Integrin signaling and cell growth control

Alan Howe, Andrew E Aplin, Suresh K Alahari and RL Juliano

Integrins contribute to cell growth by providing a physical linkage between cytoskeletal structures and the extracellular matrix, and also by participating in various signal transduction processes. The interaction of integrins with matrix ligands can generate signals in and of itself, and can also modulate signals instigated by soluble factors such as peptide mitogens. Cellular events affected by integrin-mediated signaling include motility, cell division, differentiation and programmed cell death. Elucidation of how integrin-mediated cell adhesion controls cell growth is likely to be of fundamental importance in understanding complex biological processes, such as tissue morphogenesis and tumor progression.

Address
Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, NC 27599, USA

Current Opinion in Cell Biology 1998, **10**:220–231

http://biomednet.com/elecref/0955067401000220

Abbreviations

CDK	cyclin-dependent kinase
ECM	extracellular matrix
EGF	epidermal growth factor
FAK	focal adhesion kinase
ILK	integin-linked kinase
JNK	c-Jun amino-terminal kinase
MAPK	mitogen-activated protein kinase
MEK	MAPK kinase
PDGF	platelet-derived growth factor
PI 3-K	phosphoinositide 3-OH kinase
PIP_2	phosphatidyl-4,5-bisphosphate
Rb	retinoblasoma
RTK	receptor tyrosine kinase

Introduction

The integrin family of cell surface receptors has long been known to have an essential role in the physical aspects of cell adhesion: they are the principal receptors for extracellular matrix (ECM) proteins and serve as transmembrane bridges between the ECM and actin-containing filaments of the cytoskeleton. The organization of integrin-associated actin structures is regulated by complex mechanisms governed by members of the Rho family of Ras-related GTPases. Indeed, the very ability of integrins to bind ECM ligands is also regulated by Rho and Ras family members, as well as by other proteins found within the membrane, within the cytoskeleton, or in direct association with the integrins themselves. Coordinate regulation of integrin-binding affinity and actin filament dynamics is of fundamental importance not only to cell adhesion, but to overall cellular architecture and cell motility, and to integrin-related signaling events as well.

The field of adhesion research has been energized recently by the realization that integrins and other adhesion receptors such as cadherins, selectins and immunoglobulin-family receptors, have a vital role in signal transduction processes. Integrin-mediated signaling can be roughly divided into two descriptive categories. The first is 'direct signaling', in which ligation and clustering of integrins is the only extracellular stimulus. Thus, adhesion to ECM proteins can activate cytoplasmic tyrosine kinases (e.g. focal adhesion kinase [FAK]) and serine/threonine kinases (such as those in the mitogen-activated protein kinase [MAPK] cascade), induce ionic transients (e.g. Ca^{2+}, Na^{+}/H^{+}), and stimulate lipid metabolism (e.g. phosphatidylinositol-4,5-bisphosphate (PIP_2) synthesis). Although many such direct signaling events have been described [1•], the mechanisms underlying these events, as well as their biological role, have not been fully resolved. The second category of integrin signaling is 'collaborative signaling', in which integrin-mediated cell adhesion modulates signaling events initiated through other types of receptors, particularly receptor tyrosine kinases (RTKs) that are activated by polypeptide growth factors. Several potential mechanisms for integrin regulation of growth factor signaling have emerged recently, with the locus of regulation placed either at the level of RTK activation or within the downstream signaling cascade. In all cases, however, integrin-mediated adhesion seems to be required for efficient transduction of signals with origins at the cell surface and targets in the cytosol or nucleus.

Integrin-mediated cell adhesion impacts on two key aspects of growth regulation. First, integrin-mediated adhesion can influence the activity of the basal cell-cycle machinery, consisting of various cyclin-dependent kinase (CDK) complexes. Second, integrin-mediated anchorage is also a key regulator of apoptosis. This last aspect has been covered in detail recently in this series [2•], but will be revisited briefly here. Presumably, the integrin signaling events mentioned above are implicated in anchorage regulation of the cell cycle and of apoptosis; however, the mechanistic linkages are just beginning to emerge.

Regulation of integrin–cytoskeletal complexes

Integrin signaling and signal modulation involves the productive engagement of integrins with their ECM ligands, lateral clustering of integrins in the plane of the membrane, and the formation of organized complexes between integrins and cytoskeletal proteins. Quite often, the connection between integrins and the actin cytoskeleton occurs in structures known as focal adhesions, which contain a complex mixture of structural and signaling proteins [3•]. Thus, an important consideration in understanding integrin signaling is the process underlying the formation of integrin–cytoskeletal complexes.

Like many other receptors, integrins can exist in various states of ligand-binding ability. Modulation of the strength of integrin–ligand interactions can occur through regulating the binding activity of individual integrins (affinity modulation or integrin 'activation') as well as through integrin clustering (avidity modulation). Integrin-binding affinity is controlled by biochemical events within the cell, including the activity of small GTPases of the Rho and Ras families (Figure 1). For example, transfection with a constitutively active form of R-Ras increases the binding affinity of the integrins αvβ3, α4β1, and α5β1 [4•]. In contrast, transfection of activated H-Ras, or of its downstream kinase Raf-1, inhibits the ability of co-expressed αIIbβ3 to become activated [5••]. In another example, β1 and β2 integrin-mediated adhesion in lymphoid cells seems to be regulated via the Rho GTPase [6], although it is not clear if changes in affinity or in avidity are involved. Recently, Keely *et al.* [7•] have reported that activated forms of Rac and Cdc42 dramatically increase the α2β1-mediated motility and invasiveness of breast epithelial cells in a manner dependent on phosphoinositide 3-OH kinase (PI 3-K), implicating these additional Rho family members as regulators or mediators of integrin activities.

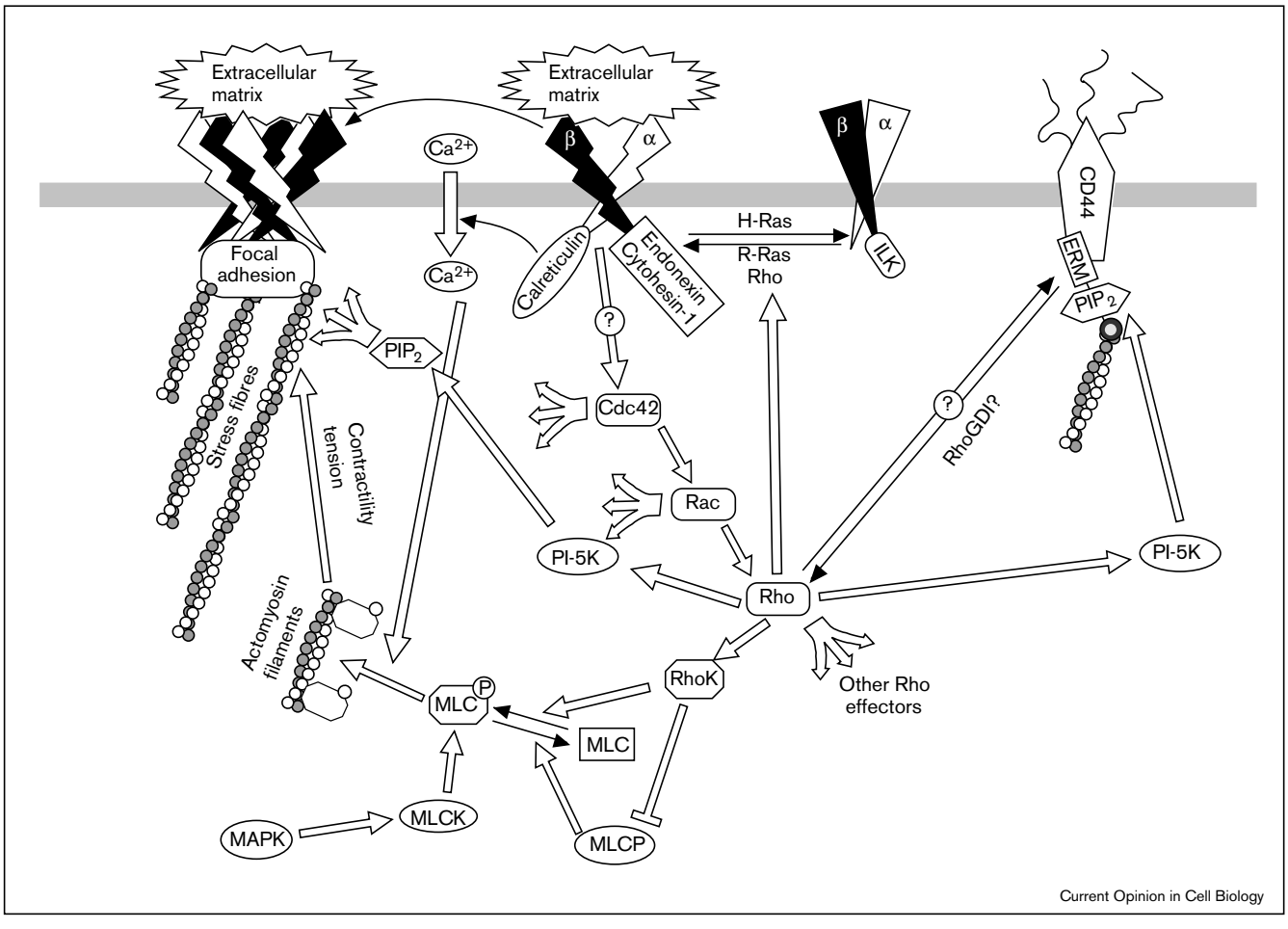
Another important set of regulators of integrin function is comprised of a variety of proteins that bind directly to integrin cytoplasmic or extracellular domains. Several of these molecules can modulate integrin affinity and/or integrin interaction with the cytoskeleton (Table 1). For example, overexpression of cytohesin-1, a β2-subunit-binding protein, can enhance αLβ2 binding to its ligand, ICAM-1 [8•], while the β-subunit-binding integrin-linked kinase (ILK) is likely to negatively regulate integrin–cytoskeletal association [9•]. An α-subunit-binding protein, calreticulin, was recently shown to be essential for effective integrin-mediated cell adhesion [10•], and may act by modulating an integrin-triggered influx of extracellular Ca^{2+} and subsequent Ca^{2+}-dependent cytoskeletal events (see Figure 1). In addition to interactions occurring within the cytoplasm, it is also clear that integrin functions can be modulated by proteins that interact with integrin extracellular and/or transmembrane domains (Table 1); this is likely to be a topic of increasing importance. At this point it is not known whether there are any links between the effects of the various integrin-binding proteins and Rho/Ras mediated regulation of integrin affinity and/or avidity. However, it is interesting to note that cytohesin-1 seems to be a nucleotide exchange factor [11].

