Integrin signaling revisited

Martin A. Schwartz

Adhesion to the extracellular matrix (ECM) is a crucial regulator of cell function, and it is now well established that signaling by integrins mediates many of these effects. Ten years of research has seen integrin signaling advance on many fronts towards a molecular understanding of the control mechanisms. Most striking is the merger with studies of other receptors, the cytoskeleton and mechanical forces within the general field of signaling networks.

When I wrote a *Trends in Cell Biology* review on integrin signaling in 1992[1], a 3000-word article could cover the entire subject without omitting any key references. In the year 2000 alone, a literature search on 'integrin' plus 'signal transduction' yielded 480 references. Given the impossibility of covering more than a sliver of what's been written, I have chosen to revisit the topics discussed in 1992, several of which seem to have developed in interesting ways.

A good deal of this expansion has been lateral. Even beyond my fairly wild dreams, integrins and integrin signaling have found their way into nearly every biological process. Integrin signals evidently play important roles in transplantation, angiogenesis, viral and bacterial infections, immune recognition, development, atherogenesis and nearly every other complex physiological or pathological process in vertebrate organisms[2–4]. Luckily, I feel no obligation to review this vast literature. However, a second kind of expansion still complicates the task. Integrin signaling has undergone a remarkable merger with other areas of signaling, particularly those involving the cytoskeleton and growth factor/cytokine receptors[5,6]. This trend in cell biology reflects the general paradigm shift towards understanding signal transduction in terms of spatially organized complex networks.

Tyrosine kinases

In 1992, focal adhesion kinase (FAK) had just been identified as a protein tyrosine kinase activated by integrin-mediated adhesion and localized to sites of adhesion. FAK has since emerged as a remarkably complex and interesting molecule. The reader is also directed to earlier reviews that cover FAK biochemistry in greater detail[7,8]. FAK is essential for multicellular life, as, in its absence, mice die early during embryogenesis. Even cells isolated from FAK$^{-/-}$ embryos show severe defects in cytoskeletal organization and motility. These defects appear to be due to a surprising number of molecular mechanisms. FAK$^{-/-}$ cells show abnormal regulation of the small GTPase Rho[9], of the mitogen-activated protein kinase (MAPK) Erk[10] and abnormal signaling through the platelet-derived growth factor (PDGF) receptor[11]. Additional mechanisms involve the Etk tyrosine kinase and the transcription factor Stat1, both of which appear to be downstream of FAK and to contribute to cell migration[12,13]. The adaptor protein p130^{cas} also binds to FAK and has been linked to activation of the small GTPase Rac to promote motility. This diversity of downstream pathways that converge on cell migration suggests that FAK is a central coordinator of this process. FAK has also been implicated in cell-cycle regulation through activation of both the Erk and JNK pathways. And FAK plays an important role in mediating integrin-dependent cell survival, possibly through phosphoinositide (PI) 3-kinase or JNK[7,8,14,15].

Multiple physical associations of FAK with other signaling molecules appear to mediate these multiple effector pathways. Proteins that bind to autophosphorylated FAK through their Src-homology 2 (SH2) domains include c-Src family kinases, GRB7 (Ref. 16), phospholipase Cγ1 (Ref. 17) and PI 3-kinase. p130^{cas} and a GTPase-activating protein (GAP) for Rho named GRAF bind to proline-rich sequences in the C-terminus of FAK through their Src-homology 3 (SH3) domains. Paxillin, talin and STAT1 bind through sequences near the C-terminus. The Etk tyrosine kinase directly binds to sequences in the N-terminus, and the PDGF receptor also associates (although perhaps indirectly) with the N-terminal region. Many of the proteins phosphorylated downstream of FAK might actually be substrates of associated Src-family kinases. Indeed, it has been suggested that FAK is best regarded as an adaptor protein with kinase activity[7]. Clearly, FAK promotes assembly of signaling complexes downstream of integrins that lead to a wide range of events; however, the detailed mechanisms by which particular partners induce specific downstream events are not well understood. An overview of what is known is presented in Fig. 1.

The Src tyrosine kinases, in addition to their role as cofactors for FAK-dependent responses, can be activated independently of FAK and contribute to a distinct set of responses. Src tyrosine kinases mediate phosphorylation of the adaptor Shc, which provides a separate link to the Ras/Erk pathway (reviewed in Ref. 18). They also phosphorylate Jab-1, which is a component of the nuclear signalosome that regulates the proteasome-dependent degradation of a number of nuclear proteins[19]; this pathway also contributes to integrin regulation of c-Fos.

