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*Topical Review*

# Integrin Signaling: Tyrosine Phosphorylation Events in Focal Adhesions

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## Introduction

Adhesive interactions between cells and the surrounding meshwork of extracellular matrix (ECM) proteins plays a vital role in numerous complex biological processes that include cell migration, cell proliferation and differentiation, cell survival, blood clotting and inflammatory responses. While the overall phenomenology of ECM effects on cell behavior is relatively well known, the biochemical and molecular bases for these effects have remained elusive. It is clear that many of the interactions between cells and the ECM are mediated by the integrin family of cell surface receptors [51, 93]. Binding of integrins to the ECM drives the formation of complex protein structures which tether actin stress fibers to the cytoplasmic face of the plasma membrane, and which are termed focal adhesions (Fig. 1). In these sites, the cytoplasmic domains of the integrins associate with the cytoskeletal proteins talin and α-actinin, serving to link the ECM to the actin cytoskeleton [10, 54]. Focal adhesions, however, play more than a structural role in anchoring the cell to the ECM, as ligand binding of integrins leads to activation of a range of biochemical signaling events, including elevation of intracellular pH and $Ca^{2+}$, activation of protein kinases, changes in lipid metabolism and, ultimately, changes in gene expression [105]. Some of the integrin-mediated signal transduction pathways, such as tyrosine phosphorylation, have been shown to be initiated from focal adhesions, whereas others may precede focal adhesion formation and be responses to integrin occupancy or clustering. The notion that focal adhesions are major sites of signal transduction is supported by the identification of multiple signaling proteins at these sites. This review focuses on recent evidence concerning the role of tyrosine phosphorylation events in integrin-mediated signaling. For a more comprehensive discussion on integrin signaling, the reader is referred to several recent excellent reviews [9, 21, 105, 123].

**Key words:** Extracellular matrix — Focal adhesion — Integrin — Tyrosine phosphorylation

## Focal Adhesions are Sites for Integrin Signaling

The focal adhesions of tissue culture cells provide a convenient model for analysis of the molecular basis of cell adhesion. These sites have been recognized for decades as sites of tight structural attachment of the cell membrane to the underlying substrate. Although classical focal adhesions can be found only in cultured cells, focal adhesions appear to be morphologically and functionally analogous to sarcolemmal dense plaques of smooth muscle cells in vivo [23]. On the outside of the cell membrane, ECM components, such as fibronectin, vitronectin, laminins and collagens are found. The primary transmembrane components of focal adhesions are integrins, a large family of transmembrane heterodimers [51]. Members of this protein superfamily act as receptors for ECM components on the outside of the cell, and interact with the cytoskeletal components of the focal adhesions inside the cells [95]. For the past decade, the number of proteins identified at the focal adhesion cytoplasmic face has expanded greatly. Analysis of their physical interactions has lead to the proposal of several models to describe the molecular links that anchor actin to the plasma membrane at these sites. Currently, it is thought that multiple mechanisms may exist to connect the actin cytoskeleton to integrins.

