[Frontiers in Bioscience 4, d102-113, January 15, 1999]

FOCAL ADHESION KINASE IN INTEGRIN-MEDIATED SIGNALING

Leslie A. Cary and Jun-Lin Guan

Department of Molecular Medicine, Cornell University, Ithaca, NY 14853, USA

TABLE OF CONTENTS

1. ABSTRACT

Integrins serve as adhesion receptors for extracellular matrix proteins and also transduce biochemical signals into the cell. These signaling events regulate such cellular processes as proliferation, apoptosis, migration and spreading. Focal adhesion kinase (FAK) is an important protein tyrosine kinase which mediates several integrin signaling pathways. Putative mechanisms of integrin-mediated FAK activation and localization to focal adhesions are discussed here. FAK interacts with a number of signaling and cytoskeletal proteins, including Src, phosphatidylinositol 3-kinase, Grb2, p130^{Cas} and paxillin. Both the mechanisms and outcomes of these interactions are also presented. Finally, FAK's roles in the regulation of several integrin-mediated cellular events are discussed, including the promotion of cell migration, proliferation and spreading, and the prevention of cell apoptosis.

2. INTRODUCTION

Integrins are a family of cell surface receptors for extracellular matrix (ECM) proteins, such as fibronectin, vitronectin, collagen and laminin (1). They are heterodimeric glycoproteins, with at least 16 alpha subunits and 8 beta subunits identified so far which can combine to form at least 20 different receptors. In addition to serving as ECM adhesion molecules, integrins can also transduce biochemical signals into the cell to affect various proteins and second messengers, such as tyrosine kinases, serine/threonine kinases, lipid mediators, low molecular weight GTPases, and intracellular calcium fluxes. These signaling pathways regulate a variety of cellular functions, including spreading, proliferation, apoptosis and migration (2, 3, 4, 5). Defining and characterizing these signaling pathways is central to understanding the mechanisms of integrin-regulated cellular functions.

FAK (6, 7) is a critical mediator of integrin signaling. It is a cytosolic protein tyrosine kinase (PTK) which, in cells plated on an appropriate ECM substrate, is colocalized with integrins to focal adhesions. FAK is expressed in a variety of species, including human (8), rodent (7), chicken (6) and *Xenopus* (9, 10), indicating that it is evolutionarily conserved. In developing rodent embryos it is expressed beginning at approximately day 7.5 (11), and in adult tissues it is primarily detected in brain, lung, heart and testes (7, 11). Unlike many other cytosolic PTKs, FAK does not contain an SH2 (src-homology 2) or SH3 (src-homology 3) domain, but it does have SH2 and SH3 domain-interacting phosphotyrosines and proline-rich regions, respectively (see figure 1). FAK demonstrates increased kinase activity (12, 13) and tyrosine phosphorylation (7, 12, 14, 15, 16, 17) upon integrin activation in a variety of cell types, with the major site of phosphorylation identified as Y397 both *in vivo* and *in vitro* (18, 19, 20). Furthermore, phosphorylation at this site is mediated by FAK itself in an autophosphorylation mechanism. It is believed that FAK is the first identified member of a novel family of PTKs, based on the identification of a structurally related gene known as Pyk2, CAKbeta or RAFTK (21, 22, 23). In addition, other products of the FAK gene have been identified which are likely generated by alternative splicing, including a FAK-related non-kinase (FRNK), consisting of the C-terminal domain of FAK alone (24), and a series of FAK gene products which are preferentially expressed in brain tissues (25, 26, 27). Although its predominant role is in integrin signal transduction, FAK also responds to other

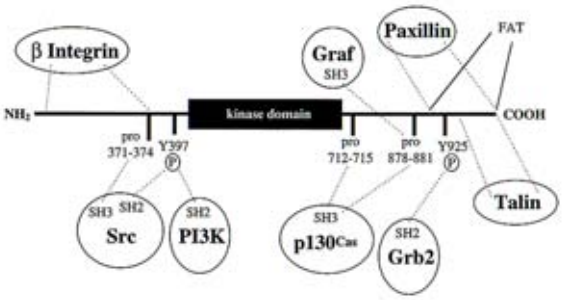

Figure 1. FAK-interacting proteins. FAK contains a central protein tyrosine kinase domain which is flanked by regions showing no homology to previously identified proteins. Its major autophosphorylation site is Y397, which serves as a binding site for the SH2 domains of Src and PI 3-kinase (PI3K). The SH3 domain of Src also participates in its interaction with FAK by binding a proline-rich region upstream of Y397. Phosphorylation of Y925 by Src mediates FAK interaction with Grb2. A proline-rich region at amino acids 712-715 serves as the primary binding site for the SH3 domain of $p130^{Cas}$, with a second proline-rich region at amino acids 878-881 contributing to this interaction. This second proline-rich region has been mapped as a binding site for the SH3 domain of Graf. The paxillin binding sequence in FAK overlaps completely with the focal adhesion targeting (FAT) sequence, and within this region lies the talin-binding sequence. Finally, a FAK sequence in its amino-terminal domain binds integrin beta subunits *in vitro*.

extracellular stimuli (28); however, space does not permit a discussion of these pathways here.

Although much is known about the role of FAK in integrin signaling, several crucial questions remain unanswered. First, the mechanism of FAK activation by integrins is not clear, although it is known to be specifically dependent on the integrin beta subunit (14, 29). There are currently two models describing beta integrin-mediated FAK activation. Direct binding of FAK to beta integrin cytoplasmic tails has been demonstrated in an *in vitro* system (30). However, concerns are raised based first on the lack of evidence for this interaction in other systems, and secondly on the differential mapping of the FAK binding site from the FAK activation site on the beta integrin (14). These results suggest that direct binding of FAK to the beta integrin is insufficient for its activation. A second model has been proposed involving the indirect association of FAK with the beta integrin through talin (31, 32). *In vivo* FAK association with talin as well as direct binding *in vitro* has been demonstrated (33). This model of FAK activation is believed to be the most likely based on several observations: first, FAK activation is dependent on the integrity of the actin cytoskeleton, as demonstrated by its disruption using cytochalasin D (16, 34); second, FAK binding to talin is not dependent on its catalytic activity; and finally, the talin binding site on the beta integrin overlaps with the site required for FAK activation (33).

Additionally, the mechanism of FAK localization to focal adhesions is not entirely clear. The FAK sequence required for this event was defined as the focal adhesion targeting (FAT) sequence, a 159 amino acid stretch located in the C-terminal domain of the protein (35) which was later redefined to a 148 amino acid stretch (36). Significant data exists to suggest that FAK localization is mediated by binding to paxillin through this FAT sequence. At least 17 different truncation mutants and 6 different point mutants of FAK have correlative abilities to localize to focal adhesions and bind paxillin (36, 37). The only data which sheds doubt on this model is that a FAK construct with a C-terminal epitope tag is localized to focal adhesions but does not bind paxillin (36). It is possible that this interaction is only destabilized by this epitope tag; however, because of this result it remains unclear whether paxillin mediates FAK localization to focal adhesions.

3. DISCUSSION

3.1. FAK-associated proteins and proposed functions

The major site of FAK phosphorylation, Y397, serves as a binding site for the SH2 domains of Src (19, 20) and phosphatidylinositol 3-kinase (PI 3-kinase) (38). This phosphotyrosine is required for FAK functions in many different integrin signaling pathways, indicating the importance of FAK association with these two proteins. FAK associates with a number of other signaling and cytoskeletal molecules (see figure 1) which will be described here, including Grb2, $p130^{Cas}$(crk-associated substrate), and paxillin. Both the mechanisms of FAK association with these proteins and the functional outcomes of these interactions in integrin-initiated signaling pathways will be discussed. Tensin is also believed to associate with FAK based on its tyrosine phosphorylation correlative with that of FAK (39, 40) which is likely mediated by Src (41). Tensin demonstrates properties of both an actin cytoskeletal protein and an integrin-activated signaling molecule (39, 40, 42, 43). However, neither the mechanism nor the functional outcome of FAK's putative interaction with tensin is clear. FAK also binds a recently identified protein called Graf (GTPase regulator associated with FAK), which is mediated by the SH3 domain of Graf and a proline-rich region on FAK (44). The demonstrations that Graf can localize with the actin cytoskeleton and that its GAP domain stimulates the GTPase activity of Rho and Cdc42 suggest that Graf may regulate the actin cytoskeleton through these proteins (44). Furthermore, Graf is believed to be a substrate of mitogen-activated protein (MAP) kinase, suggesting that it may respond to mitogenic or other stimuli (45). However, the functional role of FAK/Graf association remains a mystery, particularly since Graf is not expressed in most of the cell lines where FAK's role has been well characterized (45). Therefore the putative roles of both tensin and Graf in FAK signaling pathways will not be addressed here.

3.1.1. Src family members

The Src family of cytosolic protein tyrosine kinases (46) function intimately with FAK in integrin signaling pathways. Furthermore, the original identification of FAK as a protein with increased tyrosine phosphorylation in v-Src-transformed cells (6, 13, 47) suggests that FAK may mediate some of the oncogenic

functions of Src. The major autophosphorylation site on FAK, Y397, serves as a binding site for the Src SH2 domain (19, 20), and in fact association of FAK with Src family members has been demonstrated in a number of *in vivo* and *in vitro* systems (19, 20, 48, 49, 50). Although Y397 mediates the principle interaction of FAK with Src, a proline-rich region upstream of this phosphotyrosine may contribute to their association by binding the SH3 domain of Src (51). Consistent with this observation, the binding of a Src-SH2 construct to FAK is weaker than that of a Src-SH3-SH2 construct (49).

