ELSEVIER

Biochimica et Biophysica Acta 1540 (2001) 1–21

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.bba-direct.com

Review

Biochemical signals and biological responses elicited by the focal adhesion kinase

Michael D. Schaller *

Department of Cell and Developmental Biology, Lineberger Comprehensive Cancer Center, Center for Thrombosis and Hemostasis and Comprehensive Center for Inflammatory Disorders, 534 Taylor Hall, CB 7090, University of North Carolina, Chapel Hill, NC 27599, USA

Received 12 March 2001; received in revised form 28 May 2001; accepted 29 May 2001

Abstract

The focal adhesion kinase, FAK, is an important component of an integrin-dependent signaling pathway, which functions to transmit signals from the extracellular matrix into the cytoplasm. FAK is an essential gene product, since the *fak*−/− mouse exhibits embryonic lethality. A number of important biological processes, including cell motility and cell survival, are controlled by integrin-dependent signals and FAK has been implicated in regulating these processes. This review will focus upon recent findings providing insight into the mechanisms by which FAK transmits biochemical signals and elicits biological effects.

Keywords: Focal adhesion kinase; Integrin; Src; Rho; Migration; Apoptosis; Cancer

1. Overview

The focal adhesion kinase, FAK, is a cytoplasmic protein tyrosine kinase that discretely localizes to regions of the cell that attach to the extracellular matrix, called focal adhesions [1–4]. Many stimuli can induce tyrosine phosphorylation and activation of the catalytic activity of FAK, including growth factors, neuropeptides, reagents that stimulate G-protein coupled receptors and mechanical stimuli. However, the major mode of regulation is via integrin-dependent adhesion to the extracellular matrix and FAK is an integral component of the integrin-signaling pathway.

The biological functions of FAK have been explored by perturbing endogenous FAK signaling and by overexpression of FAK. Inhibition of endogenous FAK signaling results in reduced motility and in some cases induces cell death [5,6]. Conversely, enhancing FAK signaling by exogenous expression increases cell motility and can promote cell survival in the absence of a signal from the extracellular matrix [7,8]. Thus, FAK has been implicated in controlling cell motility and transmitting a cell survival signal from the extracellular matrix. Given the important role of FAK in the control of these fundamental biological properties, there is considerable interest in the mechanisms by which FAK signals.

FAK contains large N- and C-terminal domains that flank the catalytic domain (see Fig. 1). There are six sites of tyrosine phosphorylation. The major site of autophosphorylation, tyrosine 397, lies just upstream of the kinase domain and is a docking site for the SH2 domains of a number of proteins

* Fax: +1-919-966-1856.
E-mail address: crispy4@med.unc.edu (M.D. Schaller).

0167-4889/01/$ – see front matter
PII: S0167-4889(01)00123-9

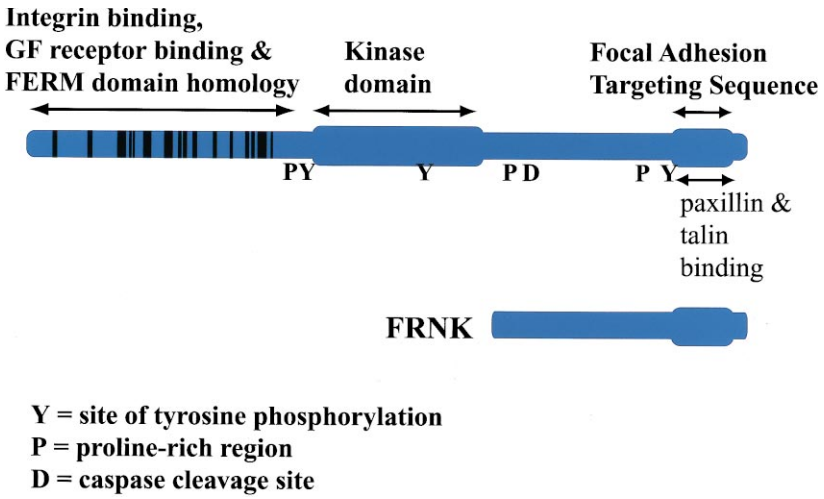

Fig. 1. Domain structure of FAK. The organization of the N-terminal, kinase and C-terminal domains are shown. The N-terminus interacts with integrins and growth factor receptors. The FERM domain, regions of homology with band 4.1 and ezrin/radixin/moesin proteins, is indicated by black bars in the N-terminus. The C-terminal focal adhesion targeting (FAT) sequence, which contains paxillin and talin binding sites, is also shown. Proline rich sequences (P), sites of tyrosine phosphorylation (Y) and a caspase cleavage site (D) are illustrated. The domain structure of FRNK, an autonomously expressed FAK variant that can function as a dominant negative mutant, is also shown.

including the Src family of tyrosine kinases [9]. The five other sites of phosphorylation on FAK are likely phosphorylated by bound Src kinases [10]. Two phosphorylation sites in the activation loop of the catalytic domain, tyrosines 576 and 577, are regulatory sites of phosphorylation that enhance catalytic activity. Phosphorylation of tyrosine 925, located in the C-terminal domain of FAK, creates a binding site for the SH2 domain of Grb2 and presents one mechanism of activation of the Ras/MAPK pathway by FAK [11]. Several proline-rich sequences in the C-terminal domain of FAK serve as binding sites for SH3 domain containing proteins and mediate binding to p130^{cas} and GRAF [12,13].

The focal adhesion targeting (FAT) sequence of FAK is located at the C-terminus and mediates the localization of FAK to focal adhesions [14]. This region also contains binding sites for two focal adhesion-associated proteins, paxillin and talin [15,16]. The precise mechanism of targeting is not established. The C-terminal noncatalytic domain of FAK, called FRNK, is autonomously expressed in some cell types and is frequently used as a dominant negative FAK variant.

FAK has been implicated in directing the tyrosine phosphorylation of several substrates, including p130^{cas} and paxillin [7,17,18]. These substrates might be directly phosphorylated by FAK or indirectly by Src, which has been recruited into complex with FAK. Upon phosphorylation of both p130^{cas} and paxillin, adaptor proteins like Crk and Nck can be recruited into complex with these proteins. These adaptor proteins bring other signaling proteins, e.g., C3G, into the complex to facilitate the generation of downstream signals. In addition to tyrosine phosphorylation of downstream substrates, the recruitment of adaptor/signaling molecules, like Grb2 and PI3K, into complex with FAK is also a very important mechanism of signaling [11,19].

A second PTK, that shares structural and sequence homology with FAK is Pyk2/CAKβ/RAFTK/CadTK/FAK2. CAKβ binds to a number of proteins in common with FAK including Src family kinases and cytoskeletal proteins like paxillin. The subcellular localization of CAKβ is variable but is generally described as diffusely cytoplasmic. CAKβ is regulated by a number of stimuli that elevate cytoplasmic levels of calcium. Thus FAK and CAKβ respond to different stimuli, but may engage similar cellular components to transmit downstream signals. Since CAKβ has recently been reviewed [1,20], this topic will not be addressed further in this review.

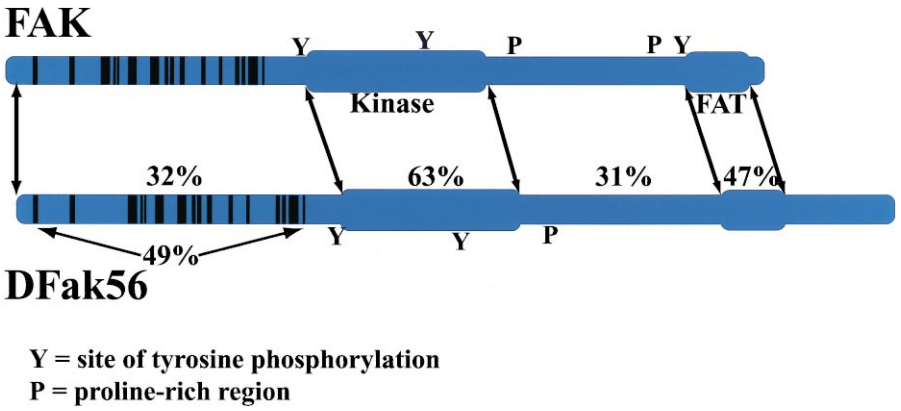

Fig. 2. Sequence homology between human and *Drosophila* FAK sequences. A schematic illustration of human FAK and *Drosophila* FAK, DFak56, is shown. Black bars denote the N-terminal FERM domain. The kinase domain, FAT sequence, major tyrosine phosphorylation sites (Y) and proline-rich sequences (P) are indicated. The numbers reflect the percentage of amino acid identity between the different domains of human and *Drosophila* FAK. The percentage of identical amino acids in the FERM domains is indicated below the cartoon of *Drosophila* FAK.

2. FAK: sequence conservation and functions of domains

2.1. Evolutionary conservation of FAK sequence and function

The *Drosophila* homologue of FAK, called DFak56, was recently isolated and exhibits approximately 33% amino acid identity with human FAK [21–23]. DFak56 exhibits the same organization of domains as FAK, with a central catalytic domain flanked by N- and C-terminal noncatalytic domains (see Fig. 2). However, DFak56 contains a large C-terminal extension beyond the C-terminal FAT sequence of FAK. DFak56 shares a number of biological properties with FAK including subcellular localization to focal adhesions and cell adhesion-dependent regulation of tyrosine phosphorylation [21–23].

Naturally, the tyrosine kinase domain is most highly conserved between DFak56 and human FAK exhibiting 63% identity. The FAT sequence is also highly conserved showing 47% identity, whereas the N-terminal domain and the region between the catalytic domain and FAT sequence of DFak56 are only 32% and 31% identical to human FAK. Not surprisingly, the major autophosphorylation site is conserved in DFak56, as are the regulatory tyrosine residues in the catalytic domain. Although DFak56 contains a tyrosine residue corresponding to tyrosine 925 in human FAK, the flanking residues suggest that DFak56 does not bind to Grb2 via this site. The first proline-rich sequence in the C-terminal domain of FAK, which is the major $p130^{cas}$ binding site, is conserved in DFak56, but the second proline sequence in the C-terminal domain, which is the major GRAF binding site, is not conserved.

It is anticipated that FAK functions in integrin signaling in *Drosophila*. This postulate remains to be rigorously tested, but initial results are consistent with this hypothesis. β integrin defects in the *Drosophila* wing cause blisters to form. Similarly, overexpression of DFak56 in the wing induces blisters [22]. However, the most exciting prospect from this observation is that DFak56 overexpression induces a readily scored phenotype in flies. This system will allow an analysis of genetic interactions between DFak56 and other genes implicated in integrin signaling. Further, this model system may be modified as a genetic screen for mutations that result in suppression of the phenotype and thus facilitating the identification of new genes that may function in FAK signaling.

2.2. The N-terminal domain of FAK

The N-terminal domain of FAK exhibits sequence homology with the band 4.1 protein/ERM (ezrin, radixin and moesin) proteins within a region known as the FERM domain [24] [25]. This homology is likely to be significant for FAK function, since it is

highly conserved evolutionarily. Whereas the N-terminal domains of DFak56 and human FAK are only 32% identical, sequences within the FERM domain exhibit 49% identity (see Fig. 2). The FERM domains of talin and the ERM proteins mediate interactions with the cytoplasmic domains of transmembrane receptors [26–29]. While the function of the N-terminus of FAK has not been definitively established, similar functions have been proposed.

The first function proposed for the N-terminal domain of FAK was binding to the cytoplasmic tails of β integrin subunits, since FAK could complex with peptides mimicking β integrin cytoplasmic domains in vitro [30]. FAK was shown to co-cluster with integrins upon capping with integrin antibodies under conditions where most focal adhesion-associated proteins, most notably paxillin and talin, failed to co-cluster [31]. Further, FAK and β1 integrin subunits were recently reported to coimmunoprecipitate and thus may associate in vivo [32,33]. However, the function of this interaction remains to be established. This interaction is clearly dispensable for localization of FAK and mutational analyses of both FAK and the β3 integrin subunit demonstrate that this interaction is not required for cell adhesion dependent tyrosine phosphorylation of FAK [34,35].