Talin is a major structural element of focal adhe-

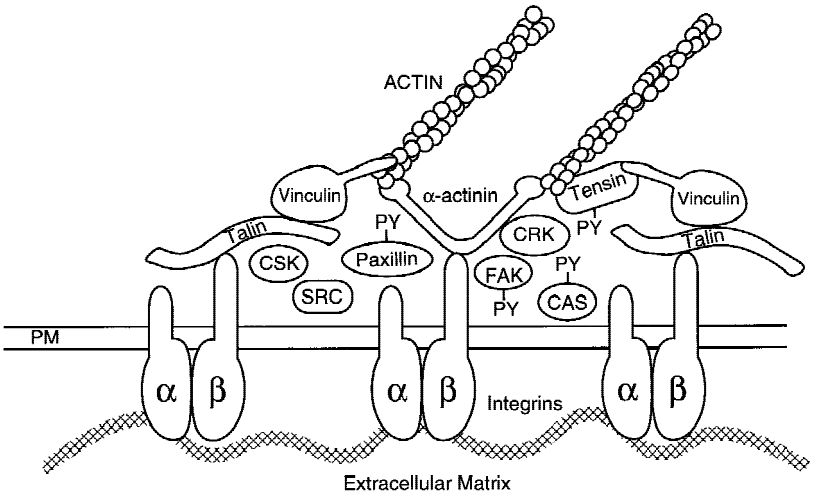


**Fig. 1.** A model showing how focal adhesion components may link actin stress fibers to sites of cell-ECM adhesion. A putative direct link from integrin to actin directly via talin is not shown. The illustration has been simplified and only includes proteins that are the topic of this review article; *see* main text for additional references. PM, plasma membrane; PY, phosphorylated tyrosine.

sions, and it is also the first cytoskeletal protein that was shown to directly interact with the cytoplasmic domain of an integrin [49]. Talin also binds to actin [37, 59, 78], thus providing a linkage from integrins to the actin cytoskeleton. α-actinin, an actin-cross-linking protein, has also been shown to bind to the cytoplasmic tail of the β-subunit of integrin [83]. Both talin and α-actinin were shown to bind to integrins with relatively low affinity in vitro, and it has been difficult to demonstrate that these interactions also occur in vivo. Coimmunoprecipitation of α-actinin and β2 integrin in neutrophils was nevertheless recently demonstrated by Pavalko and coworkers [85]. In another approach, Lewis and Schwartz [66] examined colocalization of proteins with transfected integrins that had been clustered with antibody-coated beads. Deletion of the last 13 residues at the C-terminus of the β1 cytoplasmic domain inhibited colocalization of talin and actin with clustered integrins. Interestingly, codistribution of α-actinin was blocked only after deletion of an additional 15 residues. These experiments would suggest that talin binding to integrins is required for attachment of actin and that α-actinin alone is insufficient to recruit actin to clustered integrins.

Vinculin is one of the most abundant focal adhesion proteins, interacting with both talin and α-actinin [10, 54]. Recently, a cryptic binding site for actin was located on vinculin [55, 57], suggesting that vinculin can also bind directly to actin if its conformation is favorable. Johnson and Craig demonstrated that an intramolecular, head-tail interaction exists within vinculin that masks binding sites for both talin and actin in vinculin's head and tail domains, respectively. Recent work by Gilmore and Burridge [34] and Critchley and coworkers [118] demonstrated that exposure of these sites in vinculin may be an important event in the assembly of focal adhesions. These two groups showed that the head-tail interaction of vinculin could be dissociated by acidic phospholipids and most effectively by $PIP_2$, which directly binds to the hinge region between the head and tail domains [56]. Importantly, $PIP_2$ levels are elevated in response to integrin-mediated cell adhesion in a Rho-dependent manner [19]. The small GTP-binding protein Rho in turn has a central role in a recent intriguing model for focal adhesion assembly proposed by Burridge and coworkers [20]. In this model, ligand-bound integrins in quiescent cells couple physically to actin microfilaments via proteins such as talin or α-actinin. The integrins are not clustered, however, and the actin filaments associated with these integrins are under little or no tension, owing to inactive conformation of myosin. Once the cells are stimulated with components of serum, such as lysophosphatidic acid, Rho becomes activated. Rho activation leads to myosin light chain phosphorylation, which turns on myosin function. As an end result, myosin filament assembly will generate a force to align actin filaments, and the tension generated will be transmitted to the integrins in the membrane, leading to their aggregation. Rho activity also elevates intracellular levels of $PIP_2$, which in turn enhances the binding of α-actinin [29] and vinculin (*see above*) to actin. $PIP_2$ also stimulates dissociation of two monomeric actin-binding proteins, profilin and gelsolin, from actin, thereby promoting actin polymerization [53, 65].