A major aspect of the function of Rho family GTPases involves working with integrins to regulate the assembly of actin-containing cytoskeletal structures, with Cdc42, Rac, and Rho controlling the formation of filopodia, lamellipodia, and stress fibers and focal adhesions, respectively [12•]. The assembly of mature focal adhesions and fully developed stress fibers requires both Rho GTPases (in part, for generation of contractility) and integrin-mediated anchorage (for counter-tension) (see below and Figure 1). However, a certain amount of actin organization can occur independently of integrin-mediated attachment; specifically, Rac can polymerize actin in lamellipodia and Rho can induce actinomyosin bundles in the cytoplasm in the absence of integrin engagement [13]. An important aspect of Rho-mediated cytoskeletal organization has come from studies involving Rho-kinase, a Rho-responsive serine/threonine kinase that phosphorylates and inactivates the myosin light chain phosphatase (MLCP), and also directly phosphorylates the myosin light chain [14•] (see Figure 1). Enhanced light-chain phosphorylation leads to activation of myosin ATPase activity and actinomyosin contractility. It has been proposed that this contractility generates tension which contributes to stress fiber formation and induces aggregation of integrins into focal adhesion structures [3•], thus providing a neat synthesis of current structural and biochemical observations concerning assembly of these structures.

In addition to their roles in assembly of actin cytoskeletal structures, Rho family members can stimulate signal transduction cascades leading to activation of transcription factors such as ATF2, c-Jun, SRF, and Elk [14•]. Interestingly, experiments using effector domain mutants of Rac and Cdc42 that differentially alter their interaction with various downstream effectors have shown that activation of the cascade(s) leading to transcriptional activation is not essential for mitogenesis and transformation [15,16]. This might suggest that it is the ability of Rho family proteins to work with integrins to assemble cytoskeletal complexes that is most important for their function in cell growth and transformation. Realistically, however, the correlation between Rho family cytoskeletal reorganization and transforming ability is less than perfect [14•].

In summary, the organization and function of integrin-mediated adhesion sites and actin-based cytoskeletal assemblies involves a complicated interplay between multiple structural and regulatory proteins (Figure 1). This includes Rho and Ras family GTPases, proteins that bind directly to integrins, the structural elements of focal contacts such as talin and vinculin, as well as the components of the actinomyosin contractile machinery. Given the complex skein of events linking integrins, Rho-family molecules, and the actinomyosin-based cytoskeleton, it seems reasonable to ask whether integrin ligation and/or clustering can affect the activation state of Rho GTPases. Evidence for this has been elusive; however, work in progress suggests that Cdc42 may be activated through integrins (M Schwartz, personal communication). This would provide a satisfying closure of the loop between the key players involved in integrin activation, adhesion and cytoskeletal assembly.

Figure 1



Regulation of integrin–cytoskeletal complexes. In this figure activated integrins are depicted with a 'lightning bolt' like structure. Integrin affinity and avidity are regulated, in part, by proteins that interact directly with integrin cytoplasmic domains, such as cytohesin-1 and β3-endonexin. Some integrin-binding proteins, (e.g. calreticulin, ILK) may exert their effects by modulating adhesion-triggered events that impinge upon integrin–cytoskeletal interactions (such as Ca^{2+} influx). Biochemical pathways involving various members of the Ras superfamily of GTPases also regulate integrin affinity. For example, both R-Ras- and Rho-mediated pathways have been implicated in integrin activation, while activity of H-Ras (and its downstream effector Raf) can oppose integrin activation. However, the various mechanisms involved are not fully understood. The Rho GTPase is of particular importance in the formation of higher-order integrin–cytoskeletal structures,such as focal adhesions and stress fibers, and thus is likely to function through avidity modulation. An important effector for Rho in this regard is PI 5-kinase, the principal mediator of PIP_2 synthesis. PIP_2 binding is a regulatory mechanism common to several proteins involved in assembly of the actin cytoskeleton and associated structures, including vinculin, α-actinin, gelsolin, and profilin. Another important mechanism in Rho-mediated formation of integrin-associated structures is the generation of tension through actinomyosin-based contractility, which is accomplished through the activity of Rho kinase. Rho kinase phosphorylates and inactivates myosin light chain phosphatase (MLCP), and also directly phosphorylates the myosin light chain at the same site targeted by myosin light chain kinase (MLCK). The net result is hyperphosphorylation of the myosin light chain (MLC) which stimulates its myosin ATPase activity, leading to actinomyosin contractility and formation of focal adhesions and stress fibers. Recently, MAPK activity was shown to phosphorylate and activate MLCK, raising the intriguing possibility that Rho- and MAPK-mediated signals may cooperate to stimulate contractility. The ability of Rho, as well as Rac, to instigate actin reorganization also appears to involve moesin and possibly other members of the ezrin/radixin/moesin (ERM) family of proteins [82•]. ERM proteins mediate the attachment of actin microfilaments to transmembrane proteins including CD44 and ICAMs [83]. Communication between ERM proteins and Rho may occur through PI 5-kinase, as PIP_2-binding by ERM proteins regulates ERM–actin interaction, placing ERMs downstream of Rho. However, ERMs may also lie upstream of Rho, as radixin and other ERMs bind the Rho GDP-dissociation inhibitor (RhoGDI), precluding its association with Rho, which in turn allows for Rho GTP loading and activation [84]. This illustrates what is emerging as an important aspect of integrin function, namely the interaction/communication between integrins and other cell surface adhesion receptors.

Integrin-mediated activation of the MAPK cascade

Two sets of direct integrin-mediated signaling events have attracted a great deal of attention recently; the first is activation of the cytoplasmic tyrosine kinase FAK, while the second is activation of the MAPK cascade. The autophosphorylation of FAK was among the first integrin-mediated signaling event to be identified. Over the last few years we have learned a good deal about FAK, its protein binding partners, and its relationship

Table 1

Integrin-binding proteins.