A number of reports have suggested that FAK functions downstream of growth factor receptors, since tyrosine phosphorylation of FAK is stimulated by treatment of cells with growth factors. Recent evidence, which will be discussed below, has implicated FAK as an important element in coordinating biochemical signals and biological responses following stimulation of growth factor receptors and integrins. A recent study has suggested a mechanism for linkage of FAK to growth factor receptor signaling. In several cell systems FAK was found to coimmunoprecipitate with the EGF receptor or the PDGF receptor following stimulation with their cognate ligand [36]. The N-terminal domain of FAK mediates this interaction and mutants of FAK that exhibit reduced receptor binding are impaired in their ability to coordinate this signal into a biological response [36]. Perhaps related to the observed interaction of FAK with the EGF and PDGF receptors is the recent finding that FAK can coimmunoprecipitate with a number of other receptor tyrosine kinases including ErbB2, ErbB3, the IGF1 receptor, and Eph2A [37–40]. Although these are very intriguing findings, there are still a number of outstanding issues regarding these observation. It remains to be established whether the interaction between FAK and the EGF/PDGF receptors is direct or indirect and the molecular details of the binding site remain to be elucidated. Further, the molecular mechanism of the interaction of FAK with the other receptor tyrosine kinases has not been determined. It will be interesting to see if FAK associates with these other receptors through its N-terminal domain and if the FERM homology domain mediates these interactions.

2.3. *Tyrosine 397: site of autophosphorylation and docking site for SH2 domains*

The major site of autophosphorylation of FAK is tyrosine 397, a residue that lies immediately N-terminal to the catalytic domain. This residue is vital for the biochemical and biological functions of FAK. Although Src was the first SH2 domain-containing protein found to bind to the autophosphorylation site of FAK, recent evidence demonstrates that the SH2 domains of PI3-kinase, PLCγ and Grb7 also bind to the FAK autophosphorylation site [41–43]. Shc also associates with FAK and tyrosine 397 is required for binding the Shc SH2 domain, but the binding site for Shc remains to be conclusively identified [44]. Thus there are at least four distinct signaling molecules recruited into complex with a single docking site on FAK (see Fig. 3). It is not possible for multiple SH2 domain-containing proteins to simultaneously dock with a single tyrosine phosphorylated binding site. Therefore autophosphorylation must promote the assembly of a number of distinct FAK containing signaling complexes. These individual complexes could be assembled into higher order complexes in focal adhesions, facilitating the colocalization of a number of distinct signaling molecules. This might provide a mechanism for coordinating signaling through multiple pathways. Alternatively, the colocalization of complexes may promote further interplay between FAK-binding partners. For example, recruitment of PI3-kinase and PLCγ into complex with FAK could facility the generation of $PI(3,4,5)P_3$ by PI3-kinase, which then serves as a docking site for the PH domain of PLCγ.

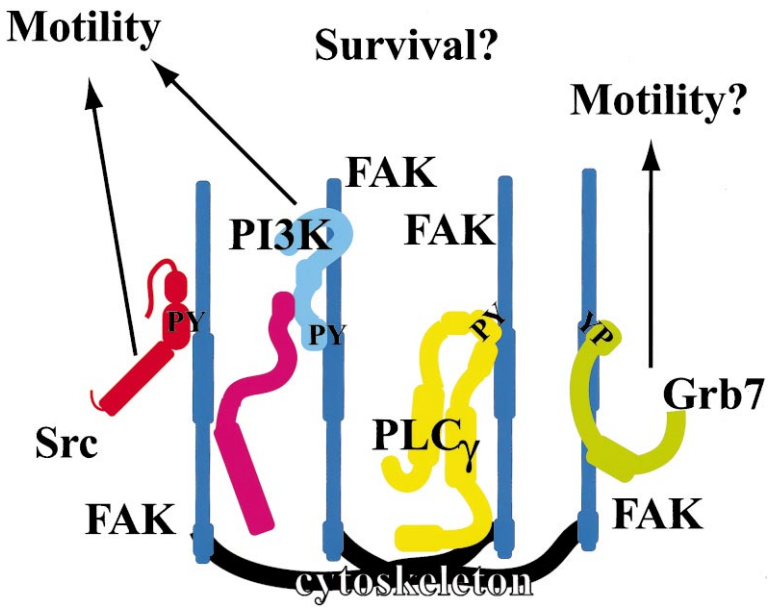

Fig. 3. Multiple signaling proteins dock at Tyrosine 397 of FAK. The single major autophosphorylation site of FAK binds to at least four different SH2 domain containing signaling molecules: Src, PI3-kinase, PLCγ and Grb7. Src and PI3-kinase function in the regulation of motility, as may Grb7. Some of these proteins also function in transmitting a cell survival signal, but the critical FAK-binding partners have not been absolutely identified.

2.4. *The C-terminal domain and focal adhesion targeting*

The correct subcellular localization of FAK is essential for its function. Mutants of FAK that fail to localize to focal adhesions are not regulated in response to cell adhesion [34,45]. Further, displacement of endogenous FAK from focal adhesions by overexpression of the C-terminal non-catalytic domain of FAK, inhibits tyrosine phosphorylation of FAK and impairs its biological functions [46–50]. Given the importance of this region of FAK for its function, there is considerable interest in elucidating the mechanism of focal adhesion targeting.

Two focal adhesion-associated proteins, paxillin and talin, have been identified as binding partners for the FAT sequence of FAK and each has been proposed as the mediator of FAK localization. The N-terminus of paxillin contains five copies of a motif, the LD motif, that functions in mediating protein–protein interactions [51]. Two of these motifs mediate the interaction of paxillin with FAK and are also involved in binding vinculin [52,53]. In the FAT sequence of FAK are two short, noncontiguous sequences that exhibit sequence homology with the region of vinculin that binds paxillin [54]. Mutation of residues within these sequences, PBS1 (for paxillin binding site 1) and PBS2 (paxillin binding site 2), disrupt paxillin binding and focal adhesion targeting [54]. These results suggest that binding to paxillin mediates targeting of FAK to focal adhesions. However, other evidence suggests that this may not be the case. Although there is sequence conservation of PBS1 and PBS2 in *Drosophila* FAK, there are residues in each site that have been implicated in paxillin binding but are not conserved in DFak56 [21–23]. While the *Drosophila* homologue of paxillin might contain complementary mutations to preserve the interaction with these altered FAK sequences, it is also possible that other FAK sequences play an important role in paxillin binding. Additional mutational analysis of FAK has revealed other residues outside of PBS1 and PBS2 that are also essential for paxillin binding [45]. The most significant finding was that a number of FAK mutants that cannot bind paxillin could still localize to focal adhesions [16,45]. Thus, paxillin binding is not essential for focal adhesion localization of FAK. However, it may be noteworthy that all of the mutants described to date that fail to target to focal adhesions are also unable to bind to paxillin [45,54].

Talin has also been suggested to target FAK to

focal adhesions. This conclusion is based upon the observation that CAKβ, which is generally diffusely localized in the cell, fails to coimmunoprecipitate with talin, whereas the focal adhesion-associated FAK can coimmunoprecipitate with talin [55]. However, it is also possible that CAKβ fails to coimmunoprecipitate with talin because it is localized differently in the cell, i.e., the talin-binding activity of CAKβ has not been assessed in vitro. Further, the C-terminal domain of CAKβ can localize to focal adhesions when expressed autonomously [56,57]. Finally, the subcellular localization of some deletion mutants of FAK does not correlate with talin-binding activity [15]. Thus talin binding also fails to explain the ability of FAK to target to focal adhesions.

Is it possible to reconcile these data and formulate a hypothetical mechanism for focal adhesion targeting of FAK? Perhaps there is another FAK-binding partner that has not been identified and this unknown protein functions to target FAK to focal adhesions. More likely, there is more than one independent mechanism for targeting FAK to focal adhesions, e.g., via talin binding or via paxillin binding. Thus paxillin-binding mutants may target to focal adhesions by interacting with talin, and paxillin may mediate the focal adhesion localization of FAK mutants defective for talin binding. This theory remains to be tested, but could potentially resolve the discrepancy in the literature.

One other intriguing observation is that the C-terminal domain of FAK can be expressed as an autonomous protein, FRNK. The expression of endogenous FRNK was first observed in primary CE cells [58]. Two major transcripts are detected in CE cells, a 4.5 kb mRNA that encodes FAK and a 2.5 kb mRNA that encodes FRNK [58]. The shorter mRNA is transcribed from an alternate promoter located within an intron of the FAK gene [59]. Whereas FAK is broadly expressed in cells and tissues, FRNK expression is quite restricted. FRNK expression has been detected in avian and murine lung tissue and in murine smooth muscle, most notably in the smooth muscle of the vasculature [59,60]. Interestingly, FRNK expression is elevated in arterial smooth muscle following vascular injury [60]. FRNK has been utilized as a dominant negative mutant to inhibit FAK signaling in a number of studies (e.g., see [46,47,61,62]). Exogenous expression of FRNK may inhibit endogenous FAK by displacing FAK from focal adhesions and/or titrating critical signaling partners away from FAK. The function of endogenous FRNK has not been established. FRNK may function as a natural competitive inhibitor of FAK or FRNK might augment a noncatalytic function of FAK, e.g., by providing a focal adhesion docking site for a FAK/FRNK-binding partner.

3. Activation of FAK and Src

3.1. How is FAK activated after integrin stimulation?

The co-localization of FAK with integrins in focal adhesions is a prerequisite for cell adhesion-dependent activation of FAK signaling. Mutants of FAK that are unable to localize to focal adhesions fail to become tyrosine phosphorylated in response to cell adhesion to fibronectin [34,45]. Additional evidence for the importance of localization in signaling comes from the analysis of CAKβ. The subcellular localization of CAKβ is generally diffuse and tyrosine phosphorylation of CAKβ is weakly stimulated upon cell adhesion [55,56,63]. Chimeric FAK/CAKβ proteins, in which the FAT sequence of FAK is substituted for the corresponding sequence of CAKβ, very efficiently target to focal adhesions and become prominently tyrosine phosphorylated in response to cell adhesion [55,63]. These findings demonstrate that localization is a critical requirement for regulation of FAK by cell adhesion.

The actin cytoskeleton is also essential for tyrosine phosphorylation of FAK, since cytochalasin D abolishes tyrosine phosphorylation of FAK in response to many stimuli, including integrin-dependent cell adhesion [64,65]. Members of the Rho family of GTP-binding proteins are key regulators of the actin cytoskeleton, and Rho itself promotes contractility and the formation of stress fibers and focal adhesions [66]. Several lines of evidence suggest that activation of Rho induces tyrosine phosphorylation of FAK, whereas inhibition of Rho blocks the tyrosine phosphorylation of FAK in response to certain stimuli [67–70]. These data suggest tyrosine phosphorylation of FAK may be controlled by Rho-dependent alterations of the actin cytoskeleton. The activity of Rho has been measured and found to transiently decline

upon cell adhesion and then increase above basal levels observed in cells in suspension [71]. The finding that Rho activity decreases upon adhesion to fibronectin seems at odds with the hypothesis that Rho stimulates tyrosine phosphorylation of FAK, since FAK tyrosine phosphorylation increases upon cell adhesion to fibronectin. However, the reduction in Rho activity is only several-fold and the residual active Rho could still potentially function to induce tyrosine phosphorylation of FAK upon cell adhesion. For example, localization of the active subpopulation of Rho to sites of adhesion could promote contractility and localized clustering of cytoskeletal components, including FAK, leading to FAK autophosphorylation and activation of FAK signaling pathways.