Aggregation of integrins is a cornerstone in further assembly of focal adhesions. If clustering of integrins is inhibited, focal adhesion assembly is prevented. On the other hand, clustering of integrins from outside with e.g., antibodies, in combination with ligand occupancy, induces colocalization of many focal adhesion molecules [75, 76]. Assembly of cytoskeletal molecules in focal adhesions is accompanied by recruitment of signaling components. By clustering of integrins with beads coated with anti-integrin antibodies or integrin ligands, Miyamoto and coworkers have identified a striking number of signaling molecules that associate with clustered integrins [76]. Assembly of the signaling molecules

takes place in a tyrosine phosphorylation-dependent manner, and several protein tyrosine kinases and their putative target proteins have been identified in focal adhesions following integrin ligand binding and clustering. In the following discussion, I will concentrate on signaling involving tyrosine phosphorylation in focal adhesions.

## FAK and Src Kinases and Their Target Molecules: A Central Role in Integrin Signaling?

Demonstration that the oncogenic v-Src tyrosine kinase localizes within focal adhesion first drew attention to these places as potential sites for tyrosine kinase-regulated signaling [81, 92]. Subsequently, it was shown by using immunofluorescence techniques that tyrosine-phosphorylated proteins in normal fibroblasts are enriched in focal adhesions [72]. The view that tyrosine phosphorylation plays a role in signal transduction in cell-matrix interactions was reinforced with the biochemical findings that ligand-engagement and clustering of integrins leads to a rapid tyrosine phosphorylation of intracellular proteins in several cell types [26, 36, 41, 64]. Since integrins do not possess intrinsic enzymatic activities, these results implied that an integrin-activated tyrosine kinase(s) must be present and active in focal adhesions. Indeed, coincident with these studies was the discovery of a cytoplasmic tyrosine kinase that localizes to focal adhesions, termed as focal adhesion kinase (FAK or $p125^{FAK}$) [43, 96]. Importantly, FAK was shown to become tyrosine phosphorylated and activated in response to integrin ligand binding and clustering [11, 40, 43, 63, 68, 115]. The discovery of FAK galvanized the integrin signaling field, and progress toward elucidating tyrosine kinase signaling events triggered by integrin-mediated cell adhesion has considerably accelerated in recent years.

### FAK: Molecular Structure, Focal Adhesion Targeting and Enzymatic Activation

The molecular structure of FAK is unusual among the families of protein tyrosine kinases. It can be divided into three domains (N-terminal, kinase, and C-terminal domains), each consisting of approximately 400 amino acids (Fig. 2) [43, 96]. Unlike many other cytoplasmic tyrosine kinases, FAK does not have Src homology 2 or 3 (SH2 or SH3) domains, which mediate specific interactions with phosphotyrosine-containing or proline-rich sequences, respectively [86]. A comparison of deduced amino acid sequences from different species reveals a remarkable high degree of identity, suggesting that the cellular function of FAK is highly conserved in evolution. In contrast to its recently identified close relative, Pyk2, which displays a restricted tissue distribution, FAK is widely expressed [44].

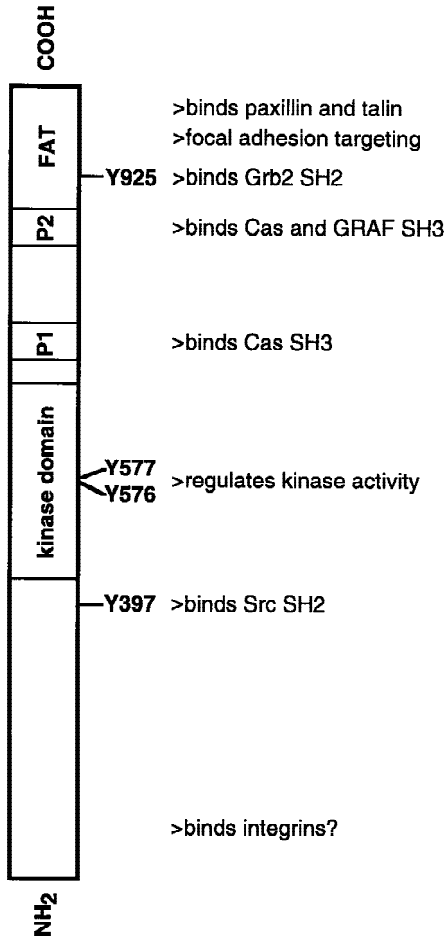


**Fig. 2.** FAK structure and domain organization. Four of the six known in vivo phosphorylation sites are indicated (Y) along with their proposed functional roles. Two proline-rich sites (P1 and P2) and their SH3-domain containing interacting partners are shown. The N-terminus of FAK contains a putative integrin-binding site, whereas the C-terminus is known to interact with paxillin and talin. FAT, focal adhesion targeting domain.