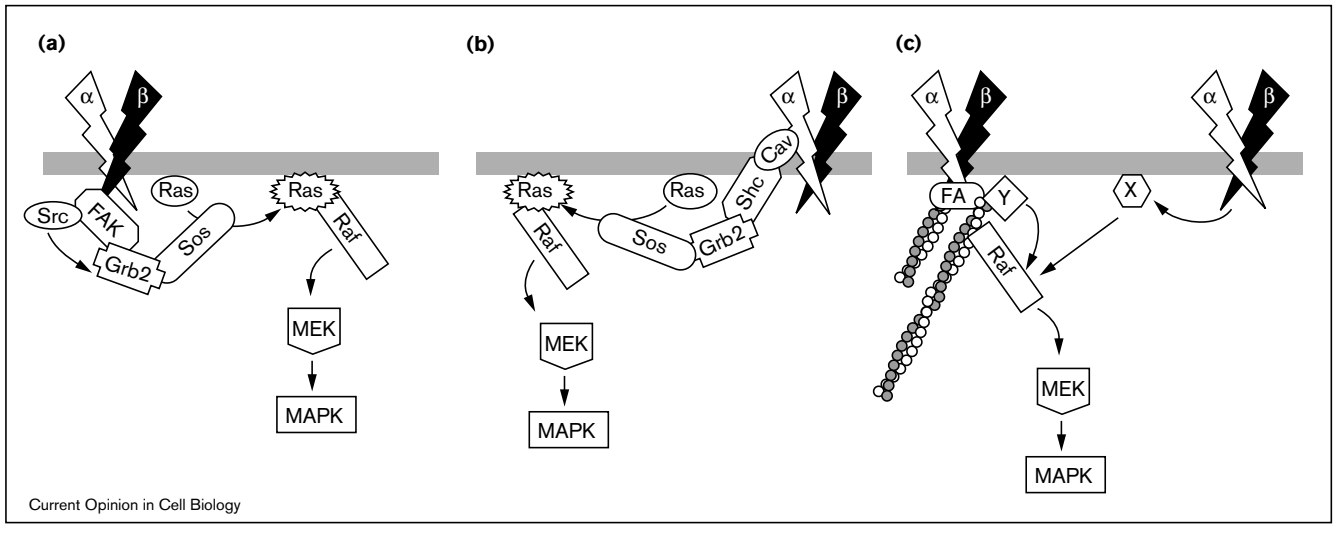
Binding target	Binding protein	Characteristics	Function	Reference
β subunits				
β2	Cytohesin-1	PH- and SEC7 domains	Enhances αLβ2 binding to ICAM-1	[8•]
β3	Endonexin	Small cytoplasmic protein	Affinity modulation	[85]
β4	p27BBP	Intermediate filament association	?	[86•]
β	ILK-1	Serine-threonine kinase	Negatively modulates integrin–cytoskeleton interactions?	[9]
β	ICAP1	Phosphoprotein	Affinity modulation? Integrin–cytoskeleton modulation?	[87]
β	CD98	Transmembrane protein	Affinity modulation	[88]
α subunits				
Multiple αs	Calreticulin	Calcium regulatory protein	Modulates integrin-triggered Ca^{2+} influx and Ca^{2+}-dependent cytoskeletal events	[10•]
αIIb	CIB	Similarities to calmodulin and calcineurin	Affinity modulation?	[89]
Trans-membrane domain	TM4 proteins	Four membrane-spanning helices	Signal transduction?	[90]

to Src-family kinases [17•]. It is apparent that FAK, Src, and the adaptor proteins $p130^{CAS}$ and paxillin form a distinct quaternary signaling and structural unit at cell adhesion sites whose assembly is normally initiated by autophosphorylation of FAK [17•,18]. However, the biological role of FAK remains elusive. At one point FAK activation was thought to be important in focal contact assembly; however, the advent of $FAK^{-/-}$ cells with relatively normal focal contacts [19•], as well as other observations [20,21] suggests a limited role for FAK in stress fiber and focal contact assembly. Instead, it seems likely that FAK and its associated proteins have a key role in cytoskeletal turnover and cell motility [22,23], and may also be important mediators of cell survival (see below).

The clustering of integrins, caused either by antibodies or by adhesion to substratum-bound ECM ligands, leads to the activation of elements of the MAPK cascade. However, the mechanistic basis of integrin-mediated MAPK activation is uncertain, with three models currently vying for support. The first model posits a close parallel with the mechanism used by many growth factors to activate MAPK. In this model FAK would substitute for the growth factor RTK; thus the pathway would proceed from FAK, to SH2-domain adaptor proteins, to guanine nucleotide exchange factors, to Ras, and thence to the downstream kinase cascade of Raf-1, MEK, and MAPK (Figure 2a). There is a good deal of evidence in support of a key role for FAK in integrin-mediated MAPK activation. Adhesion-mediated autophosphorylation of FAK leads to Src recruitment, further tyrosine phosphorylation of FAK, and of $p130^{CAS}$ and the binding of SH2-domain proteins including Shc and the Grb2/Sos adaptor protein-exchange factor complex [24•,25–27]. The formation of the FAK/Src/CAS/Grb2 assembly suggests the possibility of futher signaling to MAPK. In addition, one study showed that overexpression of FAK led to a Src- and Ras-dependent activation of MAPK [25]. However, a number of other studies militate against a direct involvement of FAK in integrin-mediated MAPK activation. Thus, overexpression of a constitutively activated form of FAK in epithelial cells failed to activate MAPK [28•], while overexpression of a dominant-negative form of FAK in fibroblasts blocked FAK activation, but failed to block MAPK activation [29•]. One concern with all of these studies is whether significant overexpression of a putative signaling protein can give a quantitatively inaccurate impression of its true physiological role.

Another study also argues against a role for FAK in MAPK activation, and instead suggests a novel alternative mechanism for integrin signaling (Figure 2b). Thus, the work by Wary *et al.* [30••] delineated a pathway leading from integrins, to the SH2- and PTB(phosphotyrosine binding)-domain-containing adaptor protein Shc, to nucleotide exchange factors and Ras, and thence to the downstream kinases of the MAPK cascade. There is no evident role for FAK in this proposed pathway. An important aspect of this work is the suggestion that integrins signal via an interaction of the external and transmembrane domains of their α subunits with the membrane protein caveolin, which in turn interacts with Shc. This is quite distinct from other models where the cytoplasmic tails of integrins are postulated to have a key role in signaling. Interestingly, a recent report has linked caveolin overexpression to an abrogation of

Figure 2



Mechanisms of integrin-mediated activation of the MAPK cascade. Evidence suggests at least three mechanisms through which integrin-mediated cell adhesion can trigger MAPK activation. The mechanism depicted in **(a)** involves an approximate recapitulation of growth factor/RTK signaling, with FAK acting as a surrogate tyrosine kinase domain. In this model, integrin engagement leads to FAK autophosphorylation on Tyr397, generating a binding site for the SH2 domain of Src. Src then phosphorylates FAK at several positions including Tyr925, which serves as a point of purchase for SH2-containing adaptor proteins such as Grb2. Binding of Grb2 to FAK results in membrane localization of Sos, a guanine nucleotide exchange factor, which in turn promotes GTP loading and activation of Ras. Activated Ras binds Raf and localizes it to the membrane, where it is activated by a complex and poorly understood mechanism. Once activated, Raf phosphorylates and activates the kinase MEK, which then does the same to MAPK. However, substantial evidence indicates that integrin-mediated MAPK activation can occur independently of FAK. **(b)** One mechanism for FAK-independent activation of MAPK by integrins involves the interaction of integrin α subunits with the membrane protein caveolin (Cav). Interestingly, this interaction involves the transmembrane and extracellular juxtamembrane domains, rather than the cytoplasmic domain, of the α subunit. The Shc adaptor protein associates, perhaps indirectly, with caveolin, and is tyrosine phosphorylated and recruited into integrin-associated complexes. Tyrosine-phosphorylated Shc is then bound by a Grb2–Sos complex, and activation of MAPK occurs through the canonical Ras-mediated pathway. **(c)** There is substantial evidence for Ras-independent mechanisms for integrin-mediated MAPK activation. It is well established that membrane localization of Raf is important for activation, but the membrane-associated component(s) responsible are as yet unidentified. Furthermore, it is also known that once activated, Raf exists in a detergent–insoluble membrane/cytoskeletal complex. Although the mechanism of Ras-independent, integrin-mediated MAPK activation is unclear, integrin-mediated cell adhesion may regulate the unknown, membrane-associated machinery responsible for Raf activation (X), simply present Raf to that machinery, or may effect a distinct signaling event (Y) that leads to Raf activation.

