

Integrin signaling to the actin cytoskeleton

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Integrin engagement stimulates the activity of numerous signaling molecules, including the Rho family of GTPases, tyrosine phosphatases, cAMP-dependent protein kinase and protein kinase C, and stimulates production of PtdIns(4,5)P₂. Integrins promote actin assembly via the recruitment of molecules that directly activate the actin polymerization machinery or physically link it to sites of cell adhesion.

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Abbreviations

Arp2/3	actin-related protein 2/3
cAMP	cyclic AMP
CHO	Chinese hamster ovary
Crk	chicken tumor virus 10 regulator of kinase
DOCK180	180-kDa protein downstream of CRK
ECM	extracellular matrix
FAK	focal adhesion kinase
GEF	guanine nucleotide exchange factor
GAP	GTPase-activating protein
GDI	guanine nucleotide dissociation inhibitor
ILK	integrin-linked kinase
MAP	mitogen-activated protein
PAK	p21-activated protein kinase
PtdIns(4,5)P₂	phosphatidylinositol-4,5-bisphosphate
PI3K	particularly interesting new Cys-His protein
PIP 5-kinase	phosphatidylinositol 4-phosphate, 5-kinase
PIX	PAK-interacting exchange factor
PKA	cAMP-dependent protein kinase
PKC	protein kinase C
PTP	protein tyrosine phosphatase
SCAR	suppressor of cAMP receptor
SHP-2	Src homology region 2 containing PTP-2
SHPS-1	SHP substrate-1
SFK	Src family kinase
WAVE	WASP family verprolin-homologous protein
WASP	Wiskott-Aldrich syndrome protein

Introduction

Twelve years ago, the discovery that integrin engagement stimulates tyrosine phosphorylation of several proteins ushered in an era of extensive research on the signaling

that occurs downstream from integrins. Many signaling pathways that emanate from integrin engagement or clustering have been identified and the pace of discovery in this field has not slowed down. This is not surprising given that adhesion to the extracellular matrix (ECM) influences the growth, differentiation, survival, morphology and migratory properties of cells. We suspect that more pathways remain to be uncovered. In this brief review we will restrict our focus to a few selected topics, particularly concentrating on the signaling pathways downstream from integrin engagement that impact on the organization of the cytoskeleton and on cell migration.

Three dimensions versus two

Although there is a long history of studying the behavior of cells in collagen gels, most work on integrins has involved cells grown on 2D surfaces coated with ECM components derived from serum; these components may be synthesized by the cells themselves or applied by the experimenter. In such cultures, integrins are prominently concentrated in matrix adhesions, which include focal complexes, focal adhesions and fibrillar adhesions. These structures have been discussed elsewhere [1]. Briefly, focal complexes are small transient adhesions at the cell periphery, regulated by Rac or Cdc42. Under the influence of RhoA activity and tension, focal complexes grow in size to become focal adhesions — larger, more stable structures. Fibrillar adhesions — adhesions made to fibronectin fibrils — contain the $\alpha 5 \beta 1$ integrin and a subset of the proteins found in focal adhesions [2]. Of these structures, focal adhesions are often the most pronounced in 2D cultures but are rarely seen *in vivo* and are much less apparent in cells growing in 3D ECMs [3]. Focal adhesions continue to provide a valuable model for studying the organization of and signaling from relatively stable integrin aggregates, but attention has recently been directed to studying integrin organization and signaling in 3D situations. Unlike cells on 2D surfaces, which have a spread morphology, fibroblasts in 3D matrices develop elongated or stellate morphologies and migrate more rapidly. These cells develop 3D-matrix adhesions that resemble fibrillar adhesions, both in their dimensions and in that the integrin $\alpha 5 \beta 1$ is present, but unlike fibrillar adhesions these matrix adhesions are rich in paxillin, focal adhesion kinase (FAK) and phosphotyrosine [3]. Surprisingly, phosphorylation of the major FAK phosphorylation site (Y397) was not detected, suggesting that the signaling pathways downstream from integrins may differ in 2D and 3D cultures [3].

The physical state of the matrix affects the structure of the adhesions and the morphology of cells, and it is

possible that this is a major factor contributing to the differences between 2D and 3D cultures. On rigid 2D surfaces focal adhesions are favored [4,5], whereas fibrillar adhesions develop when a pliable fibronectin matrix is remodeled to form fibrils [5]. In 3D collagen gels, fibroblasts at low density display a stellate or dendritic morphology and adhesions to the matrix appear to be diffusely distributed over the cell surface [6[•]]. At high cell density fibroblasts contract the collagen gels, thereby increasing their rigidity. Under these conditions, structures similar or equivalent to focal adhesions develop in a RhoA-dependent manner [6[•]]. These results raise the possibility that the development of a rigid matrix resulting from the initial contractile activity of the cells leads to subsequent isometric tension that may somehow elevate RhoA activity.

