PERGAMON

Progress in Biophysics & Molecular Biology 71 (1999) 435–478

Progress in Biophysics & Molecular Biology

Signaling through focal adhesion kinase

David D. Schlaepfer *, Christof R. Hauck, David J. Sieg

The Scripps Research Institute, Department of Immunology, IMM26, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA

Abstract

Integrin receptor binding to extracellular matrix proteins generates intracellular signals via enhanced tyrosine phosphorylation events that are important for cell growth, survival, and migration. This review will focus on the functions of the focal adhesion kinase (FAK) protein-tyrosine kinase (PTK) and its role in linking integrin receptors to intracellular signaling pathways. FAK associates with several different signaling proteins such as Src-family PTKs, $p130^{Cas}$, Shc, Grb2, PI 3-kinase, and paxillin. This enables FAK to function within a network of integrin-stimulated signaling pathways leading to the activation of targets such as the ERK and JNK/mitogen-activated protein kinase pathways. Focus will be placed on the structural domains and sites of FAK tyrosine phosphorylation important for FAK-mediated signaling events and how these sites are conserved in the FAK-related PTK, Pyk2. We will review what is known about FAK activation by integrin receptor-mediated events and also non-integrin stimuli. In addition, we discuss the emergence of a consensus FAK substrate phosphorylation sequence. Emphasis will also be placed on the role of FAK in generating cell survival signals and the cleavage of FAK during caspase-mediated apoptosis. An in-depth discussion will be presented of integrin-stimulated signaling events occurring in the FAK knockout fibroblasts (FAK^-) and how these cells exhibit deficits in cell migration. FAK re-expression in the FAK^- cells confirms the role of this PTK in the regulation of cell morphology and in promoting cell migration events. In addition, these results reinforce the potential role for FAK in promoting an invasive phenotype in human tumors.

Keywords: FAK; Pyk2; C-Src; ERK2; Cell migration

* Corresponding author. Tel.: +1-619-784-8207; fax: +1-619-784-8227; e-mail: dschlaep@scripps.edu.

0079-6107/99/$ - see front matter
PII: S0079-6107(98)00052-2

1. Introduction

Interaction of cells with extracellular matrix (ECM) proteins generates intracellular signals important for growth, survival, and migration. The integrin family of transmembrane receptors have long been recognized for their structural roles in linking ECM proteins with the cellular actin cytoskeleton in the regulation of cell shape and tissue architecture. Recent developments within the field of integrin biology have shown that integrin receptors can initiate signal transduction events that affect many aspects of cell growth (for recent reviews see Schwartz et al., 1995; Burridge and Chrzanowska-Wodnicka, 1996; Giancotti, 1997; Aplin et al., 1998). In a variety of cell types, enhanced tyrosine phosphorylation of signaling proteins is a common response to integrin receptor clustering after binding to ECM proteins. However, since transmembrane integrin receptors do not possess catalytic activity, the signals initiated by ECM-integrin interactions must be transduced into cells through the activation of integrin-associated proteins. Whereas evidence is emerging that integrin receptors can cocluster with (Miyamoto et al., 1996; Schneller et al., 1997) and affect the cellular response to growth factor receptor protein-tyrosine kinases (PTKs) (for a review see Sastry and Horwitz, 1996), a variety of nonreceptor PTKs such as the Abl, Syk, FAK and Src-family PTKs (for recent reviews see Guan, 1997; Hanks and Polte, 1997; Parsons and Parsons, 1997; Schlaepfer and Hunter, 1998) can be rapidly activated by integrin receptor clustering or cell binding to ECM proteins.

This review will focus on the functions of the focal adhesion kinase (FAK) and its role in linking integrin receptors to intracellular signaling pathways. It should be emphasized that integrins connect to a number of different signaling proteins and that FAK can be considered as one part of this network. In adherent cell types, FAK colocalizes with integrin receptors at cell-substratum contact sites termed focal adhesions and FAK was originally named as such for its hypothesized role as the important PTK involved in forming these structures (Schaller et al., 1992). Indeed, cellular focal adhesions contain structural proteins associated with the actin cytoskeleton and signaling proteins that in many cases are modified by tyrosine phosphorylation. In addition, treatment of cells with tyrosine phosphatase inhibitors leads to enhanced focal contact formation whereas pharmacological tyrosine kinase inhibitors prevent their formation (for a review see Burridge et al., 1997). Although correlative evidence points to a role for FAK in focal adhesion formation (for a review see Parsons, 1997), fibroblasts isolated from the FAK knockout mouse (FAK^-) exhibit enhanced focal contact formation in cell culture supporting a role for FAK in the dynamic regulation of these structures (Ilic et al., 1995b).

