily. It associates strongly with β3 integrins and enhances their function; IAP also associates with Gi (109). Recent evidence suggests that the IAP, Gi, β3 integrin complex is associated in a cholesterol-rich membrane domain that may serve as a supramolecular signaling complex (110). Several additional reports have implicated cytoskeletal components in various aspects of signaling mediated by GPCRs (111–113).

These various observations linking integrins and their associated cytoskeletal entities with GPCR signaling are very consistent with the emerging concept that localization of signaling components is vitally important. For example, a recent review recounts numerous instances where GPCR oligomerization, membrane targeting, or cytoskeletal association have important impacts on the functions of heterotrimeric G protein–based signaling cascades (114). Given emerging information, one could easily visualize interesting possibilities for the association of integrins, cytoskeletal adaptor proteins, and G protein-based signaling proteins into multiprotein-signaling complexes localized at specific sites on the cell membrane.

Integrin and Cytoskeletal Modulation of Signaling through Cytokine Receptors

Integrin-mediated cell adhesion can influence signaling processes initiated by cytokines and their receptors in several different ways. One well-established example concerns interleukin-1 (IL-1) dependent signaling to NFkB and to MAP kinases (115, 116). More recent studies indicate that IL-1 receptor clusters at focal adhesion sites in a multiprotein complex that also contains the Rho GTPase; indeed direct association of Rho with the IL-1 receptor cytoplasmic domain has been

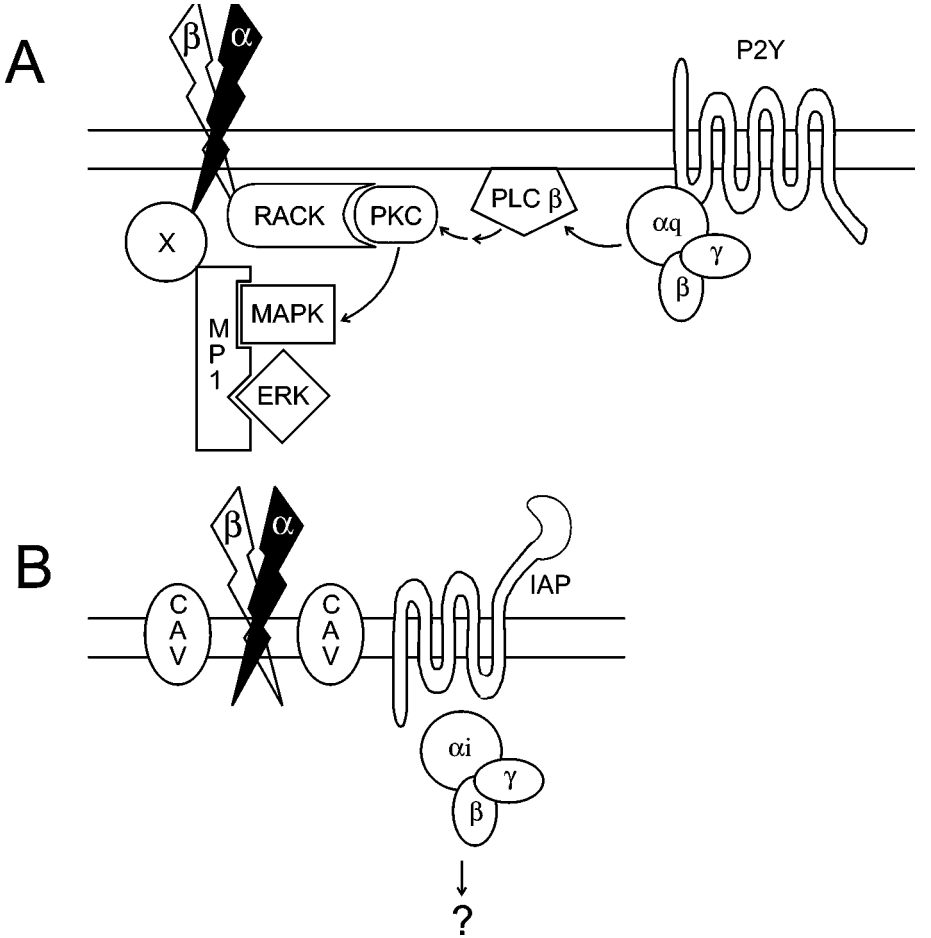


Figure 5 Integrins regulate signaling by G protein–coupled receptors. (*A*) Integrins have been shown to regulate the ability of Gq-coupled receptors to signal into the Erk/MAPK kinase pathway. One hypothesis is that integrins or their cytoskeletal partners associate with scaffolding proteins such as RACK-1 and MP1 to increase encounters between the active signaling components such as PKC and Mek. (*B*) The $\alpha v\beta 3$ integrin associates with G proteins via caveolin and integrin-associated protein (IAP).

detected (117). Furthermore, the recruitment of the IL-1 receptor association kinase into focal adhesions seems to be required for IL-1-dependent activation of Erk, whereas disruption of the actin cytoskeleton prevents IL-1 activation of Erk (118). Thus there is strong evidence for a role for integrin-dependent cytoskeletal assemblies in IL-1-mediated signaling. Other examples of association of cytokine receptors with the actin cytoskeleton have also been observed (119).