The outcome of Src binding to FAK is the phosphorylation of several tyrosine residues in FAK as well as in several FAK-associated proteins. In addition to Y397, FAK tyrosines 407, 576, 577, 861 and 925 have been mapped as phosphorylation sites. Of these, Y397 is the only residue clearly phosphorylated by FAK itself, while the others are phosphorylated by Src. The phosphorylation of Y576 and Y577 in the kinase domain of FAK positively regulates FAK catalytic activity (52). The roles of Y407 phosphorylation (52) or Y861 phosphorylation (53) are not clear, but are suggested to mediate binding to SH2 domains based on their neighboring residues. Finally, phosphorylated Y925 has been mapped as a binding site for the SH2 domain of the Grb2 adaptor protein which is believed to partially mediate activation of the Erk (extracellular-regulated kinase) family of MAP kinases (see below).

Of the known FAK-associated proteins, paxillin and $p130^{Cas}$ tyrosine phosphorylation are mediated at least in part by FAK-associated Src. Their phosphorylation in response to FAK expression is dependent on FAK Y397 (54, 55, 56), and while it is increased in $Csk^{-/-}$ cultured cells and reduced in $Src^{-/-}$ cells, it is not affected in $FAK^{-/-}$ cells (41, 55, 57). Because Pyk2 expression is increased in $FAK^{-/-}$ cells (58), it is possible that this homolog or other proteins may compensate for the absence of FAK in some functions. Although paxillin phosphorylation has been demonstrated by FAK *in vitro* (54, 59), because a recombinant source of FAK was not used it is not clear whether FAK-associated Src might be responsible for this phosphorylation. On the other hand, it is clear that $p130^{Cas}$ can be directly phosphorylated by FAK *in vitro* at the Src-binding YDYVHL sequence (60); therefore, the outcome of this event is the recruitment of Src to allow for complete $p130^{Cas}$ phosphorylation by this kinase. Src is therefore the kinase necessary for most if not all of FAK-promoted paxillin and $p130^{Cas}$ tyrosine phosphorylation in these complexes.

The outcome of Src phosphorylation of FAK or associated proteins is not always clear. FAK Y397 is crucial for many of the established biological roles of FAK, including cell migration (50), cell cycle progression (61) and prevention of anoikis, a form of detachment-induced apoptosis (62). More specifically (see below), Src binding to Y397 mediates phosphorylation of FAK Y925, binding to Grb2, and partial Erk activation by integrins. Substantial evidence demonstrates that Src binding to Y397 is necessary for FAK-promoted cell migration through its phosphorylation of $p130^{Cas}$ in this complex. Likewise, it is suggested that Src-mediated phosphorylation of paxillin in this complex mediates the rescue of FRNK-inhibited cell spreading. Characterization of Src's role by binding FAK Y397 is further complicated by the binding of an additional protein (PI 3-kinase) to this site. Clearly, Src is crucial for many of FAK's functions in integrin signal transduction through its phosphorylation of FAK and associated proteins.

3.1.2. PI 3-kinase

PI 3-kinase is an important lipid kinase which has recently been demonstrated to play a role integrin signal transduction. Some of its lipid products can act as second messengers to regulate protein kinase C (PKC) family members (63, 64) as well as the Akt kinase (65). PI 3-kinase is a heterodimeric enzyme, comprised of a 110 kDa catalytic subunit (p110) and an 85 kDa regulatory subunit (p85). In addition to its p110 binding site, the p85 subunit contains SH2 and SH3 domains which mediate its interactions with other cellular proteins. Association of FAK with PI 3-kinase in response to integrin activation has been demonstrated in both platelets (66) and fibroblasts (67). In addition, FAK/PI 3-kinase association in fibroblasts is stimulated by cell treatment with platelet-derived growth factor (PDGF) (68), suggesting a mechanism of cross-talk between integrin and growth factor signaling pathways. However, while it was suggested that p85 binding to FAK in platelets is dependent on the SH3 domain of p85, substantial evidence demonstrates that in fibroblasts it is mediated by FAK tyrosine phosphorylation. Furthermore, FAK Y397 was mapped as both an *in vitro* and *in vivo* binding site for PI 3-kinase through one or both SH2 domains of p85 (38). As discussed above, this phosphotyrosine also serves as a Src binding site; neither the mechanisms nor ramifications of two proteins binding to the same site on FAK are clear.

The requirement for FAK Y397 for its roles in cell proliferation, apoptosis and migration suggests that PI 3-kinase binding to FAK may be involved in any or all of these events. Because substantial data demonstrate a role for PI 3-kinase in preventing cell apoptosis through its downstream mediator Akt (69), the possibility that FAK/PI 3-kinase association regulates apoptosis should be emphasized, although there is no specific information available about this putative pathway. A role for PI 3-kinase in regulating cell motility has also been demonstrated in a number of studies. Growth factor-stimulated cell migration is dependent on PI 3-kinase (70, 71, 72, 73, 74), as is integrin $alpha_6beta_4$-stimulated carcinoma cell invasion (75) and chinese hamster ovary (CHO) cell migration on fibronectin (76). Cell migration stimulated by Rac or Cdc42 is also dependent on PI 3-kinase, and a constitutively active PI 3-kinase construct promotes cell migration on collagen (77). In addition, treatment of NIH 3T3 cells with purified $PI(3,4,5)P_3$, a product of PI 3-kinase, increases their migration (78). Finally, recent evidence demonstrates specifically that PI 3-kinase association with FAK plays a positive role in cell migration. A FAK point mutation proximal to Y397 which selectively disrupts its binding to p85 but not Src abolishes

FAK-promoted cell migration (76). The putative role of FAK/PI 3-kinase binding in other integrin-regulated signaling pathways will likely be an area of future study.

3.1.3. Grb2

The demonstrations that Grb2 binds directly to FAK at Y925 in response to integrin activation and that this site is phosphorylated by Src are intriguing (79, 80). They suggest that FAK acts in a manner analogous to a growth factor receptor in order to recruit Grb2 and Sos to the plasma membrane and initiate a Ras/MAP kinase pathway. However, while FAK expression in HEK 293 cells enhances fibronectin-stimulated Erk activation, this effect is dependent on FAK Y397 but only partially on FAK Y925 (81, 82). It is therefore believed that Grb2 binding to FAK mediates only a minor pathway downstream of FAK to activate Erks, with the major pathway initiated by FAK/Src-mediated tyrosine phosphorylation of Shc and its association with Grb2 (82) and another minor pathway mediated by $p130^{Cas}$ phosphorylation and recruitment of an SH2 domain-containing protein (83). In addition, a role for FAK/Grb2 binding in FAK-regulated cell migration has been ruled out (56), and although FAK/Grb2 association is detected in human monocytes treated with macrophage colony-stimulating factor (M-CSF), the role of their interaction in this system is not clear (84). Presently, therefore, the only established role of Grb2 binding to FAK is in the partial regulation of MAP kinases by integrins.

3.1.4. $p130^{Cas}$

$P130^{Cas}$ was identified as a protein with increased tyrosine phosphorylation in cells transformed by either the v-src or v-crk oncogenes (47, 85, 86, 87), and as expected it also demonstrates association with both of these proteins (85, 88). Cloning of $p130^{Cas}$ cDNA demonstrated that it is an adaptor protein, containing one SH3 domain and multiple tyrosines which are in consensus for SH2-binding sites (85). Most importantly, $p130^{Cas}$ contains multiple tyrosines in the YxxP motif, which are consensus Crk SH2-binding sites, and this YxxP repeat region within $p130^{Cas}$ is referred to as the substrate domain. Binding to Src is mediated by two independent sites within the C-terminal domain of $p130^{Cas}$: a Src SH2-binding phosphotyrosine (Y762) in the sequence YDYVHL which is outside of the substrate domain, and a Src SH3-binding proline-rich region (89). Two homologs of $p130^{Cas}$ have been identified, each by two independent groups. The first was named both Efs (90) and Sin (91), while the second was named both HEF-1 (92) and CasL (93). Both homologs are structurally similar to $p130^{Cas}$, containing an SH3 domain as well as multiple YxxP motifs. Efs/Sin is believed to play a role in some aspect of signaling by Src family members, while HEF-1/CasL is suggested to play roles in T cell receptor-mediated functions and/or regulation of the actin cytoskeleton. The relationship between the functions of $p130^{Cas}$ and these homologs remains to be determined.