The C-terminal domain of FAK plays a role in recruiting FAK to focal adhesions; Hildebrand et al. [46] identified a region in the C-terminus if FAK, specifically in residues 856-1012, that was both necessary and sufficient for efficient localization of FAK to focal adhesion sites. Recently, Hildebrand et al. [47] and Tachibana et al. [106] demonstrated that FAK forms complexes with the focal adhesion protein paxillin through its C-terminal domain. The same domain was also shown to mediate FAK binding to talin [16]. It remains unclear, however, whether talin and paxillin play a role in recruiting FAK to focal adhesions. Interaction with talin alone may not be sufficient for FAK localization to focal adhesions, because a FAK mutant in which amino acids 853 to 963 are deleted does not localize to focal adhesions [46] al-

though it could bind to talin in vitro [16]. Similarly, FAK localization to focal adhesions appears to be independent of its binding to paxillin because a carboxy-terminal epitope-tagged FAK localizes to focal adhesions although it does not associate with paxillin. Finally, Miyamoto and colleagues found that simple aggregation of integrins was sufficient to co-cluster FAK but not talin or paxillin [75]. Interestingly, certain cells express a truncated form of FAK, which has been named FRNK (for FAK-related non-kinase). FRNK is identical to the C-terminal domain of FAK; it lacks a kinase domain, and is thus not catalytically active [97]. However, FRNK localizes to focal adhesions. As described below, overexpression of FRNK has been shown to result in a dominant-negative effect on endogenous FAK, and therefore FRNK provides a useful tool in dissecting the function of FAK in intracellular signaling [91].

The N-terminal domain of FAK has been shown to interact directly with a peptide mimicking the cytoplasmic domain of β1 integrin cytoplasmic domains in vitro [99]. The significance of this interaction is not obvious because this region does not appear to be involved in targeting FAK to focal adhesions. It has been recently suggested that FAK interaction with the integrin cytoplasmic domain would be required for or enhance the catalytic activity of FAK through oligomerization [33]. Recent results by LaFlamme and coworkers [108], however, demonstrate that the in vitro FAK binding domain within the β integrin cytoplasmic domain is neither required nor sufficient for triggering FAK phosphorylation. The significance of the observed interaction therefore remains unknown.

The mechanism of FAK activation by integrins is poorly understood, but it is clear that this process is tightly coupled to the assembly of focal adhesions and associated stress fibers. In addition to integrin-mediated cell adhesion, several growth factors and cytokines that affect the cytoskeleton have an effect on FAK activation [124]. Conversely, treatment of cells with cytochalasin D, which selectively disrupts F-actin filaments, can block FAK phosphorylation and activation by integrins or other stimuli (for references *see* [39]. As mentioned above, results by Miyamoto et al. [76] suggest a crucial role for integrin aggregation in FAK activation. A simple model, which is analogous to receptor tyrosine kinase activation [113], would have integrin clustering triggering dimerization of FAK molecules to permit transphosphorylation and activation. Evidence is still missing as to how FAK dimerization would take place; the role for direct binding of FAK to integrin cytoplasmic domains was discussed above. Aggregation of FAK in response to integrin clustering could also be mediated indirectly via talin, as talin binds to both integrins and FAK, but this seems unlikely in the light of results obtained by Miyamoto et al. (*see above*). In addition to FAK, another focal adhesion molecule, namely tensin, has been shown to cocluster with integrins in response to mere aggregation of integrins [76]. It remains to be seen what is the possible role for tensin in integrin-induced activation of FAK.