Integrin-mediated regulation of Rho family GTPases

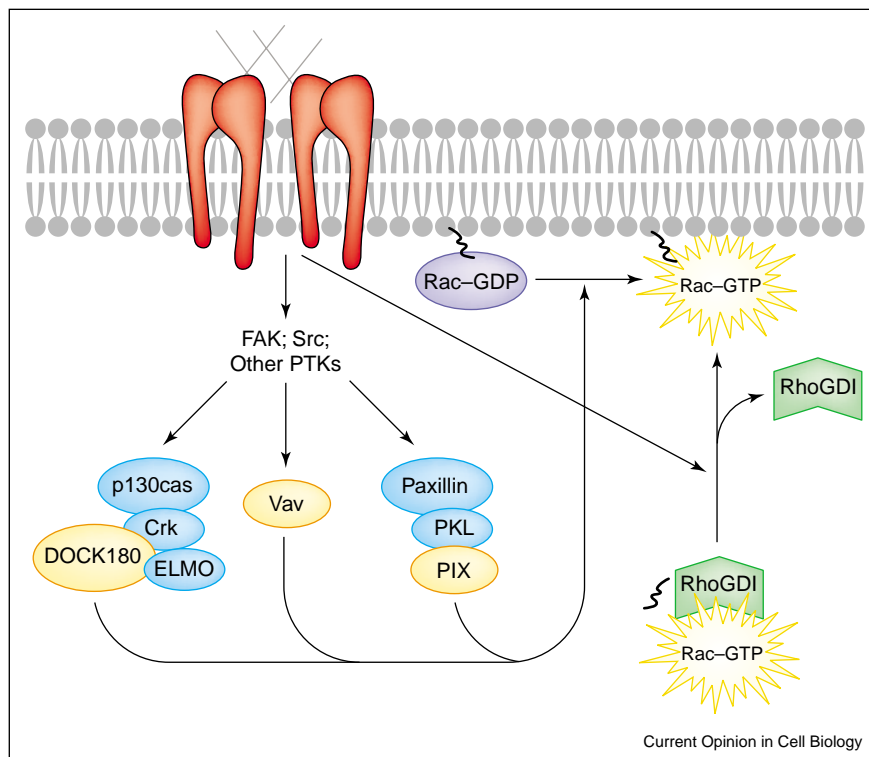
With respect to cytoskeletal organization and cell migration, signaling from integrin-mediated adhesion is typically characterized by two phases. Early adhesion is associated with pathways that stimulate protrusion whereas mature adhesions are associated with the devel-

opment of tension. The early phase leads to Rac and Cdc42 activation and to actin polymerization. The later phase leads to RhoA activation, increased contractility and the transmission of tension to the sites of integrin ligation. These pathways are often antagonistic and the biphasic nature and timing of this response can be a source of complexity and confusion.

Regulation of Rac and Cdc42

During adhesion and spreading on an ECM, cells extend filopodia and lamellipodia, structures regulated by Cdc42 and Rac, respectively. Integrin-mediated adhesion activates Cdc42 and Rac [7] and for Rac this requires an intact β integrin subunit [8,9]. Rho family GTPases are active when GTP-bound and inactive when bound to GDP. Activation is catalyzed by guanine nucleotide exchange factors (GEFs) and inactivation is promoted by GTPase-activating proteins (GAPs) that stimulate the intrinsic GTPase activity of the Rho proteins. One example of a GEF activated downstream from integrin engagement is Vav1 [10], but its expression is restricted to hematopoietic cell types. However, the closely related GEF Vav2 is widely distributed and an obvious candidate for activation downstream from integrins (Figure 1). Using tyrosine

Figure 1



Integrin-mediated activation of Rac and Cdc42. In response to integrin engagement, several tyrosine kinases are activated, including FAK and Src. These tyrosine kinases phosphorylate substrates, leading to the activation of Rac and Cdc42 (only Rac is indicated). The phosphorylated proteins include the following: GEFs (yellow circles), which activate Rac and Cdc42, such as Vav; adaptor protein complexes (blue circles) such as paxillin and PKL or p130Cas, Crk, and ELMO that bind GEFs such as PIX or DOCK180. Alternatively, integrins trigger translocation of RhoGDI-bound Rac-GTP to the plasma membrane where active Rac is liberated and available to interact with effectors.

phosphorylation as an indicator of activation, evidence was presented that Vav2 is activated downstream from growth factor receptors but not from integrins [11,12]. Nevertheless, a dominant-negative form of Vav2 blocked lamellipodium formation and spreading on fibronectin, which is consistent with Vav2 having a role in Rac activation following integrin engagement [13]. Interestingly, an elevation in tyrosine phosphorylation was not seen in response to adhesion, which is consistent with the earlier work and suggests that changes to multiple phosphorylation sites might have masked an elevation in phosphorylation of the site(s) associated with activation, or that Vav2 activation occurs by other means.