Integrin ligation has been reported to influence the synthesis of certain cytokines (120), or of certain cytokine receptors. An interesting example of the latter situation concerns transforming growth factor (TGF) β receptor in breast carcinoma cells. Overexpression of the $\alpha 5\beta 1$ integrin leads to upregulation of TGFβR expression and establishment of a growth inhibitory autocrine loop for TGFβ (121). Conversely, TGFβ treatment of NRK fibroblasts leads to upregulation of $\alpha 5\beta 1$ expression and a loss of anchorage dependence of growth. (122). Another very interesting aspect of integrin-cytokine interplay concerns the proteolytic processing of cytokine precursors. Thus latent forms of TGFβ have been shown to be ligands for the $\alpha v\beta 1$ and $\alpha v\beta 6$ integrins (123, 124). The association of latent TGFβ with $\alpha v\beta 6$ leads to a spatially restricted activation of the cytokine, presumably an important aspect of the autocrine and paracrine functions of this molecule. A very significant aspect of integrin-cytokine interplay occurs in the context of hematopoiesis. Both soluble cytokines and adhesive association with stromal cells and the extracellular matrix play important roles in the differentiation of blood cell precursors. For example, in human blood progenitor cells, integrin engagement opposes the growth promoting effects of IL-3 and stem cell factor (125). Similarly, signaling through Stat 3 can increase expression of integrins and oppose erythropoietin-induced proliferation in myeloid 32D cells (126).

REGULATION OF SIGNALING CASCADES BY CELL-CELL ADHESION RECEPTORS

Signaling by Cadherins/β-Catenin

The cadherins are an important family of calcium dependent cell-cell adhesion molecules that play a key role in developmental processes and in the maintenance of tissue architecture (31, 127–129). In addition to a structural role, cadherins have also been implicated in the regulation of signaling events (7, 130). Here I review recent developments concerning cadherin related signaling, while early literature on this topic has been discussed in the reviews cited immediately above.

Over the past few years a fascinating picture has emerged linking cadherins and β-catenin to the Wnt/wingless signaling pathway. This pathway plays a key role in control of differentiation and development, as well as in malignancy (131, 132). A very abbreviated recapitulation of current understanding of these events is as follows. The β-catenin protein exists in three pools within the cell, bound to cadherins at the membrane, in the nucleus associated with members of the LEF/TCF family of transcription factors, and in the cytoplasm where it can associate in a complex with the adenomatous polyposis coli (APC) tumor suppressor gene product and with axin/conductin. The nuclear pool of β-catenin/LEF regulates transcription of genes associated with cell cycle control and with epithelial differentiation

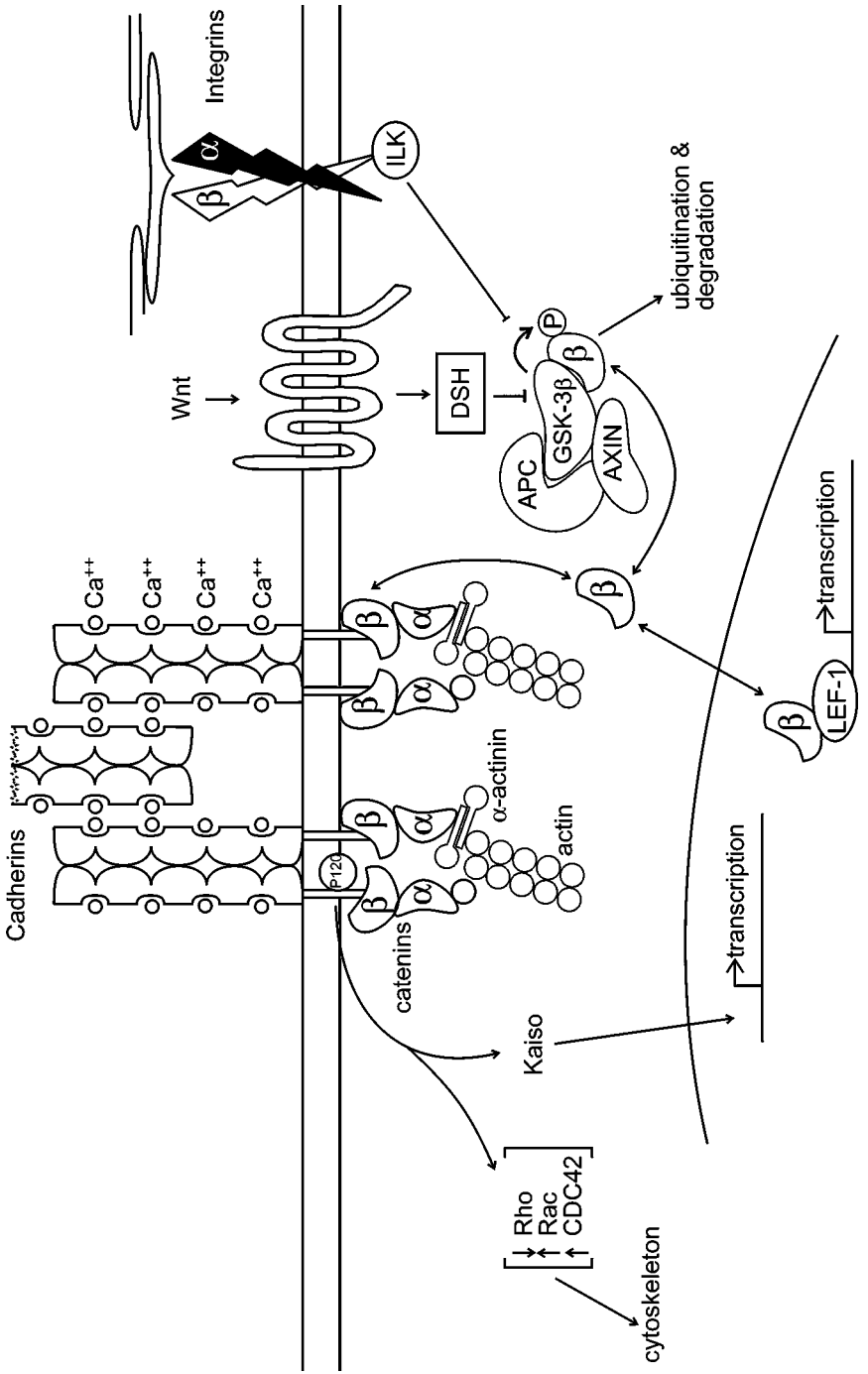
(Figure 6). For example, an important target of the LEF/β-catenin complex is the promoter of the cyclin *D1* gene (133).