Other tyrosine kinases regulated by integrins include syk, c-Abl and receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) and c-Met. Activation of syk is a very early event following stimulation of the integrin αIIbβ3 in platelets or β2 integrins on leukocytes (reviewed in Ref. 20). Unlike FAK, syk activation does not depend on the actin

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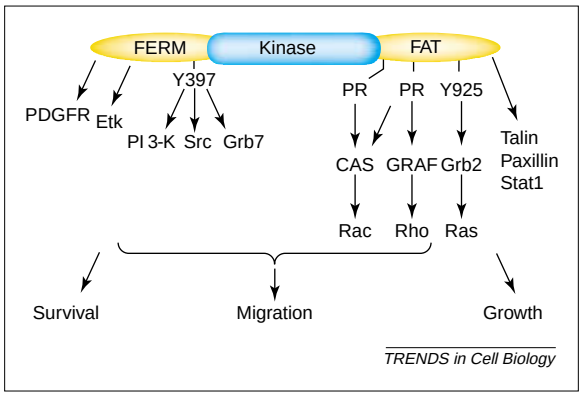

Fig. 1. Focal adhesion kinase (FAK). FAK has multiple binding partners, many of which appear to function as downstream effectors. A large number of these proteins, including the platelet-derived growth factor receptor (PDGFR), Etk, phosphoinositide (PI) 3-kinase, Grb7, p130^{cas} (CAS), GRAF, Stat1 and Erk appear to contribute to cell migration. Additionally, FAK can contribute to cell growth and survival, most likely through pathways that regulate Erk, JNK and PI 3-kinase. FAK domains include the N-terminal FERM (band 4.1, ezrin, radixin and moesin) domain and a FAT (focal adhesion targeting) sequence.

cytoskeleton. The Syk kinase contributes to integrin-mediated gene expression in monocytes and to the spreading of platelets. This last effect is most likely due to phosphorylation by syk of the Rac nucleotide exchange factor Vav1 (Ref. 21). c-Abl contributes to the integrin activation of Erk (Ref. 22) and serves to inhibit cell migration through an inhibitory phosphorylation of the SH2/SH3 domain adaptor protein c-Crk, leading to decreased assembly of a complex between Crk and p130^{cas} and decreased activation of Rac (Ref. 23). Integrins also induce transactivation of c-met and EGFR tyrosine kinase growth factor receptors in the absence of their growth factor ligands[24,25]. This process appears to be crucially dependent on the level of growth factor receptor, being apparent only at high levels of expression. Transactivation of these receptors contributes to integrin activation of the Ras–Erk pathway[24] and to tumorigenesis[25].

Phosphoinositides

The 1992 review described work from my laboratory that revealed how integrins could modulate the ability of growth factors to stimulate phosphoinositide turnover. We found that phosphatidylinositol (4,5)-bisphosphate (PIP_2) levels declined in nonadherent cells and that phospholipase C could still be activated by growth factors but to little effect in the absence of its substrate (reviewed in Ref. 26). We subsequently reported that the small GTPase Rho could activate the phosphatidylinositol 4-phosphate 5-kinase (PIP 5-kinase) responsible for synthesis of PIP_2. Rho also bound directly to the PIP 5-kinase, although this binding did not require GTP loading of Rho and did not increase the enzymatic activity of the PIP 5-kinase. Carpenter and coworkers reported that Rac bound PIP 5-kinase in a similar manner and that, despite the absence of direct stimulation of enzymatic activity, this interaction stimulates PIP_2 synthesis in activated platelets and subsequent actin polymerization[27].

The role of Rho in regulating PIP 5-kinase remained puzzling and somewhat controversial. A recent paper showed that Rho kinase played a key role in PIP 5-kinase activation, thereby providing a link between GTP loading and stimulation[28]. However, analyses of Rho and Rac activation in response to integrin stimulation do not show a tight correlation between their GTP loading and PIP 5-kinase activity[29,30]. This discrepancy might be resolved by recent work showing that integrins regulate GTPase function at a second step – that of membrane translocation. Rac in non-adherent cells could undergo GTP loading but failed to interact with effectors because it failed to associate with membranes[30]. Rho and Cdc42 evidently behave similarly (M. del Pozo and M. Schwartz, unpublished). This effect appears to account for nearly complete shut-off of Rac pathways in non-adherent cells. As membrane translocation should be crucial for PIP 5-kinase to interact with its substrate, this effect might account for a significant portion of the regulation of PIP_2 synthesis by integrins, which could involve both Rho and Rac. As PIP_2 is also a key regulator of the actin cytoskeleton, a substrate for phosphoinositide 3-kinase and a binding site for some pleckstrin-homology domains[31], integrin-dependent changes in PIP_2 levels or synthesis could have pleiotropic effects on cell signaling and membrane–protein interactions.

