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COMMENTARY

Focal adhesion kinase: at the crossroads of signal transduction

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SUMMARY

Morphogenetic processes during development, including cell migration, depend on signals from both the extracellular matrix (ECM) and soluble signaling factors. Extensive evidence has shown that the nonreceptor tyrosine kinase, focal adhesion kinase (FAK), is activated in response to both kind of signal. The most definitive evidence that FAK is directly downstream of signals initiated by the ECM comes from comparing the phenotypes of mice deficient for FAK and the ECM molecule, fibronectin: in both cases embryos die at about E8.5 and display almost identical severe vascular and other mesodermal defects. It is now clear that there are additional FAK-like proteins, indicating the existence of a FAK family. Furthermore, FAK is not located at adhesive sites in all cells where it is expressed. This, plus extensive data indicating that FAK becomes activated in response to several soluble signaling factors, suggests that the FAK family may be at the crossroads of multiple signaling pathways that affect cell and developmental processes.

Key words: Focal adhesion kinase, Signaling, Cell motility

INTRODUCTION

In the early 1990s, efforts to characterize v-*src* substrates in v-*src*–transformed chicken embryonic fibroblasts resulted in isolation of several candidates. Most of them were molecules found in close association with the cytoskeleton (Kanner et al., 1990). One of these proteins, a 120 kDa molecule, had tyrosine kinase activity and became phosphorylated on tyrosine in response to both oncogene- and extracellular matrix (ECM)-integrin–dependent stimulation (Guan and Shalloway, 1992). Further excitement was aroused by the finding that phosphorylation, stimulated by the small neuropeptides bombesin, endothelin and vasopressin, is linked directly to the same 120 kDa molecule (Zachary and Rozengurt, 1992), dubbed focal adhesion kinase (FAK), since it has tyrosine kinase activity and in cultured cells is present in cellular focal adhesions.

The cDNAs encoding chicken (Schaller et al., 1992), mouse (Hanks et al., 1992), human (André and Becker-André, 1993) and *Xenopus* (Zhang et al., 1995) FAK have been isolated, and the deduced amino acid sequences showed identity of over 90%. FAK is expressed in embryonic stem (ES) cells (Ilić et al., 1995a), and ubiquitously throughout development (Furuta et al., 1995), in all adult tissues examined (André and Becker-André, 1993; Hanks et al., 1992; Kanazawa et al., 1995), and in many cell lines (Kornberg et al., 1992; Matsumoto et al., 1994; Zhang et al., 1994). Several studies have shown that FAK is heavily phosphorylated throughout development (Maher and Pasquale, 1988; Turner et al., 1993). All these data suggest the importance of this molecule, which functions as ‘a point of convergence in the action of neuropeptides, integrins and oncogenes’ (Zachary and Rozengurt, 1992).

STRUCTURAL PROPERTIES OF THE FAK FAMILY

The highest expression of FAK is seen in the brain (André and Becker-André, 1993; Grant et al., 1995; Hanks et al., 1992), in testes (Hanks et al., 1992) and in osteoclasts (Berry et al., 1994). Although FAK localizes to focal adhesion sites in fibroblastic cell lines, FAK is not restricted to such sites in neural tissue or osteoclasts (Berry et al., 1994; Burgaya et al., 1995; Grant et al., 1995). In astroglia the majority of FAK is associated with the cytoskeleton, forming a filamentous pattern, while in neurons FAK is distributed throughout the cell (Zhang et al., 1994; Burgaya et al., 1995; Grant et al., 1995; W. L. Klein, personal communication). Several truncated forms, including some that lack focal adhesion targeting (FAT) sequences responsible for localization of FAK in focal adhesions, are present in the human brain, although their roles are not clear (André and Becker-André, 1993). The most recent findings link membrane depolarization of neuronal cells and increase in FAK phosphorylation (Derkinderen et al., 1996; Siciliano et al., 1996). All these results point to more complex roles for FAK that are not necessarily restricted to sites of cell adhesion.

FAK is distinct from other non-receptor protein tyrosine kinases (NRPTKs). Its catalytic domain is flanked by amino- and carboxy-terminal domains of about 400 amino acids each.