The binding of Wnt/wingless activates heptahelical transmembrane receptors of the frizzled family; this signal is transmitted through the disheveled protein to glycogen synthetase kinase 3β(GSK3β), which is then inhibited. The role of active GSK3β is to phosphorylate β-catenin while it is bound in the APC/axin complex, triggering its subsequent ubiquitination and proteosome mediated degradation. Thus Wnt signaling inhibits degradation of β-catenin and leads to its nuclear accumulation and role in transcription. The impact of cadherins on this signaling pathway has been somewhat controversial, with many investigators believing that the function of β-catenin in cadherin-based adhesive junctions was quite distinct from its role in the Wnt signaling pathway (reviewed in 7). However, recent evidence has suggested that cadherins can influence the Wnt signaling pathway, essentially by competing for the pool of β-catenin (134, 135).

An interesting emerging aspect of cadherin/catenin/Wnt signaling is the realization that elements of this signaling pathway are associated with cytoskeletal components and that the signals may affect cell morphology as well as growth and differentiation. In addition to the well-known β-catenin/α-catenin/actin complex, the APC protein has been found to associate with microtubule tips, whereas a second *Drosophila APC* gene product (APC2) associates with assembling actin filaments (136). The disheveled protein has also been found to associate with actin filaments and with focal adhesion sites, where it may complex with paxillin and ILK (137). Thus, once again, as in the case of integrin signaling, linkages between signaling molecules and the cytoskeleton have emerged as an important aspect of cadherin-related signaling cascades.

Signaling roles for several cadherins have been observed that are not obviously connected to the Wnt pathway. Thus N-cadherin has been implicated in signaling in muscle cell differentiation (138). Homotypic interactions of this same cadherin also elevate levels of the cyclin-dependent kinase inhibitor p27 and thus promote cell cycle arrest (139). E-cadherin-mediated cell-cell interactions also upregulate p27 (140). An interesting recent observation is that the β-catenin-associated

Figure 6 Cadherins regulate signaling in the Wnt pathway. The ability of β-catenin to traffic to the nucleus and participate in transactivation of genes is controlled by the size of its cytoplasmic pool. This in turn is regulated both by binding of β-catenin to cadherins at cell-cell adhesion sites and by the Wnt pathway. Wnt signals (probably via G-proteins, see 146a) through Disheveled to inhibit GSK-3β, which otherwise would phosphorylate β-catenin bound in the APC/axin complex, thus triggering its proteolytic degradation. ILK, an integrin-associated kinase, can also inhibit GSK-3β, thus linking the Wnt/β-catenin pathway to integrins. Cadherins also signal via other pathways. For example, p120-catenin signals to Rho GTPases and to the transcription factor Kaiso.


Integrins
α
β
ILK
Wnt
DSH
P
β
GSK-3β
APC
AXIN
ubiquitination &
degradation
Ca++
Ca++
Ca++
Ca++
Cadherins
β
α
β
α
β
α
α-actinin
actin
P120
catenins
Kaiso
Rho
Rac
CDC42
cytoskeleton
β
LEF-1
transcription
transcription

protein axin can serve as a scaffold for components of the c-Jun kinase (Jnk) pathway; this seems to be independent of axin's role in the Wnt pathway (141). Homotypic interactions of E-cadherins have also been reported to link to a signaling pathway leading to activation of PI-3-kinase and protein kinase-B (142). The cadherin/PI-3-kinase connection has also been observed in some very exciting studies concerning cadherins in vascular endothelial cells. Thus, two groups have established a linkage between cadherin-mediated cell interactions and signaling by the VEGF receptor (VEGFR-2) (143, 144). Interestingly, VE-cadherin in endothelial cells seems to play a key role in endothelial cell survival via formation of a complex with VEGFR-2, β-catenin, and PI-3-kinase (144). Cadherins also signal to the assembly of microtubules, although the underlying mechanisms are unclear (145).

An exciting new development is the work of Meigs et al. (146), who find that cadherin cytoplasmic tails can directly interact with the α subunit of the G12 family of heterotrimeric G proteins. Activation of the G protein can displace β-catenin, leading to transcriptional activation. This study provides a novel connection between G protein–signaling pathways and the cadherin/catenin system. Other recent work has confirmed the long-suspected role of G-proteins in signaling from the frizzled family of Wnt receptors to the β-catenin pathway (146a). Another very interesting development concerns the signaling role of p120-catenin. Overexpression of this protein can lead to decreased RhoA activity, increased Rac and CDC42 activity, and dramatic changes in cell morphology (32). A linkage has also been established between p120-catenin and Kaiso, a transcription factor (147), suggesting yet another pathway between cadherins and the nucleus.