Other pathways downstream of integrins that lead to Rac activation may also be involved. Both p130cas and paxillin associate with FAK and both have been linked to Rac activation. Tyrosine phosphorylation of p130cas promotes a complex of Crk, ELMO and DOCK180 [14–18,19**] (Figure 1). Recent work has revealed that DOCK180 is a Rac GEF, even though it lacks the Dbl-homology/pleckstrin-homology tandem domains characteristic of conventional Rho-family GEFs [19**,20**]. Another complex also associates with paxillin: this complex includes PKL (GIT) and Pak-interacting exchange factor (PIX), the latter being a conventional Rac GEF (reviewed in [21]) (Figure 1). Deciphering the relative importance of the different pathways that potentially lead to Rac activation following integrin engagement will be important.

Integrin-mediated activation of Rac and other Rho-family proteins may involve not only GEF activation but also targeting of the GTP-bound protein to sites of adhesion. Del Pozo and coworkers have found that a fraction of active Rac is sequestered by RhoGDI and that this active Rac is selectively released close to sites of integrin-mediated adhesion, allowing it to interact with effectors in this region of the cell [22**]. It will be interesting to determine how this is achieved and whether localized release of active Rho family members from RhoGDI is a general mechanism.

Regulation of RhoA

Integrin engagement leads to a transient depression in RhoA activity [23] and it has been argued that this promotes lamellipodial extension during cell migration [24]. The dip in RhoA activity requires Src, FAK and p190RhoGAP [25,26]. A role for paxillin phosphorylation has also been indicated in this decrease in RhoA activity. When two of the paxillin phosphorylation sites (Y31 and Y118) were mutated, the depression in RhoA activity was abolished and the cells showed premature formation of stress fibers [27*]. These authors demonstrated that the phosphorylation of these two tyrosines, which is induced by integrin-mediated adhesion, generates a binding site for p120RasGAP, displacing it from its binding partner p190RhoGAP. Evidence was presented that p190Rho-

GAP freed from p120RasGAP was activated and hence contributed to the decrease in RhoA activity [27*]. However, whether the interaction of p120RasGAP with p190RhoGAP inhibits or increases the latter's activity remains controversial. Interestingly, the decrease in RhoA activity is seen even with cells in suspension that bind soluble-peptide integrin ligands [25], a situation in which FAK does not become activated and paxillin does not become phosphorylated on these tyrosine residues. This suggests that the phosphorylation of paxillin cannot be the sole mechanism of regulation and that phosphorylation of p190RhoGAP may also be important [25]. Nevertheless, paxillin phosphorylation may contribute to the depression of RhoA activity when cells adhere to fibronectin, which results in a more robust inhibition of the RhoA response than is seen in cells in suspension stimulated with soluble ligands.

Examination of the time-course of RhoA activity in response to cells adhering to fibronectin reveals that the initial dip is followed by activation [23]. Engagement of non-integrin receptors such as syndecan-4 may contribute to this response [28–30], but integrins have also been observed to contribute to activation. Here, different responses have been observed with different integrins. O'Connor and colleagues observed that engagement or clustering of $\alpha 6 \beta 4$ resulted in stimulation of RhoA activity, in contrast to the depression induced by clustering $\beta 1$ integrins [31]. Engagement of $\alpha \nu \beta 3$ on astrocytes by Thy-1 was shown to stimulate assembly of focal adhesions and stress fibers, which is consistent with RhoA activation occurring downstream from this integrin [32]. Direct evaluation of the effect of $\beta 1$ and $\beta 3$ integrins on RhoA activity was performed in Chinese hamster ovary (CHO) cells in which these integrins were overexpressed [33*]. In this system, overexpression of $\beta 3$ resulted in a pronounced increase in Rho-GTP levels when the cells were plated on fibronectin or fibrinogen, whereas $\beta 1$ overexpression had no effect. Somewhat surprisingly, expression of a $\beta 1/\beta 3$ chimera in which a heptapeptide sequence from the $\beta 1$ extracellular-I-domain-like structure was replaced by the equivalent sequence from the $\beta 3$ integrin resulted in stimulation of Rho activity [33*]. A different result was found using cells deficient in $\beta 1$ integrins [34**]. Using either GD25 or GE11 cells, re-expression of $\beta 1$ subunits stimulated RhoA activity, whereas $\beta 3$ had no effect. Although the results were the opposite in the two studies, both studies found that the extracellular domain was critical. A possible explanation for the opposite results is the different cell types used by these two groups. It is easy to imagine that the requirements of a particular integrin may differ in different cell types, and that in some situations, but not others, it is advantageous for the integrin to be coupled to Rho activation. The large number of RhoGEFs and their variable expression in different cell types may provide cell-type specificity when coupling integrins to Rho

activation. The significance of the extracellular domains of the integrins in this coupling remains unclear.