Unlike the other NRPTKs, it contains neither the Src homology 2 or 3 (SH2 or SH3) domains, which mediate protein-protein interaction, nor a myristylation site that anchors proteins to the membrane. In some cases, alternative splicing of the *fak* gene leads to the autonomous expression of the carboxy-terminal part of FAK (Schaller et al., 1993). This isoform is named FAK-related non-kinase (FRNK), since it does not have catalytic activity. The independent expression of C-terminal domains has been observed in other classes of signaling proteins, but the existence of FAK and FRNK is the first example among NRPTKs. FRNK has FAT sequences responsible for focal adhesion targeting. Therefore, it is proposed that FRNK functions as a negative regulator of FAK localization in focal adhesions (Richardson and Parsons, 1996), since the binding of FRNK on the FAK docking site at the focal adhesions would prevent binding of full-length FAK. However, it seems that FRNK is not the only regulator of FAK localization, since FRNK has not been detected in all cells that express FAK (André and Becker-André, 1993; Ilić et al., 1995b).

The existence of molecules related to FAK became evident with the discovery and characterization of cell adhesion kinase (CAKβ) (PYK2/RAFTK; Fig. 1) (Avraham et al., 1995; Lev et al., 1995; Sasaki et al., 1995). CAKβ has 61% sequence identity with FAK in the catalytic domain and 42% and 36% identity in the N-terminal and C-terminal non-catalytic domains, respectively. The leucine residue in the kinase domain that is absolutely conserved in other protein tyrosine kinases is replaced by isoleucine in both FAK (Ile547) and CAKβ (Ile 550) (Avraham et al., 1995; Hanks et al., 1992; Lev et al., 1995; Sasaki et al., 1995). The sequence marking the autophosphorylation site in FAK (Tyr397), Tyr-Ala-Glu-Ile, is conserved in CAKβ (Tyr404). This sequence, as well as the autophosphorylation site on Src-family kinases (Tyr-Thr-Ala-Arg), has been identified as a high-affinity binding site for the Csk SH2 domain, suggesting that Csk might bind directly to FAK-family kinases and regulate their activation, as it does in the case of Src-family kinases (Sabe et al., 1994; Tremblay et al., 1996). The carboxy-terminal domain of CAKβ has three regions of high homology with FAK, although they are in reverse order on the molecule (residues 699-720, 747-777 and 788-799 on CAKβ are highly homologous to 861-882, 711-741 and 684-705 on FAK). Two of these regions (861-882 and 711-741 on FAK) are proline-rich and may therefore bind SH3 groups of molecules such as p130Cas, p105Cas-L or Graf, the *G*TPase *r*egulator *a*ssociated with *F*AK (Polte and Hanks, 1995; Minegishi et al., 1996; Parsons, 1996). Finally, the region encompassing residues 869-999 on CAKβ is highly homologous (61% identity) with the region on FAK (913-1043) that contains the binding site for paxillin and that is required for targeting FAK to focal adhesion sites. Sequences of that region, termed FAT (Richardson and Parsons, 1996), are also well preserved in CAKβ; however, CAKβ was not found in focal contact sites, at least not in mouse embryonic fibroblasts and human cytotrophoblasts. Instead, CAKβ is present in a perinuclear area in a punctate pattern (Fig. 2).

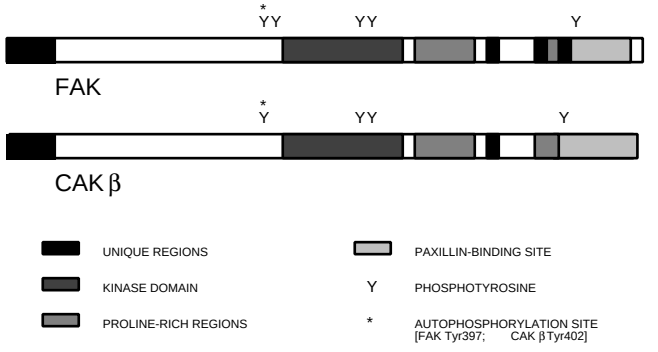

Fig. 1. Structural properties of FAK family members.