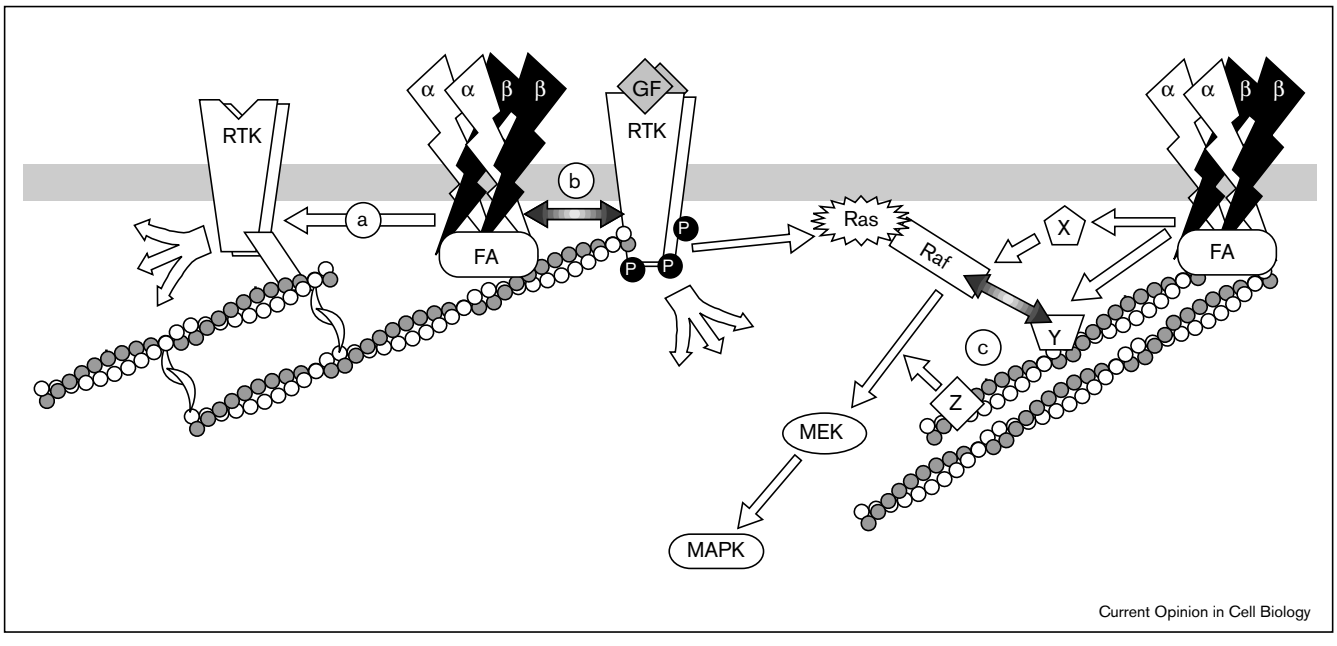
anchorage-independent cell growth [31], lending further support to a possible connection between integrins and caveolin.

Both of the models described above include Ras as a critical link between integrins and MAPK. This connection has also been supported by other studies using N17 Ras as a dominant negative to block adhesion activation of MAPK [31,32•,33]. However, studies from our laboratory, using the amino-terminal domain of Raf as a powerful and specific dominant-negative inhibitor of Ras signaling, indicate the existence of a Ras-independent component of integrin-mediated MAPK activation [34•]. Additional studies have shown that integrin-mediated adhesion can activate versions of Raf-1 that have mutations in their Ras-binding domain (A Howe and RL Juliano, unpublished observations), further supporting the notion of a Ras-independent aspect of integrin signaling. The mechanism underlying the putative Ras-independent integrin signaling pathway to MAPK is unclear. The biochemistry of Raf activation is complex and not fully understood [35•]; however, a key aspect of the process is the Ras-mediated recruitment of Raf-1 to the plasma membrane. Our observations suggest that some component of the integrin–cytoskeletal complex found at adhesion sites may be able to partially or fully substitute for Ras in the recruitment of Raf-1 to the membrane environment (Figure 2c).

In summary, the mechanistic basis for the commonly observed integrin-mediated activation of MAPK is somewhat controversial at present. Some of the observational differences may be due to the existence of several overlapping signaling pathways, with one pathway or another predominating in a particular cell type or experimental situation.

Perhaps even more important than the mechanistic details of the integrin–MAPK direct signaling pathway is consideration of its biological significance. To a substantial degree, integrins trigger a set of downstream events (activation of Raf-1, MEK, MAPK) similar to those triggered by peptide mitogens. However, integrin-mediated adhesion itself does not result in mitogenesis (the key role of

Figure 3



Integrin modulation of growth factor signaling. This figure depicts three mechanisms whereby integrin-mediated cell adhesion can affect signals elicited by growth factor receptors. **(a)** Engagement of integrins can lead to a ligand-independent increase in RTK (e.g. PDGFβ-R) tyrosine phosphorylation; this is inhibited by cytochalasin D, suggesting that at least some level of cytoskeletal organization is required. Beyond this, nothing is known about the mechanism underlying the effect. **(b)** Integrins can physically associate with growth factor receptors (evidenced by co-immunoprecipitation and immunofluorescent co-localization), although the interaction (symbolized by the two-headed arrow) may involve other proteins. **(c)** Integrins can modulate signaling effectors downstream of RTKs. In some cases, when cells are anchored to ECM proteins, the signaling cascade instigated by peptide growth factors is unbroken between the RTK and MAPK. However, in non-adherent cells, signal transmission is interrupted, with the breach occurring at the level of Raf or MEK. The mechanism by which integrin-mediated cell adhesion permits efficient signal transduction is currently unclear, but may involve regulation of an anchorage-dependent co-activator (e.g. the membrane-associated Raf activator [X]) or establishment of architectural scaffolds (Y,Z) which provide efficient spatial arrangement of signaling components.

adhesion in collaborating with soluble growth factors will be considered below). Within tissues, most cells have their integrins continually engaged with the surrounding ECM; thus major global changes in MAPK activity within the cell due to integrins are unlikely. However, cells do make and break adhesions with the ECM during cell migration and tissue remodeling; thus local changes in MAPK activity within the cytoplasm may be quite likely. This hints at the possibility that a key biological role for integrin-triggered MAPK activation may be local regulation of adhesion, contractility and cell movement, rather than global regulation of mitogenic signaling. Supporting this view is the exciting observation that MAPK can regulate myosin light chain kinase [36•] and thus influence actinomyosin contractility (Figure 1). The observations of Hughes *et al.* [5••], discussed above, suggests a possible feedback loop between integrin-mediated adhesion and the MAPK cascade that may also act locally to regulate cell adhesion. However, it also remains possible that a low level of MAPK activity, due to breaking and reforming of integrin-mediated adhesions, may play a permissive part in nuclear events leading to cell growth.

Modulation of growth factor signaling by integrins

In addition to directly generating signals, integrins can modulate signaling responses to soluble growth factors and differentiation-inducing agents (Figure 3). The best known incarnation of this is the phenomenon of anchorage-dependent growth, which has been studied for nearly 30 years. Recently, many aspects of anchorage control of cell growth have been attributed to integrin-mediated adhesive interactions with the ECM, thus placing anchorage dependence in a biochemical context [37•,38•]. Modultion of growth factor signaling by adhesion has been observed in a number of cell types, indicating that the phenomenon seems to be quite general [1•].

The most direct mechanism for integrin modulation of mitogen signaling involves binding and activation of RTKs by integrins. Thus, Sundberg and Rubin [39] have described an integrin-mediated activation of the platelet-derived growth factor (PDGF)-β receptor that is independent of ligand. Although this may seem somewhat surprising, other adhesion receptors (NCAM,

N-cadherin) have also been reported to activate RTKs in a ligand-independent fashion [40]. Ligand-dependent functional associations between integrins and RTKs have also been reported [1•]. For example, Schneller *et al.* [41•] recently found that a highly tyrosine-phosphorylated fraction of PDGF receptor associated with αvβ3, and that adhesion to vitronectin, a ligand for this integrin, potentiated mitogenic signaling. Clustering of integrins by beads coated with anti-integrin antibody has been reported to result in co-clustering of epidermal growth factor (EGF) receptor, receptor activation, and enhanced EGF-dependent activation of MAPK [42•]. In the two studies mentioned above, it is unclear whether the integrins and RTKs associate directly or whether other proteins are involved, as seems more likely. It is intuitively satisfying to think of RTKs associating with integrins or with other components of integrin adhesion sites, thus increasing the probability of RTK dimerization and activation, and enhancing the efficiency of signal transduction; however, the generality of this mechanism is uncertain at present.

In the examples described thus far, integrin regulation of growth factor signaling occurred at the level of the RTK. However, other studies have reported that integrin engagement can affect events further down the mitogenic signaling cascade. For example, Lin *et al.* [43•] showed that integrin-mediated cell adhesion influenced the efficiency of signal transduction between RTKs and MAPK. In comparing suspension cells with integrin-anchored cells, upstream events taking place at the membrane were similar, including RTK tyrosine phosphorylation and GTP loading of Ras; however, the activation of Raf and of the downstream kinases MEK and MAPK were markedly impaired. This suggests that integrin-mediated adhesion enhances the efficiency of the MAPK cascade by participating in Raf recruitment and activation. Observations from another group have also suggested that integrin-dependent adhesion regulates events in the cytoplasmic arm of the MAPK cascade; however, in this case the break in the signaling pathway was found to occur at the level of MEK rather than Raf [44•]. An interesting (though unsubstantiated) possibility is that one or more components of integrin adhesion sites act as a scaffold to organize elements of the cytoplasmic MAPK cascade in a manner that mimics the function of the Ste7p protein in the yeast MAPK cascade [45].