An important event in the enzymatic activation of FAK is phosphorylation of the tyrosine residues in the activation loop. These residues are not sites of autophosphorylation of FAK, but are phosphorylated by Src family kinases [10]. Pharmacological inhibitors of Src have been used to assess the role of Src in tyrosine phosphorylation of FAK in several signaling scenarios. Inhibition of Src signaling impairs tyrosine phosphorylation of tyrosine residue 925 of FAK following adhesion to fibronectin [65]. Inhibition of Src also inhibits tyrosine phosphorylation of FAK, specifically at activation loop residues, in response to bombesin stimulation of Swiss 3T3 cells [72]. Further, genetic analyses have also implicated Src in the tyrosine phosphorylation of FAK following integrin-dependent cell adhesion. *Src*−/− fibroblasts are slightly defective and fibroblasts deficient for the *src*, *fyn* and *yes* genes exhibit a dramatic defect in the cell adhesion-dependent tyrosine phosphorylation of FAK [73,74]. Analysis of FAK tyrosine phosphorylation in these cells using phospho-specific antibodies has not been reported; hence the role of Src in promoting phosphorylation of distinct tyrosine residues in FAK has not been addressed genetically. Nevertheless, these results support the hypothesis that Src is required for maximum induction of FAK tyrosine phosphorylation in response to cell adhesion.

Src is proposed to bind to the autophosphorylation site of FAK to facilitate Src-dependent phosphorylation of other tyrosine residues on FAK. However, there is evidence from *src*-transformed fibroblasts that activated Src mutants can phosphorylate the FAK 397F mutant, which is defective for Src binding [75,76]. The presence of a Src SH3 binding site in FAK complicates this interpretation since there might still be a weak interaction [77]. These results suggest that in the context of *src*-transformed cells, Src does not have to stably dock with FAK to induce its tyrosine phosphorylation. In terms of integrin-dependent signaling in fibroblasts, this question has been addressed by characterizing FAK mutants using phospho-specific antibodies. An autophosphorylation site mutant of FAK, which fails to associate with Src, exhibits a very dramatic reduction in phosphorylation of activation loop residues [76]. This observation suggests that in response to cell adhesion, the association of Src with FAK is required for Src-dependent tyrosine phosphorylation of the activation loop of FAK. A FAK mutant with phenylalanine substitutions for the activation loop sites of tyrosine phosphorylation shows substantial tyrosine phosphorylation of the major autophosphorylation site of FAK, albeit slightly reduced [76]. This suggests that the cell adhesion-dependent tyrosine phosphorylation of the autophosphorylation site of FAK occurs independently of activation loop phosphorylation, and presumably independently of Src kinases. These observations suggest that cell adhesion-dependent tyrosine phosphorylation of FAK occurs in two phases. The first, autophosphorylation phase, occurs in the absence of Src and results in the tyrosine phosphorylation of FAK residue 397. The second phase occurs following recruitment of Src into complex with FAK and results in phosphorylation of FAK at other tyrosine residues leading to the full activation of FAK and transmission of downstream signals.

3.2. How is Src activated after integrin stimulation?

Src is negatively regulated by phosphorylation of a C-terminal tyrosine residue by the Csk tyrosine kinase [78]. This modification promotes an intramolecular interaction between the C-terminus of Src and its SH2 domain and a second intramolecular interaction involving the SH3 domain [78]. These intramolecular interactions stabilize Src in an inactive conformation. Two major mechanisms of Src activation can be envisioned. First, dephosphorylation of

the negative regulatory site could activate Src and second activation could be achieved by disruption of the low affinity intramolecular SH2 and SH3 interactions by the presentation of high affinity ligands for these binding sites. Both mechanisms have been proposed for the activation of Src following engagement of integrins by their ligands.

There are a number of ligands for the SH2 and SH3 domains of Src in focal adhesions. The major autophosphorylation site of FAK and a tyrosine phosphorylation site on $p130^{cas}$ are Src SH2 domain ligands [9,79]. In addition, there are Src SH3 domain binding sites in paxillin, $p130^{cas}$ and FAK [77,79,80]. In both FAK and $p130^{cas}$, the docking sites for the Src SH2 and SH3 domains are near one another suggesting that both domains of Src may be simultaneously engaged. Src can be potently activated by disruption of both of its intramolecular inhibitory interactions by competition with the SH3 domain and SH2 domain binding sites from either FAK or a $p130^{cas}$-related protein, Sin1 [77,81]. Thus this mechanism of activation following tyrosine phosphorylation of FAK and $p130^{cas}$ seems plausible. This mechanism is unlikely to account for the activation of Src following stimulation of Swiss 3T3 cells with neuropeptides, since cytochalasin D treatment, which abrogates FAK tyrosine phosphorylation, has no effect upon Src activation [82]. In contrast to this observation, cytochalasin D treatment of NIH 3T3 partially inhibits Src activation in response to cell adhesion [65]. This finding demonstrates that Src can be activated independently of FAK but also suggests that a FAK-dependent pathway is required for maximal stimulation of Src activity following cell adhesion.

There is also evidence that tyrosine phosphatases may function in the activation of Src following cell adhesion. Overexpression of a catalytically inactive mutant of PTP1B in L cells inhibits cell spreading and reduces cell adhesion-dependent tyrosine phosphorylation [83]. Src exhibits lower activity in these cells suggesting that the mutant PTP1B may inhibit the endogenous wild-type PTP1B and block Src activation [83]. In these cells there is a small amount of PTP1B that can be coimmunoprecipitated with β1 integrin and a small amount of PTP1B may localize to focal adhesions [83]. Shp2 might also function in the regulation of Src following cell adhesion. *Shp2*−/− fibroblasts exhibit reduced spreading on fibronectin and reduced activation of Src upon adhesion to fibronectin [84]. Thus Shp2 is also a candidate as the regulatory phosphatase that activates Src upon cell adhesion. While these initial studies support the role of PTP1B and Shp-2 in the activation of Src following cell adhesion, further analyses are required to firmly establish these functions. In contrast, there are a number of studies strongly suggesting that PTPα may serve to activate Src following cell adhesion.

PTPα might function as a positive regulator of Src since overexpression of PTPα activates Src in vivo and induces cellular transformation [85,86]. Several recent reports have begun to assess the role of PTPα in the regulation of Src activity following cell adhesion. Overexpression of wild-type PTPα in A431 cells induces dephosphorylation of Src and an elevation in the catalytic activity of Src [87]. PTPα expression also promotes assembly of a complex between FAK and Src, a complex that normally forms in response to integrin-dependent cell adhesion, and promotes tyrosine phosphorylation of paxillin, a focal adhesion-associated protein [87]. Exogenously expressed PTPα mutants were shown to localize to focal adhesions in NIH 3T3 cells, and thus PTPα might localize to sites of integrin signaling [88]. *PTPα*−/− fibroblasts were recently derived and shown to have reduced Src family kinase activity and exhibit a delay in spreading, a defect that is shared with Src deficient cells [89]. Some insight into the mechanism of regulation of Src by PTPα has recently been obtained. A C-terminal tyrosine in PTPα is a site of phosphorylation and appears to serve as a docking site for the SH2 domain of Src [90]. This interaction might disrupt the intramolecular inhibitory interactions within Src exposing the C-terminal regulatory phosphotyrosine residue for dephosphorylation, in addition to recruiting Src into complex with the phosphatase.

The evidence described suggests that FAK can become autophosphorylated initially in the absence of Src (Fig. 4, models A and C). While Src might be activated by complex formation with FAK and disruption of intramolecular inhibitory interactions, this mode of activation is not likely the major mechanism of Src activation (Fig. 4, model A). The actions of protein tyrosine phosphatases, currently the odds-on

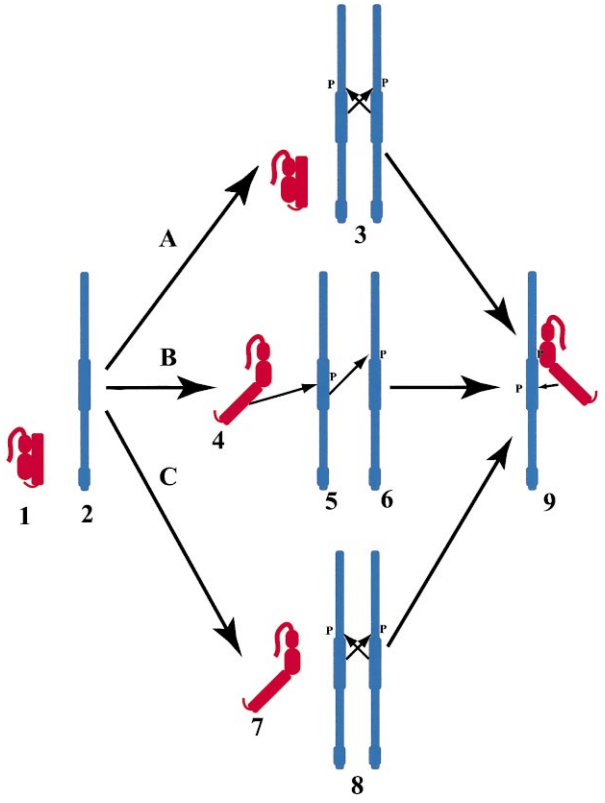

Fig. 4. Models of Src and FAK activation. In suspended cells Src is inactive (1) and FAK is in its dephosphorylated state (2). Three possible models for the activation of these kinases and assembly into a signaling complex can be proposed. (A) FAK clustering might be sufficient to induce transphosphorylation at tyrosine 397 (3). The inactive Src protein might be recruited into complex with FAK, with the high affinity SH2 and SH3 binding sites in FAK disrupting the intramolecular inhibitory interactions in Src, resulting in the activation of Src and assembly of the FAK/Src signaling complex (9). (B) Src might be initially activated by a phosphatase (4). Activated Src could phosphorylate FAK on the activation loop residues (5), activating FAK and promoting autophosphorylation at tyrosine 397 (6). Activated Src could then be recruited into complex with activated FAK to form a signaling complex (9). (C) Src and FAK could independently be activated (7,8) and then activated Src and FAK assembled into a signaling complex.

favorite being PTPα, seem to activate Src in response to cell adhesion (Fig. 4, models B and C). Once FAK and Src are independently activated, complex formation occurs. This may help stabilize Src in its active conformation. In addition Src catalyzes tyrosine phosphorylation of FAK on additional residues, elevating the kinase activity of FAK, creating binding sites for other signaling molecules and promoting the transmission of downstream signals.

4. Inhibition of FAK signaling

4.1. FIP200: a negative regulator of FAK?

A new binding partner that may be involved in negative regulation of FAK signaling was recently isolated [91]. The protein, FIP200, was isolated from a yeast two-hybrid screen using the N-terminal and catalytic domains of CAKβ as bait. The FIP200 binding site was mapped to the catalytic domain of CAKβ, and FIP200 can inhibit the catalytic activity of CAKβ in vitro and block CAKβ signaling in vivo. FIP200 was also shown to bind to FAK and to inhibit the catalytic activity of FAK in vitro. Thus this protein may be involved in regulating both members of the FAK family of tyrosine kinases.

4.2. FAK and protein tyrosine phosphatases

Since tyrosine phosphorylation of FAK regulates its catalytic activity and association with other signaling molecules, dephosphorylation of tyrosine residues is potentially a very important mechanism in regulating FAK signaling. A number of tyrosine phosphatases have been suggested as regulators of FAK, including PTP-PEST [92] and PTP1B [93]. However, there is more compelling evidence that Shp-2 might regulate FAK tyrosine phosphorylation, and the most convincing evidence to date suggests that PTEN catalyzes dephosphorylation of FAK.