As will be discussed, FAK associates with a number of different signaling proteins which enables this PTK to couple to a variety of different intracellular signaling pathways. Although the exact mechanisms leading to FAK kinase activation are not well defined, enhanced FAK tyrosine phosphorylation events are an important link between integrin receptors and the activation of downstream targets such as the ERK and JNK/mitogen-activated protein kinase cascades. FAK connections to phosphatidylinositol 3′-kinase may provide an essential anchorage-dependent survival signal in the prevention of cell apoptosis and in addition, caspase cleavage of FAK during the initial stages of cell apoptosis can generate a FAK protein fragment that can promote cell death. FAK also plays a role in early mammalian development during the processes of gastrulation. Homozygous mutations in murine FAK result in

embryonic lethality and cells isolated from these embryos exhibit morphological alterations and migration defects in cell culture. This cellular phenotype seems to stem from an inability to efficiently remodel or turnover the contacts at the cell-substratum interface in the FAK^- cells.

We will review what is known about the integrin-stimulated signaling events in the FAK^- fibroblasts and the effects of either transient (Sieg et al., 1998) or stable (discussed herein) FAK protein re-expression in these cells. Emerging evidence supports the hypothesis that FAK does indeed play an important role in the regulation of focal contact structures with respect to cell shape and during the processes of cell migration. With respect to human cancer, the accumulated findings on the role of FAK in the processes of cell survival and cell migration suggest that elevated FAK expression in tumors of high invasive potential may directly contribute to the metastatic phenotype.

2. Structural domains of FAK

2.1. FAK isoforms generated by alternative splicing

FAK is a nonreceptor and nonmembrane associated PTK which does not contain Src homology 2 (SH2) or SH3 protein interaction domains. The centrally located kinase domain of FAK is flanked by large N- and C-terminal noncatalytic domains (Fig. 1). FAK homologs have been identified in human (Whitney et al., 1993), mouse (Hanks et al., 1992), chicken (Schaller et al., 1992), and frog (Hens and DeSimone, 1995) and they share over 90% amino acid sequence identity. In addition, a *Drosophila* FAK cDNA has been isolated and it exhibits a similar domain structure as other FAK homologs (R. Palmer, personal communication). Several FAK isoforms can be produced as a result of the use of alternative promoters and through alternative splicing (Fig. 1). For instance, chicken fibroblasts express an N-terminally truncated form of FAK termed FRNK (FAK-related nonkinase), whose mRNA is generated from a promoter located within an intron lying downstream of the catalytic domain (Schaller et al., 1993). Alternatively-spliced mouse FAK mRNAs have been identified that have variations in kinase domain residues (Eide et al., 1995) and rat brain contains FAK transcripts (termed FAK^+) encoding a 3 amino acid insertion (Pro–Trp–Arg) within the C-terminal domain (Burgaya and Girault, 1996). An antigenically-distinct FAK isoform termed FAKB also has been described (Kanner et al., 1994) however its existence has not been confirmed by DNA sequencing information.

These different isoforms may be important for the regulation of FAK biological activity, since FRNK can act as a negative regulator of full-length FAK (Richardson and Parsons, 1996) and FAK^+ can be differentially activated by specific cellular stimuli (Derkinderen et al., 1996). Additional FAK alternatively-spliced isoforms termed FAK box 6, box 7 and box 28 yield proteins with amino acid insertions surrounding the FAK autophosphorylation site at Tyr-397 (Burgaya et al., 1997) (see Fig. 1). The presence of boxes 6 or 7 within FAK yield proteins that exhibit increased in vitro kinase activity potentially through subtle conformation and changes as will be discussed in Section 3.3. Many of these FAK isoforms are specifically expressed in brain tissues suggesting that neuronal FAK may exhibit specific and unique properties.