## Interactions of FAK with Signaling Molecules

For many receptor tyrosine kinases, ligand binding triggers autophosphorylation, and the resulting phosphotyrosines in the receptor cytoplasmic domain function as binding sites for SH2-domain containing signaling molecules. A similar situation has been found for FAK. The major, and maybe the only, site of autophosphorylation in FAK both in vitro and in vivo is Tyr-397 [98]. The importance of the Tyr-397 autophosphorylation is that phosphorylation of this site creates a high-affinity binding site for the SH2-domains of Src-family kinases, which have been shown to complex with FAK in cells [22, 98, 102]. This interaction may release autoinhibition of the Src family kinases by displacement of the C-terminal regulatory tyrosine residue from the SH2-domain, resulting in enzymatic activation of the Src kinases [8]. One consequence of the FAK-Src interaction is that Src further phosphorylates FAK on additional tyrosine residues and leads to full activation of FAK [12]. To date, five additional phosphoacceptor sites have been identified in FAK: tyrosines 407, 576, 577, 861 and 925 [44]. Maximal kinase activity of FAK requires phosphorylation of Tyr-576 and Tyr-577, which appear to be part of a regulatory region in FAK known as the ''activation loop'' [12]. While the significance of the phosphoacceptor sites Tyr-407 and Tyr-861 is not known, phosphorylation of Tyr-925 results in binding to the SH2-domain of Grb2 [102, 103]. Grb2 further binds to the GDP/GTP exchange protein Sos which activates Ras [25]. Therefore, FAK may be a link to the integrin-mediated Ras/Erk kinase activation observed by a number of investigators [18, 77, 102, 104, 125]. Recent evidence indicates that other signaling cascades initiated by integrin-mediated adhesion, such as those mediated by the adapter protein Shc, may also trigger Erk activation [67, 104, 117]. Autophosphorylated FAK is also known to interact with and possibly activate phosphatidylinositol 3′-kinase (PI 3-kinase) [17] and with Csk, a negative regulator of Src family kinases [4, 94]. FAK is thus linked to a number of signaling pathways through its interactions with SH2-domain containing signaling molecules.

FAK also binds to several signaling molecules in a phosphorylation-independent manner. As mentioned above, the C-terminus of FAK interacts with paxillin, which has emerged as a likely relevant signaling partner for FAK (*see later*). The two short proline-rich stretches in the C-terminal flanking region of FAK have received

attention as putative mediators of protein interactions through binding to SH3-domains. Indeed, a recently described protein, designated GRAF (for GTPase regulator associated with FAK), contains an SH3-domain, and binds to the C-terminal proline-rich region in FAK [48]. GRAF is homologous to GTPase activating proteins for members of the Rho family and was shown to stimulate the GTPase activity of two members of this family, Cdc42 and Rho, but not Rac [48]. Thus, GRAF may be a regulator of the Cdc42-Rac-Rho cascade in integrin signaling. Both of the proline-rich regions of FAK have been found to interact with a novel docking protein Cas ($p130^{Cas}$, Crk-associated substrate) [45, 89]. Similar to paxillin, Cas may be a target for FAK kinase activity, and is a likely candidate for mediating effects of FAK on intracellular signaling events.

**Table.** Interactions between focal adhesion components[a]

| | Integrin | FAK | Src | Tensin | Paxillin | Cas |
|---|---|---|---|---|---|---|
| Integrin | | 99 | | | 99 | |
| Talin | 49[b] | 16 | | | | |
| α-actinin | 83 | | | | | |
| Vinculin | | | | 71 | 112 | |
| FAK | 99 | | 22, 98 | | 47, 106 | 45, 89 |
| Src | | 22, 98 | | | 100, 119 | 80 |
| Csk | | 4, 94 | 94 | | 94 | |
| Tensin | | | | | | 70, 89 |
| Paxillin | 99 | 47, 106 | 100, 119 | | | |
| Cas | | 45, 89 | 80 | 70, 89 | | |
| PI 3-kinase | | 17 | 30, 88 | 2 | | |
| Crk | | | | | 5, 100 | 5, 114 |
| Nck | | | | | | 101 |