Another important issue concerns cross talk between cadherins and other families of adhesion receptors and how these interactions regulate cell-cell junction formation and/or the signaling properties of cadherins. One very important aspect is the relationship between integrin-linked kinase (ILK) (148) and the Wnt/β-catenin signaling pathway (Figure 6). ILK is an ankyrin-repeat containing serine/threonine kinase, whose overexpression can contribute to cell cycle progression and transformation. When expressed in epithelial cells, ILK can lead to disruption of cell-cell adhesion and conversion to a mesenchymal phenotype. This is due to ILKs' ability to regulate the β-catenin signaling pathway (149) and occurs via phosphorylation of GSKβ3 leading to nuclear translocation of β-catenin. ILK can also phosphorylate PKB, a kinase that is implicated in cell survival pathways, perhaps acting in concert with PINCH, an adaptor protein that seems important in the subcellular localization of ILK (150). A number of other linkages between cadherins and other adhesion receptors have been established. This includes several functional connections with β1 or β3 integrins (151–153), as well as a relation with the endothelial Ig CAM protein PECAM-1 (154). In summary, it seems evident that there are extensive interactions between cadherins and signaling components, as well as with other adhesion receptors, particularly integrins. This may serve to reciprocally regulate the signaling functions of the different cell-cell and cell-matrix

receptor families, and thus allow smooth control of cell shape, cell motility, and overall tissue organization.

Signaling by Ig CAMs, Selectins, and Proteoglycans

IG CAMS Members of the Ig CAM family of cell-cell adhesion molecules are known to play important roles in signaling. As mentioned in the Overview, key receptors in the immune system are members of this family (10). In addition, a number of distinct Ig CAM subfamilies play vital roles in neuronal development (11).

NCAM and L1 are important members of a large group of neuronal Ig CAMs whose signaling properties have been extensively studied (34, 155). However, there has been quite a bit of controversy with regard to the mechanism of signaling by these proteins. When NCAM is engaged in homotypic interactions, for example during neurite extension, it triggers a signaling process that involves a phospholipase C, production of diacylglycerol and eicosanoids, activation of calcium channels, and increases in intracellular calcium. This sequence of events also occurs upon activation of FGF-receptor (FGFR); and it has been suggested that NCAM and L1 activate intracellular signaling events via direct interaction with the FGFR (156–158). However, the NCAM/FGFR concept has also been criticized on the basis of a number of inconsistencies (34) and may need to be reinterpreted based on the observation that DN-FGFR binds FRS2 proteins, which can disrupt the functions of various FGFR signaling pathways (155, 159). Another concept for signaling by NCAM and L1 has linked these molecules to intracellular tyrosine kinases. Thus, the 140-kDa isoform of NCAM has been reported to associate with p59Fyn and with FAK (160), while previous work had shown that L1 associates with Src rather than Fyn (161). Recruitment of the intracellular tyrosine kinases then leads to activation of the Erk/MAP kinase cascade, but not to activation of Jnk (162). L1 may need to be internalized in order to trigger the Src/Erk pathway, since transfection of a mutant form of dynamin blocked the signaling ability of L1 (163). Similar observations concerning the importance of L1 internalization to its signaling role have been reported by another group (164). Signaling downstream of neural Ig CAMs not only impacts on morphological changes, but can also affect transcription factor activation and gene induction (165). Although controversies remain concerning the precise mechanisms involved, it is clear that neural Ig CAM molecules such as NCAM and L1 signal through the Erk/MAP kinase pathway in order to carry out their role in neurite outgrowth (166) and that tyrosine kinases act upstream of activation of the Erk cascade. Thus, in broad outline, there are many similarities between signaling mediated by neural Ig CAMs and the integrin-signaling events described above.

PECAM-1 (CD31) is a homotypic Ig CAM receptor found on endothelial cells, platelets, and some types of leukocytes. It is involved in the formation of junctions between endothelial cells and in the extravasation of leukocytes (7, 167). It is

clear that PECAM-1 is capable of signal transduction; for example, ligation of this protein can activate the $\alpha v\beta 3$ integrin (168). Recently some novel insights into PECAM-1 function have developed. One interesting finding is that the cytoplasmic domain of PECAM-1 can bind to certain catenin family members, molecules usually thought to be associated with cadherins. This may be important in helping to organize PECAM-1 into cell junctional sites. Another exciting concept derives from the recognition that the cytoplasmic domain of PECAM-1 contains two ITIMs (immunoreceptor tyrosine-based inhibitory motifs) that serve as docking sites for the dual SH2 domains of certain phosphatases. The SHP-1 and SHP-2 tyrosine phosphatases are known to bind to PECAM-1 (169), as does the inositol phosphatase SHIP (170). Based on such observations, Newman (171) has suggested that PECAM-1 is a member of a large subfamily of the Ig superfamily that contains ITIMs that serve as inhibitory receptors by virtue of their ability to efficiently recruit phosphatases. Thus these proteins would provide negative signals to counterbalance the signals provided by cell surface receptors that are, or that recruit, tyrosine kinases.

Yet another interesting group of homotypic Ig CAMs is a set of molecules whose expression is altered during epithelial tumor progression. CEA is the prototypic carcinoembryonic antigen, which is expressed in a controlled manner during development but which shows disregulated increases in expression in many tumors. CEA is a member of a small subfamily including two proteins that associate with the membrane via GPI (glycosylphosphatidyl-inositol) linkages (CEA, CEACAM6) and one that has a transmembrane domain (CEACAM1) (172). CEA has been proposed to contribute to tumor progression by prolonging cell survival in the presence of differentiation signals (173). Furthermore, CEA and CEACAM6 seem to be able to block "anoikis," that is, apoptosis due to loss of integrin-mediated matrix anchorage, whereas CEACAM1 failed to have this effect (172). In fact, CEACAM1 has been reported to have a tumor inhibitory effect, possibly due to the presence of an ITIM in its cytoplasmic domain and consequent recruitment of the SHP-1 and SHP-2 tyrosine phosphatases (174). Deleted in colon carcinoma (DCC) is another extremely interesting Ig CAM. As its name implies, loss of expression of the *DCC* gene plays a critical role in colon tumor progression. Although first observed in the colon, the expression of DCC is widespread, and one of its most interesting roles is as a receptor for members of the netrin family of axon guidance molecules (13, 175). However, DCC like CEA seems also to play an important role in the regulation of epithelial cell growth and survival. Overexpression of DCC has been reported to reduce growth and tumorigenicity of colon carcinoma cells (176). Thus CEA and DCC, both markers associated with carcinoma progression, seem to play opposing roles in the regulation of growth and survival processes in epithelial cells.