There is currently some controversy about the precise site of integrin regulation of the RTK–Ras–MAPK pathway. However, it is quite conceivable that integrins may regulate this pathway at several levels, with the predominant locus of regulation differing under various experimental conditions. In addition to the examples above, there have been several other recent reports concerning cooperation between integrins and soluble growth, motility or differentiation factors [46•,47–50]; however, there have also been counterexamples where such cooperation was not observed [51].

In summary, it has recently become evident that integrin-dependent adhesive structures often have a major role in modulating the efficiency of growth factor signaling through RTKs and the MAPK cascade. This may be of great importance in anchorage regulation of the cell cycle and of apoptosis, but details of the connections between the signaling cascades and cell growth have yet to be resolved. The mechanistic aspects of the integrin-mediated signal modulation process also remain rather hazy at present, but deserve intensive scrutiny because of the biological significance of this set of events. In a teleological sense it is not surprising that a cell would use both positional information about its relation to the ECM, as well as information about the availability of growth factors, to determine when to enter the cell cycle. What is interesting about this process is the mechanism for coordinating biochemical and positional signals. It is conceivable that nature might have evolved two quite distinct signaling pathways for soluble factors and for positional cues; however, what seems to exist is a situation whereby adhesion receptors provide positional information by modulating the signaling cascades used by receptors for soluble factors. Nonetheless, it seems reasonable to keep an open mind about the possibility of growth regulatory signaling that is unique to adhesion receptors. One interesting and plausible mechanism concerns direct mechanical links between the cytoskeleton and functional complexes in the nucleus; this is discussed in detail in the review by D Ingber and co-workers (see this issue, pp 232–239).

In addition to modulating the activity of growth factor receptors, a growing body of literature demonstrates that integrins can regulate and be regulated by other cell adhesion molecules, including cadherins [52], selectins [53], and other integrins [54] (these are a few selected recent examples). Communication between different families of adhesion molecules is also suggested by the existence of common cytoplasmic regulators. In this way, Rho GTPases, the well established regulators of integrin-associated actin structures, are also proving to be essential regulators of cadherin-mediated cell–cell adhesion [55,56]. It seems likely that several types of cell-surface adhesion receptors, each recognizing a unique facet of adjacent cells or matrix, will conspire to govern cell growth and division in response to mitogens. Indeed, the dominance of contact inhibition of growth over the mitogenic permissivity afforded by anchorage to ECM attests to a higher order of growth regulation by the cellular microenvironment.

Integrins, cell adhesion and cell-cycle control

One of the defining characteristics of transformed cells is that they can respond to soluble growth factors and

proceed through the cell cycle while in suspension, whereas normal cells require anchorage to a substratum. In recent years some of the molecular and biochemical events underlying anchorage dependence of cell-cycle control have begun to come to light, suggesting an important role for integrin-mediated cell adhesion [37•].

A key event in the G1 phase of cell-cycle progression is hyperphosphorylation of the retinoblastoma (Rb) protein, leading to release of E2F-family transcription factors from their complex with Rb; in normal cells this event requires both soluble mitogens and cell anchorage [37•,57–59]. The precise identity of the cyclin–CDK complexes that regulate Rb phosphorylation in response to adhesion is somewhat uncertain at this point. Some reports indicate that the expression of cyclin D1 mRNA and protein is strongly adhesion dependent [37•,60•], and emphasize a key role for cyclin D1–CDK4,6 complexes [61]. However, another study found no difference in levels of cyclin D1 and D3 or of CDK4 or CDK6 proteins, nor in cyclin D–CDK6 kinase activity, in anchored compared with suspended cells [62•]. Several investigators have observed anchorage dependence of the activity of cyclin E–CDK2 complexes due to changes in the levels of associated CDK inhibitor proteins such as p21 and p27. In addition, in some cell types cell-cycle progression seems to be blocked later on in the cycle due to cyclin A-dependent functions [37•].

In suspended cells, the expression of p21 is increased and the turnover of p27 is decreased [49,59,60•]; this leads to an increase in the amount of p21 and p27 associated with CDK complexes and thus a reduction in kinase activity. A recent report has suggested that p53 becomes activated in response to disruption of cell–ECM interactions, leading to a p53-mediated induction of p21 expression and subsequent G1 arrest; interestingly, in $p53^{-/-}$ fibroblasts, disruption of cell anchorage did not affect p21 levels (as expected) but rather induced p27 [63]. In epithelial cells a novel connection has emerged between anchorage control of cell cycle and of apoptosis [64]. Thus, loss of anchorage resulted in reduced G1 CDK activity and hypophosphorylation of Rb; in contrast to the situation in fibroblasts, accumulation of hypophosphorylated Rb triggered apoptosis rather than cell-cycle arrest. In epithelial cells lacking functional Rb anchorage-dependent apoptosis was not observed, while overexpression of Rb enhanced the apoptotic effect. This study illustrates that cell anchorage can affect growth control pathways very differently in various cell lineages. Recently, there have been a few direct studies of the interplay between oncogenes and anchorage in cell-cycle regulation [58,65]. These studies have further emphasized the complexity of the process, with the presence of the oncogene affecting both the timing and the controlling mechanism of cell-cycle traverse.

At present, there is a poorly explored 'gray zone' between our growing understanding of integrin-mediated signaling pathways and our understanding of how anchorage influences the components of the cell-cycle machinery. Presumably, the fact that integrin-mediated adhesion can strongly influence the transduction efficiency of signaling cascades triggered by soluble mitogens will provide an important link to anchorage regulation of the cell cycle. However, the precise connections remain to be elucidated.

Integrin-mediated adhesion and apoptosis

Programmed cell death or apoptosis is an important regulator of the growth of both normal and transformed cells. Recently it has become clear that integrin-mediated cell anchorage has a vital role in the control of apoptosis mark 1[2•]; indeed a new term 'anoikis' has been coined to describe programmed cell death caused by loss of anchorage. As the role of integrins in apoptosis has been explored over the last couple of years, there has also been remarkable progress in understanding other elements of the pathway. Thus, the caspase family of proteases has been shown to have a key role in programmed cell death [66•]; these enzymes are usually thought to be downstream effectors, but it is becoming clear that they can also modulate upstream events in cell death pathways. Different members of the Bcl-2 family of proteins either positively or negatively regulate apoptosis; a major aspect of this involves control of caspase activity [67•]. Activation of the Jun-kinase (JNK) cascade, one of three mammalian signaling pathways related to the MAPK cascade, has been shown to lead to apoptosis in some cell types [68]. Finally, activation of PI 3-K has been shown to block apoptosis; this is mediated through Akt, a cytosolic protein kinase which is a target for PI 3-K products, and which seems to be a key antagonist of programmed cell death [69•]. A picture is now beginning to emerge that links these various actors in controlling the anchorage regulation of apoptosis. However, as seen below, this area of research is not free of controversy.

A couple of years ago it was shown that FAK has an important role in anchorage regulation of programmed cell death. Thus, expression of an activated form of FAK in epithelial cells blocked anoikis [28•]. Further, inhibition of FAK function by microinjection of an antibody [70], or inhibition of FAK expression using antisense oligonucleotides [71], triggered apoptosis in fibroblasts and tumor cells respectively. While the precise mechanism is unresolved, one interesting possibility relates to the ability of FAK to associate with PI 3-K [72]. It has been shown that cell adhesion can activate PI 3-K, probably by a Ras-dependent mechanism [33,73••]. Further, expression of constitutively activated forms of PI3 kinase or Akt blocked anoikis in epithelial cells, while use of drugs that inhibit PI 3-K enhanced anoikis, but this could be overcome by Akt [73••]. These observations suggest an anti-apoptotic pathway that leads from integrin engagement, to FAK, to PI 3-K, and thence to Akt. The mechanism by which active Akt blocks apoptosis is not entirely clear; one exciting possibility is that Akt

phosphorylates the pro-apoptotic Bcl-2 family protein BAD, causing it to be sequestered by 14-3-3 proteins [74]. The role of PI 3-K and Akt in regulation of apoptosis is discussed in more detail by J Downward in this issue (pp 262–267).