Integrins and protein tyrosine phosphatases

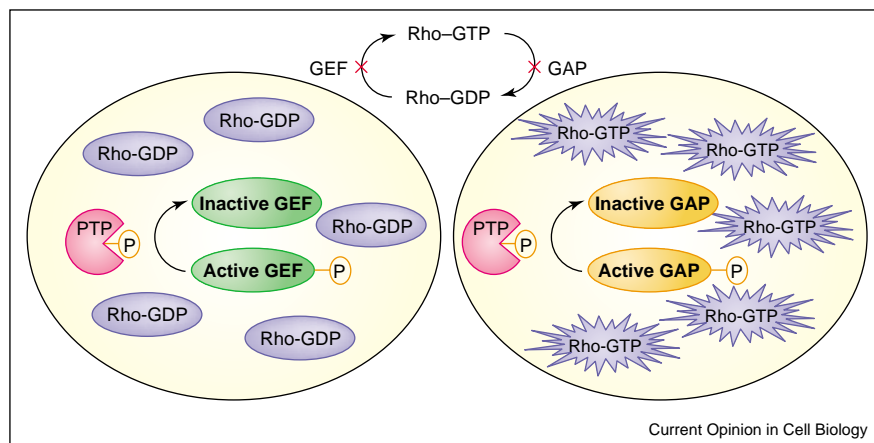
Integrin-mediated adhesion induces the tyrosine phosphorylation of many proteins. The consequences of this tyrosine phosphorylation and the kinases involved have commanded much attention. By comparison, the protein tyrosine phosphatases (PTPs) have been much less studied, although several recent papers suggest that this is changing. Early work indicated that integrin-mediated adhesion results in a bulk inhibition of PTP activity that parallels the increase in tyrosine phosphorylation seen in response to adhesion [35]. Sastry and coworkers, however, have found that PTP-PEST is stimulated upon integrin-mediated adhesion [36[•]]. This PTP had previously been shown to act on various focal adhesion targets such as p130cas [37] and paxillin [38], but not on FAK. Both overexpression and deletion of PTP-PEST inhibit cell migration [39,40], suggesting that a fine balance in the level of tyrosine phosphorylation of relevant substrates regulates cell migration. PTP-PEST overexpression inhibits protrusive activity and this has been related to a depression in Rac activity [36[•]]. As mentioned above, the tyrosine phosphorylation of both p130cas and paxillin has been linked to Rac activation, and so PTP-PEST may be affecting Rac activity by dephosphorylating these known targets.

One PTP that has been associated both with integrin-mediated signaling and with regulating RhoA activity is SHP-2 (Src homology region 2 containing PTP-2). Perturbation of SHP-2 levels or activity has effects on adhesion, cytoskeletal organization and cell migration [41–45].

Conflicting results have been obtained with respect to SHP-2's effect on Rho activity, with some groups detecting activation [45,46] and others inhibition [47,48]. It is difficult to reconcile these differences; however, it is possible that in different cellular contexts SHP-2 acts on different targets that influence RhoA activity in opposite directions (Figure 2). Recent work has identified p190BRhoGAP as a potential target for SHP-2 [49^{••}]. Here tyrosine phosphorylation has been associated with increased GAP activity and so the action of SHP-2 to decrease p190 activity will result in elevated RhoA activity [49^{••}]. By contrast, the activity of some GEFs (e.g. the Vav family, PDZ-RhoGEF and leukemia-associated Rho-GEF) is stimulated by tyrosine phosphorylation [50–52]. Although Vav2 has broad specificity for Rho family GTPases *in vitro*, *in vivo* the phenotype resulting from activated Vav2 varies with cell type, often suggesting increased Rac activity but at other times increased RhoA activity as well [12]. SHP-2 will exert an inhibitory effect on RhoGEFs that are stimulated by tyrosine phosphorylation.

One of the substrates for SHP-2 is the transmembrane protein SHP substrate 1 (SHPS-1, also known as SIRP α 1), which becomes tyrosine-phosphorylated in response to integrin-mediated adhesion by FAK and Src family kinases [53]. SHPS-1 binds SHP-2, thereby targeting SHP-2 to the membrane, where it may act on other tyrosine-phosphorylated proteins. Expression of a truncated form of SHPS-1 lacking most of the cytoplasmic domain and unable to bind SHP-2 results in cells with increased stress fibers [45]. Contrary to expectations these cells exhibit reduced rather than elevated RhoA activity [45]. The reason for this paradoxical result has not been resolved.