SELECTINS Although the role of selectins and their glycoprotein counterreceptors in regulating the traffic of leukocytes between the bloodstream and tissues is now very well understood (39, 177), there is still relatively little known about signaling

by selectins. Some of the earlier studies on this topic have been reviewed elsewhere (7, 41, 177). These studies had implicated both nonreceptor tyrosine kinases such as c-Src, as well as the MAP kinase cascade in selectin signaling. More recently, studies have focused on the role of selectins in triggering the activation of $\beta 2$ family integrins. Thus, the tethering of neutrophils on E-selectin ectopically expressed in a tissue culture cell monolayer led to enhanced $\beta 2$ integrin-mediated binding to coexpressed ICAM-1 (178); this was blocked by inhibitors of the Erk/MAP kinase cascade. This indicates that the glycoprotein counterreceptor for E-selectin on neutrophils can trigger integrin activation in these cells by a MAPK-dependent pathway. A parallel set of observations (179) indicated that engagement of the selectin ligand PSGL-1 on leukocytes led to activation of $\beta 2$ integrins, a process blocked by tyrosine kinase inhibitors. A similar but distinct picture arises when L-selectin, which is expressed on neutrophils, is cross-linked by antibodies (180). In this case, $\beta 2$ integrins are also activated, but the process is blocked by inhibition of the p38 MAP kinase, and not the Erk/MAP kinase. Thus, several reports agree that ligation of either leukocyte selectins or selectin counterreceptors can signal the activation of $\beta 2$ integrins; however, the details of the signaling pathways are still preliminary. Selectin signaling involving elements of MAP kinase cascades also occurs in other cell types (181, 182).

PROTEOGLYCANS Several forms of transmembrane proteoglycans, particularly members of the syndecan family, can engage in signal transduction (183). A major development in this area was the discovery by Couchman and colleagues that the cytoplasmic domain of syndecan-4 can associate with protein kinase C and regulate its activity (184). This has led to further insights into the role of syndecans in signaling, particularly with regard to cooperation between integrins and syndecans in regulation of cytoskeletal structures (185). In some cell types, syndecans 1 and 4 become tyrosine phosphorylated on their cytoplasmic domains upon cell adhesion (186); this could also support a role for syndecans in signaling. In addition to syndecans, other transmembrane proteoglycans have been implicated in signaling events. Thus MCSP is a chondroitin sulfate-type proteoglycan that has been linked to cosignaling with integrins in melanoma cells (187). A very exciting recent observation is that syndecan 3 expression in the brain may control the binding of a key regulator of hunger, the anti-satiety peptide, to its transmembrane receptor, the melanocortin-4-receptor (187a). This unanticipated physiological role for a syndecan underscores the potential for cooperation between adhesion receptors and more conventional signaling receptors.

In summary, Ig CAMs, selectins, and transmembrane proteoglycans have all been linked to various signaling processes. In the latter two cases, it is still very early in terms of working out the details of the transduction pathways. Studies on signaling by Ig CAMs are more mature, but much remains to be learned. As in the case of integrins, these cell-cell adhesion molecules seem to project into the same signaling pathways that are activated by growth factor receptors; triggering of MAP kinase cascades is a common observation.

SIGNALING AND CYTOSKELETAL SCAFFOLDS

Current Concepts Regarding Signaling Scaffolds

A major focus of current research on signal transduction concerns the roles of so-called scaffold molecules in organizing and regulating signaling processes. As alluded to several times in this review, cell adhesion receptors and their associated cytoskeletal partners seem to influence various signaling pathways largely by acting as scaffolds. Here I explore this theme in greater detail. An interesting overview of the relationship between cytoskeletal architecture and regulation of tissue growth and differentiation is provided in a recent review (188). Many of the processes discussed therein may relate to the ability of actin- and tubulin-based structures to serve as signaling scaffolds.

Scaffolds can serve a variety of purposes in signal transduction processes. For example, receptor tyrosine kinases provide a prime example of how a scaffold (in this case the RTK itself) can allow a single input to ramify into several signaling pathways. Thus, when EGF triggers the ErbB1/ErbB3 RTK heterodimer, signals proceed via Grb2 to the MAPK pathway, via p85 to the PI-3-kinase/PKB pathway, and via PLCγ to the IP3/calcium pathway (189).