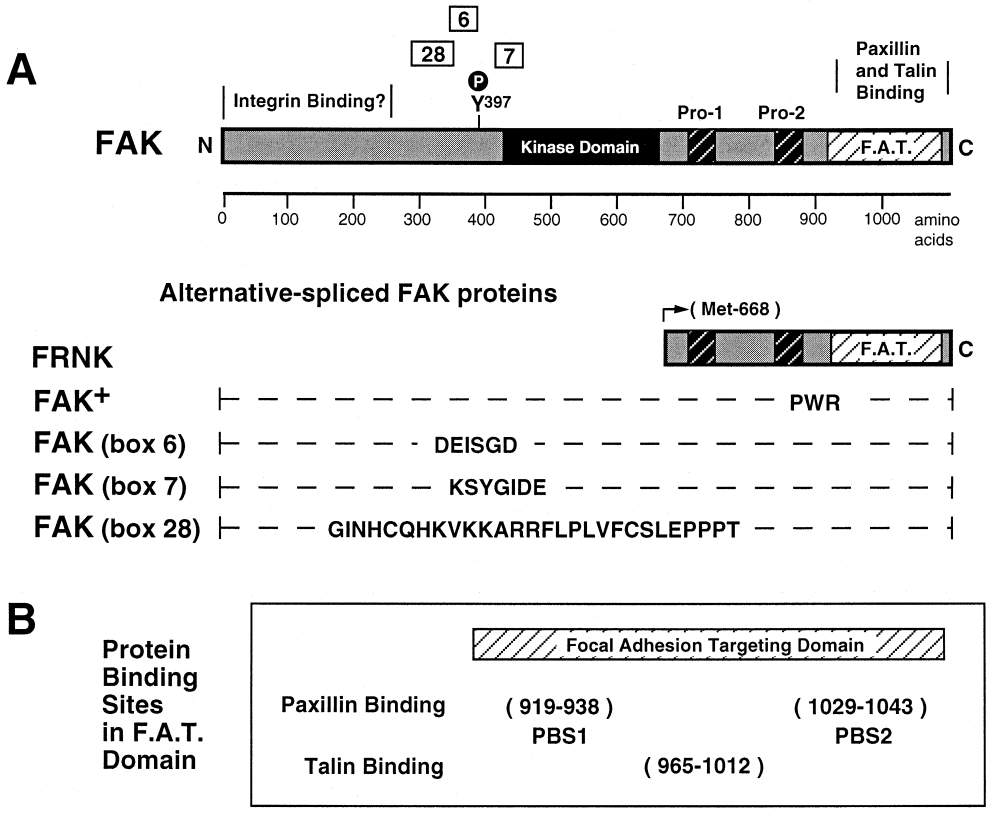

Fig. 1. FAK structural features and alternate splice forms. (A) FAK contains a central kinase domain flanked by large N- and C-terminal domains. Undefined residues within the FAK N-terminal domain may associate with integrin receptors and the tail region of the FAK C-terminal domain contains residues important for focal adhesion targeting (FAT). The major FAK autophosphorylation site is at Tyr-397. Alternate-spliced FAK isoforms (box 6, 7, and 28) contain amino acid insertions surrounding the FAK Tyr-397. Other FAK isoforms include FRNK which is the C-terminal noncatalytic domain of FAK and FAK$^+$ which contains a short 3 amino acid insertion as indicated. Two Proline-rich regions in the FAK C-terminal domain are sites for SH3 domain-containing protein binding. (B) As indicated, the FAT domain contains regions important for paxillin (PBS, paxillin binding sequences) and talin binding.