An important role has been demonstrated for $p130^{Cas}$ in integrin signal transduction pathways. It is localized to focal adhesions in rat fibroblasts (87, 94) as well as in COS cells plated on fibronectin, where complete $p130^{Cas}$ localization is dependent on its SH3 domain, substrate domain, and Src-binding sites (95). Integrin activation induces tyrosine phosphorylation of $p130^{Cas}$ on various ECM proteins (86, 87, 94, 96), as well as in cells activated by anti-integrin antibodies (96, 97, 98). Further evidence for its role in integrin signal transduction comes from the demonstration that $p130^{Cas}$ binds FAK directly through its SH3 domain (88, 94, 99). There are two proline-rich regions in FAK which participate in binding to $p130^{Cas}$. The major $p130^{Cas}$-binding site, identified first, is located at amino acids 712-715 (99). A second site at amino acids 878-881 also contributes to this interaction, and both sites must be mutated in order to completely abolish $p130^{Cas}$ binding to FAK (94, 100). Interestingly, $p130^{Cas}$ is a substrate for several protein tyrosine phosphatases, including PTP-PEST (101), the *Yersinia* YopH protein (102) and PTP1B (103, 104). At least in the case of PTP1B, the SH3 domain of $p130^{Cas}$ mediates direct binding to the phosphatase, which may indicate a competitive interaction between FAK and PTP1B for binding $p130^{Cas}$.

Because $p130^{Cas}$ phosphorylation is increased by integrin activation and other FAK-activating stimuli and because it binds directly to FAK, a reasonable speculation is that FAK mediates $p130^{Cas}$ tyrosine phosphorylation. Indeed, FAK overexpression in either rat fibroblasts (55) or CHO cells (56) increases $p130^{Cas}$ phosphotyrosine levels. As discussed above, Src is primarily responsible for tyrosine phosphorylation of $p130^{Cas}$ in this complex (55). Although FAK does phosphorylate $p130^{Cas}$ directly, this event allows for Src binding to $p130^{Cas}$ and subsequently its complete phosphorylation of $p130^{Cas}$ at sites in the substrate domain (60). This model predicts that integrin-mediated phosphorylation of $p130^{Cas}$ by the FAK/Src complex results in its association with Crk. In fact, *in vivo* $p130^{Cas}$/Crk association as well as *in vitro* binding of $p130^{Cas}$ to several signaling proteins has been demonstrated by cell adhesion (55). In addition, several protein tyrosine phosphatases interact with $p130^{Cas}$, as discussed above, and are believed to be responsible for the downregulation of these $p130^{Cas}$-mediated signaling pathways through their dephosphorylation of $p130^{Cas}$ and/or associated proteins.

The functional role of $p130^{Cas}$ binding to FAK is in the regulation of cell motility, as demonstrated in several ways in CHO cells overexpressing FAK (56). Furthermore, the mechanism of $p130^{Cas}$ function in this system is believed to involve tyrosine phosphorylation of $p130^{Cas}$ by FAK-associated Src and subsequent recruitment of an SH2 domain-containing signaling molecule to $p130^{Cas}$. Correlative with this, $p130^{Cas}$ has also been demonstrated to play a role in COS cell migration, as expression of either $p130^{Cas}$ or Crk increases cell migration, which is dependent on their respective binding sites (105). Whether FAK/Src/$p130^{Cas}$-promoted migration in CHO cells is mediated by $p130^{Cas}$/Crk association remains to be determined. Other SH2 domain-containing proteins bind $p130^{Cas}$ in a FAK- or adhesion-dependent manner, such as PI 3-kinase (55, 56), and therefore could also regulate cell motility by $p130^{Cas}$.

If and when $p130^{Cas}$ does regulate cell migration through Crk, the mechanisms involved are not clear. Interestingly, either cell attachment or Src transformation increases the amount of FAK/$p130^{Cas}$ complexes in detergent-insoluble subcellular fractions (100). Similarly, Crk transformation increases the amount of cytoskeletal-associated $p130^{Cas}$ (106). These results suggest that $p130^{Cas}$ may control cell motility through regulation of the cytoskeleton. The Crk-binding protein DOCK180 is implicated in this mechanism, as it is suggested to regulate actin cytoskeleton dynamics (107, 108). Because the FAK/Src/$p130^{Cas}$ complex regulates the Erk family of MAP kinases (83), and Erks have been demonstrated to directly phosphorylate and activate myosin light chain kinase (109), regulation of the actin cytoskeleton in this way is another possibility. However, a role for Erks in FAK-promoted CHO cell migration has been ruled out, because FAK overexpression does not promote Erk activation, nor does a specific MEK inhibitor affect cell migration in this system (56). Furthermore, COS cell migration by $p130^{Cas}$ expression is not inhibited by coexpression of dominant-negative Ras (105). Erks are therefore not believed to mediate cell migration by $p130^{Cas}$. Another possible mechanism of $p130^{Cas}$-mediated cell migration is through the Rho family of GTPases, since Crk is suggested to mediate some of its cell morphological effects through activation of Rho signaling pathways (110) which are known to regulate specific actin cytoskeletal functions (111, 112). It should be noted that the major pathway downstream of Crk that has been identified results in activation of the JNK (Jun N-terminal kinase) family of MAP kinases (113, 114); however, there is little evidence to suggest that JNK may regulate cell motility. In conclusion, $p130^{Cas}$ clearly regulates cell motility through its associations with FAK and Crk; however, while many pathways downstream of these proteins have been identified, it is not clear which are mediating the effects on cell motility.

3.1.5. Paxillin

Paxillin was first identified as a cytoskeletal protein with increased tyrosine phosphorylation in v-Src transformed fibroblasts (115). Its roles in integrin signal transduction and/or cytoskeletal organization are demonstrated based on its localization to focal adhesions, binding to vinculin, and tyrosine phosphorylation concomitantly with FAK upon integrin activation (116, 117). The latter result also suggests that paxillin may be a substrate of FAK or a FAK-associated kinase. Analysis of paxillin cDNA (117) reveals many putative binding sites for other proteins, including several SH2 domain-binding phosphotyrosines, SH3 domain-binding proline-rich regions, and four LIM domains which have been shown to mediate protein dimerization (118, 119). In various *in vitro* or *in vivo* binding assays, paxillin binds vinculin (116, 117), integrin beta subunits (30, 120), Src family members (54, 121), FAK (36, 37, 117), Csk (54), Crk (54, 122) and the tyrosine phosphatase PTP-PEST (123). Because of its lack of enzymatic activity, paxillin is generally believed to act as a scaffolding protein in focal adhesions by mediating interactions with other signaling and/or cytoskeletal proteins.

The mechanism of paxillin localization to focal adhesions is not clear, although at least two binding proteins have been ruled out (124). In this study, it was demonstrated that the third LIM domain of paxillin (LIM3) was necessary for paxillin localization, with LIM2 possibly contributing to this effect. However, both the vinculin and FAK binding sites were mapped to paxillin's LD motifs (short stretches named for their repeats of leucine and aspartic acid), sequences which are well separated from LIM2 and LIM3. Therefore, paxillin is targeted to focal adhesions by a LIM domain-mediated interaction and not by binding vinculin or FAK, which is consistent with the model of FAK localization to focal adhesions by its binding to paxillin. The protein(s) which bind to these LIM domains to mediate paxillin localization have not yet been identified. Furthermore, phosphorylation of various serine and threonine residues within LIM2 and LIM3 affects paxillin localization in a transient manner (125), but the kinase(s) responsible for these phosphorylation events are also not known.

The roles of FAK association with paxillin are not completely clear. Substantial evidence demonstrates that paxillin recruits FAK to focal adhesions by its direct binding to the FAT sequence, thus in this model, paxillin is upstream of FAK. However, many studies suggest that paxillin may also be a downstream mediator of FAK signal transduction pathways. Because tyrosine phosphorylation of paxillin occurs concomitantly with FAK, it is believed to occur in a FAK-dependent manner. Indeed, increased tyrosine phosphorylation of paxillin has been demonstrated as a result of FAK overexpression in CEF cells (54). However, several lines of evidence suggest that Src rather than FAK is responsible for paxillin phosphorylation (41, 54, 57). *In vitro* phosphorylation of paxillin by FAK has been demonstrated in two independent studies, with a major site of phosphorylation mapped to Y118 in both (54, 59). However, in neither study was a recombinant source of FAK used; thus it is possible that phosphorylation of paxillin Y118 is mediated by FAK-associated Src. In any case, phosphorylation of paxillin at Y118, whether by FAK or by Src, is not necessary for efficient binding to FAK (59), but is suggested to create a binding site for Crk, as this tyrosine fits the consensus YxxP motif (YSFP in paxillin) for binding the Crk SH2 domain. Indeed, paxillin phosphorylated as a result of FAK overexpression does demonstrate increased *in vitro* binding to a Crk SH2 construct (54), and stable *in vivo* association of paxillin and Crk has also been demonstrated by coimmunoprecipitation in Crk-transformed cells (122). The biological role of paxillin Y118 phosphorylation and binding to Crk is not clear, although roles in both paxillin localization to focal adhesions (59, 125) and cell adhesion to fibronectin (125) have been ruled out by studies with a paxillin Y118F mutant. Recently, a direct interaction between paxillin and PTP-PEST has been demonstrated (123); dephosphorylation of paxillin and/or associated proteins by this phosphatase is suggested as an important regulatory mechanism of paxillin-mediated signaling pathways.