[a] For a more complete presentation, please *see* ref. 9
[b] Numbers refer to citation numbers used in the references

## Target Effectors of the FAK-Src Complex: Tensin, Paxillin and Cas

In addition to FAK, several other focal adhesion proteins have been identified as becoming tyrosine-phosphorylated upon integrin-mediated cell adhesion; among these are tensin, paxillin and Cas [6, 11, 82, 87, 116]. All the three molecules can be classified as docking molecules with no enzymatic activity that are capable of mediating numerous protein-protein interactions. These interactions are described in the Table, and for more details, the reader is referred to the original articles. Tensin has characteristics of both a structural and a signaling molecule; it binds to vinculin and actin [71, 120, 121] but it also contains an SH2-domain [24], which suggests that it may bind specific phosphotyrosine residues in other molecules. Paxillin also binds to vinculin [112] and, in addition, it interacts with a number of protein tyrosine kinases, including FAK (*see above*), Src [111, 119], and Csk [4, 94, 100]. Similar to paxillin, Cas also interacts with FAK (*see above*) and Src [80, 100], but Cas may also recruit protein tyrosine phosphatases to focal adhesions [31, 69]. The fact that tensin, paxillin and Cas become tyrosine-phosphorylated in an integrin-dependent manner suggests that they may mediate signaling complex formation with SH2-domain containing molecules, as well. Indeed, tyrosine-phosphorylated paxillin and Cas are known to bind the adapter protein Crk, which consists of SH2- and SH3-domains [5, 100, 114]. SH3-domains of Crk in turn mediate interaction with two guanine nucleotide exchange factors for members of Ras family, namely Sos and C3G [74, 109]. As described below, formation of these multi-protein complexes, which is partially controlled by tyrosine phosphorylation events, appears to be crucial in the regulation of several important cellular functions.

Integrin-mediated tyrosine phosphorylation of tensin, paxillin and Cas coincides with FAK activation, and it therefore seems feasible to assume that these molecules might be target molecules for the kinase activity of FAK. Indeed, FAK has been shown to phosphorylate both Cas [89, 101, 114] and paxillin [3, 100] in vitro. FAK appears to regulate tyrosine phosphorylation of paxillin and Cas also in vivo: overexpression of FAK stimulates paxillin tyrosine phosphorylation in chicken embryo fibroblasts [100] while expression of a constitutive active form of FAK results in a constitutive tyrosine phosphorylation of Cas [114]. Furthermore, overexpression of FRNK, which in a dominant-negative manner interferes with FAK function, inhibits tyrosine phosphorylation of paxillin and tensin [91]. Studies examining Cas phosphorylation in cell lines deficient in various protein tyrosine kinases indicate a major role for Src-family kinases, however. The adhesion-dependent tyrosine phosphorylation of Cas appears to be normal in cell lines established from FAK knockout embryos, but it is substantially reduced in cells isolated from Src knockout embryos [7, 42, 114]. Also implicating Src kinases, tyrosine phosphorylation of Cas is enhanced in cells lacking Csk [114]. Similarly, the phosphotyrosine content of tensin and paxillin is not diminished in cell derived from FAK knockout embryos [42, 52], and it is increased in cells lacking Csk [79, 110]. It is possible, however, that another FAK-related kinase, such as Pyk2, could compensate for the FAK deficiency. The likeliest explanation may be that FAK and Src coordinately regulate the tyrosine phosphorylation events in focal adhesions. It has been suggested that the role of FAK would be to recruit Src family kinases to FAK via autophosphorylation of Tyr-397, and that the Src family kinases would then catalyze the tyrosine phosphorylation of Cas and paxillin [90, 100, 114]. Along these lines, Tachibana and coworkers recently demonstrated that FAK's role is to initiate the tyrosine phosphorylation of Cas by directly