Figure 2



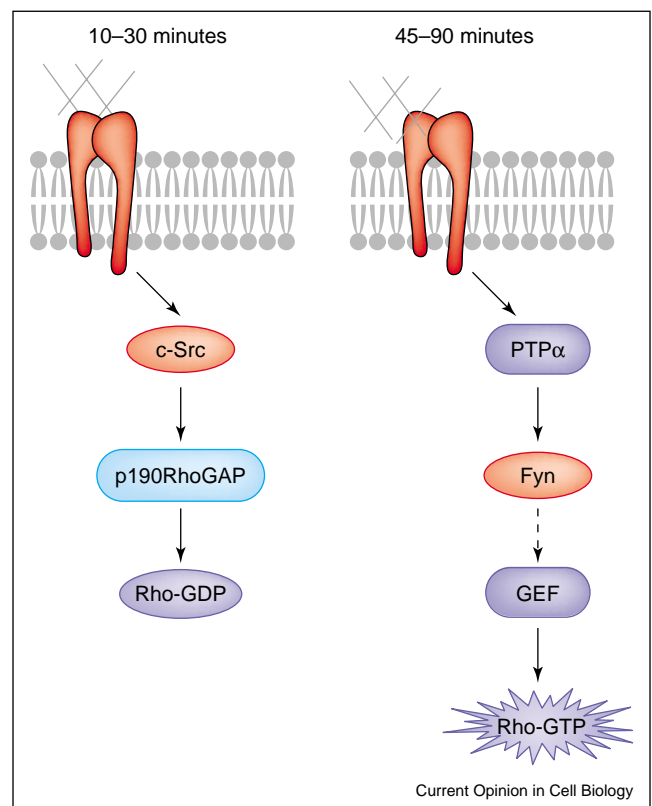
Model for the potential regulation of Rho activity by tyrosine phosphatases. Left: a tyrosine phosphorylated activated GEF (green ovals) is dephosphorylated by a tyrosine phosphatase (shown in pink). The GEF is unable to catalyze the exchange of GTP for GDP and Rho is left in the inactive GDP-bound form (purple circles). Right: the phosphatase dephosphorylates a GAP (orange ovals), inactivating it, and Rho-GTP levels accumulate (purple starbursts).

In some situations, engagement of integrins with their ligands promotes integrin association with lipid rafts (reviewed in [54]). SHP-2 is targeted to rafts in response to integrin binding to its ligands [55**]. Significantly, the use of a double palmitoylation signal to target SHP-2 to lipid rafts in cells in suspension stimulates FAK tyrosine phosphorylation and other pathways normally triggered by integrin engagement [55**]. Targeting SHP-2 to lipid rafts also affected Rho activity: the resting level of active Rho was elevated, but a dip in activity was still observed in response to adhesion to fibronectin. Interestingly, when a catalytically dead form of SHP-2 was targeted to lipid rafts, the level of Rho activity returned to more normal levels, but the adhesion-induced dip was abolished. These results led the authors to conclude that SHP-2 can function as both a positive and negative regulator of RhoA activity [55**]. The elevation of Rho activity by SHP-2 in lipid rafts could be explained by the regulation of p190RhoGAP phosphorylation and activity [49**]. However, the fact that catalytically inactive SHP-2 blocks the adhesion-induced dip in Rho activity suggests that p190RhoGAP is not becoming activated in this situation. It would be possible to explain this observation if, in response to integrin engagement, SHP-2 promotes Src activation by removing Src's inhibitory C-terminal phosphate [43]. The catalytically inactive SHP-2 would block this pathway and thereby prevent the tyrosine phosphorylation of p190RhoGAP and consequent depression of Rho activity. The different observations obtained with SHP-2 illustrate the complexity of these pathways and how PTPs may act at multiple sites in a pathway, often generating paradoxical results (illustrated in Figure 2).

PTP α , another PTP implicated in promoting cell spreading [56] and found in focal adhesions [57], removes inhibitory phosphates from the C-terminal tyrosines of Src family kinases (SFKs) [56,58]. Cells lacking PTP α reveal decreased FAK phosphorylation, particularly on tyrosine 397, the autophosphorylation site, leading to the suggestion that PTP α activity lies between integrin engagement and FAK activation in the pathway [59*]. Strong support for this idea comes from another study in which PTP α has been found to physically associate with the integrin $\alpha\beta3$ but not with $\alpha5\beta1$ [60**]. This work built on earlier studies showing that $\alpha\beta3$ integrin signaling via a Src family kinase is involved in the reinforcement of integrin–cytoskeleton linkages [61]. The decrease in focal adhesions seen in the PTP α null cells, together with their decreased development of force transmitted to $\alpha\beta3$ integrins [60**], suggests that PTP α may normally contribute to the activation of RhoA downstream from $\alpha\beta3$ integrin engagement. In preliminary work, our laboratory has confirmed that PTP α null cells exhibit decreased RhoA–GTP levels when plated on fibronectin (Ellerbroek and Burrige, unpublished observations). As mentioned earlier, in some cells $\beta3$ integrin engagement activates RhoA [33*] and it seems likely this occurs via