The SH2 domain containing tyrosine phosphatase, Shp-2, has been proposed to regulate tyrosine phosphorylation of FAK in multiple scenarios, although the regulation of FAK by Shp-2 may be complicated. Several receptor tyrosine kinases have been reported to reduce tyrosine phosphorylation of FAK, including the insulin receptor, IGF-1R and EphA2 [39,40]. The IGF-1R and EphA2 can coimmunoprecipitate with both FAK and Shp-2 and overexpression of a catalytically inactive Shp-2 mutant in MCF-7 cells can block dephosphorylation of endogenous FAK in response to IGF-1 [39]. In contrast, PDGF stimulation of porcine aortic endothelial cells induces activation of FAK and a PDGF receptor mutant that cannot bind to Shp-2 fails to induce FAK activation in response to PDGF [94]. Thus Shp-2 has been proposed to inhibit signaling by promoting FAK de-

phosphorylation and to activate FAK in response to different stimuli.

Shp-2 may function in regulating FAK signaling upon cell adhesion since Shp-2 deficient fibroblasts exhibit a defect in spreading and motility, which are biological responses that are regulated by FAK [84,95]. However, further experimentation to define the biochemical consequences of altering Shp-2 function has not yielded consistent results. In one instance, tyrosine dephosphorylation of FAK was reportedly impaired when Shp-2-deficient cells were taken into suspension, suggesting that Shp-2 might function in dephosphorylating FAK upon de-adhesion from the extracellular matrix [95]. A second report describes impaired tyrosine phosphorylation of FAK upon adhesion of Shp-2-deficient cells to the extracellular matrix suggesting the Shp-2 promotes tyrosine phosphorylation of FAK [84]. This defect in FAK tyrosine phosphorylation upon cell adhesion is ascribed to a defect in activation of Src. In a third study, expression of a catalytically defective Shp-2 mutant in Rat-1 cells, which might be expected to exhibit the same effect as the Shp-2 deficiency, has no effect upon FAK phosphorylation when cells are taken into suspension and shows an increase in FAK tyrosine phosphorylation when cells are plated on fibronectin [96]. While Shp-2 is intriguing as a candidate regulatory phosphatase for FAK, no consistent theme regarding the effects of Shp-2 upon FAK has yet emerged from these investigations, making it difficult to definitively assign a function for Shp-2 in regulating FAK dephosphorylation.

The strongest evidence to date suggests that PTEN may catalyze dephosphorylation of FAK. This is a very intriguing hypothesis since PTEN was originally isolated as a tumor suppressor gene, which may have implications for FAK in the development of cancer [97,98]. PTEN exhibits three different types of phosphatase activity. Originally shown to be a protein tyrosine phosphatase, PTEN was later shown to also exhibit serine/threonine phosphatase activity in vitro and most recently to function as a lipid phosphatase [99–101]. The lipid phosphatase activity of PTEN is directed against phosphatidyl-inositols containing phosphate at the D3 position. Consequently, PTEN functions to degrade the reaction product of PI3-kinase and was shown to function as a negative regulator of PI3-kinase signaling in vivo [102].

There are several lines of evidence implicating PTEN in the regulation of tyrosine phosphorylation of FAK. Overexpression of PTEN in either NIH 3T3 cells or U87-MG cells, which are PTEN deficient, causes a reduction in tyrosine phosphorylation of FAK [103–105]. Expression of a catalytically defective mutant of PTEN induces a small increase in tyrosine phosphorylation of FAK and the mutant PTEN protein coimmunoprecipitates with FAK [103,105]. Interestingly, catalytically defective PTEN variants do not associate with a FAK mutant containing a phenylalanine for tyrosine substitution at residue 397 (the major autophosphorylation site of FAK), suggesting that PTEN may target this important phosphorylation site for dephosphorylation [105]. Further, in an in vitro assay, recombinant PTEN can dephosphorylate FAK protein that has been subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred and immobilized to a membrane [103]. Expression of PTEN in NIH 3T3 cells or U87-MG cells induces a number of phenotypic changes that are similar to phenotypes associated with inhibition of FAK signaling, including reduced spreading, motility and invasion [103,104]. Simultaneous overexpression of FAK with PTEN reverted these phenotypes consistent with the hypothesis that PTEN induced these phenotypes by dephosphorylation of FAK and inhibition of FAK signaling. Although these experiments suggest that FAK is a PTEN substrate, other studies have failed to provide similar evidence. Analysis of *PTEN*−/− ES cells and fibroblasts revealed no increase in tyrosine phosphorylation of FAK, contrary to the predicted result if FAK was a major PTEN substrate [106,107]. In addition, overexpression of PTEN in U87MG cells does not always lead to FAK dephosphorylation [108].

The activity of PTEN as a protein phosphatase has received much less scrutiny than its role as a lipid phosphatase. One specific PTEN mutation that is associated with human disease, G129E, abrogates lipid phosphatase activity, but has little effect upon its protein phosphatase activity [99]. This finding suggests that the lipid phosphatase activity of PTEN is essential in its role as a tumor suppressor. In support of this hypothesis, wild-type PTEN rescues a number of cellular phenotypes exhibited by cancer cells containing PTEN lesions, whereas the

G129E mutant does not [102,109]. However, these experiments demonstrate the importance of lipid phosphatase activity without assessing the role of protein phosphatase activity. In fact, the G129E variant of PTEN functions as well as the wild-type PTEN to inhibit cell spreading and invasion of U87-MG cells, demonstrating that PTEN exhibits some biological activity in the absence of lipid phosphatase activity [103,104]. Further investigation of PTEN is clearly required to fully define the role of its protein phosphatase activity in tumor suppression and the role of PTEN in regulating tyrosine phosphorylation of FAK. It is interesting to note that one downstream effector of FAK signaling is PI3-kinase. PTEN may utilize its protein phosphatase activity to dephosphorylate FAK, thus inhibiting the activation of PI3-kinase, and its lipid phosphatase activity to degrade any products of PI3-kinase that are generated (Fig. 5). In this fashion PTEN could serve as a very potent inhibitor of PI3-kinase signaling following integrin-dependent cell adhesion.

Although there are clearly some provocative candidate protein tyrosine phosphatases that may catalyze dephosphorylation of FAK, this aspect of FAK regulation is only beginning to be elucidated. The fact that multiple tyrosine residues of FAK are phosphorylated raises the possibility that distinct protein tyrosine phosphatases may be responsible for dephosphorylation of different sites. Since different phosphorylation sites function to regulate catalytic activity and protein–protein interactions, site-specific dephosphorylation of FAK may be an effective mechanism to modulate some aspects of FAK signaling independently from others. The study of tyrosine dephosphorylation of FAK will undoubtedly provide an area of fruitful investigation in the near future.

4.3. Proteolytic cleavage of FAK

While dephosphorylation of FAK may function to curb signaling, an equally effective, albeit more dramatic, mechanism of terminating FAK signaling is proteolytic cleavage. Proteolysis of FAK was first observed in chicken embryo fibroblasts transformed by the *v-src* or *myc* oncogenes [110,111]. Cleavage of FAK was subsequently observed in a number of other cell types exposed to different stimuli [112–117]. In each of these experimental systems FAK is similarly

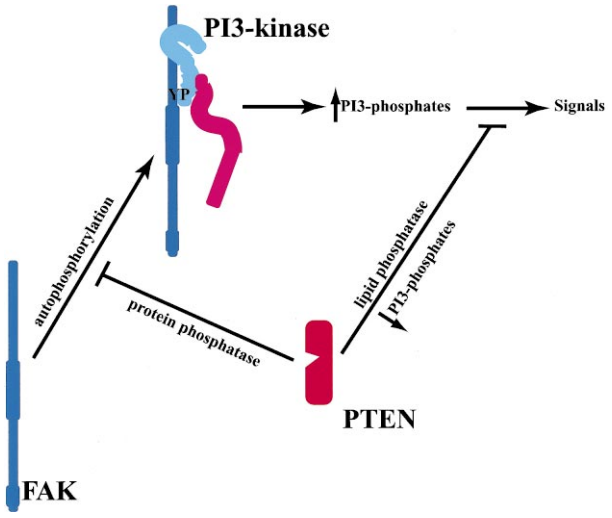

Fig. 5. PTEN regulation of FAK and PI3-kinase signaling. Autophosphorylation of FAK promotes complex formation with PI3-kinase, which likely results in the generation of PI3-phosphates in the membrane. PTEN can dephosphorylate FAK to block association with PI3-kinase. PTEN can also dephosphorylate PI3-phosphates to terminate signaling downstream of PI3-kinase. Thus PTEN might intervene at two positions within this signaling cascade.

cleaved into a large N-terminal fragment of 85–100 kDa that is subsequently cleaved into smaller fragments and a C-terminal fragment of approximately 35 kDa. Through the use of pharmacological inhibitors and in vitro cleavage assays, several proteases have been implicated in FAK cleavage. Caspases 3 and 7 have been identified as major enzymes involved in cleavage of FAK, whereas caspase 6 might play a minor role [112,113,118]. In other situations calpain has been implicated as the protease responsible for FAK cleavage [114,115,119].

The major caspase 3 cleavage site has been identified as a DQTDS sequence beginning at amino acid 772 in FAK [118]. However, this cleavage site is not highly conserved amongst vertebrate FAK sequences and is more divergent in the sequence of *Drosophila* FAK. Although this site is not conserved in murine FAK, apoptotic stimuli still induce proteolytic cleavage of FAK in mouse cells to generate fragments similar, but not identical, in size to the fragments generated in human cells [118]. It is striking that different proteases are induced by different stimuli to cleave FAK generating similar proteolytic fragments. It is of further interest to note that FAK cleavage occurs in different species, yet the one pro-

tease cleavage site that has been mapped is not evolutionarily conserved. Perhaps the target site for cleavage by these various proteases is a region of FAK that is exposed and particularly susceptible to cleavage.

What are the consequences of FAK cleavage? The major site of cleavage of FAK in response to these stimuli separates the catalytic domain of FAK from the FAT sequence. The enzyme activity of cleaved FAK would be mislocalized and therefore ineffective at transmitting signals. The more interesting possibility, that the cleavage fragments of FAK might actively transmit aberrant signals that promote cell cycle arrest or induce apoptosis has also been suggested. Overexpression of a C-terminal FAK fragment, similar to the C-terminal product of cleavage, can induce apoptosis and overexpression of a FAK mutant that fails to localize to focal adhesions can inhibit cell cycle progression [49,50] [120]. Whether cleavage of endogenous FAK simply terminates signaling or generates fragments that actively promote cell death remains to be established.

5. FAK signaling

As outlined in the overview, there are a number of biochemical pathways that can be activated downstream of FAK. These include signaling components that are well known from the field of growth factor receptor signaling; the PI3-kinase, PLCγ and MAP kinase pathways. FAK can also transmit downstream signals by tyrosine phosphorylation of FAK-associated adaptor proteins, paxillin and $p130^{cas}$, which in turn recruit additional signaling molecules into complex resulting in activation of downstream pathways. These signaling pathways have been reviewed both in the context of FAK signaling and otherwise and will not be extensively discussed here [1,121–125]. However, recent findings regarding FAK signaling and the MAP kinase pathway merit some discussion.

5.1. FAK and MAP kinases

Several mechanisms can be utilized by FAK to stimulate MAP kinase signaling. FAK can be phosphorylated on tyrosine 925 to create a Grb2 binding site and recruitment of a Grb2/SOS complex is one mechanism that FAK may use to activate Ras and the MAP kinase pathway [11]. FAK contains a binding site for the $p130^{cas}$ adaptor protein and tyrosine phosphorylation of $p130^{cas}$ can result in the recruitment of the Crk and Nck adaptor proteins. Since these adaptor proteins can bind SOS, this provides a second mechanism for stimulation of Ras/MAP kinase signaling [73]. Finally, FAK activation can promote tyrosine phosphorylation of Shc, which in turn recruits Grb2 and SOS into complex promoting the activation of Ras and the MAP kinase pathway [44]. Thus there are apparently multiple mechanisms that FAK could employ to induce the activation of MAP kinase (see Fig. 6).