Another important purpose of scaffolding molecules is to prevent cross talk between different pathways that share components. A good example derives from the mating pathway in yeast, where the Ste5 scaffold protein binds the MAPKKK Ste11, the MAPKK Ste7, and the MAPK Fus3. However, Ste11 also functions in the osmotic stress response pathway in yeast. Thus the Ste 5 scaffold helps to isolate the mating and osmotic responses (190). Scaffold molecules for MAPK pathways also exist in mammalian cells. Thus JIP-1 provides a scaffold for the Jnk pathway by binding a MAPKKK (MLK, mixed-lineage kinase), a MAPKK (MKK7), and Jnk itself (191). Similarly, MP1 provides a scaffold for the Erk pathway by binding a MAPKK (Mek1) and a MAPK (Erk1) (101). Recently, another scaffold for the Jnk pathway has been defined as β-arrestin 2, which binds the MAPKKK ASK1, the MAPKK MKK4, and Jnk3 (192). An interesting aspect of the β-arrestin 2 scaffold is that it causes relocalization of the signaling components from the cytoplasm to the plasma membrane and thence to endosomes; this process seems to be critical to the signaling events in this pathway.

Since scaffold proteins cause cosegregation of components of MAPK pathways, it has often been assumed that this results in signal amplification. However, recent analyses have suggested that the situation is far more complex and that either enhancement or inhibition of signaling can occur, depending on the relative amounts of the active signaling components and the scaffold (193, 194). The presence of an excess of scaffold molecules can lead to the separation of the active components into nonfunctional complexes, and thus to reduction of signaling activity.

Localization of signaling components to specialized cellular structures is another important function for scaffold proteins. Perhaps the best examples of this relate to signaling structures in the central nervous system or sensory organs.

Thus, the multi-PDZ domain protein InaD plays a key role in organizing the visual signaling transducisome in *Drosophila* (195, 196). Another important PDZ domain-based signaling entity involves ligand-gated ion channels located in neural synapses and their associated postsynaptic density molecules. For example, the NR2 subunit of the N-methyl-D-aspartate (NMDA) receptor (a calcium permeant channel) binds to the multi-PDZ domain protein PSD-95. This molecule also associates with downstream signaling components including neural nitric oxide synthetase, a calcium regulated enzyme, as well as with SynGAP, a regulator of Ras activity, thus bringing these and other effectors into the postsynaptic density (197, 198). The NR1 subunit of the NMDA receptor also participates in signal complex assembly and can link to the cytoskeleton via association with α-actinin. In addition, NR1 binds Yotiao, which is a protein kinase A anchoring protein (AKAP), thus localizing protein kinase A to the postsynaptic density. Yotiao also binds the protein phosphatase PP1, which can inactivate the NMDA receptor (197, 199). Thus the postsynaptic density is comprised, in part, of an elaborate network of signaling proteins colocalized via various scaffolding molecules with the ligand-gated ion channels at the synapse.

No discussion of signaling scaffolds would be complete without mention of caveolae. These cholesterol-rich membrane "rafts" are organized by the caveolin 1 and 2 proteins. Caveolae seem to sequester both large and small GTPases, as well as Src-family kinases, in their inactive configurations. Thus, caveolae may serve to preassemble signaling complexes at the plasma membrane where they may subsequently be efficiently activated (200, 200a).

In summary, scaffold proteins can serve a variety of purposes including signal diversification, signal amplification, prevention of cross talk, anchoring of signaling components at specialized subcellular sites, and relocalization of active molecules during the signaling process.

The Cytoskeleton as a Signaling Scaffold

There is ever-increasing support for the concept that the cytoskeleton is a critical scaffold for various signal transduction pathways. A very comprehensive review has cataloged a large number of instances where signaling components are associated with actin- or tubulin-based structures, or where such structures influence the activity of the signaling molecule (201). Table 3 (available as Supplemental Material: follow the Supplemental Material link on the Annual Reviews homepage at http://www.annualreviews.org/) lists a number of more recent examples of cytoskeletal effects on signal transduction events. These multiple examples of cytoskeletal effects on signaling probably reflect a number of underlying mechanisms, most of which remain obscure. Here I would like to propose a mechanistic rationale for one important example of cytoskeletal effects on signaling. This concerns the ability of integrins and associated cytoskeletal components to regulate the linkage between upstream and downstream events in the receptor tyrosine kinase/Ras/MAPK pathway (see Sec. 3 and Figure 4).

I have previously shown that the signal from receptor tyrosine kinases to the Erk/MAPK is blocked below the level of Ras in cells deprived of their integrin-mediated anchorage to the substratum (88). The disruption of actin filaments with cytochalasin D (93) or the "unloading" of mechanical stress from cells in 3-dimensional matrices (202) have similar effects. The common theme seems to be a substantial perturbation of the organization of the actin filament system, particularly cortical actin (93). Further mechanistic insights into these events have been gleaned through some very recent studies from my laboratory. Thus, we found that either inhibition of protein kinase A (PKA) or activation of p21 activated kinase (PAK) permitted persistent signaling to MAPK in cells held in suspension (203). Furthermore, activated PKA can phosphorylate and inactivate PAK, while the effects of PKA inhibition or PAK activation on signaling to MAPK could be completely reversed by treatment with cytochalasin D. Our interpretation of these results is that PAK assembles—while PKA disassembles—actin-based structures required for efficient signaling. This view is compatible with evidence showing that PAK plays a key role in regulating actin assembly (67, 204), while PKA activation is known to disrupt actin filaments (4). One important aspect of PKA's effects on the cytoskeleton may be mediated through inhibition of PAK (203).