Both FAK and CAKβ can be activated by a G-protein-coupled bradykinin receptor and by phorbol esters, and both link these receptors with the MAPK pathway (Dikic et al., 1996). However, in other cases, the activation mechanisms and effectors of these two kinases appear to be quite distinct. In contrast to FAK, CAKβ is not activated by cell-ECM interactions with the exception of megakaryocytes (Li et al., 1996). On the other hand, CAKβ, but not FAK, is involved in regulation of potassium channels and may modulate the action of voltage-gated calcium channels that are regulated by the intracellular calcium concentration (Lev et al., 1995).

ACTIVATION AND PHOSPHORYLATION OF FAK

In fibroblasts, tyrosine phosphorylation of FAK requires that cells are adherent to the ECM and is reduced in suspended cells (Burridge et al., 1992). FAK activation and phosphorylation apparently depend on clustering of integrins, and can be seen when suspended cells are incubated with anti-integrin antibodies or replated on ECM substrates. Under these conditions, FAK becomes highly tyrosine-phosphorylated and shows high kinase activity. Enhanced phosphorylation and activation does not occur when cells are plated on poly-L-lysine, indicating that specific signals from ECM, transduced by integrins, can trigger FAK phosphorylation and activation. In contrast, FAK is constitutively phosphorylated in suspended, freshly isolated mouse thymocytes, and clustering of integrins on their surface leads not to an increase, but to a decrease in FAK phosphorylation and activity (Kanazawa et al., 1996). This interesting exception indicates that FAK could have other roles too. However, evidence for the presence of an ECM-integrin-FAK cascade has been fortified with an in vitro demonstration of the ability of FAK and paxillin to bind directly to peptides mimicking β integrin cytoplasmic domains (Schaller et al., 1995b). The N-terminal portion of FAK has been suggested to contain the putative integrin binding site.

FAK has five tyrosine phosphorylation sites (see note added in proof) (Tyr397, 407, 576, 577 and 925; Fig. 1) (Calalb et al., 1995; Schlaepfer et al., 1995). Tyr397 is an autophosphorylation site that generates a high-affinity binding site for the SH2 domain of Src-family NRPTKs (Cobb et al., 1994; Schaller et al., 1994; Eide et al., 1995; Cary et al., 1996). The interaction of Src with the FAK autophosphorylation site then leads to phosphorylation of Tyr407, 576 and 577, which maximizes the kinase activity of FAK in vitro (Calalb et al., 1995). Interaction with Src also results in the phosphorylation of Tyr925 on FAK, which creates a Grb2-binding site and therefore links FAK to the Ras/mitogen-activated protein kinase (MAPK) pathway (Schlaepfer et al., 1995). These data suggest that phosphorylated FAK can bind and integrate multiple signaling pathways in response to signals from the external environment.

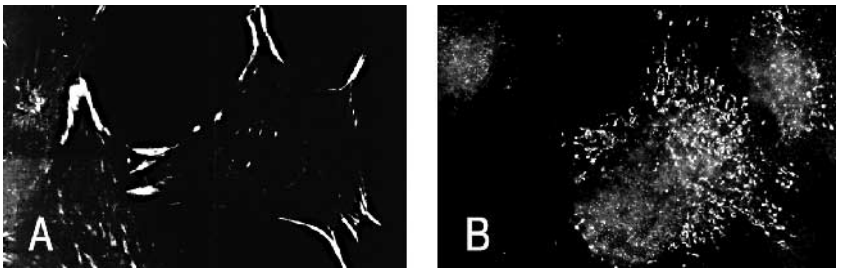

Fig. 2. Intracellular distribution of FAK family members in mouse embryonic fibroblasts. (A) FAK is localized in focal adhesions and (B) CAKβ around the nucleus.