Identification of a functional role for paxillin downstream of FAK is complicated by the lack of

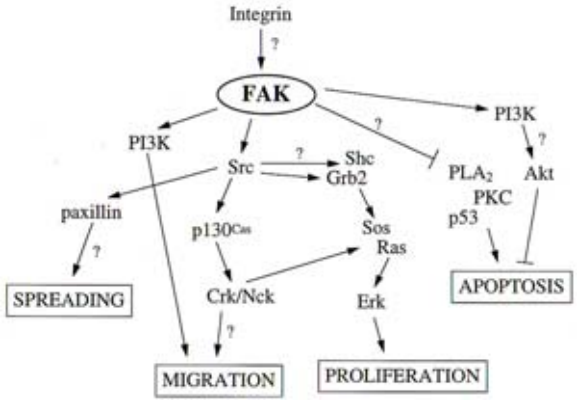

Figure 2. Integrin signal transduction pathways mediated by FAK. FAK has been demonstrated to play a role in several different integrin-mediated cellular events, including the promotion of cell migration, proliferation and spreading, and the prevention of cell apoptosis. Both established and putative signaling pathways downstream of FAK which regulate these processes are shown here. Question marks (?) indicate unclear mechanisms and/or pathways. Two pathways have been described downstream of FAK which promote cell migration: the first occurs through Src-mediated phosphorylation of FAK-associated $p130^{Cas}$ and may require Crk downstream of $p130^{Cas}$, and the second is mediated by PI 3-kinase (PI3K) association with FAK. Three independent pathways downstream of the FAK/Src complex regulate the Erk family of MAP kinases, which are predicted to regulate cell proliferation. The major pathway involves the promotion of Grb2/Shc association by an unknown mechanism, with minor pathways occurring through FAK binding to Grb2 and $p130^{Cas}$. FAK clearly prevents apoptosis, although the mechanisms by which it does so are less clear than the pathways described above. It is suggested that PI3K and possibly Akt are downstream of FAK in this pathway. A separate pathway involving PLA_2, PKC and p53 has also been described. Finally, FAK-promoted cell spreading is believed to occur through Src-dependent phosphorylation of paxillin in this complex.

identification of a functional role for paxillin itself. Recently, expression of paxillin mutants targeting the serine/threonine phosphorylation sites in the LIM domains were shown to affect cell adhesion to fibronectin (125). To date, this is the best established functional role for paxillin, although it is not clear whether its association with FAK and/or Src may be involved in this process. Because paxillin phosphorylation is believed to be mediated by Src, and because FAK's Src-binding site (Y397) is crucial for many of its established functional roles, including cell proliferation, migration, and prevention of apoptosis, it is possible that paxillin mediates some of these events by the FAK/Src complex. However, paxillin tyrosine phosphorylation by FAK overexpression in CHO cells is only partially dependent on Y397 (50). Furthermore, the role of FAK Y397 in this system is believed to be necessary for Src-mediated phosphorylation of $p130^{Cas}$ (56) and for an independent PI 3-kinase mediated pathway (76). Therefore we conclude that paxillin at best is only partially responsible for FAK-promoted cell migration. More convincing is the suggestion that FAK/Src-mediated phosphorylation of paxillin mediates the rescue of FRNK-inhibited cell spreading, since paxillin phosphorylation correlates completely with this phenotype (126). Paxillin is therefore believed to localize FAK to focal adhesions, and may play additional roles downstream of FAK in the regulation of cell spreading or other events.

3.2. Integrin-regulated functions mediated by FAK

Many FAK-associated proteins have been described, and in some cases the biochemical signal transduction pathways resulting from these interactions have been identified. Furthermore, many functional roles for FAK in integrin signaling processes are known. It was originally predicted that FAK plays a role in integrin-mediated cell adhesion, spreading or focal adhesion formation; however, little evidence exists to date to support this hypothesis. Instead, roles for FAK have been identified in the positive regulation of cell proliferation and cell migration, and the prevention of cell apoptosis. The signaling pathways responsible for these events will be described here (see figure 2).

3.2.1. Cell adhesion and spreading

FAK was identified as a protein tyrosine kinase which demonstrates increased kinase activity and tyrosine phosphorylation in response to integrin activation, e.g. by cell attachment to ECM proteins. Because of the strong correlation between FAK regulation and these integrin-dependent events, it was predicted that FAK mediates these functions. However, there is little evidence to support this hypothesis, although many studies have examined this possibility. There is no direct evidence to demonstrate a role for FAK in regulating integrin-mediated cell adhesion. Cultured cells from $FAK^{-/-}$ cells show little or no differences in adhesion to fibronectin compared to $FAK^{+/+}$ cells (57); likewise, CHO cells which stably overexpress FAK demonstrate no altered cell adhesion to fibronectin (50). However, some studies do indicate that FAK may mediate cell spreading on ECM proteins. Although FAK expression in various cultured cells does not affect cell morphology or spreading (35, 50), $FAK^{-/-}$ cells are more poorly spread when plated on fibronectin than their $FAK^{+/+}$ counterparts (57). Less direct evidence for a FAK role in cell spreading comes from studies with the C-terminal FAK-related non-kinase, FRNK (24). Expression of FRNK in chicken embryo fibroblast cells results in delayed cell spreading on fibronectin, i.e. FRNK-expressing cells spread more slowly, but eventually their morphology is indistinguishable from that of control cells (127). Furthermore, this reduced cell spreading correlates to some degree with reduced tyrosine phosphorylation of FAK and can be overcome by coexpression of wild-type FAK. From these results it is believed that FRNK acts as a competitive inhibitor of FAK, and therefore this phenotype reflects a FAK-mediated function. In addition, cell spreading in this system was shown to correlate with paxillin but not tensin phosphorylation (126). It is therefore suggested that phosphorylation of paxillin or other proteins by the FAK/Src complex regulates cell spreading.

3.2.2. Cell proliferation

Several studies demonstrate a role for FAK in regulating cell growth. This was first suggested based on studies using a FRNK-related FAK construct. When microinjected into cells this construct inhibits DNA synthesis, presumably through competitive inhibition of FAK (128). Recently, inducible expression of FAK was demonstrated to positively regulate cell cycle progression (61). While wild-type FAK increases DNA synthesis and accelerates the G_1/S transition, a dominant-negative FAK construct inhibits these steps, and these effects correlate with expression of cyclin D1 and the cdk inhibitor p21. Furthermore, these FAK-mediated cell cycle effects are dependent on Y397 and are believed to require Src and/or PI 3-kinase binding to this site. In addition, FAK is predicted to mediate integrin-dependent regulation of the Erk family of MAP kinases through several pathways (81, 82, 83), and these pathways are suggested to act synergistically with mitogenic signaling pathways to regulate cell growth (129, 130, 131, 132). Interestingly, $FAK^{-/-}$ cells do not demonstrate reduced proliferation (57), which is likely a result of compensation by the FAK-related protein Pyk2 (58). Together, these results demonstrate that FAK positively regulates cell cycle progression and cell proliferation. Future studies will likely determine the mechanisms of FAK function in these pathways.

3.2.3. Cell apoptosis

In several studies FAK has been demonstrated to protect cells from a form of apoptosis known as anoikis, which is induced by cell detachment from the ECM. Expression of an activated, membrane-targeted FAK construct called CD2FAK (18) in epithelial cells prevents anoikis (62). Conversely, inhibition of FAK either by treatment of tumor cell lines with FAK antisense oligonucleotides (133) or by microinjection of CEF cells with an anti-FAK monoclonal antibody (134) induces apoptosis. In addition, various cell lines induced to undergo apoptosis demonstrate concomitant FAK proteolysis (135, 136, 137), and several caspases, apoptosis-specific proteolytic enzymes, have been shown to cleave FAK at specific sites *in vitro* (136, 138). Cleavage of FAK is predicted to generate FRNK-like polypeptides, so that not only are the levels of full-length FAK reduced in apoptotic cells, but they are replaced by a competitive inhibitor of FAK. The mechanisms of cell survival by FAK are not clear. Because Y397 of FAK is required for its prevention of anoikis, FAK association with Src and/or PI 3-kinase is predicted to be necessary (62). Recent evidence also suggests that FAK prevents apoptosis through a pathway involving phospholipase A_2 (PLA_2), PKC and p53, although the pathways from FAK to these proteins are not clear (139).

3.2.4. Cell migration

Perhaps the best defined role for FAK in integrin-mediated cellular functions is in the promotion of cell migration. This was first suggested by the demonstration that a tyrosine kinase was required for endothelial cell migration and that the activity of FAK was correlated with this event (140). More direct evidence for FAK's role in cell motility comes from several different studies. Inhibition of FAK by microinjection of a FRNK-related construct results in decreased endothelial cell motility (128). A FAK homozygous knockout in mice is embryonic lethal, with embryos showing abnormalities in mesoderm development, and cells cultured from these $FAK^{-/-}$ embryos display decreased motility *in vitro* (57). Conversely, stable overexpression of FAK in CHO cells results in increased cell motility on fibronectin, which is dependent on both Y397 (50) and the $p130^{Cas}$ proline-rich binding site (56). FAK is believed to regulate CHO cell migration through at least two distinct pathways: one involving tyrosine phosphorylation of FAK-associated $p130^{Cas}$ by Src, which binds FAK at Y397 (56), and the other involving a distinct pathway initiated by PI 3-kinase binding to FAK Y397 (76). Interestingly, the FAK homolog Pyk2 can substitute for FAK in the regulation of MAP kinases but not in the regulation of cell migration (58).