The role of FAK in the activation of MAP kinase in response to integrin engagement has been controversial. Clearly there are other signaling pathways that integrins can utilize to induce activation of MAP kinase, since MAP kinase activation can be dissociated from tyrosine phosphorylation of FAK [126–128]. On the other hand, the expression of FAK can enhance cell adhesion-dependent activation of MAP kinase and it has been reported that inhibition of FAK signaling blocks MAP kinase activation in response to integrin-dependent signaling in epithelial cells [1,129]. These conflicting reports suggest that under certain conditions FAK is a major regulator of MAP kinase activation following integrin stimulation, whereas under other conditions other signaling pathways to MAP kinase are dominant. One interesting suggestion is that the expression of B-Raf might be the key difference between these individual experiments [128]. It was proposed that FAK signaling via $p130^{cas}$/Crk/C3G leads to the activation of Rap1, B-Raf, MEK and finally MAP kinase in cells expressing B-Raf [128]. However, in cells lacking B-Raf this signaling pathway is inoperative, and other mechanisms for the activation of MAP kinase are employed. It was further proposed that FAK induced MAP kinase activation was a later event in integrin signaling. Thus activation of FAK-independent MAP kinase activation pathways would produce a transient activation and the additional engagement of the FAK-dependent pathway would produce a prolonged MAP kinase response. According to this theory, FAK might play an important role in controlling the kinetics of MAP kinase

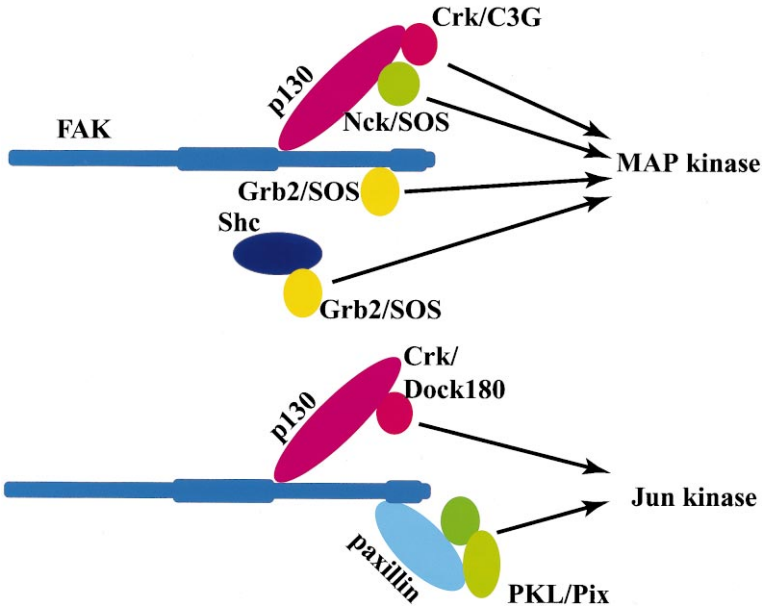

Fig. 6. Multiple FAK signaling pathways can converge on MAP kinase and JNK. Several pathways are proposed to link FAK to the activation of MAP Kinase. (1) The C-terminus of FAK contains a binding site that can recruit Grb2/SOS. (2) Tyrosine phosphorylation of Shc can be induced by FAK leading to the assembly of a Shc/Grb2/SOS complex. (3) $p130^{cas}$, which associates with FAK, becomes tyrosine phosphorylated, creating binding sites for the Crk and Nck adaptor proteins. This promotes recruitment of Nck/SOS and Crk/C3G complexes. The SOS and C3G exchange factors can activate GTP-binding proteins leading to activation of MAP kinase. Multiple pathways also lead to JNK signaling. Tyrosine phosphorylation of $p130^{cas}$ can also recruit Crk/Dock 180 complexes that may signal through Rac to activate JNK. A phosphotyrosine independent linkage to JNK, via the paxillin/PKL/PIX complex has also been proposed.

activation, which might have a profound effect upon the ultimate biological response to the signal.

In addition to activation of the MAP kinase pathway, there is some indication that integrin-dependent adhesion causes activation of the JNK pathway, and that FAK might play a role in this pathway as well [130–132]. FAK may utilize different mechanisms to activate MAP kinase and JNK since a catalytically inactive FAK mutant fails to activate MAP kinase, but still activates JNK [131]. In this system, using an activated, membrane-bound FAK construct, the linkage to JNK was proposed to occur via recruitment of paxillin, and an associated exchange factor for Rho family proteins, into complex with FAK. This mechanism of JNK activation occurs independently of tyrosine phosphorylation. There are also other phosphotyrosine-dependent mechanisms that FAK could use to stimulate the JNK pathway, e.g., via $p130^{cas}$/Crk/Dock180 and stimulation of Rac [50]. Further experimentation is required to define the mechanisms that FAK might utilize to stimulate JNK activity in response to integrin engagement.

The role of FAK in regulating MAP kinases discussed in the above paragraphs relates to MAP kinase activation in response to integrin-dependent cell adhesion. One other interesting aspect of integrin signaling is the synergistic activation of the MAP kinase signaling pathway when both the integrins and growth factor receptors are simultaneously stimulated [133,134]. Recent evidence suggests that FAK may function in the crosstalk between these two receptor classes. Expression of a CD2/FAK chimera that is constitutively active can substitute for integrin-dependent adhesion and promote maximal MAP kinase activation in response to PDGF [48]. Further, *fak*−/− cells exhibit a defect in PDGF- and serum-induced MAP kinase activation and overexpression of dominant negative mutants of FAK in NIH3T3 cells blocks serum induced activation of MAP kinase [48,135]. FAK has also been implicated in the activation of MAP kinase following stimula-

tion of the urokinase-type plasminogen activator receptor [136]. One other interesting observation is that inhibition of FAK signaling by a dominant negative variant has a major effect upon the duration of MAP kinase activation following PDGF stimulation of smooth muscle cells [135]. Inhibition of FAK in this case produced a transient activation of MAP kinase following PDGF stimulation, rather than the more prolonged response seen in control cells. This observation is interesting in light of the previous proposition that FAK might control the temporal response of MAP kinase induced by integrin signaling. In contrast to the evidence provided in these studies, one other report disputes the role of FAK in the activation of MAP kinase in response to PDGF in fibroblasts [128]. Thus, this effect may also exhibit a cell type dependency. The cells utilized in this latter analysis also do not require FAK for the stimulation of MAP kinase in response to integrin-dependent cell adhesion.

Although the controversy regarding the role of FAK in MAP kinase activation remains to be resolved, two of these recent findings are particularly intriguing. First, FAK may function to coordinate MAP kinase signaling following costimulation of integrins and growth factor receptors. Second, FAK may control the temporal response of MAP kinase to stimuli. These findings suggest that FAK may play a somewhat unique function in controlling MAP kinase stimulation.

6. Biological responses to FAK signals

6.1. FAK, Rho and the regulation of focal adhesion turnover

Hypotheses about the role of FAK in focal adhesion assembly/disassembly have dramatically changed over the years. FAK was originally thought to function in the assembly of focal adhesions. However, a study performed using *v-src* transformed fibroblasts suggested that FAK might perform the opposite function, i.e., promote the disassembly of focal adhesions [110]. This theory was partially corroborated with studies using *fak*−/− fibroblasts, since these cells exhibit larger focal adhesions than the control FAK expressing fibroblasts [5]. This hypothesis has now been very elegantly confirmed by imaging a GFP-α-actinin chimeric protein in spreading cells [137]. In spreading, control fibroblasts, GFP-α-actinin assembles into focal adhesions which then undergo disassembly. In contrast, in spreading *fak*−/− cells, GFP-α-actinin assembles into focal adhesions, which remain intact for the duration of the experiment [137]. These results provide very strong support for FAK as a regulator of disassembly of focal adhesions.

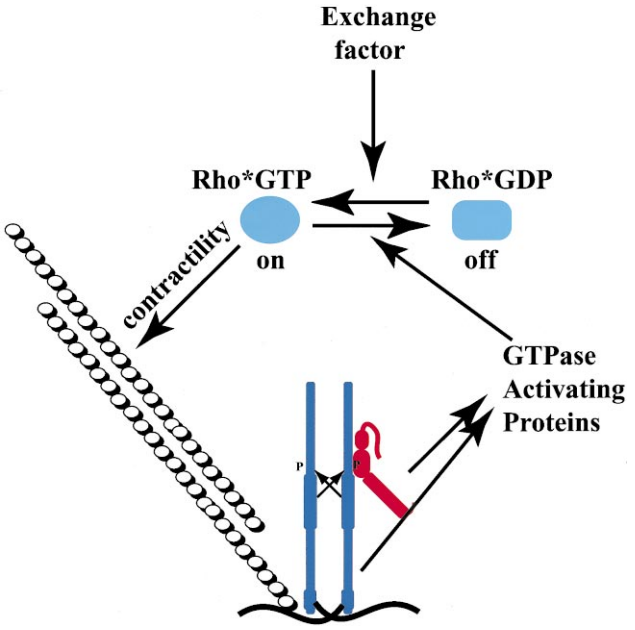

Fig. 7. FAK and Rho; interdependent regulation. Rho is active when bound to GTP and inactive in its GDP bound form. Exchange factors promote GTP binding to Rho, which induces contractility. This induces assembly of stress fibers and focal adhesions, promoting tyrosine phosphorylation of FAK. FAK binds a GTPase activating protein (GAP), called GRAF, which may promote hydrolysis of GTP bound to Rho. FAK and Src have both been implicated in inhibiting Rho activity upon cell adhesion. There is also evidence that a second GAP, RhoGAP, functions in inhibiting Rho following cell adhesion.

The intriguing possibility that FAK may control focal adhesion disassembly by downregulating Rho activity has been proposed [137]. Whereas fibroblasts exhibit a transient reduction in the activity of Rho upon adhesion to fibronectin, *fak*−/− fibroblasts exhibit no reduction in Rho activity [71,137]. Since Rho activity is important for FAK activation, this may be a negative feedback mechanism to inhibit Rho signaling (see Fig. 7). Further the morphological differences observed between wild-type and *fak*−/−

fibroblasts, including the characteristic appearances of their focal adhesions, are apparently due to different levels of Rho activity. Inhibition of Rho causes *fak*−/− cells to resemble wild-type fibroblasts, whereas introduction of an activated Rho construct into normal fibroblasts induces a morphology resembling *fak*−/− cells [137]. These data suggest that FAK regulates focal adhesion turnover by modulating Rho activity.

The mechanism by which FAK inhibits Rho signaling has not been addressed, but could involve the FAK-binding partner GRAF, a negative inhibitor of Rho [12]. In an interesting parallel study, the Src family of tyrosine kinases has been implicated in the inhibition of Rho activity upon engagement of integrins [138]. The results of this study further suggest that RhoGAP may play a role in inhibiting the activity of Rho upon adhesion to fibronectin [138]. The results of the two studies together might suggest a role for the FAK/Src complex in the feedback to inhibit Rho activation, an experimentally testable hypothesis using a reconstitution approach in *fak*−/− cells.

6.2. FAK and cell motility

Earlier experiments demonstrated that FAK signaling regulates the motility of cells in response to an extracellular matrix protein stimulus, a process called haptotaxis [5,8]. More recent experiments have focused upon identification of FAK-regulated signaling pathways that may be involved in controlling motility. The major autophosphorylation site of FAK, tyrosine 397, is absolutely required for enhancing cell motility [8,139,140]. The original interpretation of this observation was that Src recruitment into complex with FAK was required for cell motility. However, the discovery that the SH2 domains of additional signaling molecules also directly associate with the autophosphorylation site of FAK has forced reconsideration of this conclusion. In support of a role for Src, transient expression of exogenous FAK in *fak*−/− fibroblasts enhanced cell motility, whereas coexpression of the Src inhibitory kinase, Csk, reduced the ability of FAK to promote cell migration [140]. A likely downstream target for FAK/Src tyrosine phosphorylation in the cell migration pathway is p130^{cas}. This FAK-associated protein is tyrosine phosphorylated following cell adhesion and has independently been implicated in cell motility [141]. A FAK mutant, defective for binding p130^{cas} fails to induce tyrosine phosphorylation of p130^{cas} and is also defective for inducing cell migration [140,142]. Furthermore, overexpression of a fragment of p130^{cas}, predicted to function as a dominant negative mutant and inhibit the recruitment of endogenous p130^{cas} to FAK, impairs FAK-dependent cell migration [142]. These observations support a role for Src and p130^{cas} in the promotion of cell motility by FAK.