a PTP α /SFK pathway. At first, this seems to conflict with the idea that SFKs downstream from integrin engagement depress RhoA activity via p190RhoGAP [25]; however, an explanation is suggested by the finding of von Wichert and coworkers that it is Fyn rather than Src that becomes activated by PTP α downstream of $\alpha\beta3$ integrin occupancy [60**]. These investigators found that overexpression of Src in cells expressing PTP α actually depressed focal-adhesion formation, whereas this was not seen with Fyn [60**]. Together these results suggest a model in which the initial depression of RhoA activity occurs via integrin-mediated activation of Src leading to elevated p190RhoGAP activity, whereas the slower increase in RhoA activity occurs as a result of PTP α 's activation of Fyn (Figure 3). Presumably this involves a RhoGEF that is stimulated by tyrosine phosphorylation. It will be interesting to determine whether other integrins may also couple to PTP α in some situations or cell types, and whether this may account for the elevation of RhoA

Figure 3



Role of Src family kinases in the inhibition and reactivation of Rho activity. During the first 10–30 minutes of adhesion via some integrins, the activity of Rho is transiently suppressed. The pathway to inhibition involves c-Src-dependent phosphorylation and activation of p190RhoGAP. This GAP triggers the hydrolysis of GTP bound to Rho rendering it inactive. With other integrins, or with the same integrins at later times (45–90 minutes) of adhesion, the levels of Rho–GTP increase as a result of PTP α activating the tyrosine kinase, Fyn, which presumably phosphorylates and activates a RhoGEF.

activity downstream from $\alpha 5\beta 1$ integrins observed by Danen and coworkers [34**].

Integrin regulation of cAMP/PKA

The activity of cAMP-dependent protein kinase (PKA) regulates the cytoskeleton both by inhibiting contractility and by stimulating protrusion. PKA can inhibit actin-myosin contractility in several ways. It can phosphorylate the $G\alpha_{13}$ subunit of heterotrimeric G proteins, leading to decreased downstream RhoGEF activation [62]; it can directly phosphorylate the C terminus of RhoA, resulting in an increased binding to RhoGDI and thereby terminate RhoA activity [63,64]; and finally, it can phosphorylate and inactivate myosin light chain kinase [65]. All these behaviors lead to decreased actin-myosin contractility. In addition, PKA has been shown to phosphorylate and inhibit vasodilator-stimulated phosphoprotein (VASP) [66] and p21-activated protein kinase (PAK) [67], and to activate Rac1 and Cdc42 [68,69]. Increased levels of cAMP or activation of PKA in cells inhibit RhoA activation [70] and lead to loss of stress fibers and focal adhesions [63]. Conversely, inhibition of PKA results in formation of stress fibers [71,72] and allows adhesion-independent mitogen-activated protein (MAP) kinase activation [66].

While PKA regulates adhesion, integrin ligation and cell adhesion in turn are potent regulators of PKA activity. Detachment of cells causes a transient activation of PKA [66], probably through a relaxation-dependent mechanism [73]. The detachment-dependent activation of PKA prevents anchorage-independent activation of MAP kinase by inactivating PAK [67]. PKA activity in suspended cells, however, returns to baseline levels within 60–90 minutes. Somewhat surprisingly, adhesion will also activate PKA [66,68]. The time-course of this correlates with the activation of Rac and Cdc42 [7,74], the inactivation of RhoA [23], and the timing of membrane protrusions during cell spreading. Similarly, ligand-dependent clustering of $\beta 1$ integrins by function-blocking $\beta 1$ antibodies or soluble-peptide integrin ligands induces activation of PKA [75,76]. As with the detachment-dependent activation of PKA, the attachment-dependent activation of PKA is transient [66,68] and the later reduction in PKA activity allows cells to form stress fibers and focal adhesions, adhere firmly, and sustain survival signals, possibly through MAP kinase signaling [67,71,72]. The deactivation, at least in endothelial cells, is matrix- and integrin-dependent: integrins $\alpha 1\beta 1$, $\alpha 2\beta 1$, or $\alpha 5\beta 1$ can support inactivation but $\alpha 6\beta 1$ or $\alpha V\beta 3$ cannot [71,72].