A recent observation shows that FAK can be cleaved by caspases [75]; this may suggest a positive feedback loop whereby initiation of apoptosis activates capsases and shuts down the FAK–PI 3-K–Akt anti-apoptotic pathway. Interestingly, a recent study has implicated Pyk2 as a pro-apoptotic molecule [76]; the Pyk2 effect on apoptosis could be blocked by activated Akt, indicating that it lies on the same pathway. Pyk2 is a cytosolic tyrosine kinase that has a strong resemblance to FAK, but seems to have a different role in cells [77].

Another possible arm of the anoikis pathway has also been explored, one that involves caspases, Bcl-2 and the JNK cascade [78]. A recent report elucidates part of the mechanism, demonstrating that loss of matrix anchorage activates a caspase that cleaves and activates MEKK-1, an upstream kinase in the JNK pathway [79•]; when overexpressed in cells, the MEKK-1 cleavage product triggers apoptosis. Integrin anchorage prevents MEKK-1 cleavage by maintaining the expression of Bcl-2, an anti-apoptotic protein that blocks caspase activation. These observations suggest a pathway that links integrins, Bcl-2, caspases, and activation of the MEKK-1–JNK cascade as regulators of anchorage-dependent apoptosis. However, this view has been criticized on the basis of recent evidence that the correlation between JNK activation and anoikis can be uncoupled, and that a dominant-negative form of a JNK kinase failed to block anoikis [80]. One possible way to reconcile these very different sets of observations is to suggest that MEKK-1 may exert its apoptotic effect by a mechanism that is independent of JNK activation.

Control of anoikis may vary substantially from one cell lineage to another and be influenced by the presence of active oncogenes. For example, in epithelial cells, which are very subject to anoikis, the Ras oncogene strongly antagonizes this process, probably by activating Akt [2•,73••]. In contrast, fibroblasts normally do not undergo apoptosis upon loss of anchorage; rather they arrest in G1 [37]. However, when fibroblasts are transformed by the Myc/Ras or E1A/Ras sets of oncogenes, the cells now readily undergo anoikis [81]. This illustrates the important implications of cell type and transformation status on the regulation of apoptosis.

In summary, integrin-mediated cell anchorage is known to regulate a complex set of events that impact programmed cell death pathways. Current evidence suggests the possible existence of two different pathways controlled by anchorage. The first involves integrins, FAK, PI 3-K and Akt; the second involves integrins, Bcl-2, caspases and MEKK-1. However, as we learn more about events in various cell types, linkages between these pathways may become more apparent.

Conclusions

The functional status (activation) of integrins is regulated by complex interactions with a number of cytosolic, cytoskeletal and membrane-bound proteins. Integrin activation and engagement with ECM ligands directly activates signaling pathways and also modulates pathways triggered by other agents, particularly polypeptide growth factors. These events are likely to be very important in the anchorage regulation of cell-cycle progression and of apoptosis. However, the mechanistic basis of integrin signaling and signal modulation, as well as how these processes impinge on cyclin–CDK complexes and on the machinery for programmed cell death, have yet to be fully elucidated. Thus, the study of integrin-mediated signaling is likely to be an important area of research for some time to come.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. • Aplin AE, Howe A, Alahari SK, Juliano RL: **Signal transduction and signal modulation by cell adhesion receptors.** *Pharmacol Rev* 1998, in press.

A broad-ranging review of signaling processes mediated by integrins, cadherins, selectins, and immunoglobulin family-CAMs.

2. • Frisch SM, Ruoslahti E: **Integrins and anoikis.** *Curr Opin Cell Biol* 1997, **9**:701-706.

A timely review of anchorage regulation of apoptosis.

3. • Burridge K, Chrzanowska-Wodnicka M: **Focal adhesions, contractility, and signaling.** *Annu Rev Cell Dev Biol* 1996, **12**:463-519.

A thought-provoking review on the structure and formation of focal contacts, suggesting the role of actomysosin-generated tension.

4. • Zhang Z, Vuori K, Wang H-G, Reed JC, Ruoslahti E: **Integrin activation by R-ras.** *Cell* 1996, **85**:61-69.

The first report of G-protein regulation of integrin affinity by the process of 'inside-out' signaling.

5. •• Hughes PE, Renshaw MW, Pfaff M, Forsyth J, Keivens VM, Schwartz MA, Ginsberg MH: **Suppression of integrin activation: a novel function of a Ras/Raf-initiated MAP kinase pathway.** *Cell* 1997, **88**:521-530.

An important paper that implicates elements of the Ras/MAPK pathway in modulation of integrin affinity.

6. Laudanna C, Campbell JJ, Butcher EC: **Role of Rho in chemoattractant-activated leukocyte adhesion through integrins.** *Science* 1996, **271**:981-983.

7. • Keely PJ, Westwick JK, Whitehead IP, Der CJ, Parise LV: **Cdc42 and Rac 1 induce integrin-mediated cell motility and invasiveness via PI 3-kinase.** *Nature* 1997, **390**:632-636.

An interesting study illustrating the collaborating role of integrins and Rho-family proteins in control of tumor progression.

8. • Kolanus W, Nagel W, Schiller B, Zeitlmann L, Godar S, Stockinger H, Seed B: **αLβ2 integrin/LFA1 binding to ICAM1 induced by cytohesin-1, a cytoplasmic regulatory molecule.** *Cell* 1996, **86**:233-242.

The first report of a cytoplasmic protein that binds to integrin cytoplasmic tails and modulates integrin affinity.

9. • Hannigan GE, Leung-Hagesteijn C, Fitz-Gibbon L, Coppolino MG, Radeva G, Filmus J, Bell JC, Dedhar S: **Regulation of cell adhesion and anchorage-dependent growth by a new β1-integrin-linked protein kinase.** *Nature* 1996, **379**:91-96.

The initial description of an integrin-regulated kinase that may have a vital role in the control of cell growth and differentiation.

10. • Coppolino MG, Woodside MJ, Demaurex N, Grinsten S, St-Arnaud R, Dedhar S: **Calreticulin is essential for integrin-mediated calcium signalling and cell adhesion.** *Nature* 1997, **386**:843-847.

This work demonstrates that a protein that binds to alpha subunit cytoplasmic tails can have a striking effect on integrin mediated adhesion and signaling.

11. Meacci E, Tsai S-C, Adami R, Moss J, Vaughan M: **Cytohesin-1, a cytosolic guanine nucleotide-exchange protein for ADP ribosylation factor.** *Proc Natl Acad Sci USA* 1997, **94**:1745-1748.

12. • Tapon N, Hall A: **Rho, Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton.** *Curr Opin Cell Biol* 1997, **9**: 86-92.

An excellent review of Rho-family GTPases.

13. Machesky LM, Hall A: **Role of actin polymerization and adhesion to extracellular matrix in Rac- and Rho-induced cytoskeletal reorganization.** *J Cell Biol* 1997, **138**:913-926.

14. • Van Aelst L, D'Souza-Schorey D: **Rho GTPases and signaling networks.** *Genes Dev* 1997, **11**:2295-2322.

A excellent and very comprehensive review of Rho-family GTPases.

15. Lamarche NN, Tapon L Stowers PD, Burbelo P, Aspenstron T, Bridges J, Chant A, Hall A. **Rac and cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade.** *Cell* 1996, **87**:519-29.

16. Westwick JK, Lambert QT, Clark GJ, Symons M, Van Aelst L, Pestell RG, Der CJ: **Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-independent pathways.** *Mol Cell Biol* 1997, **17**:1324-1335.

17. • Hanks SK, Polte TR: **Signaling through focal adhesion kinase.** *BioEssays* 1997, **19**:137-145.

An up to date and useful review of FAK and its role in focal contacts; this includes considerable detail that is omitted in the discussion of FAK in the current article.

18. Schlaepfer DD, Broome MA, Hunter T: **Fibronectin-stimulated signaling from a focal adhesion kinase-c-Src complex: involvement of the Grb2, p130cas, and Nck adaptor proteins.** *Mol Cell Biol* 1997, **17**:1702-1713.

19. • Ilic D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T *et al.*: **Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice.** *Nature* 1995, **377**:539-544.

This 'knockout' study is still a key to understanding the role of FAK.

20. Lyman S, Gilmore A, Burridge K, Gidwitz S, White GC II: **Integrin-mediated activation of focal adhesion kinase is independent of focal adhesion formation or integrin activation. Studies with activated and inhibitory beta3 cytoplasmic domain mutants.** *J Biol Chem* 1997, **272**:22538-22547.