The implications for FAK's role in integrin-mediated cell motility are several. The first is in the regulation of cell migration steps required for proper embryonic development at approximately day 8 of mouse development. FAK expression is detected starting at about day 7.5 (11), while $FAK^{-/-}$ mice are embryonic lethal at about day 8.5 with defects that suggest abnormalities in mesoderm migration (57). Another is in tumor cell invasion and metastasis because FAK expression is correlated with more invasive human tumors (8, 141), although it is not clear whether FAK expression in these tumors is a cause or a consequence of their increased invasiveness. Finally, FAK expression correlates with keratinocyte migration in repairing burn wounds, suggesting that FAK-mediated motility may also be important for wound healing processes (142).

4. ACKNOWLEDGMENTS

Research in the authors' laboratory is supported by NIH grants R01GM48050 and R01GM52890. Jun-Lin Guan is an Established Investigator of the American Heart Association. We thank Dong Cho Han, Molly Ingersoll, Heinz Reiske and Ji-He Zhao for critical reading of the manuscript.

5. REFERENCES

1. R.O. Hynes: Integrins: versatility, modulation and signaling in cell adhesion. *Cell* 69, 11-25 (1992)
2. E. Ruoslahti & J.C. Reed: Anchorage dependence, integrins, and apoptosis. *Cell* 77, 477-478 (1994)
3. D.A. Lauffenburger & A.F. Horwitz: Cell migration: a physically integrated molecular process. *Cell* 84, 359-369 (1996)
4. R.K. Assoian: Anchorage-dependent cell cycle progression. *J. Cell Biol.* 136, 1-4 (1997)
5. M.E. Bottazzi & R.K. Assoian: The extracellular matrix and mitogenic growth factors control G1 phase cyclins and cyclin-dependent kinase inhibitors. *Trends Cell Biol.* 7, 348-352 (1997)
6. M.D. Schaller, C.A. Borgman, B.S. Cobb, A.B. Reynolds & J.T. Parsons: $pp125^{FAK}$, a structurally distinctive protein tyrosine kinase associated with focal

adhesions. *Proc. Natl. Acad. Sci. USA* 89, 5192-5196 (1992)
7. S.K. Hanks, M.B. Calalb, M.C. Harper & S.K. Patel: Focal adhesion protein tyrosine kinase phosphorylated in response to cell spreading on fibronectin. *Proc. Natl. Acad. Sci. USA* 89, 8487-8491 (1992)
8. T.M. Weiner, E.T. Liu, R.J. Craven & W.G. Cance: Expression of focal adhesion kinase gene and invasive cancer. *Lancet* 342, 1024-1025 (1993)
9. M.D. Hens & D.W. DeSimone: Molecular analysis and developmental expression of the focal adhesion kinase pp125^{FAK} in *Xenopus laevis*. *Dev. Biol.* 170, 274-288 (1995)
10. X. Zhang, C.V. Wright & S.K. Hanks: Cloning of a *Xenopus laevis* cDNA encoding focal adhesion kinase (FAK) and expression during early development. *Gene* 160, 219-222 (1995)
11. T.R. Polte, A.J. Naftilan & S.K. Hanks: Focal adhesion kinase is abundant in developing blood vessels and elevation of its phosphotyrosine content in vascular smooth muscle cells is a rapid response to angiotensin II. *J. Cell. Biochem.* 55, 106-119 (1994)
12. L. Lipfert, B. Haimovich, M.D. Schaller, B.S. Cobb, J.T. Parsons & J.S. Brugge: Integrin-dependent phosphorylation and activation of the protein tyrosine kinase pp125^{FAK} in platelets. *J. Cell Biol.* 119, 905-912 (1992)
13. J.-L. Guan & D. Shalloway: Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. *Nature* 358, 690-692 (1992)
14. J.-L. Guan, J.E. Trevithick & R.O. Hynes: Fibronectin/integrin interaction induces tyrosine phosphorylation of a 120-kDa protein. *Cell Regul.* 2, 951-964 (1991)
15. L. Kornberg, S.E. Earp, C.E. Turner, C. Procktop & R.L. Juliano: Signal transduction by integrins: increased protein tyrosine phosphorylation caused by clustering of beta$_1$ integrins. *Proc. Natl. Acad. Sci. USA* 88, 8392-8396 (1991)
16. K. Burridge, C.E. Turner & L.H. Romer: Tyrosine phosphorylation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. *J. Cell Biol.* 119, 893-903 (1992)
17. L. Kornberg, H.S. Earp, J.T. Parsons, M. Schaller & R.L. Juliano: Cell adhesion or integrin clustering increases phosphorylation of a focal adhesion-associated tyrosine kinase. *J. Biol. Chem.* 267, 23439-23442 (1992)
18. P.-Y. Chan, S.B. Kanner, G. Whitney & A. Aruffo: A transmembrane-anchored chimeric focal adhesion kinase is constitutively activated and phosphorylated at tyrosine residues identical to pp125^{FAK}. *J. Biol. Chem.* 269, 20567-20574 (1994)
19. M.D. Schaller, J.D. Hildebrand, J.D. Shannon, J.X. Fox, R.R. Vines & J.T. Parsons: Autophosphorylation of the focal adhesion kinase, pp125^{FAK}, directs SH2-dependent binding of pp60^{src}. *Mol. Cell. Biol.* 14, 1680-1688 (1994)
20. B.L. Eide, C.W. Turck & J.A. Escobedo: Identification of Tyr-397 as the primary site of tyrosine phosphorylation and pp60^{src} association in the focal adhesion kinase, pp125^{FAK}. *Mol. Cell. Biol.* 15, 2819-2827 (1995)
21. S. Avraham, R. London, Y. Fu, S. Ota, D. Hiregowdara, J. Li, S. Jiang, L.M. Pasztor, R.A. White, J.E. Groopman & H. Avraham: Identification and characterization of a novel related adhesion focal adhesion kinase (RAFTK) from megakaryocytes and brain. *J. Biol. Chem.* 270, 27742-27751 (1995)
22. S. Lev, H. Moreno, R. Martinez, P. Canoll, E. Peles, J.M. Musacchio, G.D. Plowman, B. Rudy & J. Schlessinger: Protein tyrosine kinase PYK2 involved in Ca^{2+}-induced regulation of ion channel and MAP kinase functions. *Nature* 376, 737-745 (1995)
23. H. Sasaki, K. Nagura, M. Ishino, H. Tobioka, K. Kotani & T. Sasaki: Cloning and characterization of cell adhesion kinase beta, a novel protein-tyrosine kinase of the focal adhesion kinase subfamily. *J. Biol. Chem.* 270, 21206-21219 (1995)
24. M.D. Schaller, C.A. Borgman & J.T. Parsons: Autonomous expression of a noncatalytic domain of the focal adhesion-associated protein tyrosine kinase pp125^{FAK}. *Mol. Cell. Biol.* 13, 785-791 (1993)
25. F. Burgaya & J.A. Girault: Cloning of focal adhesion kinase, pp125FAK, from rat brain reveals multiple transcripts with different patterns of expression. *Brain Res. Mol. Brain Res.* 37, 63-73 (1996)
26. P. Derkinderen, M. Toutant, F. Burgaya, M. Le Bert, J.C. Siciliano, V. de Franciscis, M. Gelman & J.-A. Girault: Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* 273, 1719-1722 (1996)
27. F. Burgaya, M. Toutant, J.-M. Studler, A. Costa, M. Le Bert, M. Gelman & J.-A. Girault: Alternatively spliced focal adhesion kinase in rat brain with increased autophosphorylation activity. *J. Biol. Chem.* 272, 28720-28725 (1997)
28. M.A. Schwartz, M.D. Schaller & M.H. Ginsberg: Integrins: emerging paradigms of signal transduction. *Annu. Rev. Cell Dev. Biol.* 11, 549-599 (1995)
29. S.K. Akiyama, S.S. Yamada, K.M. Yamada & S.E. LaFlamme: Transmembrane signal transduction by integrin cytoplasmic domains expressed in single-subunit chimeras. *J. Biol. Chem.* 269, 15961-15964 (1994)
30. M.D. Schaller, C.A. Otey, J.D. Hildebrand & J.T. Parsons: Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. *J. Cell Biol.* 130, 1181-1187 (1995)
31. A. Horwitz, K. Duggan, C. Buck, M.C. Beckerle & K. Burridge: Interaction of plasma membrane fibronectin receptor with talin- a transmembrane linkage. *Nature* 320, 531-533 (1986)
32. P. Tapley, A. Horwitz, C. Buck, K. Duggan & L. Rohrschneider: Integrins isolated from Rous sarcoma virus-transformed chicken embryo fibroblasts. *Oncogene* 4, 325-333 (1989)
33. H.-C. Chen, P.A. Appeddu, J.T. Parsons, J.D. Hildebrand, M.D. Schaller & J.-L. Guan: Interaction of focal adhesion kinase with cytoskeletal protein talin. *J. Biol. Chem.* 270, 16995-16999 (1995)
34. J. Sinnett-Smith, I. Zachary, A.M. Valverde & E. Rozengurt: Bombesin stimulation of p125 focal adhesion kinase tyrosine phosphorylation. *J. Biol. Chem.* 268, 14261-14268 (1993)
35. J.D. Hildebrand, M.D. Schaller & J.T. Parsons: Identification of sequences required for the efficient