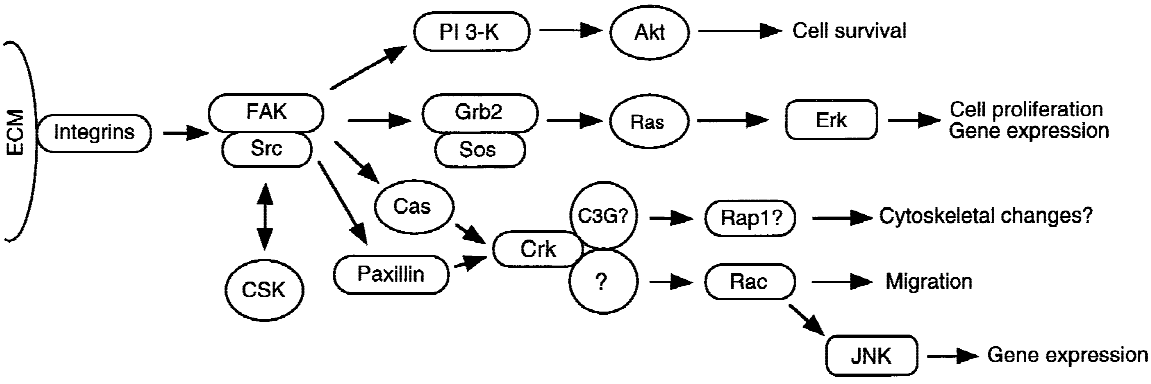


**Fig. 3.** Integrin signaling pathways through the FAK-Src complex. A putative model of how activation of downstream signaling pathways leads to changes in cellular functions is shown. *See* the main text for details.

phosphorylating Cas at the Src SH2-binding site. Upon binding to this site, Src family kinases would then carry out the bulk of tyrosine phosphorylation on Cas [107]. A similar coordinated action of the FAK-Src complex may well be applicable for the other phosphorylation events in focal adhesions.

## Biological Effects of FAK, Src and Docking Protein Complexes in Focal Adhesions

As a result of the tyrosine phosphorylation events, FAK and Src kinases and the docking proteins residing in focal adhesions form a network of protein-protein interactions that connect to multiple downstream biochemical signaling pathways (Fig. 3). Although our understanding of the exact molecular nature and biological significance of such signaling is still in its infancy, a number of recurrent themes are beginning to emerge.

Compelling studies have emphasized a role for the FAK-Src complex in controlling cell spreading and migration. Fibroblasts derived from Src −/− mice have been shown to spread more slowly on fibronectin than control cells [58]. Similarly, overexpression of Csk which negatively regulates Src has been shown to inhibit HeLa cell spreading [4]. Overexpression of FRNK also inhibits cell spreading, suggesting that FAK is involved in controlling this pathway [91]. The dominant negative effect of FRNK on cell spreading can be rescued by coexpression of Src, demonstrating the significance of cooperation between the bipartite kinase complex of FAK and Src [90]. The importance of FAK in modulating cytoskeleton is underscored by the observation that cells isolated from FAK −/− embryos appear rounded and show reduced rates of migration [52]. The migration defect may contribute for the embryonic lethality of the FAK knockout, as FAK −/− embryos exhibit a phenotype indicative of aberrant cell migration during gastrulation [52]. In another study, microinjection of a fusion protein corresponding to FRNK has been shown to reduce cell motility [35]. In both of these studies, lack or inhibition of the FAK function did not result in a reduction in focal adhesions; on the contrary, FAK −/− cells exhibit an unusually large number of centrally located small focal adhesions. These experiments therefore suggest that FAK signaling is not required for focal adhesion assembly or maintenance, but rather for focal adhesion turnover required for cell migration. The observations that overexpression of FAK increases migration of CHO cells [13] and that FAK expression in vivo is increased in rapidly migrating and invasive cells [1, 32, 84] are also consistent with a role of FAK in the dynamic regulation of focal adhesions. Formation of the FAK-Src kinase complex appear to be of importance in cell migration, as overexpression of a form of FAK in which Tyr-397 is mutated does not induce a migratory phenotype [13].