2.2. Focal adhesion targeting (FAT) domain

In fibroblasts, the localization of FAK with integrin receptors at cell substratum contact sites is mediated by sequences within the tail of the C-terminal domain of FAK. This stretch of ~160 amino acids can function independently to localize fusion proteins to focal contact sites (Hildebrand et al., 1993). Contained within the focal adhesion targeting domain are binding sites for proteins such as paxillin and talin. Paxillin is an adaptor protein containing different interaction domains such as a proline-rich site for SH3 domain binding, four N-terminal domain LD motifs which contribute to FAK binding, and four zinc-finger LIM domains which are important for paxillin targeting to focal contacts (Brown et al., 1996). Talin is a structural protein that can associate with β-integrin cytoplasmic tails. In vitro binding analyses have

localized the talin binding region to residues 965–1012 of the FAK FAT domain (Chen et al., 1995) (Fig. 1). Mutagenesis and in vitro binding analyses have identified two regions flanking the talin binding site in the FAT domain which are important for both FAK localization to focal contacts and paxillin binding (Tachibana et al., 1995). These sites have been termed paxillin binding sequences (PBS1 and PBS2). Interestingly, PBS2 shares sequence similarity to a contiguous stretch of 22 amino acids in the structural protein vinculin which also binds to paxillin in vitro (Wood et al., 1994).

Although it has been hypothesized that paxillin recruits FAK to focal contact sites (Tachibana et al., 1995), other reports have shown FAK localization to integrin clusters/focal adhesions in the absence of paxillin association (Hildebrand et al., 1995; Miyamoto et al., 1995b). FAK also contains undefined sequences within its N-terminal domain that can bind peptides derived from β-integrin cytoplasmic domains (Schaller et al., 1995) which may act to stabilize FAK associations with integrins in focal contacts. Therefore, it is likely that multiple protein binding interactions with proteins such as paxillin, talin, and integrins act to recruit and stabilize FAK localization to focal contacts.

2.3. Comparisons with the FAK-related PTK, Pyk2

FAK and a second nonreceptor PTK variously called proline-rich tyrosine kinase 2 (Pyk2) (Lev et al., 1995), cell adhesion kinase β (CAKβ) (Sasaki et al., 1995), related adhesion focal tyrosine kinase (RAFTK) (Avraham et al., 1995), or calcium-dependent protein-tyrosine kinase (CADTK) (Yu et al., 1996) define a new subfamily of nonreceptor PTKs. To avoid nomenclature confusion, we will refer to this FAK-related protein as Pyk2 (Fig. 2). Pyk2 is highly expressed in cells of the central nervous system and is also found in cells of hematopoietic lineage. Pyk2 (112 kDa) is slightly smaller than FAK (119 kDa), both proteins contain unique sequences within the first 100 amino acid residues, and Pyk2 and FAK share ~45% overall sequence similarity (Fig. 2). Pyk2 and FAK sequence conservation is maximal within the central kinase domains and surrounding the conserved tyrosine phosphorylation sites which are also binding sites for SH2 domain containing proteins (Table 1). The C-terminal domain regions of FAK and Pyk2 also contain two conserved proline-rich domains which are also binding sites for SH3 domain containing proteins (Fig. 2 and Table 1). Pyk2 mRNA transcripts are subject to alternative splicing in the region between the two proline-rich domains yielding a Pyk2-variant with a 42 amino acid deletion (Dikic and Schlessinger, 1998; Li et al., 1998; Xiong et al., 1998).

The Pyk2 C-terminal tail region contains consensus paxillin binding sequences (PBS1, 875–894 and PBS2, 985–999) (Fig. 2) and Pyk2 can associate with paxillin (Salgia et al., 1996a; Li and Earp, 1997), or with the paxillin-related proteins Hic-5 (Matsuya et al., 1998), or with leupaxin (Lipsky et al., 1998) in lysates from a variety of cell types. Although there is strong evidence to support the association of Pyk2 with paxillin, immunofluorescent localization studies have shown that both endogenous Pyk2 (Sasaki et al., 1995; Matsuya et al., 1998; Sieg et al., 1998; Zheng et al., 1998) or recombinant Pyk2 (Schaller and Sasaki, 1997) only weakly associate with focal contacts in adherent cell types whereas paxillin exhibits strong localization to these structures. Interestingly, the Pyk2 C-terminal domain (sequences related to FRNK) strongly localized to focal contacts when exogenously-expressed in chicken fibroblasts (Schaller