Proximal signals

The proximal mechanisms by which integrins signal constitute crucially important unknowns to which we have little insight. Lacking enzymatic activity, integrins must associate with other proteins, essentially adaptors, to trigger signals. The major area of progress has been the identification of such adaptors.

Integrin cytoplasmic domains bind directly to several cytoskeletal proteins that might associate with signaling molecules (reviewed in Ref. 32). For example, the β1A, β3 and β1D cytoplasmic domains bind to talin; the β1A tail binds to α-actinin; β1A, β2 and β7 tails bind to filamin; and the α4 tail binds to paxillin. Filamin and paxillin are excellent candidates for mediating signaling effects as they associate with many other adaptor and signaling molecules (reviewed in Refs 33 and 34). A serine/threonine integrin-linked kinase (ILK) was also reported to bind to the β1A integrin cytoplasmic domain (reviewed in Ref. 32) and to localize to focal adhesions[35]. This protein was proposed to function as a regulator of anchorage-dependent growth. ILK is evidently an important protein as its mutation results in a severe phenotype in *Drosophila*, where the cytoskeleton detaches from the integrins in several tissues[36]. However, more recent work has cast some doubt upon whether the direct binding of ILK to integrins mediates its localization to focal contacts or is necessary for its function[36,37].

Integrin cytoplasmic domains also bind directly to tyrosine kinases. FAK was reported to bind to peptides from the integrin β1 subunit (reviewed in Refs 7 and 8). This binding, however, involves the N-terminus of FAK rather than the C-terminal domain that targets it to focal adhesions and has been controversial. Recently, good evidence has been obtained that FAK binds directly to the β5 cytoplasmic

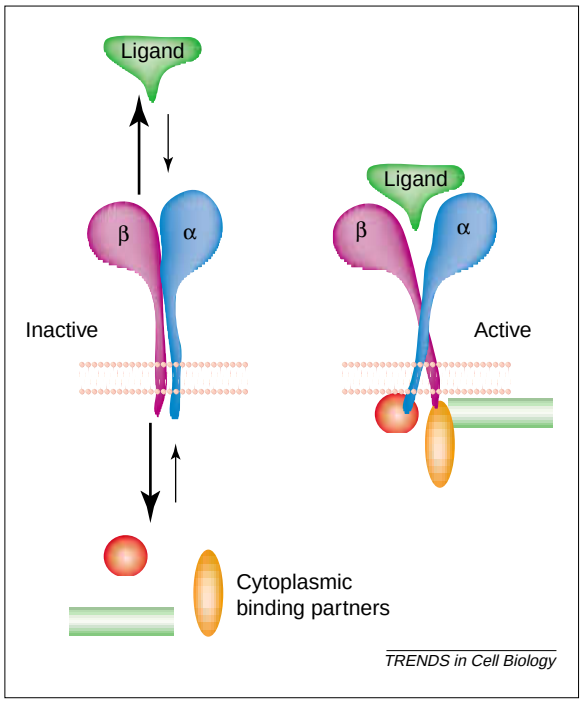

Fig. 2. Bidirectional signaling. For inactive integrins, the extracellular domain has a low affinity for extracellular matrix (ECM) ligands, while the α and β cytoplasmic domains associate with each other to induce a low affinity for intracellular cytoskeletal and signaling binding partners. Activated or occupied integrins have a high affinity for ECM ligands outside the cell, while disruption of the intramolecular tail interactions increases the affinity for intracellular components. Intracellular and extracellular events are therefore coupled through cooperative effects on conformation.

domain following FAK phosphorylation, which is crucial for β5-mediated cell migration[38]. The tyrosine kinase syk binds directly to the β3 cytoplasmic domain[39]. Syk is activated following ligation of β3 integrins; this event is very rapid and, as discussed above, has characteristics suggestive of a relatively direct event[20,21]. Clustering and occupancy of this integrin might therefore directly induce syk activation.