Despite the mapping of the tyrosine phosphorylation sites on FAK, and identification of several interacting proteins, its phosphorylation and activation cycle is still unclear. The autophosphorylation site on FAK (Tyr397) appears to be phosphorylated even in quiescent, adherent cells even though FAK is probably not always playing an active role in integrating signaling pathways in such cells. This suggests that the availability of Src, or the conformation of FAK, and not only the phosphorylation state of Tyr397 on FAK, regulates the binding of Src-family kinases and subsequent FAK activation. A change in the conformation of FAK and the subsequent phosphorylation of other tyrosines such as Tyr925 could lead to an active role for FAK in the integration of multiple pathways. There are, however, no insights into how FAK conformation might be regulated. Also, the downstream targets for FAK activity have not been identified definitively; most data are obtained from in vitro phosphorylation experiments. Paxillin and tensin have been suggested as FAK substrates (Richardson and Parsons, 1996). However, these molecules are phosphorylated equally well in a FAK-deficient fibroblast cell line (Ilić et al., 1995b). Paxillin binds to both Src and Csk (Bellis et al., 1995; Schaller and Parsons, 1995a). Csk, by virtue of its ability to inactivate Src by phosphorylating its C-terminal Tyr (Cooper and Howell, 1993), may play an indirect role in regulating FAK activity and/or FAK function as an integrator of distinct multiple pathways. Bringing Csk into close proximity to Src would trigger Src inactivation and subsequent downregulation of the whole FAK activation complex, and downregulation of FAK phosphotyrosine content. Csk-deficient cells have malformed focal adhesions, whereas Csk/Src double mutants are partially rescued for this phenotype (Thomas et al., 1995). Furthermore, overexpressed Csk is localized predominantly in focal adhesions (Bergman et al., 1995). Taken together, Src-family NRPTKs and Csk, together with paxillin, are likely to be critical players in regulating FAK activation and signal transduction in focal adhesion sites (Tremblay et al., 1996) (Fig. 3).

FAK AS A PLATFORM FOR THE ASSEMBLY OF SIGNALING CASCADES

All available information suggests that FAK is at a crossroad for multiple signaling pathways. This is dramatically illustrated by studying the effects on cytoskeletal organization of soluble as well as ECM-associated activators of FAK. As indicated previously, several bioactive peptides (endothelin, bombesin) and serum factors such as lysophosphatidic acid (LPA) activate FAK via their specific cell surface receptors (Imamura et al., 1996; Zachary and Rozengurt, 1992). Furthermore, growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) trigger dramatic effects on the organization of the actin cytoskeleton. The effects of PDGF and LPA, and perhaps the other factors as well, on cytoskeletal organization are regulated in a highly specific manner by small G-proteins of the Rho family (Nobes and Hall, 1995). Rho activity links the assembly of focal adhesions and actin stress fibers to LPA and bombesin. Rac regulates formation of lamellopodia and ruffles, linking PDGF, EGF and insulin activity to the polymerization of actin at the plasma membrane. Cdc42 triggers formation of filopodia. The effects of LPA on both FAK activation and focal contact formation are mediated specifically by Rho, as evidenced by their inhibition by the ADP-ribosyltransferase C3 exoenzyme from *Clostridium botulinum*, which blocks activation of RhoA (Rankin et al., 1994). This suggests that in the cellular response to LPA, activation of RhoA could be upstream of FAK activation, although the existence of two independent pathways cannot be excluded.

The relationship of Rho function to FAK activation in response to integrin-triggered focal contact assembly remains controversial. Data from many laboratories suggest that replating fibroblasts on fibronectin in the absence of serum is sufficient to activate FAK and form focal contacts. However, the work of Hotchin and Hall (1995) suggests that in Swiss 3T3 cells, which can be serum starved extremely effectively, adhesion per se (to fibronectin, for example) does not promote maximum cell spreading or the formation of focal contacts. Introduction of constitutively active Rho in such cells, however, does permit focal contact formation in cells plated in the absence of serum. This suggests that Rho is an intermediary in integrin-regulated cytoskeletal reorganization and perhaps FAK activation as well. On the other hand, it is possible that for complete spreading and cytoskeletal reorganization, signals from both ECM, via integrins, and soluble factors, via activated G-proteins, are needed.