21. Defilippi P, Venturino M, Gulino D, Duperray A, Boquet P, Fiorentini C, Volpe G, Palmieri M, Silengo L, Tarone G : **Dissection of pathways implicated in integrin-mediated actin cytoskeleton assembly. Involvement of protein kinase C, Rho GTPase, and tyrosine phosphorylation.** *J Biol Chem* 1997, **272**:21726-21734.

22. Richardson A, Parsons T: **A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125FAK.** *Nature* 1996, **380**:538-540.

23. Cary LA, Chang JF, Guan J-L: **Stimulation of cell migration by overexpression of focal adhesion kinase and its association with Src and Fyn.** *J Cell Sci* 1996, **109**:1787-1794.

24. • Schlaepfer DD, Hanks SK, Hunter T, van der Geer P: **Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase.** *Nature* 1994, **372**:786-791.

The first report suggesting that FAK could recruit Grb2 and thus possibly signal to the MAPK pathway.

25. Schlaepfer DD, Hunter T: **Focal adhesion kinase overexpression enhances Ras-dependent integrin signaling to ERK2/mitogen-activated protein kinase through interactions with and activation of c-Src.** *J Biol Chem* 1997, **272**:13189-13195.

26. Polte TR, Hanks SK: **Complexes of focal adhesion kinase (FAK) and Crk-associated substrate (p130(Cas)) are elevated in cytoskeleton-associated fractions following adhesion and Src transformation. Requirements for Src kinase activity and FAK proline-rich motifs.** *J Biol Chem* 1997, **272**:5501-5509.

27. Vuori K, Hirai H, Aizawa S, Ruoslahti E: **Introduction of p130cas signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases.** *Mol Cell Biol* 1996, **16**:2606-2613.

28. • Frisch SM, Vuori K, Ruoslahti E, Chan-Hui P Y: **Control of adhesion-dependent cell survival by focal adhesion kinase.** *J Cell Biol* 1996, **134**:793-799.

This study showed that FAK has a key role in anoikis; it also suggested that FAK might not be involved in adhesion regulation of MAPK.

29. • Lin TH, Aplin AE, Shen Y, Chen Q, Schaller M, Romer L, Aukhil I, Juliano RL: **Integrin-mediated activation of MAP kinase is independent of FAK: Evidence for dual integrin signaling pathways in fibroblasts.** *J Cell Biol* 1997, **136**:1385-1395.

This report supports the existence of integrin signaling paths to MAPK that do not involve FAK. It makes use of the C-terminal domain of FAK as a dominant negative for FAK activation.

30. •• Wary KK, Mainiero F, Isakoff SJ, Marcantonio EE, Giancotti FG: **The adaptor protein Shc couples a class of integrins to the control of cell cycle progression.** *Cell* 1996, **87**:733-743.

This report indicates the existence of a novel and important signaling pathway from integrins, through caveolin and Shc, and then to the Ras/MAPK cascade.

31. Engleman JA, Wykoff CC, Yasuhara S, Song KS, Okamoto T, Lisanti MP: **Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth.** *J Biol Chem* 1997, **272**:16374-16381.

32. • Clark EA, Hynes RO: **Ras activation is necessary for integrin-mediated activation of extracellular signal-regulated kinase 2 and cytosolic phospholipase A2 but not for cytoskeletal organization.** *J Biol Chem 1996*, **271**:14814-14818.

This report provided evidence in favor of a role for Ras in integrin signaling to MAPK. It makes use of N17 Ras as a dominant negative.

33. King WG, Mattaliano MD, Chan TO, Tsichlis PN, Brugge JS: **Phosphatidyl 3-kinase is required for integrin-stimulated AKT and Raf-1/mitogen-activated protein kinase pathway activation.** *Mol Cell Biol* 1997, **17**:4406-4418.

34. • Chen Q, Lin TH, Der CJ, Juliano RL: **Integrin-mediated activation of MEK and mitogen-activated protein kinase is independent of Ras.** *J Biol Chem* 1996, **271**:18122-18127.

This report provided evidence in favor of a Ras-independent pathway for integrin signaling to MAPK. It makes use of an amino-terminal domain of Raf as a dominant negative.

35. • Cutler RE, Morrison DK: **The complexity of Raf regulation.** *Curr Opin Cell Biol* 1997, **9**:174-179.

This is a timely review of the intricacies of Raf activation.

36. • Klemke RL, Cai S, Giannini AL, Gallagher PJ, de Lanerolle P, Cheresh DA: **Regulation of cell motility by mitogen-activated protein kinase.** *J Cell Biol* 1997, **137**:481-492.

An important study showing that MAPK can regulate MLCK and thus influence actinomyosin contractility and cell motility.

37. • Assoian RK: **Anchorage-dependent cell cycle progression.** *J Cell Biol* 1997, **136**:1-4.

An important review of the role of anchorage in regulation of the cell-cycle machinery.

38. • Schwartz MA: **Integrins, oncogenes and anchorage independence.** *J Cell Biol* 1997, **139**:575-578.

An unique and interesting viewpoint on the relationship between oncogenes and anchorage regulation of cell growth.

39. Sundberg C, Rubin K: **Stimulation of β1 integrins on fibroblasts induces PDGF independent tyrosine phosphorylation of PDGF β-receptors.** *J Cell Biol* 1996, **132**:741-752.

40. Saffell JL, Williams EJ, Mason IJ, Walsh FS, Doherty P: **Expression of a dominant negative FGF receptor inhibits axonal growth and FGF receptor phosphorylation stimulated by CAMs.** *Neuron* 1997, **18**:231-242.

41. • Schneller M, Vuori K, Ruoslahti E: **αvβ3 integrin associates with activated insulin and PDGFβ receptors and potentiates the biological activity of PDGF.** *EMBO J* 1997, **16**:5600-5607.

An excellent recent example of integrin modulation of the activation of a RTK (PDGFb receptor). It also recapitulates earlier work on integrin effects on insulin receptor and IRS-1.

42. • Miyamoto S, Teramoto H, Gutkind JS, Yamada KM: **Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors.** *J Cell Biol* 1996, **135**:1633-1642.

A key early report demonstrating that integrin clustering could enhance growth factor signaling.

43. Lin TH, Chen Q, Howe A, Juliano RL: **Cell anchorage permits**
• **efficient signal transduction between Ras and its downstream kinases.** *J Biol Chem* 1997, **272**:8849-8852.

44. Renshaw M W, Ren X-D, Schwartz, MA: **Growth factor activation**
• **of MAP kinase requires cell adhesion.** *EMBO J* 1997, **16**:5592-5599.
Lin *et al*., 1997 [43•] and the paper above investigate the role of integrin-mediated anchorage in regulating downstream events in the MAPK cascade triggered by peptide mitogens. They come to somewhat different specific conclusions, but both show integrins affecting coupling between upstream and downstream events.

45. Faux MC, Scott JD: **Molecular glue: kinase anchoring and scaffold proteins.** *Cell* 1996, **85**:9-12.

46. Sastry SK, Lakonishok M, Thomas DA, Muschler J, Horwitz AF:
• **Integrin α subunit ratios, cytoplasmic domains, and growth factor synergy regulates muscle proliferation and differentiation.** *J Cell Biol* 1996, **133**:169-184.
One of the best developed examples of how specific integrins can modulate the effects of polypeptide growth and differentiation factors.

47. McCarthy JB, Vachhani BV, Wahl SM, Finbloom DS, Feldman GM: **Human monocyte binding to fibronectin enhances IFN-gamma-induced early signaling events.** *J Immunol* 1997, **159**:2424-2430.

48. Xu J, Clark RA: **Extracellular matrix alters PDGF regulation of fibroblast integrins.** *J Cell Biol* 1997, **132**:239-249.

49. Stromblad S, Becker JC, Yebra M, Brooks PC, Cheresh DA: **Suppression of p53 activity and p21WAF1/CIP1 expression by vascular cell integrin alphaVbeta3 during angiogenesis.** *J Clin Invest* 1996, **98**:426-433.

50. Boudreau N, Werb Z, Bissell MJ: **Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle.** *Proc Natl Acad Sci USA* 1996, **93**:3509-3513.

51. Hedin UL, Daum G, Clowes AW: **Disruption of integrin alpha 5 beta 1 signaling does not impair PDGF-BB-mediated stimulation of the extracellular signal-regulated kinase pathway in smooth muscle cells.** *J Cell Physiol* 1997, **172**:109-116.

52. Monier-Gavelle F, Duband JL: **Cross talk between adhesion molecules: control of N-cadherin activity by intracellular signals elicited by beta1 and beta3 integrins in migrating neural crest cells.** *J Cell Biol* 1997, **137**:1663-1681.