Recent results by Richardson and coworkers provide some clues as to what might be downstream of the FAK-Src kinase complex in the pathway controlling cell spreading [90]. By using the FRNK overexpressing cells as the model system, Richardson et al. found that not only does co-overexpression of Src rescue the spreading in these cells (*see above*), but so does expression of a catalytically inactive form of FAK. However, coexpression of the Tyr-397 autophosphorylation site mutant of FAK or a mutant that fails to bind to paxillin did not promote cell spreading. In every case, promotion of cell spreading correlated well with paxillin phosphorylation, and therefore the authors conclude that FAK acts as a ''switchable adapter'' that recruits Src to phosphorylate paxillin, promoting cell spreading.

Several recent studies suggest that Cas might be downstream of FAK in controlling cell migration. Cary and coworkers found that unlike overexpression of the wild-type form of FAK, overexpression of a FAK P712/715A mutant, which is not capable of interacting with Cas, does not result in enhanced cell migration [14]. Also, coexpression of Cas further increases FAK-

enhanced cell migration, whereas coexpression of a construct containing the Cas SH3-domain alone, which binds to FAK, functions as a dominant negative mutant and decreases FAK-enhanced migration. Klemke et al. in turn found that interaction of Cas with the adapter protein Crk functions as a molecular switch for induction of cell migration, and the small GTP-binding protein Rac is a necessary signaling mediator downstream of the Cas-Crk complex [62]. Importantly, dominant negative mutants of both Cas and Crk were found to block not only integrin-mediated haptotactic migration, but also cytokine-induced chemotactic migration, suggesting that these two molecules play a central role in various migration processes. Interestingly, the SH3-domain of Crk binds to the nucleotide exchange factor C3G, which is an effective activator of Rap1, a GTPase closely related to Ras [38]. The function of mammalian Rap1 is uncertain, but the functional Rap1 homologue in yeast, Bud1/Rsr1, is involved in coordinating the polarization of the actin cytoskeleton [15]. A homologous role for Rap1 in mammalian cells may involve regulation of actin cytoskeleton in processes such as cell shape changes and spreading.

Other studies have indicated the FAK-Src complex as a regulator of anchorage-independent growth and survival. Expression of a constitutive active form of FAK in epithelial cells confers resistance to detachment-induced apoptosis (''anoikis'') [27] and permits cell growth in soft agar [28]. Mutational analysis further demonstrated that FAK's autophosphorylation function was required for protection from anoikis and cell survival correlated with the FAK-Src complex formation. The role of FAK in regulating adhesion-dependent cell survival is supported by peptide microinjection experiments in which the inactivation of FAK caused apoptosis [50]. Furthermore, antisense attenuation of FAK expression in rhabdomyosarcoma tumor cells results in apoptosis associated with loss of adherence [122]. Recent results by Downward and coworkers demonstrate that PI 3-kinase and one of its downstream effector molecules, serine-threonine kinase Akt, provide survival signals in adherent epithelial cells [60]. These findings are consistent with the anti-apoptotic function of PI 3-kinase and Akt in other cell survival systems (for a review, *see* [73] and with the fact that PI 3-kinase and Akt become activated upon integrin-mediated cell attachment [60, 61]. Interestingly, autophosphorylated FAK is known to interact with and possible activate PI 3-kinase [17], and therefore FAK may mediate its effects on cell survival through the PI 3-kinase/Akt pathway.

## Conclusions

The last few years have witnessed considerable progress in the field of integrin signaling research. Particularly rapid advances have been made in dissecting the biochemical pathways utilizing tyrosine phosphorylation events as key mediators for signaling. It is likely that focal adhesions will continue to serve as a useful model for further studies regarding signaling in response to cell adhesion to ECM. On the other hand, we have just begun to understand how all these pathways influence cell survival, proliferation, migration and differentiation. It will be important to extend the studies to identify the further downstream components and nuclear events that control the cytoskeleton and the cell cycle and apoptotic machinery. An analysis of the molecular mechanisms that function downstream of integrins will be important not only to delineate a biochemical signaling pathway, but will also lead to a better understanding of such profound biological processes as normal growth regulation and anchorage dependency.

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