A different approach (Miyamoto et al., 1995a,b) emphasizes the stepwise nature of the assembly of FAK-containing signaling complexes in response to integrin ligation by antibodies or fibronectin. When integrins on fibroblasts are clustered, using beads coated with anti-β1 integrin antibodies that do not perturb function in conventional adhesion assays and do not trigger FAK activation, FAK is recruited to the bead site, but Src-family kinases and members of the Ras/MAPK cascade are not. In contrast, if beads are coated with function-perturbing anti-β1 integrin antibodies or with fibronectin, both of which are capable of activating FAK, FAK and the many cytoskeletal and signaling molecules usually found in focal contacts are recruited to the bead site. In addition, Rho and the Rho GAP-family member p190-B are also recruited to fibronectin-coated beads (Burbelo et al., 1995). The most straightforward conclusion is that FAK activation is not

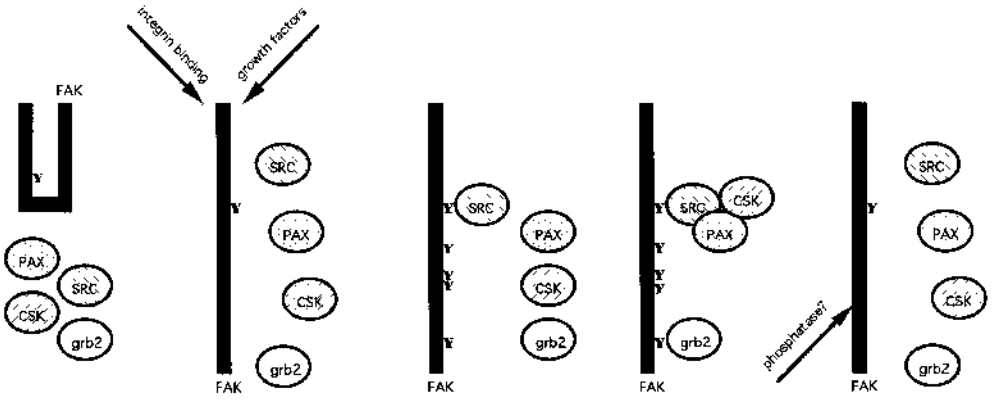

Fig. 3. Putative phosphorylation cycle of FAK. Phosphorylated tyrosines (Y) on FAK are docking sites for molecules of multiple pathways. Left to right: soluble factors and/or integrin-ECM interactions cause conformational changes in FAK and allow access of Src-family members to FAK autophosphorylation site (Tyr397). Bound Src phosphorylates other tyrosine residues on FAK. These phosphorylated tyrosines on FAK are, in turn, binding sites for molecules from distinct signaling pathways, such as Grb2, a link to Ras/MAPK pathway. Bound Src also forms a complex with paxillin. Csk, which downregulates Src activity by phosphorylating its C-terminal tyrosine, also binds paxillin. Thus recruitment of Csk, via paxillin, into close proximity with FAK-associated Src, may promote inactivation of Src. This, plus unknown signals that activate focal adhesion-associated phosphatases, could cause breakdown of the FAK-associated signaling complex.

necessary for its localization to focal contacts. However, recruitment of Src-family members and activation of the Ras/MAPK cascade does require FAK activation and phosphorylation. Finally, addition of EGF to cells that have bound fibronectin-coated or integrin-activating antibody-coated beads triggers recruitment of EGF receptors to the bead site. Together, these studies are consistent with the hypothesis that FAK plays an important role in the assembly of signaling complexes that both regulate the organization of the cytoskeleton and modulate the function of growth factors. Such a role for FAK is also supported by the fact that FAK interacts directly with kinases, such as Src-family members (Cobb et al., 1994; Eide et al., 1995; Kanazawa et al., 1996), the p85 subunit of PI3-kinase (Guinebault et al., 1995; Chen et al., 1996) and perhaps Csk (Sabe et al., 1994; Tremblay et al., 1996), and with adaptor molecules such as Grb2 (Kharbanda et al., 1995; Schlaepfer et al., 1995), paxillin (Tachibana et al., 1995; Turner and Miller, 1994), and Nck (Choudhury et al., 1996).