To glean additional understanding of the functional relationships between integrins, the cytoskeleton, and signaling components such as PKA, PAK, and MAPK, it is necessary to consider details of the architecture of the linkages between integrins and actin filaments (Figure 7*A*). A timely review provides an excellent summary of this subject (205). Briefly, integrin β subunit cytoplasmic tails can bind directly to talin, α-actinin, or filamin, each of which can bind directly to actin filaments. Talin and α-actinin can also bind to vinculin, another actin binding protein. Integrins also participate in the assembly of actin filaments regulated by profilin and the Arp 2/3 complex (68, 206). Thus, vinculin or zyxin can link α-actinin to VASP, an actin and profilin binding protein that participates in actin filament assembly, although the precise details of VASP's role are somewhat controversial (207, 208). Another possible linkage involves the integrin-binding protein ILK. This molecule binds the LIM domain protein PINCH (150), which binds the adapter protein NCK2, which likely binds to WASP, a key protein involved in organizing the actin-Arp2/3 assemblage. The formation of integrin/cytoskeletal linkages also leads to the recruitment of molecules potentially involved in signal transduction. Thus, FAK can bind via its c-terminal domain to talin, or via paxillin and vinculin to α-actinin (46, 47, 209, 210). The CDC42- and Rac-responsive kinase PAK can bind to WASP via association with the NCK adaptor protein (211, 212), or via the adaptor PKL and the GTPase PIX to paxillin (213).

How then does this complex integrin/cytoskeletal architecture enhance signaling downstream of receptor tyrosine kinases? One important possibility involves cosegregation of key signaling molecules, thus permitting more efficient interaction. The signaling cascade from RTKs to Ras to Erk/MAPK is not a simple linear pathway; rather it is part of a complex network that requires coordination of diverse components (See Figure 7*B*). Thus efficient activation of Raf-1 by Ras

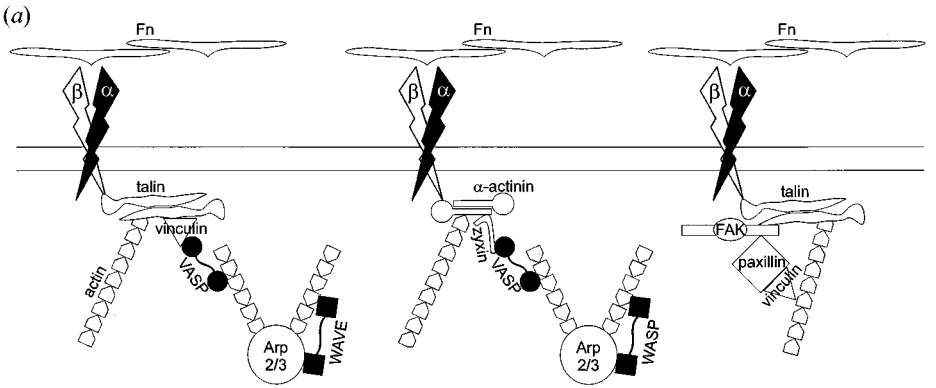


Figure 7 Cytoskeletal scaffolds in the MAP kinase cascade. (*a*) *Integrin-dependent cytoskeletal complexes*. Integrins are clustered in focal adhesion sites via interactions with matrix proteins such as fibronectin. Integrin β subunit cytoplasmic tails bind directly to talin and α-actinin; both of these molecules can link to F-actin filaments. Talin or α-actinin can also link via vinculin or zyxin to VASP, and thence to actin filaments being assembled by the Arp2/3 complex under the influence of WAVE or WASP. The FAK/paxillin complex can also bind to talin, thus indirectly associating FAK with integrins.

Con't. p. 310. (*b*) *Nonlinear signal transduction downstream of receptor tyrosine kinases*. Growth factor activation of RTKs leads to activation of Ras, recruitment and activation of Raf1, and subsequent activation of Mek and Erk. However, efficient activation of Raf requires its phosphorylation by PAK. Ras simultaneously activates PI-3-kinase, whose product PIP3 can activate guanine nucleotide exchange factors that activate the Rac GTPase. Rac then activates PAK (some of which may be bound to RTKs via an association with the NCK adaptor protein); PAK then phosphorylates Raf, thus allowing efficient signal transduction to Mek and Erk. Note that three links in this pathway are sensitive to cell anchorage: (*a*) Ras activation of Raf; (*b*) Rac activation of PAK; (*c*) PAK-NCK association (however, these links may be interdependent). (*c*) *Integrin-cytoskeletal complexes cosegregate key components of nonlinear signaling cascades*. Receptor tyrosine kinases are recruited to integrin-cytoskeletal complexes, either by association with FAK (as depicted here) or via direct binding to integrins. RTK ligation triggers the Ras/Raf/Mek/Erk cascade. Ras also activates CDC42 and Rac, which contribute to actin assembly by activating WASP and WAVE and thus the Arp2/3 complex. Rac or CDC42 also activate PAK. PAK is colocalized in the cytoskeletal complexes by associating with FAK/paxillin via PIX and PKL, or by associating with WASP via NCK and WIP. This increases opportunities for PAK-Raf interactions and thus enhanced signaling in the Erk cascade. PAK also plays a role in stabilizing actin structures by activating LIM kinase and thus inactivating its target, the actin-severing protein cofilin. The integrin-cytoskeletal complex can be disrupted by PKA, which may act by phosphorylating VASP, PAK, and other targets; PKA is localized to the complex by binding to WAVE.