Although the implications for signaling are still hypothetical, recent structural studies have provided intriguing insights. The ligand-binding domains of integrins are globular regions near the N-termini of the α and β subunits and are connected to the transmembrane domains by long stalks[40]. Structural studies have revealed large conformational changes in the ligand-binding domains upon association with ligands[41,42]. Evidence suggests that these might be conveyed to the cytoplasmic domains by a change in separation between the α and β subunits within the stalk region[40]. These studies mesh nicely with evidence that an intramolecular interaction between the α and β cytoplasmic tails can regulate the affinity of the extracellular domain for ligand[43]. The emerging picture is therefore that separation between the C-termini of the two subunits conveys a bidirectional conformational change between the ligand-binding domain and the cytoplasmic domain. Binding of ECM proteins to the N-terminal domains might cause a change in conformation to free the β tail from an association with the α tail that sterically blocks interactions or constrains its conformation, thereby promoting binding to intracellular signaling proteins. Changes in the disposition of the cytoplasmic tails could similarly alter the conformation of the ligand-binding regions, leading to changes in affinity for ECM proteins. These ideas are summarized in Fig. 2.

Interestingly, integrin transmembrane and extracellular domains also associate with other membrane proteins that might serve as adaptors to promote signaling. The first such interaction identified was between integrin β3 and integrin-associated protein (IAP or CD47)[2]. Binding is mediated by the extracellular portion of the integrin to the Ig domain of CD47 and leads to formation of a signaling complex containing heterotrimeric G_i proteins, cholesterol and, most likely, other components[44,45]. Depletion of cholesterol dissociates IAP and integrins, suggesting that transmembrane domains might participate. Integrin α2β1 also associates with IAP and, in all cases, IAP-dependent signals modulate integrin function as well as initiating other G_i-dependent events[46].

Integrins associate with tetraspanin proteins, a family of small membrane proteins with four transmembrane domains ('TM4'; reviewed in Ref. 47). Integrins α3 and α6 form a tight complex with CD151, whereas α3, α6 and αvβ3 form weaker associations with CD9, CD63 and CD81. The high-affinity interaction of CD151 with integrin α3β1 involves the extracellular regions[48]. Associations between other tetraspanins and integrins are highly sensitive to even nonionic detergents such as Triton X-100 (Ref. 45), again suggesting that transmembrane domains might be involved. TM4 protein cytoplasmic domains can associate with signaling proteins such as protein kinase C (Ref. 49), supporting a possible adaptor function. The immediate signaling events triggered by tetraspanins are unknown, but the downstream consequences include modulation of cell migration and the cytoskeleton[50].

A distinct subset of integrins consisting of αv, α5 and α1 associate with caveolin[18]. This interaction has been mapped to the integrin transmembrane and extracellular regions. The association is sensitive to cholesterol depletion, suggesting that transmembrane domains are involved. Given the participation of extracellular regions of the integrin, this association might be indirect as caveolin does not extend outside the cell. The interaction is functionally important as it is linked to phosphorylation of shc by Src-family kinases, activation of the Ras–Erk pathway, cell proliferation and survival. Caveolin again enters the picture through an association between integrins and the urokinase plasminogen activator receptor (uPAR), a GPI-linked protein that localizes to lipid rafts and caveolae[51]. Association of both β1 and β2 integrins with uPAR promotes the ligand binding function of the integrins, possibly by bringing them into proximity with other raft/caveolae components. This lateral interaction is again sensitive to depletion of cholesterol. These results are summarized in Fig. 3.

To briefly summarize, it appears that the most central signaling events shared by all or nearly all integrins originate in the β cytoplasmic domains,

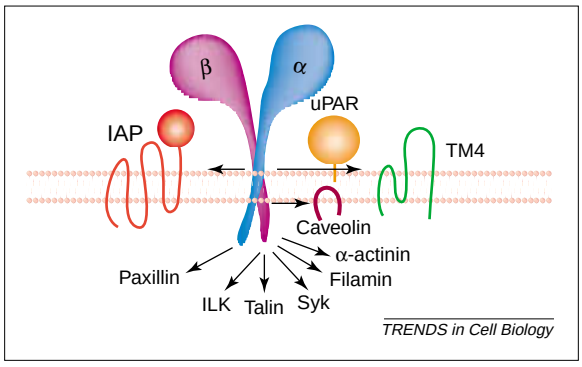

Fig. 3. Integrin binding partners. Integrins associate with other membrane proteins, including integrin-associated protein (IAP), urokinase plasminogen activator receptor (uPAR) and tetraspanin (TM4) proteins. These associations occur through extracellular and, perhaps, transmembrane regions of the molecules. Integrins also associate with the intramembrane and cytoplasmic protein caveolin – although it is not clear that this association is direct. The integrin cytoplasmic domains bind to a variety of cytoskeletal and signaling molecules, of which only those discussed in the text are shown. Abbreviation: ILK, integrin-linked kinase.

whereas α subunit and lateral associations mediate signaling and modulatory functions specific to one or a few integrin subunits. The past decade has seen the identification of many molecular interactions between integrins and other molecules, but there has been limited progress towards the important goal of understanding how integrin binding to the ECM triggers signaling. However, with so many physical protein interactions in hand, elucidating these mechanisms now seems within reach.