localization of the focal adhesion kinase, pp125^{FAK}, to cellular focal adhesions. *J. Cell Biol.* 123, 993-1005 (1993)
36. J.D. Hildebrand, M.D. Schaller & J.T. Parsons: Paxillin, a tyrosine phosphorylated focal adhesion-associated protein binds to the carboxyl terminal domain of focal adhesion kinase. *Mol. Biol. Cell* 6, 637-647 (1995)
37. K. Tachibana, T. Sato, N. D'Avirro & C. Morimoto: Direct association of pp125^{FAK} with paxillin, the focal adhesion-targeting mechanism of pp125^{FAK}. *J. Exp. Med.* 182, 1089-1100 (1995)
38. H.-C. Chen, P.A. Appeddu, H. Isoda & J.-L. Guan: Phosphorylation of tyrosine 397 in focal adhesion kinase is required for binding phosphatidylinositol 3-kinase. *J. Biol. Chem.* 271, 26329-26334 (1996)
39. S. Davis, M.L. Lu, S.H. Lo, S. Lin, J.A. Butler, B.J. Druker, T.M. Roberts, Q. An & L.B. Chen: Presence of an SH2 domain in the actin-binding protein tensin. *Science* 252, 712-715 (1991)
40. S.M. Bockholt & K. Burridge: Cell spreading on extracellular matrix proteins induces tyrosine phosphorylation of tensin. *J. Biol. Chem.* 268, 14565-14567 (1993)
41. S.M. Thomas, P. Soriano & A. Imamoto: Specific and redundant roles of Src and Fyn in organizing the cytoskeleton. *Nature* 376, 267-271 (1995)
42. S.H. Lo, P.A. Janmey, J.H. Hartwig & L.B. Chen: Interactions of tensin with actin and identification of its three distinct actin-binding domains. *J. Cell Biol.* 125, 1067-1075 (1994)
43. K.R. Auger, Z. Songyang, S.H. Lo, T.M. Roberts & L.B. Chen: Platelet-derived growth factor-induced formation of tensin and phosphoinositide 3-kinase complexes. *J. Biol. Chem.* 271, 23452-23457 (1996)
44. J.D. Hildebrand, J.M. Taylor & J.T. Parsons: An SH3 domain-containing GTPase-activating protein for Rho and Cdc42 associates with focal adhesion kinase. *Mol. Cell. Biol.* 16, 3169-3178 (1996)
45. J.M. Taylor, J.D. Hildebrand, C.P. Mack, M.E. Cox & J.T. Parsons: Characterization of Graf, the GTPase-activating protein for Rho associated with focal adhesion kinase. *J. Biol. Chem.* 273, 8063-8070 (1998)
46. S.M. Thomas & J.S. Brugge: Cellular functions regulated by Src family kinases. *Annu. Rev. Cell Dev. Biol.* 13, 513-609 (1997)
47. S.B. Kanner, A.B. Reynolds, R.R. Vines & J.T. Parsons: Monoclonal antibodies to individual tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases. *Proc. Natl. Acad. Sci. USA* 87, 3328-3332 (1990)
48. B.S. Cobb, M.D. Schaller, T.-H. Leu & J.T. Parsons: Stable association of pp60^{src} and pp59^{fyn} with the focal adhesion-associated protein kinase, pp125^{FAK}. *Mol. Cell. Biol.* 14, 147-155 (1994)
49. Z. Xing, H.-C. Chen, J.K. Nowlen, S.J. Taylor, D. Shalloway & J.-L. Guan: Direct interaction of v-Src with the focal adhesion kinase mediated by the Src SH2 domain. *Mol. Biol. Cell* 5, 413-421 (1994)
50. L.A. Cary, J.F. Chang & J.-L. Guan: Stimulation of cell migration by overexpression of focal adhesion kinase and its assocation with Src and Fyn. *J. Cell Sci.* 109, 1787-1794 (1996)
51. J.W. Thomas, B. Ellis, R.J. Boerner, W.B. Knight, G.C. White & M.D. Schaller: SH2- and SH3-mediated interactions between focal adhesion kinase and Src. *J. Biol. Chem.* 273, 577-583 (1998)
52. M.B. Calalb, T.R. Polte & S.K. Hanks: Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. *Mol. Cell. Biol.* 15, 954-963 (1995)
53. M.B. Calalb, X. Zhang, T.R. Polte & S.K. Hanks: Focal adhesion kinase tyrosine-861 is a major site of phosphorylation by Src. *Biochem. Biophys. Res. Commun.* 228, 662-668 (1996)
54. M.D. Schaller & J.T. Parsons: pp125^{FAK}-dependent tyrosine phosphorylation of paxillin creates a high-affinity binding site for Crk. *Mol. Cell. Biol.* 15, 2635-2645 (1995)
55. K. Vuori, H. Hirai, S. Aizawa & E. Ruoslahti: Introduction of p130^{Cas} signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases. *Mol. Cell. Biol.* 16, 2606-2613 (1996)
56. L.A. Cary, D.C. Han, T.R. Polte, S.K. Hanks & J.-L. Guan: Identification of p130^{Cas} as a mediator of focal adhesion kinase-promoted cell migration. *J. Cell Biol.* 140, 211-221 (1998)
57. D. Ilic, Y. Furuta, S. Kanazawa, N. Takeda, K. Sobue, N. Nakatsuji, S. Nomura, J. Fujimoto, M. Okada, T. Yamamoto & S. Aizawa: Reduced cell motility and enhanced focal contact formation in cells from FAK-deficient mice. *Nature* 377, 539-544 (1995)
58. D.J. Sieg, D. Ilic, K.C. Jones, C.H. Damsky, T. Hunter & D.D. Schlaepfer: Pyk2 and Src-family protein-tyrosine kinases compensate for the loss of FAK in fibronectin-stimulated signaling events but Pyk2 does not fully function to enhance FAK^{-} cell migration. *EMBO J.* 17, 5933-5947 (1998)
59. S.L. Bellis, J.T. Miller & C.E. Turner: Characterization of tyrosine phosphorylation of paxillin *in vitro* by focal adhesion kinase. *J. Biol. Chem.* 270, 17437-17441 (1995)
60. K. Tachibana, T. Urano, H. Fujita, Y. Ohashi, K. Kamiguchi, S. Iwata, H. Hirai & C. Morimoto: Tyrosine phosphorylation of Crk-associated substrates by focal adhesion kinase. *J. Biol. Chem.* 272, 29083-29090 (1997)
61. J.-H. Zhao, H. Reiske & J.-L. Guan: Regulation of the cell cycle by focal adhesion kinase. *J. Cell Biol.* In press (1999)
62. S.M. Frisch, K. Vuori, E. Ruoslahti & P.Y. Chan-Hui: Control of adhesion-dependent cell survival by focal adhesion kinase. *J. Cell Biol.* 134, 793-799 (1996)
63. C. Keenan & D. Kelleher: Protein kinase C and the cytoskeleton. *Cell Signal.* 10, 225-232 (1998)
64. H. Mellor & P.J. Parker: The extended protein kinase C family. *Biochem. J.* 332, 281-292 (1998)
65. J. Downward: Mechanisms and consequences of activation of protein kinase B/Akt. *Curr. Opin. Cell Biol.* 10, 262-267 (1998)
66. C. Guinebault, B. Payrastre, C. Racaud-Sultan, H. Mazarguil, M. Breton, G. Mauco, M. Plantavid & H. Chap: Integrin-dependent translocation of phosphoinositide 3-kinase to the cytoskeleton of thrombin-activated platelets involves specific interactions of p85alpha with actin filaments and focal adhesion kinase. *J Cell Biol.* 129, 831-842 (1995)