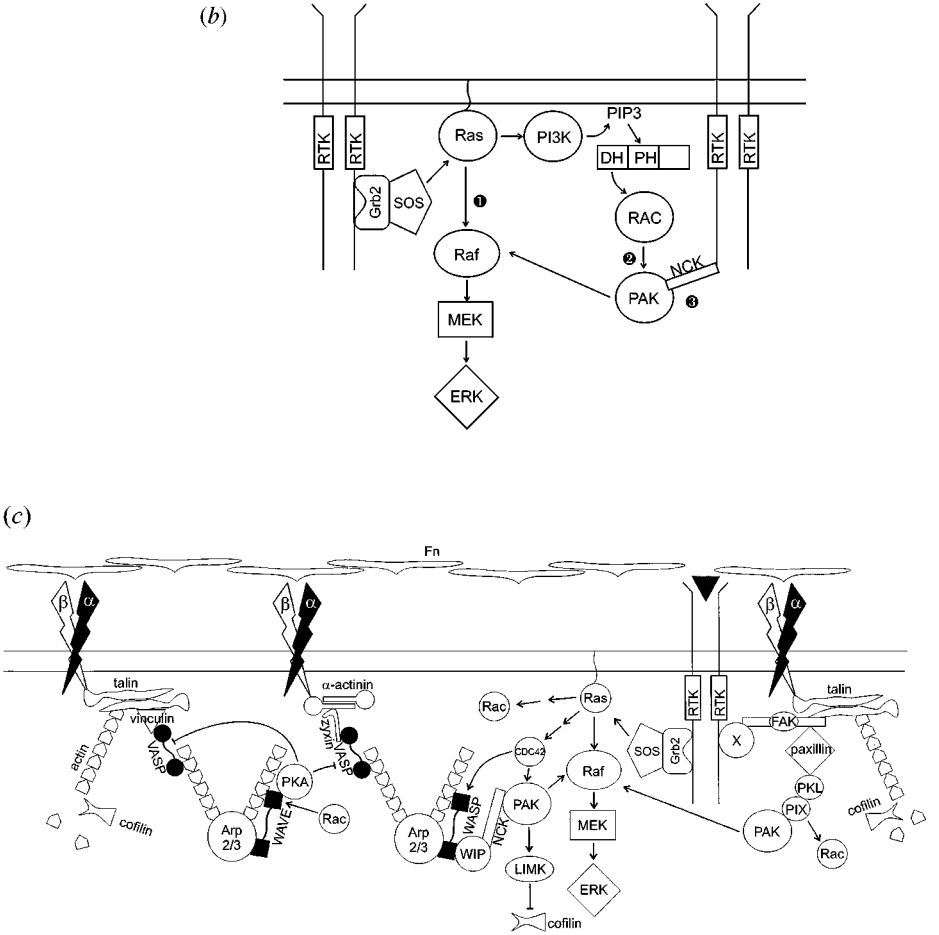


Figure 7 (*Continued*)

requires phosphorylation of Raf-1 at Ser338 by PAK (214, 215). PAK is activated by Rac, which in turn is activated by DH/PH domain exchange factors such as Vav or Sos that respond to PIP3, a product of PI-3-kinase, which in turn is activated by Ras, thus closing the circle (216). It is noteworthy that several links in this complex network have been found to depend on cell anchorage; these include the Ras-Raf connection (88), the association of Rac with PAK (76), and the association of PAK with NCK (217).

Integrin/cytoskeletal complexes can cosegregate key signaling molecules in several ways (Figure 7*C*). First, as discussed in the Integrin Modulation of Signal Transduction Cascades section above, RTKs can be recruited to these complexes either by direct interactions with integrins (83, 84) or via interactions with FAK (86). Second, the complexes provide a site for the concentration of PAK, either bound to

WASP via NCK, or bound to paxillin via PIX and PKL. Recent studies have provided clear evidence that activated PAK is associated with cytoskeletal structures (218). The close juxtaposition of PAK and the Ras-Raf complex should allow for highly efficient activation of Raf and subsequently of Mek and Erk/MAPK. Activated PAK also contributes to the stability of the integrin/cytoskeletal complexes by activating LIM kinase, which in turn suppresses the actin-severing activity of cofilin (67). However, it is interesting that the integrin/cytoskeletal signaling complex may also contain the seeds of its own destruction. Recent results demonstrate that WAVE, a member of the WASP family of actin organizing proteins, is an AKAP (219), thus bringing PKA into the vicinity of the cytoskeletal complex. Thus, activation of PKA could disassemble the complex by phosphorylation of VASP or other targets. Finally, an interesting but speculative notion is that the integrin/cytoskeletal complex may bind scaffolding proteins for the Erk/MAPK cascade such as MP1 (101) or KSR (220). While there is no direct evidence of this for Erk pathway scaffolds, there is clear evidence that RACK-1, a scaffold for activated protein kinase C, binds directly to integrin cytoplasmic tails (221). Thus, the integrin/cytoskeletal complex may provide the organizing framework that allows efficient activation of complicated, nonlinear signaling cascades such as the RTK/Ras/Erk pathway. As mentioned in the Integrin Modulation of Signal Transduction Cascades section, integrin/cytoskeletal complexes also regulate the shuttling of activated Erk to the nucleus. Thus, integrin-based structures fulfill several of the major purposes of signaling scaffolds, including signal amplification, localization of components to specialized subcellular sites (the focal contact), and relocalization of active components during the signaling process.

SUMMARY

The interaction of transmembrane cell adhesion receptors, such as integrins, cadherins, Ig CAMs, and selectins, with their macromolecular ligands in the extracellular matrix or on other cells, inevitably has profound effects on multiple cellular signaling processes. A key role for the adhesion molecules seems to be to organize membrane-proximal cytoskeletal structures that then serve as scaffolds for signaling cascades. The precise compositions of the cytoskeletal scaffolds organized by various adhesion receptors will no doubt differ. This would then provide considerable biochemical and biological diversity in terms of the qualitative and quantitative impacts on signaling cascades. The challenge for the immediate future is to dissect the detailed molecular interconnections between important signaling pathways such as the RTK/Ras/MAPK cascade and key scaffold structures such as the integrin-based focal complex.

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