Signaling networks

The past ten years has seen our view of signaling pathways evolve into an understanding that they are organized into complex networks[52]. Signaling pathways branch, as illustrated by the many events downstream of FAK, and converge, as illustrated by the many upstream pathways that can regulate PI 5-kinase, Rac or Erk. These networks allow cells to respond in a coherent fashion to multiple stimuli. Signals from integrins act upon the same pathways as receptors for growth factors, cytokines and antigens, but often do so at different steps so that the net response is synergistic. The inositol lipid pathway discussed in the 1992 *Trends in Cell Biology* review was the first such synergy identified between integrins and growth factor receptors, but it has since been joined by many others. Activation of Erk, JNK, p38, Rac, Rho, Cdc42, PI 3-kinase, NF-κB and JAK–STAT pathways by soluble factors are all heavily influenced by integrin binding[5,6]. Conversely, plating cells on ECM proteins induces a transient transactivation of growth factor receptor tyrosine kinases[24,25], while stimulation of growth factor receptors can activate integrins to initiate new ECM binding[53]. I do not know of any pathways that are dedicated to only one or the other.

Growth factors and integrins both induce assembly of multicomponent complexes containing kinases, adaptors, substrates and scaffolding proteins[52]. This trend has gone so far that one might question whether integrins and growth factor receptors are equivalent. I suggest that integrin signaling is different because of its intimate association with the actin cytoskeleton. Integrins induce assembly of actin filaments and higher-order structures such as stress fibers and focal adhesions. These effects are mediated by pathways involving Rho family GTPases and phosphoinositides as well as through actin-binding proteins such as vinculin, talin, α-actinin and actopaxin that physically link integrins to actin filaments[32,54]. Simultaneously, integrin signals such as FAK activation are dependent on the state of the actin cytoskeleton[7,8]. Thus, the actin cytoskeleton is both upstream and downstream of integrin signaling. While growth factor/cytokine receptors are not entirely independent of actin, they do not participate in the same sort of intimate relationship.

One consequence of this relationship is mechanotransduction. Physical stresses from outside the cell can be transmitted to the cytoskeleton through integrins and modify cytoskeletal organization and influence signaling, and vice versa. For example, cells can sense the degree of mechanical resistance in the surrounding matrix and regulate contractility, protrusive activity, cell migration, differentiation and growth[55–58]. Conversely, cells generate forces that regulate their own cytoskeleton[59] and are transmitted to the ECM, where they modulate its assembly[60]. Assembly of cytoskeletal structures has been implicated in the ability of integrins to enhance transmission of growth factor signals[61,62]. Integrins, so named because they physically integrate the cytoskeleton with the ECM, might in fact mediate functional integration on a much larger scale, enabling cells to modulate their behavior based on their state of adhesion, mechanical forces and the concentrations of soluble growth factors.

Perspectives

A recurring image from the past decade is the complex of cytoskeleton and signaling proteins assembled at sites of integrin–ECM adhesion (see Fig. 1 in Ref. 1). The general concept that integrin-dependent assemblies guide and organize signaling is now prevalent. But, despite its appeal, the real evidence is thin. Clearly, immunofluorescence has shown that focal adhesions contain a variety of signaling molecules. Signaling assays have shown that detaching cells from the ECM results in diminished transmission of many signals initiated by growth factor/cytokine receptors. Immunoprecipitation assays indicate that most proteins are found in multimolecular complexes. But the real workings of these intracellular assemblies and how they transmit and modulate signals remain very poorly understood.

I suggest that the elucidation of localized events that are spatially and temporally determined by large protein complexes represents the major challenge for the next decade. Tackling this problem will require sophisticated imaging and biochemical methods to reveal their structure and function within living cells. Two previously unrelated fields – adhesion/cytoskeleton and signal transduction – have grown markedly closer in the past decade; it is now apparent that they represent two sides of a single coin and that a closely integrated approach is needed to solve both problems. Sounds like fun.

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