53. Giblin PA, Hwang ST, Katsumoto TR, Rosen SD: **Ligation of L-selectin on T lymphocytes activates beta1 integrins and promotes adhesion to fibronectin.** *J Immunol* 1997, **159**:3498-507.

54. Porter JC, Hogg N: **Integrin cross talk: activation of lymphocyte function-associated antigen-1 on human T cells alters alpha4beta1- and alpha5beta1-mediated function.** *J Cell Biol* 1997, **138**:1437-1447.

55. Braga VM, Machesky LM, Hall A, Hotchin NA: **The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts.** *J Cell Biol* 1997, **137**:1421-1431.

56. Takaishi K, Sasaki T, Kotani H, Nishioka H, Takai Y: **Regulation of cell-cell adhesion by Rac and Rho small G proteins in MDCK cells.** *J Cell Biol* 1997, **139**:1047-1059.

57. Koyama H, Raines EW, Bornfeldt KE, Roberts JM, Ross R: **Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of Cdk2 inhibitors.** *Cell* 1996, **87**:1069-1078.

58. Kang JS, Krauss RS: **Ras induces anchorage-independent growth by subverting multiple adhesion-regulated cell cycle events.** *Mol Cell Biol* 1996, **16**:3370-3380.

59. Schulze A, Zerfass-Thome K, Berges J, Middendorp S, Jansen-Durr P, Henglein B: **Anchorage-dependent transcription of the cyclin A gene.** *Mol Cell Biol* 1996, **16**:4632-4638.

60. Zhu X, Ohtsubo M, Bohmer RM, Roberts JM, Assoian RK:
• **Adhesion-dependent cell cycle progression linked to the expression of cyclin D1, activation of cyclin E-cdk2, phosphorylation of the retinoblastoma protein.** *J Cell Biol* 1996, **133**:391-403.
A key analysis of the cell-cycle events regulated by cell anchorage.

61. Resnitzky D: **Ectopic expression of cyclin D1 but not cyclin E induces anchorage-independent cell cycle progression.** *Mol Cell Biol* 1997, **17**:5640-5647.

62. Fang F, Orend G, Watanabe N, Hunter T, Ruoslahti E:
• **Dependence of cyclin E-CDK2 kinase activity on cell anchorage.** *Science* 1996, **271**:499-502.
This report emphasizes the role of p21 and p27 inhibitor proteins in anchorage regulation of the cell cycle.

63. Wu R-C, Schönthal AH: **Activation of p53-p21^{waf1} pathway in response to disruption of cell-matrix interactions.** *J Biol Chem* 1997, **272**:29091-29098.

64. Day ML, Foster RG, Day KC, Zhao X, Humphrey P, Swanson P, Postigo AA, Zhang SH, Dean DC: **Cell anchorage regulates apoptosis through the retinoblastoma tumor suppressor/E2F pathway.** *J Biol Chem* 1997, **272**:8125-8128.

65. Yang JJ, Krauss RS: **Extracellular ATP induces anchorage-independent expression of cyclin A and rescues the transformed phenotype of a ras-resistant mutant cell line.** *J Biol Chem* 1997, **272**:3103-3108.

66. Porter AG, Ng P, Janicke RU: **Death substrates come alive.**
• *Bioessays* 1997, **19**:501-507.
An excellent review on caspases and their role in programmed cell death.

67. White E: **Life, death, adn the pursuit of apoptosis.** *Genes Dev*
• 1996, **10**:1-15.
An excellent review on Bcl-2 family proteins and their role in programmed cell death.

68. Kyriakis JM, Avruch J: **Protein kinase cascades activated by stress and inflammatory cytokines.** *BioEssays* 1996, **18**:567-577.

69. Franke TF, Kaplan DR, Cantley LC: **PI3K: downstream AKTion**
• **blocks apoptosis.** *Cell* 1997, **88**:435-437.
An excellent review on the PI 3-K, Akt connection to apoptosis.

70. Hungerford JE, Compton MT, Matter ML, Hoffstrom BG, Otey CA: **Inhibition of pp125^{FAK} in cultured fibroblasts results in apoptosis.** *J Cell Biol* 1996, **135**:1383-1390.

71. Xu LH, Owens LV, Sturge GC, Yang X, Liu ET, Craven RJ, Cance WG: **Attenuation of the expression of the focal adhesion kinase induces apoptosis in tumor cells.** *Cell Growth Differ* 1996, **7**:413-418.

72. Chen H-C, Guan J-L: **Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase.** *Proc Natl Acad Sci USA* 1994, **91**:10148-10152.

73. Khwaja A, Rodriguez-Viciana R, Wennstrom S, Warne PH,
•• Downward J: **Matrix adhesion and ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway.** *EMBO J* 1997, **16**: 2783-2793.
An important and convincing study of the role of PI3 kinase and Akt in anchorage regulation of programmed cell death.

74. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME: **Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery.** *Cell* 1997, **91**:231-241.

75. Wen L-P, Fahrni JA, Troie S, Guan J-L, Orth K, Rosen GD: **Cleavage of focal adhesion kinase by caspases during apoptosis.** *J Biol Chem* 1997, **272**:26056-26061.

76. Xiong W, Parsons JT: **Induction of apoptosis after expression of PYK2, a tyrosine kinase structurally related to focal adhesion kinase.** *J Cell Biol* 1997, **139**:529-539.

77. Schaller MD, Sasaki T: **Differential signaling by the focal adhesion kinase and cell adhesion kinase β.** *J Biol Chem* 1997, **272**:25319-25325.

78. Frisch SM, Vuori K, Kelaita D, Sicks S: **A role for jun-N-terminal kinase in anoikis; suppression by bcl-2 and crmA.** *J Cell Biol* 1996b, **135**:1377-1382.

79. Cardone MH, Salvesen GS, Widmann C, Johnson G, Frisch SM:
• **The regulation of anoikis: MEKK-1 activation requires cleavage by caspases.** *Cell* 1997, **90**:315-323.
An provocative study of the possible role of MEKK-1 in anchorage regulation of programmed cell death.

80. Khwaja A, Downward J: **Lack of correlation between activation of Jun-NH_2-terminal kinase and induction of apoptosis after detachment of epithelial cells.** *J Cell Biol* 1997, **139**:1017-1023.

81. McGill G, Shimamura A, Bates RC, Savage RE, Fisher DE: **Loss of matrix adhesion triggers rapid transformation-selective apoptosis in fibroblasts.** *J Cell Biol* 1997, **138**:901-911.

82. • Mackay DJG, Esch F, Furthmayr H, Hall A: **Rho- and Rac-dependent assembly of focal adhesion complexes and actin filaments in permeabilized fibroblasts: an essential role for ezrin/radixin/moesin proteins.** *J Cell Biol* 1997, **138**:927-938.
The first indication that ERM proteins might regulate the cytoskelton through Rho-family proteins.

83. Tsukita S, Yonemura S, Tsukita S: **ERM (ezrin/radixin/moesin) family: from cytoskeleton to signal transduction.** *Curr Opin Cell Biol* 1997, **9**:70-75.

84. Takahashi K, Sasaki T, Mammoto A, Takaishi K, Kameyama T, Tsukita S, Tsukita S, Takai Y: **Direct interaction of the Rho GDP dissociation inhibitor with ezrin/radixin/moesin initiates the activation of the Rho small G protein.** *J Biol Chem* 1997, **272**:23371-23371.

85. Kashiwagi H, Schwartz MA, Eigenthaler M, Davis K A, Ginsberg MH, Shattil SJ: **Affinity modulation of platelet integrin αIIbβ3 by β3 endonexin, a selective binding partner of the β3 integrin cytoplasmic tail.** *J Cell Biol* 1997, **137**:1433-1443.

86. Biffo S, Sanvito F, Costa S, Preve L, Pignatelli R, Spinardi L, Marchisio PC: **Isolation of a novel β_4 integrin-binding protein ($p27^{BBP}$) highly expressed in epithelial cells.** *J Biol Chem* 1997, **272**:30314-30321.

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

88. Fenczik CA, Sethi T, Ramos JW, Hughes PE, Ginsberg MH: **Complementation of dominant suppression implicates CD98 in integrin activation.** *Nature* 1997, **390**:81-85.

89. Naik UP, Patel PM, Parise LV: **Identification of a novel calcium-binding protein that interacts with the integrin αIIb cytoplasmic domain.** *J Biol Chem* 1997, **272**:4651-4654.

90. Berditchevski F, Tolias KF, Wong K, Carpenter CL, Hemler ME: **A novel link between integrins, transmembrane superfamily proteins (CD63 and CD81), and phosphatidylinositol 4-kinase.** *J Biol Chem* 1997, **272**:2595-2598.