FAK, MORPHOGENESIS AND MOTILITY

The fact that assembly of supramolecular complexes containing activated FAK takes place at sites of adhesion has suggested that FAK would have a primary role in the formation of focal contacts and regulation of cell motility and migration. To address these hypotheses directly, the FAK gene was targeted in mouse ES cells, and mice deficient in FAK were generated. Deletion of FAK is an early embryonic lethal mutation; embryos die at E8.0-8.5 (Furuta et al., 1995). Severe morphogenetic defects, evident primarily in axial mesodermal tissues and the cardiovascular system, are responsible for the demise of the embryos. One hypothesis that might account for the observed defects in axial mesoderm is that FAK-null mesodermal cells migrate more slowly than normal mesoderm. This could result in a relatively normal formation of anterior mesodermal tissues, which are formed first and involve shorter migration distances, and increasingly poor development of more posterior mesodermal structures that form later or require migration over longer distances. Reduced cell cycle length of cells entering the primitive streak in FAK-null embryos, or reduced survival of mesodermal cells, could also contribute to the defects seen in FAK-null animals.

The other major defect in FAK-deficient embryos is in the vascular system. Initial generation (vasculogenesis) and subsequent growth (angiogenesis) of the vasculature were severely impaired, and neither fully developed blood vessels nor a normal heart were ever formed. The dorsal aorta was present in some regions, but not in others, suggesting poor survival or defective morphogenesis of endothelium. The omphalomesenteric artery, the site of the junction between embryonic and extraembryonic vasculatures, was also not detected in mutants. Therefore, blood cells could not circulate in the embryo even though they are formed within blood islands in the yolk sac. Involvement of highly regulated cell-ECM interactions in virtually all processes required for endothelial morphogenesis, including anchorage, migration, invasion, as well as proliferation and survival (Meredith et al., 1993; Brooks et al., 1994; Risau and Flamme, 1995) strongly suggests specific roles for FAK in regulating some or all of these events. This is supported by the report that platelet-activating factor (PAF), a potent activator of angiogenesis and motility of endothelial cells, induces FAK tyrosine phosphorylation (Soldi et al., 1996).

The overall phenotype of FAK-null embryos resembles strongly that of fibronectin-deficient mice (George et al., 1993). Such similarity supports the idea that FAK and fibronectin are part of the same signaling pathway. The major intermediates between them are members of the integrin family. It is significant, therefore, that mice deficient in the $\alpha 5$ subunit of the specific integrin fibronectin receptor, $\alpha 5\beta 1$, also have a similar pattern of embryonic defects, although less severe (Yang et al., 1993). In the $\alpha 5$-null embryos, vascular abnormalities are detected, but blood does circulate through the embryo. The posterior mesoderm of these mice is also defective, although more somites are formed and heart development proceeds further than it does in the FAK-null and fibronectin-null embryos.

The finding that overexpression of FAK stimulates cell migration (Cary et al., 1996) and thorough consideration of the phenotype of FAK-null mice suggest that defects in migration could underlie all of the mesodermal abnor-malities. Indeed, FAK-null mesodermal cells moved at half the rate of

cells from wild-type embryos. Unexpectedly, FAK-null cells showed an *increased* number of focal contacts, suggesting that FAK regulates the cycle of assembly and disassembly of focal contacts, rather than being required specifically for their assembly (Ilić et al., 1995b). According to this interpretation, the absence of FAK would reduce focal adhesion turnover, resulting in a net increase in the steady-state number of focal contacts and a reduced ability to release cell processes during migration, especially at the rear of the cell. Interestingly, the focal contacts in FAK-null cells continued to show a high level of staining with anti-phosphotyrosine antibodies, indicating either that other proteins can compensate for FAK in recruiting Src-family kinases into focal contacts, or that other kinases unrelated to the Src family are present in these contacts.

Endodermal cells lacking FAK also migrated more slowly than their normal counterparts, suggesting that decreased motility is a fundamental property of the FAK-null phenotype in all cell types (Ilić et al., 1996). The apparently specific effects of FAK deficiency on mesodermal structures in early development most likely results from the fact that mesodermal cells are the most highly migratory cells in the early embryo. Another possibility is that there are other signaling pathways that can compensate for the absence of FAK in some cell lineages, and that FAK is not essential for normal morphogenesis of all cell types