Additional studies have identified other FAK-binding partners that also may function in regulating cell motility. A mutant of FAK that retains binding to the Src SH2 domain, but fails to associate with the PI3-kinase SH2 domain fails to stimulate motility when overexpressed in CHO cells [42]. This experiment identifies PI3-kinase as an effector of FAK that functions in the regulation of motility.

Grb7 is also a good candidate as a regulator of motility since it can bind FAK, is tyrosine phosphorylated in a FAK dependent manner and has been implicated in the regulation of motility [43,143]. However, its linkage to FAK-dependent motility has not been completely solidified, since Grb7 mutants can inhibit motility in *fak*−/− cells. Additional experimental evidence, as was obtained for the role of Src and PI3-kinase in FAK-dependent motility, is required to absolutely place Grb7 in a pathway downstream of FAK in the regulation of migration.

In addition to haptotactic motility, cells also exhibit chemotactic motility in response to extracellular stimuli like growth factors. Recent studies have also implicated FAK in controlling chemotactic responses in cells. Overexpression of FAK in MDCK cells increases the ability of these cells to 'scatter' in response to HGF [144]. In addition, FAK overexpression enhances the motility of MDCK cells in response to HGF in a chemotaxis assay [144]. Urokinase-type plasminogen activator stimulates motility of MCF-7 cells and inhibition of FAK signaling using a dominant negative approach inhibits the chemotactic response of the cells [136]. *Fak*−/− fibroblasts exhibit defective chemotactic responses to PDGF and EGF. Re-expression of FAK in these

cells restores the chemotactic response in these cells [36]. Thus in several different chemotactic systems, FAK has been implicated as a regulatory protein.

The mechanism(s) by which FAK controls chemotaxis has not been fully elucidated. Some experimental data suggests that similar FAK pathways are involved in the response to HGF induced chemotaxis and to haptotaxis in MDCK cells [144]. In particular, the PI3-kinase and p130^{cas} pathways have been implicated in chemotaxis since FAK mutants that do not engage these effectors fail to promote chemotaxis [144]. In contrast, FAK may function differently in the chemotactic response to EGF and PDGF in fibroblasts [36]. The autophosphorylation site of FAK is required to promote chemotaxis and recruitment of Src into complex also appears necessary since FAK-dependent chemotactic responses are blocked by overexpression of Csk [36]. However, FAK mutants that fail to bind to p130^{cas} or Grb2 are as effective as wild-type FAK at promoting chemotaxis [36]. Both the N-terminal and C-terminal domains of FAK are required for FAK to promote chemotaxis in *fak*−/− cells. Unexpectedly, the N-terminal domain of FAK, including the major autophosphorylation site, will support some of FAK's function in chemotaxis [36]. Presumably this fragment becomes tyrosine phosphorylated and recruits signaling molecules to fulfill these functions. While these studies demonstrate that FAK can function to promote chemotaxis, in addition to its well established role in haptotaxis, there are many discrepancies regarding the mechanisms involved that remain to be resolved before we understand how FAK may control chemotaxis.

6.3. FAK and the cell cycle

FAK has also been proposed to function as a positive regulator of the cell cycle. Overexpression of FAK can modestly enhance DNA synthesis in fibroblasts after the cells have been starved and stimulated with serum [120,145]. Conversely, mutants of FAK can function in a dominant negative fashion to inhibit progression through the cell cycle following serum stimulation [120,130]. While the mechanism of FAK function in controlling cell cycle progression has not been completely elucidated, there is some evidence that FAK may function to control the levels of cyclin D and the cyclin-dependent kinase inhibitor, p21 [120].

6.4. FAK and apoptosis

In many cells, the extracellular matrix transmits a cell survival signal. When cells are cultured in the absence of this survival signal, they undergo apoptosis or anoikis [146]. Inhibition of FAK can induce apoptosis and overexpression of FAK can prevent anoikis and apoptosis induced in response to other stimuli [6,7,49,50,117,147–149]. These findings support the role of FAK in transmitting a cell survival signal.

Several approaches have been taken to begin to elucidate the mechanism(s) of action of FAK in promoting cell survival. FAK mutants were utilized to demonstrate that the catalytic activity of FAK and the major autophosphorylation site of FAK (tyrosine 397) were required to protect cells from apoptosis [7,117,149]. In MDCK cells, the association of FAK with PI3-kinase was specifically shown to be a prerequisite for the inhibition of apoptosis induced by ultraviolet light [117]. In HL60 cells, tyrosine 925, which binds Grb2 and links FAK to the Ras/MAPK pathway, was also required to inhibit apoptosis [149]. Additional insights into the mechanism by which FAK regulates apoptosis come from experiments using dominant negative FAK constructs to inhibit endogenous FAK signaling. Overexpression of a GFP fusion protein containing just the C-terminal FAT sequence of FAK induced apoptosis in rabbit synovial fibroblasts, whereas overexpression of a GFP-FRNK fusion protein did not [49,50]. Further analysis using mutants of FAK and FRNK demonstrated that the p130^{cas} binding site was required to prevent apoptosis of fibroblasts in the absence of serum [50]. Additional evidence suggested that FAK could activate JNK through recruitment and tyrosine phosphorylation of p130^{cas} that in turn signals to Rac, Pak1 and MKK4 to stimulate JNK activity [50]. In this system expression of activated Akt failed to block apoptosis induced by the FAT construct suggesting that the PI3-kinase signaling alone was insufficient to block apoptosis [49,50]. An alternative mechanism for the inhibition of apoptosis by FAK was demonstrated in HL60 cells. FAK overexpression appeared to enhance NFκB ac-

tivity and stimulate expression of cIAP-1, cIAP-2 and X-IAP, which are endogenous inhibitors of caspases and thus potent inhibitors of apoptosis [149,150].

The described studies have demonstrated that FAK functions to promote cell survival in response to cell adhesion. Interestingly, some studies suggest that overexpression of FAK can protect cells from apoptosis in response to other stimuli. Some progress has been made toward defining the mechanism(s) utilized by FAK to prevent cell death, although they remain to be fully elucidated. This information could be important for the design of strategies to inhibit FAK-promoted cell survival in experimental and pathological scenarios.

6.5. FAK and cancer

FAK was isolated as a tyrosine phosphorylated substrate from cells transformed by the *v-src* oncogene [151]. The finding that Src signaling is activated in a number of human tumors suggests that any role for FAK in this system might be relevant to human disease and not restricted to model systems for transformation [152]. FAK may function to promote maximal tyrosine phosphorylation of some substrates, like paxillin, and stimulate MAP kinase activation in transformed cells [48,53]. However, inhibition of FAK using dominant negative mutants has no effect upon the phenotype of *v-src*-transformed cells [48,75].

FAK might also function downstream of integrins in some cancers since changes in the repertoire of integrins on the surface of cells can profoundly change the cancerous phenotype of cells [153–155]. Some of these changes are likely to occur due to altered signaling in these cells. FAK has not been directly implicated as a component of an altered integrin signaling pathway in transformed cells, but remains an attractive candidate.

Expression of a constitutively active, membrane-bound FAK chimera (CD2/FAK) in several cell types has elicited some of the phenotypes of cancer. MDCK cells expressing CD2/FAK have acquired the ability to grow in soft agar and form tumors in nude mice [7]. NIH3T3 cells expressing this construct have also been analyzed. Although CD2/FAK could not promote the growth of NIH3T3 cells in soft agar, nor increase the number of colonies that Ras-transformed NIH3T3 cells form in soft agar, CD2/FAK did induce an increase in the size of the colonies formed by Ras-transformed NIH3T3 cells [48]. While these studies are clearly just the first of many that will attempt to prove in principle the role FAK might play in the development of cancer, they establish that under certain circumstances, FAK signaling can contribute to the development of some of the phenotypes of cancerous cells.

FAK expression is elevated in a number of different tumors including breast, colon, prostate, thyroid, ovarian and mesenchymal tumors [156–161]. In some cases, additional copies of the FAK gene have been identified demonstrating that FAK overexpression can sometimes be due to gene amplification [162]. Early analyses had suggested that FAK overexpression might be a late alteration in the development of invasive and metastatic cancer. Further, in an in vitro model for the development of colon cancer, FAK amplification and overexpression was observed only in the later stages of carcinoma development [163]. However, recent immunohistological analyses of FAK expression in developing tumors has identified populations of cells overexpressing FAK early in the development of breast, colon and squamous cell carcinomas of the mouth [164,165]. In another study, FAK was undetectable in primary ectocervical cells or human foreskin fibroblasts, but overexpressed in these cells following immortalization by HPV or SV40 large T antigen [166]. These results suggest that FAK overexpression might be a very early event in the progression of cancer. While these studies have not irrefutably linked FAK to cancer, they certainly present FAK as a compelling candidate as a regulator of some aspects of tumor development and progression.

7. Concluding remarks

Recent studies have provided insight into the mechanisms by which FAK signaling is regulated and how FAK promotes cell motility and cell survival. Although our understanding of FAK signaling is increasing, the picture is still far from clear. The role of protein tyrosine phosphatases in inhibiting FAK signaling is likely to be important and has not been

fully explored. While some FAK signaling pathways have been implicated in controlling biological processes, other pathways remain to be tested. FAK is potentially important in the pathology of human disease, e.g., cancer, but has not been definitively linked to the development of any disease. Although we have come a long way in elucidating the biochemical and biological functions of FAK, there is still much to be learned before we fully understand the mechanisms of FAK signaling.

Acknowledgements

Thanks to Jill Broome, Veronica Niecko-Gabarra and Patrick Lyons for their comments and suggestions during the preparation of the manuscript. Work in the author's laboratory is supported by grants from the National Institutes of Health (GM57943 and CA90901).