67. H.-C. Chen & J.-L. Guan: Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. *Proc. Natl. Acad. Sci. USA* 91, 10148-10152 (1994)
68. H.-C. Chen & J.-L. Guan: Stimulation of phosphatidylinositol 3'-kinase association with focal adhesion kinase by platelet-derived growth factor. *J. Biol. Chem.* 269, 31229-31233 (1994)
69. T.F. Franke, D.R. Kaplan & L.C. Cantley: PI3K: Downstream AKTion blocks apoptosis. *Cell* 88, 435-437 (1997)
70. V. Kundra, J.A. Escobedo, A. Kazlauskas, H.K. Kim, S.G. Rhee, L.T. Williams & B.R. Zetter: Regulation of chemotaxis by the platelet-derived growth factor receptor-beta. *Nature* 367, 474-476 (1994)
71. S. Wennstrom, P. Hawkins, F. Cooke, K. Hara, K. Yonezawa, M. Kasuga, T. Jackson, L. Claesson-Welsh & L. Stephens: Activation of phosphoinositide 3-kinase is required for PDGF-stimulated membrane ruffling. *Curr. Biol.* 4, 385-393 (1994)
72. M.P. Derman, M.J. Cunha, E.J. Barros, S.K. Nigam & L.G. Cantley: HGF-mediated chemotaxis and tubulogenesis require activation of the phosphatidylinositol 3-kinase. *Am. J. Physiol.* 268, F1211-F1217 (1995)
73. M.P. Derman, J.Y. Chen, K.C Spokes, Z. Songyang & L.G. Cantley: An 11-amino acid sequence from c-met initiates epithelial chemotaxis via phosphatidylinositol 3-kinase and phospholipase C. *J. Biol. Chem.* 271, 4251-4255 (1996)
74. A. Khwaja, K. Lehmann, B.M. Marte & J. Downward: Phosphoinositide 3-kinase induces scattering and tubulogenesis in epithelial cells through a novel pathway. *J. Biol. Chem.* 273, 18793-18801 (1998)
75. L.M. Shaw, I. Rabinovitz, H.H.Wang, A. Toker & A.M. Mercurio: Activation of phosphoinositide 3-OH kinase by the $alpha_6beta_4$ integrin promotes carcinoma invasion. *Cell* 91, 949-960 (1997)
76. H.R. Reiske, S.-C. Kao, L.A. Cary, J.-L. Guan, J.-F., Lai & H.-C. Chen: Requirement of phosphatidylinositol 3-kinase in focal adhesion kinase-promoted cell migration. In preparation (1999)
77. P.J. Keely, J.K. Westwick, I.P. Whitehead, C.J. Der & L.V. Parise: Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K. *Nature* 390, 632-636 (1997)
78. M.P. Derman, A. Toker, J.H. Hartwig, K. Spokes, J.R. Falck, C.-S. Chen, L.C. Cantley & L.G. Cantley: The lipid products of phosphoinositide 3-kinase increase cell motility through protein kinase C. *J. Biol. Chem.* 272, 6465-6470 (1997)
79. D.D. Schlaepfer, S.K. Hanks, T. Hunter & P. van der Geer: Integrin-mediated signal transduction linked to ras pathway by Grb2 binding to focal adhesion kinase. *Nature* 372, 786-791 (1994)
80. D.D. Schlaepfer & T. Hunter: Evidence for *in vivo* phosphorylation of the Grb2 SH2-domain binding site on focal adhesion kinase by Src-family protein-tyrosine kinases. *Mol. Cell. Biol.* 16, 5623-5633 (1996)
81. D.D. Schlaepfer & T. Hunter: Focal adhesion kinase overexpression enhances Ras-dependent integrin signaling to ERK2/mitogen-activated protein kinase through interaction with and activation of c-Src. *J. Biol. Chem.* 272, 13189-13195 (1997)
82. D.D. Schlaepfer, K.C. Jones & T. Hunter: Multiple Grb2-mediated integrin-stimulated signaling pathways to ERK2/mitogen-activated protein kinase: summation of both c-Src- and focal adhesion kinase-initiated tyrosine phosphorylation events. *Mol. Cell. Biol.* 18, 2571-2585 (1998)
83. D.D. Schlaepfer, M.A. Broome & T. Hunter: Fibronectin-stimulated signaling from a focal adhesion kinase-c-Src complex: involvement of the Grb2, $p130^{Cas}$, and Nck adaptor proteins. *Mol. Cell. Biol.* 17, 1702-1713 (1997)
84. S. Kharbanda, A. Saleem, Z. Yuan, Y. Emoto, K.V.S. Prasad & D. Kufe: Stimulation of human monocytes with macrophage colony-stimulating factor induces a Grb2-mediated association of the focal adhesion kinase $pp125^{FAK}$ and dynamin. *Proc. Natl. Acad. Sci. USA* 92, 6132-6136 (1995)
85. R. Sakai, A. Iwamatsu, N. Hirano, S. Ogawa, T. Tanaka, H. Mano, Y. Yazaki & H. Hirai: A novel signaling molecule, p130, forms stable complexes *in vivo* with v-Crk and v-Src in a tyrosine phosphorylation-dependent manner. *EMBO J.* 13, 3748-3756 (1994)
86. Y. Nojima, N. Morino, T. Mimura, K. Hamasaki, H. Furuya, R. Sakai, T. Sato, K. Tachibana, C. Morimoto, Y. Yazaki & H. Hirai: Integrin-mediated cell adhesion promotes tyrosine phosphorylation of $p130^{Cas}$, a Src homology 3-containing molecule having multiple Src homology 2-binding motifs. *J. Biol. Chem.* 270, 15398-15402 (1995)
87. L.A. Petch, S.M. Bockholt, A. Bouton, J.T. Parsons & K. Burridge: Adhesion-induced tyrosine phosphorylation of the p130 SRC substrate. *J. Cell Sci.* 108, 1371-1379 (1995)
88. M.R. Burnham, M.T. Harte, A. Richardson, J.T. Parsons & A.H. Bouton: The identification of $p130^{Cas}$-binding proteins and their role in cellular transformation. *Oncogene* 12, 2467-2472 (1996)
89. T. Nakamoto, R. Sakai, K. Ozawa, Y. Yazaki & H. Hirai: Direct binding of C-terminal region of $p130^{Cas}$ to SH2 and SH3 domains of Src kinase. *J. Biol. Chem.* 271, 8959-8965 (1996)
90. M. Ishino, T. Ohba, H. Sasaki & T. Sasaki: Molecular cloning of a cDNA encoding a phosphoprotein, Efs, which contains a Src homology 3 domain and associates with Fyn. *Oncogene* 11, 2331-2338 (1995)
91. K. Alexandropoulos & D. Baltimore: Coordinate activation of c-Src by SH3- and SH2-binding sites on a novel, $p130^{Cas}$-related protein, Sin. *Genes. Dev.* 10, 1341-1355 (1996)
92. S.F. Law, J. Estojak, B. Wang, T. Mysliwiec, G. Kruh & E.A. Golemis: Human enhancer of filamentation 1, a novel $p130^{Cas}$-like docking protein, associates with focal adhesion kinase and induces pseudohyphal growth in Saccharomyces cerevisiae. *Mol. Cell. Biol.* 16, 3327-3337 (1996)
93. Y. Ohashi, K. Tachibana, K. Kamiguchi, H. Fujita & C. Morimoto: T cell receptor-mediated tyrosine phosphorylation of Cas-L, a 105-kDa Crk-associated substrate-related protein, and its association of Crk and C3G. *J. Biol. Chem.* 273, 6446-6451 (1998)
94. M.T. Harte, J.D. Hildebrand, M.R. Burnham, A.H. Bouton & J.T. Parsons: $p130^{Cas}$, a substrate associated with v-Src and v-Crk, localizes to focal adhesions and binds to