PUZZLES AND PERSPECTIVES

Most of the information summarized thus far suggests that the primary role of FAK is to regulate motility and focal adhesion turnover. However, the observation that expression of FAK is elevated in invasive tumors (Imamura et al., 1996; Matsumoto et al., 1994; Owens et al., 1995), its linkage via Grb2 to the Ras-MAPK pathway (Kharbanda et al., 1995; Schlaepfer et al., 1995), and its activation both by certain growth factors (Chen et al., 1996; Matsumoto et al., 1994) and by ECM (Burridge et al., 1992) all support the hypothesis that FAK may also play a role in the regulation of cell growth and/or survival. This idea is supported by recent data showing that interfering with FAK function in freshly plated fibroblasts by microinjecting anti-FAK antibodies, or with FAK-integrin interaction by microinjecting the membrane-proximal peptide from the β1 integrin cytoplasmic domain that binds FAK in vitro, results in cell death (C. Otey, personal communication). Another group (Gilmore and Romer, 1996) has shown that injection of the C-terminal part of FAK was associated with a marked decrease in DNA synthesis. Although FAK may play some role in regulating cell growth or survival when present, it is probably not required, as FAK-null cells are viable. It is likely that other molecules can compensate for FAK deficiency, at least in part. This kind of complementation is well known in the case of Src-family NRPTK-deficient mice, where the absence of a single family member results in a very mild or undetectable phenotype (Thomas et al., 1995). Another possibility is the presence of alternative pathways that become activated when the pathway involving FAK fails to function. The discovery and isolation of CAKβ has introduced the existence of a family of FAK-related molecules. The size of the family remains a question. However, the comparison of FAK and CAKβ will stimulate investigation of their specific functions and the extent to which they can compensate for one another when one of them is absent. It is likely that some of their functions are analogous or complementary, since they both have conserved docking sites for Src-family kinases, PI3-kinase, Grb2, and perhaps paxillin (Tachibana et al., 1995), as well as proline-rich regions that are proposed to bind Cas (Polte and Hanks, 1995).

In addition to understanding how FAK functions at the cell biological level, many questions remain about how FAK is activated and how its interactions with other signaling molecules are regulated in vivo. The availability of mice deficient for members of the Src family will be very helpful in unraveling FAK-Src family interactions. For example, intercrossing Fyn-deficient mice with FAK heterozygotes has provided genetic evidence for their direct interaction in vivo. Mice that are both deficient for Fyn and heterozygous for FAK showed thymic abnormalities during a critical period of immune cell maturation (Kanazawa et al., 1996), and skin pathology starting at 6-9 months (D. Ilić and S. Kanazawa, unpublished observations). Neither of these phenomena was observed in mice that were only Fyn-null or FAK heterozygous. Additional studies of crosses between FAK and Src-family mutant mice should address many questions about their interactions.

Finally, the roles of protein tyrosine phosphatases, which must be critical factors in any regulation of a signaling pathway triggered by kinases, have been greatly underappreciated. At least one phosphatase, LAR, is associated with focal adhesion sites (Serra-Pagès et al., 1995). How is its activity regulated? Does it play a role in regulating FAK signaling? Does CD45 play a role in the FAK-Fyn pathway in thymocytes?

In summary, analysis of the phenotype of FAK-deficient mice and cells, as well as those deficient in fibronectin and integrins, has helped to point out critical stages of development that depend on signals initiated by cell-ECM interactions. These studies complement earlier studies in amphibia suggesting strongly that fibronectin and signals generated by its binding to cell-surface receptors are essential for executing gastrulation normally (Ramos and DeSimone, 1996). Deletion of α5β1 integrin function (Yang et al., 1993), and that of the whole β1 family (Stephens et al., 1995), also showed that integrins are critical transducers of signals from the ECM that are required for cell migration and survival. Figuring out how FAK and FAK-family members integrate and propagate signals from the ECM, via integrins, and from soluble bioactive factors, via their specific receptors, promises to be an exciting endeavor for the future.

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Note added in proof

A sixth tyrosine phosphorylation site (Tyr681) has been reported which is a target for Src (Calalb, M. B., Zhang, X., Polte, T. R. and Hanks, S. K. (1996). Focal adhesion kinase tyrosine-861 is a major site of phosphorylation by Src. *Biochem. Biophys. Res. Commun.* **228**, 662-668).