An interplay between integrins and protein kinase C

Protein kinase C (PKC) α was one of the first signaling molecules identified in focal adhesions [77] and subsequent work has established that isoforms of PKC become activated following adhesion to the ECM and cell spreading [78–80,81*]. For example, in muscle cells activation of

PKC ϵ is followed sequentially by activation of PKC α and PKC δ [81*]. Whereas activation of PKC promotes cell spreading and focal adhesion formation in fibroblasts [28], it should be noted that PKC stimulation (e.g. by phorbol esters) induces disruption of focal adhesions in epithelial cells [82,83]. In fibroblasts, early work established that adhesion to the cell-binding domain of fibronectin mediated by $\alpha 5\beta 1$ is insufficient for cells to develop focal adhesions, but assembly of these structures could be stimulated by PKC activation or by the addition of the heparin-binding domain of fibronectin (reviewed in [84]). The relevant proteoglycan responsible for promoting focal adhesion assembly was identified as syndecan-4, a transmembrane proteoglycan that binds to the heparin-binding domain of fibronectin (reviewed in [84]). Syndecan-4 localizes to focal adhesions and PKC α binds to syndecan-4's cytoplasmic domain (reviewed in [84]). Recent work has revealed an interesting level of complexity. Whereas the integrin $\alpha 5\beta 1$ requires PKC α activation via syndecan-4 for focal adhesions to develop, the integrin $\alpha 4\beta 1$, which binds to another site in fibronectin, does not [85**]. Other interesting differences exist between these two integrins, such as the binding of paxillin by the $\alpha 4$ cytoplasmic domain [86], which prompts the question of whether the recruitment of paxillin or some other protein by $\alpha 4\beta 1$ fulfills functions that may be supplied by PKC α activation downstream from $\alpha 5\beta 1$ engagement. Downstream from syndecan-4 engagement, multiple studies have implicated RhoA activation as well as PKC activation [29,87], raising the possibility that PKC may be upstream of RhoA. This has recently been validated with the demonstration that the RhoA-GEF p115 is a substrate for and stimulated by PKC α [88].

Regulation of PtdIns(4,5) P_2 by integrin signaling

The activities of many cytoskeletal proteins are regulated by phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5) P_2). Downstream from integrins, both vinculin and talin undergo a conformational change on binding this molecule. With vinculin, this exposes cryptic binding sites for other proteins, including talin [89–91], whereas talin's interaction with PtdIns(4,5) P_2 promotes its binding to the cytoplasmic domain of $\beta 1$ integrin subunit [92]. Significantly, integrin-mediated adhesion stimulates PtdIns(4,5) P_2 synthesis [93]. In part, this may occur via stimulation of PI5-kinase by Rho and Rac, although the mechanism by which this could occur is not well understood. Recent work has revealed another pathway. Independently, two groups have found that one PIP 5-kinase splice isoform localizes to focal adhesions by binding to talin and that this interaction stimulates its activity [94**,95**]. In one case, the direct interaction was found to stimulate PIP 5-kinase activity, whereas in the other case localization to focal adhesions resulted in activation by FAK phosphorylation. This phosphorylation of PIP

5-kinase not only stimulated catalytic activity but also increased its association with talin [94^{••}]. The binding of PIP 5-kinase to talin and its consequent activation should elevate PtdIns(4,5) P_2 levels in the local region where integrins are clustered. The positive feedback mechanism suggested by these findings should contribute to the assembly of these integrin-based complexes. Indeed, expression of a kinase-dead form of the relevant PIP 5-kinase isoform blocked recruitment of FAK to focal adhesions. The local increase in PtdIns(4,5) P_2 in the vicinity of integrin engagement may also stimulate actin polymerization, as discussed below.

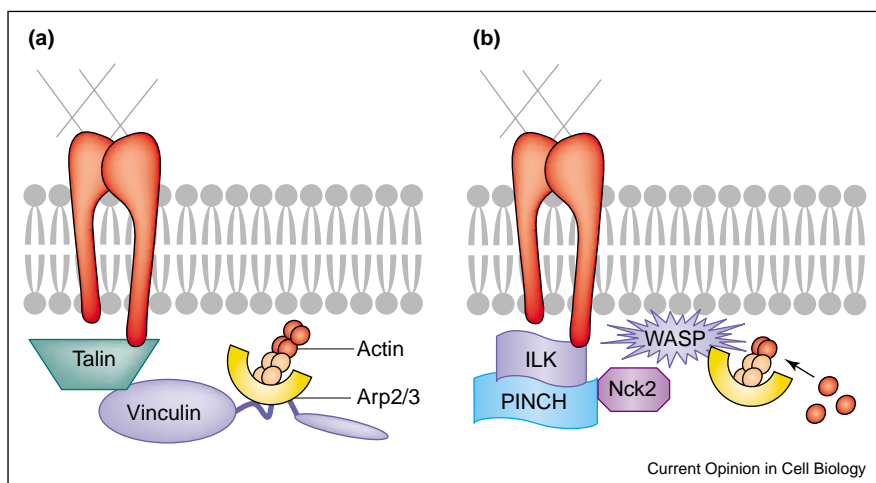
Regulation of actin assembly by integrins