focal adhesion kinase. *J. Biol. Chem.* 271, 13649-13655 (1996)
95. T. Nakamoto, R. Sakai, H. Honda, S. Ogawa, H. Ueno, T. Suzuki, S. Aizawa, Y. Yazaki & H. Hirai: Requirements for localization of $p130^{Cas}$ to focal adhesions. *Mol. Cell. Biol.* 17, 3884-3897 (1997)
96. K. Vuori & E. Ruoslahti: Tyrosine phosphorylation of $p130^{Cas}$ and cortactin accompanies integrin-mediated cell adhesion to extracellular matrix. *J. Biol. Chem.* 270, 22259-22262 (1995)
97. L. Petruzzelli, M. Takami & R. Herrera: Adhesion through the interaction of lymphocyte function-associated antigen-1 with intracellular adhesion molecule-1 induces tyrosine phosphorylation of $p130^{Cas}$ and its association with c-CrkII. *J. Biol. Chem.* 271, 7796-7801 (1996)
98. S.N. Manie, A. Astier, N. Haghayeghi, T. Canty, B.J. Druker, H. Hirai & A.S. Freedman: Regulation of integrin-mediated $p130^{Cas}$ tyrosine phosphorylation in human B cells. *J. Biol. Chem.* 272, 15636-15641 (1997)
99. T.R. Polte & S.K. Hanks: Interaction between focal adhesion kinase and Crk-associated tyrosine kinase substrate $p130^{Cas}$. *Proc. Natl. Acad. Sci. USA* 92, 10678-10682 (1995)
100. T.R. Polte & S.K. Hanks: Complexes of focal adhesion kinase (FAK) and Crk-associated substrate ($p130^{Cas}$) are elevated in cytoskeleton-associated fractions following adhesion and Src transformation. *J. Biol. Chem.* 272, 5501-5509 (1997)
101. A.J. Garton, A.J. Flint & N.K. Tonks: Identification of $p130^{Cas}$ as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. *Mol. Cell. Biol.* 16, 6408-6418 (1996)
102. D.S. Black & J.B. Bliska: Identification of $p130^{Cas}$ as a substrate of *Yersinia* YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J.* 16, 2730-2744 (1997)
103. F. Liu, D.E. Hill & J. Chernoff: Direct binding of the proline-rich region of protein tyrosine phosphatase 1B to the src homology 3 domain of $p130^{Cas}$. *J. Biol. Chem.* 271, 31290-31295 (1996)
104. F. Liu, M.A. Sells & J. Chernoff: Protein tyrosine phosphatase 1B negatively regulates integrin signaling. *Curr. Biol.* 8, 173-176 (1998)
105. R.L. Klemke, J. Leng, R. Molander, P.C. Brooks, K. Vuori & D.A. Cheresh: CAS/Crk coupling serves as a "molecular switch" for induction of cell migration. *J. Cell Biol.* 140, 961-972 (1998)
106. M.G. Nievers, R.B. Birge, H. Greulich, A.J. Verkleij, H. Hanafusa & P.M.P. van Bergen en Henegouwen: v-Crk induced cell transformation: changes in focal adhesion composition and signaling. *J. Cell Sci.* 110, 389-399 (1997)
107. H. Hasegawa, E. Kiyokawa, S. Tanaka, K. Nagashima, N. Gotoh, M. Shibuya, T. Kurata & M. Matsuda: DOCK180, a major crk-binding protein, alters cell morphology upon translocation to the cell membrane. *Mol. Cell. Biol.* 16, 1770-1776 (1996)
108. E. Kiyokawa, Y. Hashimoto, T. Kurata, H. Sugimura & M. Matsuda: Evidence that DOCK180 up-regulates signals from the CrkII-$p130^{Cas}$ complex. *J. Biol. Chem.* 273, 24479-24484 (1998)
109. R.L. Klemke, S. Cai, A.L. Giannini, P.J. Gallagher, P. Lanerolle & D.A. Cheresh: Regulation of cell motility by mitogen-activated protein kinase. *J. Cell. Biol.* 137, 481-492 (1997)
110. Z.F. Altun-Gultekin, S. Chandriani, C. Bougeret, T. Ishizaki, S. Narumiya, P. de Graaf, P. van Bergen en Henegouwen, H. Hanafusa, J.A. Wagner & R.B. Birge: Activation of Rho-dependent cell spreading and focal adhesion biogenesis by the v-Crk adaptor protein. *Mol. Cell. Biol.* 18, 3044-3058 (1998)
111. A. Hall: Small GTP-binding proteins and the regulation of the actin cytoskeleton. *Annu. Rev. Cell Biol.* 10, 31-54 (1994)
112. N. Tapon & A. Hall: Rho, Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton. *Curr. Opin. Cell Biol.* 9, 86-92 (1997)
113. S. Tanaka, T. Ouchi & H. Hanafusa: Downstream of Crk adaptor signlaing pathway: activation of Jun kinase by v-Crk through the guanine nucleotide exchange protein C3G. *Proc. Natl. Acad. Sci. USA* 94, 2356-2361 (1997)
114. S. Tanaka & H. Hanafusa: Guanine-nucleotide exchange protein C3G activates JNK1 by a Ras-independent mechanism. *J. Biol. Chem.* 273, 1281-1284 (1998)
115. J.R. Glenney & L. Zokas: Novel tyrosine kinase substrates from Rous Sarcoma Virus-transformed cells are present in the membrane cytoskeleton. *J. Cell Biol.* 108, 2401-2408 (1989)
116. C.E. Turner, J.R. Glenney & K. Burridge: Paxillin: a new vinculin-binding protein present in focal adhesions. *J. Cell Biol.* 111, 1059-1068 (1990)
117. C.E. Turner & J. Miller: Primary sequence of paxillin contains putative SH2 and SH3 domain binding motif and multiple LIM domains: identification of a vinculin and $p125^{FAK}$ binding region. *J. Cell Sci.* 107, 1583-1591 (1994)
118. R. Feuerstein, X. Wang, D. Song, N.E. Cooke & S.A. Liebhaber: The LIM/double zinc-finger motif functions as a protein dimerization domain. *Proc. Natl. Acad. Sci. USA* 91, 10655-10659 (1994)
119. K.L. Schmeichel & M.C. Beckerle: The LIM domain is a modular protein-binding interface. *Cell* 79, 211-219 (1994)
120. T. Tanaka, R. Yamaguchi, H. Sabe, K. Sekiguchi & J.M. Healy: Paxillin association *in vitro* with integrin cytoplasmic domain peptides. *FEBS Letters* 399, 53-58 (1996)
121. Z. Weng, J.A. Taylor, C.E. Turner, J.S. Brugge & C. Seidel-Dugan: Detection of Src homology 3-binding proteins, including paxillin, in normal and v-Src-transformed Balb/c 3T3 cells. *J. Biol. Chem.* 268, 14956-14963 (1993)
122. R.B. Birge, J.E. Fajardo, C. Reichman, S.E. Shoelson, Z. Songyang, L.C. Cantley & H. Hanafusa: Identification and characterization of a high-affinity interaction between v-Crk and tyrosine-phosphorylated paxillin in CT10-transformed fibroblasts. *Mol. Cell. Biol.* 13, 4648-4656 (1993)
123. Y. Shen, G. Schneider, J.-F. Cloutier, A. Veillette & M.D. Schaller: Direct association of protein-tyrosine phosphatase PTP-PEST with paxillin. *J. Biol. Chem.* 273, 6474-6481 (1998)

124. M.C. Brown, J.A. Perrotta & C.E. Turner: Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding. *J. Cell Biol.* 135, 1109-1123 (1996)
125. M.C. Brown, J.A. Perrotta & C.E. Turner: Serine and threonine phosphorylation of the paxillin LIM domains regulates paxillin focal adhesion localization and cell adhesion to fibronectin. *Mol. Biol. Cell* 9, 1803-1816 (1998)
126. A. Richardson, R.K. Malik, J.D. Hildebrand & J.T. Parsons: Inhibition of cell spreading by expression of C-terminal domain of focal adhesion kinase (FAK) is rescued by coexpression of Src or catalytically inactive FAK: a role for paxillin tyrosine phosphorylation. *Mol. Cell. Biol.* 17, 6906-6914 (1997)
127. A. Richardson & J.T. Parsons: A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125^{FAK}. *Nature* 380, 538-540 (1996)
128. A.P. Gilmore & L.H. Romer: Inhibition of focal adhesion kinase (FAK) signaling in focal adhesions decreases cell motility and proliferation. *Mol. Biol. Cell* 7, 1209-1224 (1996)
129. X. Zhu & R.K. Assoian: Integrin-dependent activation of MAP kinase: a link to shape-dependent cell proliferation. Mol. Biol. *Cell* 6, 273-282 (1995)
130. T.H. Lin, Q. Chen, A. Howe & R.L. Juliano: Cell anchorage permits efficient signal transduction between Ras and its downstream kinases. *J. Biol. Chem.* 272, 8849-8852 (1997)
131. B.P. Eliceiri, R. Klemke, S. Stromblad & D.A. Cheresh: Integrin alpha$_v$beta$_3$ requirement for sustained mitogen-activated protein kinase activity during angiogenesis. *J. Cell Biol.* 140, 1255-1263 (1998)
132. S.M. Short, G.A. Talbott & R.L. Juliano: Integrin-mediated signaling events in human endothelial cells. Mol. Biol. *Cell* 9, 1969-1980 (1998)
133. L.-H. Xu, L.V. Owens, G.C. Sturge, X. Yang, E.T. Liu, R.J. Craven & W.G. Cance: Attenuation of the expression of the focal adhesion kinase induces apoptosis in tumor cells. *Cell Growth Diff.* 7, 413-418 (1996)
134. J.E. Hungerford, M.T. Compton, M.L. Matter, B.G. Hoffstrom & C.A. Otey: Inhibition of pp125^{FAK} in cultured fibroblasts results in apoptosis. *J. Cell Biol.* 135, 1383-1390 (1996)
135. D.H. Crouch, V.J. Fincham & M.C. Frame: Targeted proteolysis of the focal adhesion kinase pp125^{FAK} during c-Myc-induced apoptosis is suppressed by integrin signalling. *Oncogene* 12, 2689-2696 (1996)
136. L.-P. Wen, J.A. Fahrni, S. Troie, J.-L. Guan, K. Orth & G.D. Rosen: Cleavage of focal adhesion kinase by caspases during apoptosis. *J. Biol. Chem.* 272, 26056-26061 (1997)
137. B. Levkau, B. Herren, H. Koyama, R. Ross & E.W. Raines: Caspase-mediated cleavage of focal adhesion kinase pp125^{FAK} and disassembly of focal adhesions in human endothelial cell apoptosis. *J. Exp. Med.* 187, 579-586 (1998)
138. F.G. Gervais, N.A. Thornberry, S.C. Ruffolo, D.W. Nicholson & S. Roy: Caspases cleave focal adhesion kinase during apoptosis to generate a FRNK-like polypeptide. *J. Biol. Chem.* 273, 17102-17108 (1998)
139. D. Ilic, E.A.C. Almeida, D.D. Schlaepfer, P. Dazin, S. Aizawa & C.H. Damsky: Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. *J. Cell Biol.* 143, 547-560 (1998)
140. L.H. Romer, N. Mclean, C.E. Turner & K. Burridge: Tyrosine kinase activity, cytoskeleton organization, and motility in human vascular endothelial cells. *Mol. Biol. Cell* 5, 349-361 (1994)
141. L.V. Owens, L. Xu, R.J. Craven, G.A. Dent, T.M. Weiner, L. Kornberg, E.T. Liu & W.G. Cance: Overexpression of the focal adhesion kinase (p125^{FAK}) in invasive human tumors. *Cancer Res.* 55, 2752-2755 (1995)
142. R.E. Gates, L.E. King Jr., S.K. Hanks & L.B. Nanney: Potential role for focal adhesion kinase in migrating and proliferating keratinocytes near epidermal wounds and in culture. *Cell Growth Differ.* 5, 891-899 (1994)

Key Words: Extracellular matrix (ECM), Focal adhesion kinase (FAK), Integrin, Signal transduction

Send correspondence to: Dr Jun-Lin Guan, Department of Molecular Medicine, Cornell University, Ithaca, NY 14853, USA, Tel:607-253-3586, Fax::607-253-3708, E-mail: jg19@cornell.edu

Received 11/25/98 Accepted 12/15/98