References

[1] D.D. Schlaepfer, C.R. Hauck, D.J. Sieg, Prog. Biophys. Mol. Biol. 71 (1999) 435.
[2] J.L. Guan, Int. J. Biochem. Cell Biol. 29 (1997) 1085.
[3] M.D. Schaller, J. Endocrinol. 150 (1996) 1.
[4] J.T. Parsons, K.H. Martin, J.K. Slack, J.M. Taylor, S.A. Weed, Oncogene 19 (2000) 5606.
[5] D. Ilic, Y. Furuta, S. Kanazawa, N. Takeda, K. Sobue, N. Nakatsuji, S. Nomura, J. Fujimoto, M. Okada, T. Yamamoto, Nature 377 (1995) 539.
[6] J.E. Hungerford, M.T. Compton, M.L. Matter, B.G. Hoffstrom, C.A. Otey, J. Cell Biol. 135 (1996) 1383.
[7] S.M. Frisch, K. Vuori, E. Ruoslahti, P.Y. Chan-Hui, J. Cell Biol. 134 (1996) 793.
[8] L.A. Cary, J.F. Chang, J.L. Guan, J. Cell Sci. 109 (Pt 7) (1996) 1787.
[9] M.D. Schaller, J.D. Hildebrand, J.D. Shannon, J.W. Fox, R.R. Vines, J.T. Parsons, Mol. Cell. Biol. 14 (1994) 1680.
[10] M.B. Calalb, T.R. Polte, S.K. Hanks, Mol. Cell. Biol. 15 (1995) 954.
[11] D.D. Schlaepfer, S.K. Hanks, T. Hunter, P. van der Geer, Nature 372 (1994) 786.
[12] J.D. Hildebrand, J.M. Taylor, J.T. Parsons, Mol. Cell. Biol. 16 (1996) 3169.
[13] T.R. Polte, S.K. Hanks, Proc. Natl. Acad. Sci. USA 92 (1995) 10678.
[14] J.D. Hildebrand, M.D. Schaller, J.T. Parsons, J. Cell Biol. 123 (1993) 993.
[15] H.C. Chen, P.A. Appeddu, J.T. Parsons, J.D. Hildebrand, M.D. Schaller, J.L. Guan, J. Biol. Chem. 270 (1995) 16995.
[16] J.D. Hildebrand, M.D. Schaller, J.T. Parsons, Mol. Biol. Cell 6 (1995) 637.
[17] K. Vuori, H. Hirai, S. Aizawa, E. Ruoslahti, Mol. Cell. Biol. 16 (1996) 2606.
[18] M.D. Schaller, J.T. Parsons, Mol. Cell. Biol. 15 (1995) 2635.
[19] H.C. Chen, J.L. Guan, Proc. Natl. Acad. Sci. USA 91 (1994) 10148.
[20] H. Avraham, S.Y. Park, K. Schinkmann, S. Avraham, Cell Signal. 12 (2000) 123.
[21] G.L. Fox, I. Rebay, R.O. Hynes, Proc. Natl. Acad. Sci. USA 96 (1999) 14978.
[22] R.H. Palmer, L.I. Fessler, P.T. Edeen, S.J. Madigan, M. McKeown, T. Hunter, J. Biol. Chem. 274 (1999) 35621.
[23] J. Fujimoto, K. Sawamoto, M. Okabe, Y. Takagi, T. Tezuka, S. Yoshikawa, H. Ryo, H. Okano, T. Yamamoto, J. Biol. Chem. 274 (1999) 29196.
[24] A.H. Chishti, A.C. Kim, S.M. Marfatia, M. Lutchman, M. Hanspal, H. Jindal, S.C. Liu, P.S. Low, G.A. Rouleau, N. Mohandas, J.A. Chasis, J.G. Conboy, P. Gascard, Y. Takakuwa, S.C. Huang, E.J. Benz Jr., A. Bretscher, R.G. Fehon, J.F. Gusella, V. Ramesh, F. Solomon, V.T. Marchesi, S. Tsukita, S. Tsukita, K.B. Hoover, Trends Biochem. Sci. 23 (1998) 281.
[25] J.A. Girault, G. Labesse, J.P. Mornon, I. Callebaut, Trends Biochem. Sci. 24 (1999) 54.
[26] D.R. Critchley, Curr. Opin. Cell Biol. 12 (2000) 133.
[27] S. Tsukita, S. Yonemura, J. Biol. Chem. 274 (1999) 34507.
[28] P. Mangeat, C. Roy, M. Martin, Trends Cell Biol. 9 (1999) 187.
[29] A. Bretscher, Curr. Opin. Cell Biol. 11 (1999) 109.
[30] M.D. Schaller, C.A. Otey, J.D. Hildebrand, J.T. Parsons, J. Cell Biol. 130 (1995) 1181.
[31] S. Miyamoto, S.K. Akiyama, K.M. Yamada, Science 267 (1995) 883.
[32] L.M. Chen, D. Bailey, C. Fernandez-Valle, J. Neurosci. 20 (2000) 3776.
[33] K. Danker, B. Gabriel, C. Heidrich, W. Reutter, Exp. Cell Res. 239 (1998) 326.
[34] Y. Shen, M.D. Schaller, Mol. Biol. Cell 10 (1999) 2507.
[35] P.D. Tahiliani, L. Singh, K.L. Auer, S.E. LaFlamme, J. Biol. Chem. 272 (1997) 7892.
[36] D.J. Sieg, C.R. Hauck, D. Ilic, C.K. Klingbeil, E. Schaefer, C.H. Damsky, D.D. Schlaepfer, Nat. Cell Biol. 2 (2000) 249.
[37] T. Vartanian, A. Goodearl, S. Lefebvre, S.K. Park, G. Fischbach, Biochem. Biophys. Res. Commun. 271 (2000) 414.
[38] K.M. Ignatoski, T. Maehama, S.M. Markwart, J.E. Dixon, D.L. Livant, S.P. Ethier, Br. J. Cancer 82 (2000) 666.
[39] S. Manes, E. Mira, C. Gomez-Mouton, Z.J. Zhao, R.A. Lacalle, A. Martinez, Mol. Cell. Biol. 19 (1999) 3125.
[40] H. Miao, E. Burnett, M. Kinch, E. Simon, B. Wang, Nat. Cell Biol. 2 (2000) 62.
[41] X. Zhang, A. Chattopadhyay, Q.S. Ji, J.D. Owen, P.J. Ru-

est, G. Carpenter, S.K. Hanks, Proc. Natl. Acad. Sci. USA 96 (1999) 9021.
[42] H.R. Reiske, S.C. Kao, L.A. Cary, J.L. Guan, J.F. Lai, H.C. Chen, J. Biol. Chem. 274 (1999) 12361.
[43] D.C. Han, J.L. Guan, J. Biol. Chem. 274 (1999) 24425.
[44] D.D. Schlaepfer, T. Hunter, J. Biol. Chem. 272 (1997) 13189.
[45] M.A. Cooley, J.M. Broome, C. Ohngemach, L.H. Romer, M.D. Schaller, Mol. Biol. Cell 11 (2000) 3247.
[46] A. Richardson, T. Parsons, Nature 380 (1996) 538.
[47] A.P. Gilmore, L.H. Romer, Mol. Biol. Cell 7 (1996) 1209.
[48] M.W. Renshaw, L.S. Price, M.A. Schwartz, J. Cell Biol. 147 (1999) 611.
[49] D. Ilic, E.A. Almeida, D.D. Schlaepfer, P. Dazin, S. Aizawa, C.H. Damsky, J. Cell Biol. 143 (1998) 547.
[50] E.A. Almeida, D. Ilic, Q. Han, C.R. Hauck, F. Jin, H. Kawakatsu, D.D. Schlaepfer, C.H. Damsky, J. Cell Biol. 149 (2000) 741.
[51] M.C. Brown, M.S. Curtis, C.E. Turner, Nat. Struct. Biol. 5 (1998) 677.
[52] M.C. Brown, J.A. Perrotta, C.E. Turner, J. Cell Biol. 135 (1996) 1109.
[53] J.W. Thomas, M.A. Cooley, J.M. Broome, R. Salgia, J.D. Griffin, C.R. Lombardo, M.D. Schaller, J. Biol. Chem. 274 (1999) 36684.
[54] K. Tachibana, T. Sato, N. D'Avirro, C. Morimoto, J. Exp. Med. 182 (1995) 1089.
[55] C. Zheng, Z. Xing, Z.C. Bian, C. Guo, A. Akbay, L. Warner, J.L. Guan, J. Biol. Chem. 273 (1998) 2384.
[56] M.D. Schaller, T. Sasaki, J. Biol. Chem. 272 (1997) 25319.
[57] W.C. Xiong, M. Macklem, J.T. Parsons, J. Cell Sci. 111 (Pt 14) (1998) 1981.
[58] M.D. Schaller, C.A. Borgman, J.T. Parsons, Mol. Cell. Biol. 13 (1993) 785.
[59] K. Nolan, J. Lacoste, J.T. Parsons, Mol. Cell. Biol. 19 (1999) 6120.
[60] J.M. Taylor, C.P. Mack, K. Nolan, C.P. Regan, G.K. Owens, J.T. Parsons, Mol. Cell. Biol. 21 (2001) 1565.
[61] L.H. Xu, X. Yang, C.A. Bradham, D.A. Brenner, A.S. Baldwin, R.J. Craven, W.G. Cance, J. Biol. Chem. 275 (2000) 30597.
[62] J.M. Taylor, J.D. Rovin, J.T. Parsons, J. Biol. Chem. 275 (2000) 19250.
[63] C.K. Klingbeil, C.R. Hauck, D.A. Hsia, K.C. Jones, S.R. Reider, D.D. Schlaepfer, J. Cell Biol. 152 (2001) 97.
[64] L. Lipfert, B. Haimovich, M.D. Schaller, B.S. Cobb, J.T. Parsons, J.S. Brugge, J. Cell Biol. 119 (1992) 905.
[65] D.D. Schlaepfer, K.C. Jones, T. Hunter, Mol. Cell. Biol. 18 (1998) 2571.
[66] S.M. Schoenwaelder, K. Burridge, Curr. Opin. Cell Biol. 11 (1999) 274.
[67] S.T. Barry, D.R. Critchley, J. Cell Sci. 107 (Pt 7) (1994) 2033.
[68] M.J. Seckl, N. Morii, S. Narumiya, E. Rozengurt, J. Biol. Chem. 270 (1995) 6984.
[69] H.M. Flinn, A.J. Ridley, J. Cell Sci. 109 (Pt 5) (1996) 1133.
[70] F. Wang, C.D. Nobes, A. Hall, S. Spiegel, Biochem. J. 324 (Pt 2) (1997) 481.
[71] X.D. Ren, W.B. Kiosses, M.A. Schwartz, EMBO J. 18 (1999) 578.
[72] E.P. Salazar, E. Rozengurt, J. Biol. Chem. 274 (1999) 28371.
[73] D.D. Schlaepfer, M.A. Broome, T. Hunter, Mol. Cell. Biol. 17 (1997) 1702.
[74] R.A. Klinghoffer, C. Sachsenmaier, J.A. Cooper, P. Soriano, EMBO J. 18 (1999) 2459.
[75] G.W. McLean, V.J. Fincham, M.C. Frame, J. Biol. Chem. 275 (2000) 23333.
[76] P.J. Ruest, S. Roy, E. Shi, R.L. Mernaugh, S.K. Hanks, Cell Growth Differ. 11 (2000) 41.
[77] J.W. Thomas, B. Ellis, R.J. Boerner, W.B. Knight, G.C. White, M.D. Schaller, J. Biol. Chem. 273 (1998) 577.
[78] S.M. Thomas, J.S. Brugge, Annu. Rev. Cell Dev. Biol. 13 (1997) 513.
[79] T. Nakamoto, R. Sakai, K. Ozawa, Y. Yazaki, H. Hirai, J. Biol. Chem. 271 (1996) 8959.
[80] Z. Weng, J.A. Taylor, C.E. Turner, J.S. Brugge, C. Seidel-Dugan, J. Biol. Chem. 268 (1993) 14956.
[81] K. Alexandropoulos, D. Baltimore, Genes Dev. 10 (1996) 1341.
[82] J.L. Rodriguez-Fernandez, E. Rozengurt, J. Biol. Chem. 271 (1996) 27895.
[83] C.O. Arregui, J. Balsamo, J. Lilien, J. Cell Biol. 143 (1998) 861.
[84] E.S. Oh, H. Gu, T.M. Saxton, J.F. Timms, S. Hausdorff, E.U. Frevert, B.B. Kahn, T. Pawson, B.G. Neel, S.M. Thomas, Mol. Cell. Biol. 19 (1999) 3205.
[85] X.M. Zheng, Y. Wang, C.J. Pallen, Nature 359 (1992) 336.
[86] J. den Hertog, C.E. Pals, M.P. Peppelenbosch, L.G. Tertoolen, S.W. de Laat, W. Kruijer, EMBO J. 12 (1993) 3789.
[87] K.W. Harder, N.P. Moller, J.W. Peacock, F.R. Jirik, J. Biol. Chem. 273 (1998) 31890.
[88] R. Lammers, M.M. Lerch, A. Ullrich, J. Biol. Chem. 275 (2000) 3391.
[89] J. Su, M. Muranjan, J. Sap, Curr. Biol. 9 (1999) 505.
[90] X.M. Zheng, R.J. Resnick, D. Shalloway, EMBO J. 19 (2000) 964.
[91] H. Ueda, S. Abbi, C. Zheng, J.L. Guan, J. Cell Biol. 149 (2000) 423.
[92] Y. Shen, G. Schneider, J.F. Cloutier, A. Veillette, M.D. Schaller, J. Biol. Chem. 273 (1998) 6474.
[93] F. Liu, M.A. Sells, J. Chernoff, Curr. Biol. 8 (1998) 173.
[94] J.H. Qi, N. Ito, L. Claesson-Welsh, J. Biol. Chem. 274 (1999) 14455.
[95] D.H. Yu, C.K. Qu, O. Henegariu, X. Lu, G.S. Feng, J. Biol. Chem. 273 (1998) 21125.
[96] M. Tsuda, T. Matozaki, K. Fukunaga, Y. Fujioka, A. Imamoto, T. Noguchi, T. Takada, T. Yamao, H. Takeda, F. Ochi, T. Yamamoto, M. Kasuga, J. Biol. Chem. 273 (1998) 13223.
[97] J. Li, C. Yen, D. Liaw, K. Podsypanina, S. Bose, S.I. Wang, J. Puc, C. Miliaresis, L. Rodgers, R. McCombie, S.H. Bign-