Several of the prominent integrin-associated structural proteins (talin, vinculin and α -actinin) bind F-actin, but relatively little polymerization of actin has been detected in focal adhesions. As mentioned above, localized PtdIns(4,5) P_2 synthesis in focal adhesions may contribute to the assembly of protein complexes and the binding of actin at these sites. It may also promote the limited polymerization that occurs in focal adhesions by dissociating capping proteins from the barbed ends of actin filaments [96] or by other mechanisms. Much more polymerization of actin occurs at the leading edge of cells, which is also the region where integrins first engage their ligands. The major nucleator of actin polymerization is the actin-related protein 2/3 (Arp2/3) complex. Recent work has established a link between the Arp2/3 complex and new sites of integrin engagement that is mediated by the Arp2/3 complex binding to vinculin [97[•]] (Figure 4a). This interaction is transient, being confined to the newest adhesions and not seen in more mature focal adhesions.

The association is regulated by phosphatidylinositol-3-kinase and Rac activity. Cells deficient in vinculin show decreased spreading and formation of lamellipodia, phenotypes corrected by re-expression of wildtype vinculin but not of vinculin unable to bind the Arp2/3 complex [97[•]]. Although this interaction does not stimulate actin polymerization by the Arp2/3 complex, it does recruit the Arp2/3 complex to sites of integrin clustering.

Other mechanisms for linking actin polymerization to integrins have also been identified. Key regulators of the Arp2/3 complex are members of the Wiskott–Aldrich syndrome protein (WASP) family of proteins, including WASP, N-WASP and WAVE/SCAR proteins. N-WASP has been identified in β 1-integrin immunoprecipitates and was released under conditions stimulating actin polymerization and protrusion [98]. The WASP proteins stimulate the Arp2/3 complex after they have undergone a conformational change in which the C-terminal domain is exposed, enabling it to bind the Arp2/3 complex. WASP and N-WASP are activated by binding to Cdc42 or Nck, whereas WAVE/SCAR is activated downstream from Rac or Nck [99,100,101^{••}]. Nck also binds to WIP, a WASP-interacting protein that promotes actin polymerization [102]. A link between Nck and integrins has been identified via the LIM domain protein, PINCH, which binds integrin-linked kinase (ILK), which in turn associates with integrin β subunit cytoplasmic domains [103] (Figure 4b). Significantly, the action of WASP on Arp2/3-induced actin polymerization is stimulated by PtdIns(4,5) P_2 , which, as mentioned above, is synthesized by enzymes recruited to sites of integrin engagement by binding talin [94^{••},95^{••}].

Figure 4



Links between the actin polymerization machinery and integrins. **(a)** Activated Arp2/3 complex binds directly to the hinge region of vinculin, an adhesion molecule that is recruited to integrins via an interaction with talin. Binding of the Arp2/3 complex to vinculin does not stimulate the activity of the Arp2/3 complex, but rather localizes polymerization to new sites of integrin adhesion. **(b)** Actin polymerization is stimulated at sites of integrin clustering via recruitment of a complex of proteins, including ILK–PINCH and Nck. Nck binds and activates WASP proteins, which in turn recruit and activate the Arp2/3 complex.

The above observations suggest that the machinery for nucleating actin polymerization can be linked in various ways to integrins and may be particularly active where integrins are newly engaged with the ECM. Superimposed on these physical links between integrins and the Arp2/3 complex is the activation of Rac and Cdc42 downstream from integrin ligation. The local activation of these GTPases will further stimulate WASP or WAVE/SCAR in the vicinity of integrin–ligand binding. Additionally, selective release of activated Rac from the sequestering protein RhoGDI has been reported to occur where integrins mediate adhesion to the ECM [22**]. Together, these pathways should synergize to promote actin polymerization at sites of new adhesion.

Conclusions

Progress in the field of integrin-mediated signaling has been substantial in the last couple of years, but much still remains to be learned. The apparent complexity of many of the signaling pathways downstream from integrin ligation reflects in part the transition from an early response, associated with Rac/Cdc42 activation and membrane protrusion, to a late response, associated with RhoA activation and the generation of tension. The development of live cell imaging techniques should contribute to resolving many of the spatial and temporal complexities downstream from integrin engagement. Biosensors are being designed and tested that will visualize specific signaling events, such as activation of Rho GTPases, kinases and phosphatases, within living cells in real time. This technology promises to revolutionize the field by allowing signaling pathways to be visualized locally within cells as integrin ligation occurs, matures and is terminated.

Acknowledgements

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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

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