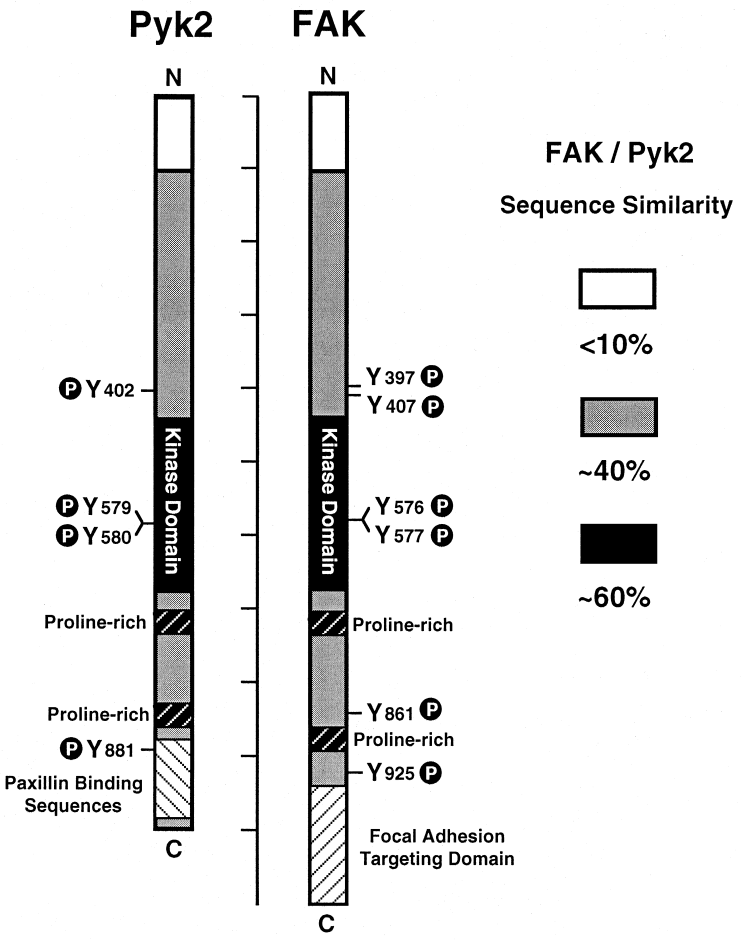

Fig. 2. FAK and Pyk2 sequence comparisons. The central kinase domains, regions surrounding phosphorylation sites (P), and proline-rich regions (Pro-1, Pro-2) show a high degree of sequence similarity between FAK and Pyk2. The six sites of FAK tyrosine phosphorylation and the analogous sites in Pyk2 are indicated. FAK Tyr-397 and Pyk2 Tyr-402 are sites for Src family PTK SH2 domain binding. The FAK Tyr-925 and Pyk2 Tyr-881 are sites for Grb2 SH2 domain binding. FAK exhibits a focal adhesion targeting domain in its C-terminal part. Although Pyk2 does not have contain a complete FAT domain as in FAK, Pyk2 contains conserved paxillin binding sequences in the C-terminal tail region. The regional sequence similarities between FAK and Pyk2 are also highlighted.

and Sasaki, 1997) and stable targeting of a chimeric Pyk2 protein to focal contacts was achieved by swapping the Pyk2 C-terminal domain with that of the FAK C-terminal domain (Zheng et al., 1998). These results would suggest that 1) the FAK and Pyk2 C-terminal domains are not equivalent in their abilities to target proteins to focal contacts and 2) that paxillin binding may be sufficient to promote localization to focal adhesions, but in the context of full-length Pyk2, it may not be enough to over-ride other potential protein binding interactions limiting the amount of Pyk2 localized to focal contact structures in cells.