er, B.C. Giovanella, M. Ittmann, B. Tycko, H. Hibshoosh, M.H. Wigler, R. Parsons, Science 275 (1997) 1943.
[98] P.A. Steck, M.A. Pershouse, S.A. Jasser, W.K. Yung, H. Lin, A.H. Ligon, L.A. Langford, M.L. Baumgard, T. Hattier, T. Davis, C. Frye, R. Hu, B. Swedlund, D.H. Teng, S.V. Tavtigian, Nat. Genet. 15 (1997) 356.
[99] M.P. Myers, I. Pass, I.H. Batty, J. Kaay, J.P. Van der Stolarov, B.A. Hemmings, M.H. Wigler, C.P. Downes, N.K. Tonks, Proc. Natl. Acad. Sci. USA 95 (1998) 13513.
[100] M.P. Myers, J.P. Stolarov, C. Eng, J. Li, S.I. Wang, M.H. Wigler, R. Parsons, N.K. Tonks, Proc. Natl. Acad. Sci. USA 94 (1997) 9052.
[101] T. Maehama, J.E. Dixon, J. Biol. Chem. 273 (1998) 13375.
[102] F. Vazquez, W.R. Sellers, Biochim. Biophys. Acta 1470 (2000) M21–M35.
[103] M. Tamura, J. Gu, K. Matsumoto, S. Aota, R. Parsons, K.M. Yamada, Science 280 (1998) 1614.
[104] M. Tamura, J. Gu, T. Takino, K.M. Yamada, Cancer Res. 59 (1999) 442.
[105] M. Tamura, J. Gu, E.H. Danen, T. Takino, S. Miyamoto, K.M. Yamada, J. Biol. Chem. 274 (1999) 20693.
[106] J. Liliental, S.Y. Moon, R. Lesche, R. Mamillapalli, D. Li, Y. Zheng, H. Sun, H. Wu, Curr. Biol. 10 (2000) 401.
[107] H. Sun, R. Lesche, D.M. Li, J. Liliental, H. Zhang, J. Gao, N. Gavrilova, B. Mueller, X. Liu, H. Wu, Proc. Natl. Acad. Sci. USA 96 (1999) 6199.
[108] D. Maier, G. Jones, X. Li, A.H. Schonthal, O. Gratzl, E.G. Van Meir, A. Merlo, Cancer Res. 59 (1999) 5479.
[109] A. Di Cristofano, P.P. Pandolfi, Cell 100 (2000) 387.
[110] V.J. Fincham, J.A. Wyke, M.C. Frame, Oncogene 10 (1995) 2247.
[111] D.H. Crouch, V.J. Fincham, M.C. Frame, Oncogene 12 (1996) 2689.
[112] L.P. Wen, J.A. Fahrni, S. Troie, J.L. Guan, K. Orth, G.D. Rosen, J. Biol. Chem. 272 (1997) 26056.
[113] B. Levkau, B. Herren, H. Koyama, R. Ross, E.W. Raines, J. Exp. Med. 187 (1998) 579.
[114] P. Cooray, Y. Yuan, S.M. Schoenwaelder, C.A. Mitchell, H.H. Salem, S.P. Jackson, Biochem. J. 318 (Pt 1) (1996) 41.
[115] N.O. Carragher, B. Levkau, R. Ross, E.W. Raines, J. Cell Biol. 147 (1999) 619.
[116] B. van de Water, J.F. Nagelkerke, J.L. Stevens, J. Biol. Chem. 274 (1999) 13328.
[117] P.C. Chan, J.F. Lai, C.H. Cheng, M.J. Tang, C.C. Chiu, H.C. Chen, J. Biol. Chem. 274 (1999) 26901.
[118] F.G. Gervais, N.A. Thornberry, S.C. Ruffolo, D.W. Nicholson, S. Roy, J. Biol. Chem. 273 (1998) 17102.
[119] N.O. Carragher, V.J. Fincham, D. Riley, M.C. Frame, J. Biol. Chem. 276 (2001) 4270.
[120] J.H. Zhao, H. Reiske, J.L. Guan, J. Cell Biol. 143 (1998) 1997.
[121] C.E. Turner, Nat. Cell Biol. 2 (2000) E231–E236.
[122] G.M. O'Neill, S.J. Fashena, E.A. Golemis, Trends Cell Biol. 10 (2000) 111.
[123] S.M. Feller, G. Posern, J. Voss, C. Kardinal, D. Sakkab, J. Zheng, B.S. Knudsen, J. Cell Physiol. 177 (1998) 535.
[124] M.J. Rebecchi, S.N. Pentyala, Physiol. Rev. 80 (2000) 1291.
[125] S.J. Leevers, B. Vanhaesebroeck, M.D. Waterfield, Curr. Opin. Cell Biol. 11 (1999) 219.
[126] T.H. Lin, A.E. Aplin, Y. Shen, Q. Chen, M. Schaller, L. Romer, I. Aukhil, R.L. Juliano, J. Cell Biol. 136 (1997) 1385.
[127] K.K. Wary, F. Mainiero, S.J. Isakoff, E.E. Marcantonio, F.G. Giancotti, Cell 87 (1996) 733.
[128] L. Barberis, K.K. Wary, G. Fiucci, F. Liu, E. Hirsch, M. Brancaccio, F. Altruda, G. Tarone, F.G. Giancotti, J. Biol. Chem. 275 (2000) 36532.
[129] M.A. Sanders, M.D. Basson, J. Biol. Chem. 275 (2000) 38040.
[130] M. Oktay, K.K. Wary, M. Dans, R.B. Birge, F.G. Giancotti, J. Cell Biol. 145 (1999) 1461.
[131] T. Igishi, S. Fukuhara, V. Patel, B.Z. Katz, K.M. Yamada, J.S. Gutkind, J. Biol. Chem. 274 (1999) 30738.
[132] P. Lebrun, V. Baron, C.R. Hauck, D.D. Schlaepfer, E. Van Obberghen, J. Biol. Chem. 275 (2000) 38371.
[133] T.H. Lin, Q. Chen, A. Howe, R.L. Juliano, J. Biol. Chem. 272 (1997) 8849.
[134] M.W. Renshaw, X.D. Ren, M.A. Schwartz, EMBO J. 16 (1997) 5592.
[135] C.R. Hauck, D.A. Hsia, D.D. Schlaepfer, J. Biol. Chem. 275 (2000) 41092.
[136] D.H. Nguyen, D.J. Webb, A.D. Catling, Q. Song, A. Dhakephalkar, M.J. Weber, K.S. Ravichandran, S.L. Gonias, J. Biol. Chem. 275 (2000) 19382.
[137] X. Ren, W.B. Kiosses, D.J. Sieg, C.A. Otey, D.D. Schlaepfer, M.A. Schwartz, J. Cell Sci. 113 (Pt 20) (2000) 3673.
[138] W.T. Arthur, L.A. Petch, K. Burridge, Curr. Biol. 10 (2000) 719.
[139] J.D. Owen, P.J. Ruest, D.W. Fry, S.K. Hanks, Mol. Cell. Biol. 19 (1999) 4806.
[140] D.J. Sieg, C.R. Hauck, D.D. Schlaepfer, J. Cell Sci. 112 (Pt 16) (1999) 2677.
[141] R.L. Klemke, J. Leng, R. Molander, P.C. Brooks, K. Vuori, D.A. Cheresh, J. Cell Biol. 140 (1998) 961.
[142] L.A. Cary, D.C. Han, T.R. Polte, S.K. Hanks, J.L. Guan, J. Cell Biol. 140 (1998) 211.
[143] D.C. Han, T.L. Shen, J.L. Guan, J. Biol. Chem. 275 (2000) 28911.
[144] J.F. Lai, S.C. Kao, S.T. Jiang, M.J. Tang, P.C. Chan, H.C. Chen, J. Biol. Chem. 275 (2000) 7474.
[145] J. Zhao, C. Zheng, J. Guan, J. Cell Sci. 113 (Pt 17) (2000) 3063.
[146] S.M. Frisch, H. Francis, J. Cell Biol. 124 (1994) 619.
[147] L.H. Xu, X. Yang, R.J. Craven, W.G. Cance, Cell Growth Differ. 9 (1998) 999.
[148] L.H. Xu, L.V. Owens, G.C. Sturge, X. Yang, E.T. Liu, R.J. Craven, W.G. Cance, Cell Growth Differ. 7 (1996) 413.
[149] Y. Sonoda, Y. Matsumoto, M. Funakoshi, D. Yamamoto, S.K. Hanks, T. Kasahara, J. Biol. Chem. 275 (2000) 16309.
[150] Q.L. Deveraux, J.C. Reed, Genes Dev. 13 (1999) 239.
[151] S.B. Kanner, A.B. Reynolds, R.R. Vines, J.T. Parsons, Proc. Natl. Acad. Sci. USA 87 (1990) 3328.

[152] J.S. Biscardi, D.A. Tice, S.J. Parsons, Adv. Cancer Res. 76 (1999) 61.
[153] E. Ruoslahti, Adv. Cancer Res. 76 (1999) 1.
[154] G.J. Mizejewski, Proc. Soc. Exp. Biol. Med. 222 (1999) 124.
[155] R.J. Sanders, F. Mainiero, F.G. Giancotti, Cancer Invest. 16 (1998) 329.
[156] P.L. Judson, X. He, W.G. Cance, L. Van Le, Cancer 86 (1999) 1551.
[157] L.V. Owens, L. Xu, G.A. Dent, X. Yang, G.C. Sturge, R.J. Craven, W.G. Cance, Ann. Surg. Oncol. 3 (1996) 100.
[158] L.V. Owens, L. Xu, R.J. Craven, G.A. Dent, T.M. Weiner, L. Kornberg, E.T. Liu, W.G. Cance, Cancer Res. 55 (1995) 2752.
[159] T.M. Weiner, E.T. Liu, R.J. Craven, W.G. Cance, Ann. Surg. Oncol. 1 (1994) 18.
[160] T.M. Weiner, E.T. Liu, R.J. Craven, W.G. Cance, Lancet 342 (1993) 1024.
[161] L. Tremblay, W. Hauck, A.G. Aprikian, L.R. Begin, A. Chapdelaine, S. Chevalier, Int. J. Cancer 68 (1996) 164.
[162] M. Agochiya, V.G. Brunton, D.W. Owens, E.K. Parkinson, C. Paraskeva, W.N. Keith, M.C. Frame, Oncogene 18 (1999) 5646.
[163] V.G. Brunton, B.W. Ozanne, C. Paraskeva, M.C. Frame, Oncogene 14 (1997) 283.
[164] L.J. Kornberg, Head Neck 20 (1998) 634.
[165] W.G. Cance, J.E. Harris, M.V. Iacocca, E. Roche, X. Yang, J. Chang, S. Simkins, L. Xu, Clin. Cancer Res. 6 (2000) 2417.
[166] S.J. McCormack, S.E. Brazinski, J.L. Moore, B.A. Werness, D.J. Goldstein, Oncogene 15 (1997) 265.