Table 1
Conservation of SH2 and SH3 binding sites in FAK and Pyk2

Site in FAK	FAK motif	Site in Pyk2	Pyk2 Motif	Kinase	Function
Tyr-397	*Y*AEI	Tyr-402	*Y*AEI	FAK/Pyk2	Src-family PTK SH2 binding
Tyr-407	*Y*TMP	N/A	N/A	Src PTKs	unknown
Tyr-576/577	ST*YY*KAS	Tyr-579/580	ED*YY*KAS	Src PTKs	regulatory/kinase activation loop
Tyr-861	*Y*QPV	N/A	N/A	Src PTKs	unknown
Tyr-925	*Y*ENV	Tyr-881	*Y*LNV	Src PTKs	Grb2 SH2 binding site
Pro-712/713	A*PP*KPSRPG	Pro-714/715	P*PP*KPSRPK	N/A	$p130^{Cas}$ SH3 binding site
Pro-876/877	PPKK*PP*RPG	Pro-859/860	PPQK*PP*RLG	N/A	$p130^{Cas}$/Graf SH3 binding sites

Consensus $p130^{Cas}$ SH3 binding motif (P-X-K-P-X-R) is underlined.

2.4. Conservation of SH2 and SH3 binding sites on Pyk2 and FAK

Analyses of FAK activation events have shown that integrin-stimulated FAK tyrosine phosphorylation is complex and occurs at six or more sites in vivo (Calalb et al., 1995; Schlaepfer and Hunter, 1996). Two sites within the FAK N-terminal domain (Tyr-397 and Tyr-407), two sites within the kinase domain activation loop (Tyr-576 and Tyr-577), and two sites within the C-terminal domain (Tyr-861 and Tyr-925) are phosphorylated in vivo (Fig. 2 and Table 1). Four of the six FAK tyrosine phosphorylation sites (Tyr-397, 576, 577, and 925) are conserved at analogous positions in Pyk2 (Tyr-402, 579, 580, and 881). When expressed in bacteria, recombinant FAK exhibits phosphorylation at Tyr-397 and therefore this site is a bona fide autophosphorylation site (Calalb et al., 1995). The motif surrounding FAK Tyr-397 (pTyr–Ala–Glu–Ile motif) fits the consensus for a Src-family SH2 domain binding site and numerous studies have shown that the phosphorylation of this site regulates Src-family PTK association with FAK in vivo (for a review see Schwartz et al., 1995).

The other FAK tyrosine phosphorylation sites have been shown to be preferentially trans-phosphorylated by Src-family PTKs in vivo (Calalb et al., 1995, 1996; Schlaepfer and Hunter, 1996). Phosphorylation of FAK Tyr-567/577 in the activation loop of the kinase domain is required for maximal FAK kinase activity (Calalb et al., 1995) and phosphorylation of FAK Tyr-925 (pTyr–Glu–Asn–Val) creates a SH2 binding site for the Grb2 small adaptor protein (Schlaepfer et al., 1994) (Table 1). Grb2 also has been shown to bind to activated Pyk2 (Lev et al., 1995; Li et al., 1996a) and mutation of the equivalent Tyr-881 site (pTyr–Leu–Asn–Val) in Pyk2 disrupts Grb2 binding (Felsch et al., 1998). As will be discussed in Section 4.1, direct Grb2 binding to FAK or Pyk2 is one of several signaling pathways contributing to the activation of downstream targets such as the ERK2/MAP kinase cascade. It should be also emphasized that FAK exhibits a high level of serine phosphorylation at multiple-sites in vivo, but the biological significance of these events has not been determined (Calalb et al., 1995; Schlaepfer and Hunter, 1996; Richardson et al., 1997b).

Another conserved feature of Pyk2 and FAK is the presence of two proline-rich motifs within the C-terminal domain which are sites for SH3-mediated binding interactions. The SH3 domain of the large adaptor protein $p130^{Cas}$ binds directly to the first proline-rich motif (residues 711–717) of FAK (Polte and Hanks, 1995; Harte et al., 1996) and further

mutagenesis studies have shown that the second proline-rich motif (residues 872–879) also contributes to $p130^{Cas}$ binding to FAK (Polte and Hanks, 1997). Pyk2 and $p130^{Cas}$ also associate (Astier et al., 1997b) and upon comparison of the two proline-rich domains from both FAK and Pyk2, a consensus $p130^{Cas}$ SH3 binding motif can be deduced (Pro-X–Lys–Pro-X–Arg) (see Table 1). Another SH3 domain-containing protein that binds to both FAK and Pyk2 is the GTPase-activating protein, Graf (Hildebrand et al., 1996; Ohba et al., 1998). In vitro binding assays indicate that the SH3 domain of Graf may prefer the second proline-

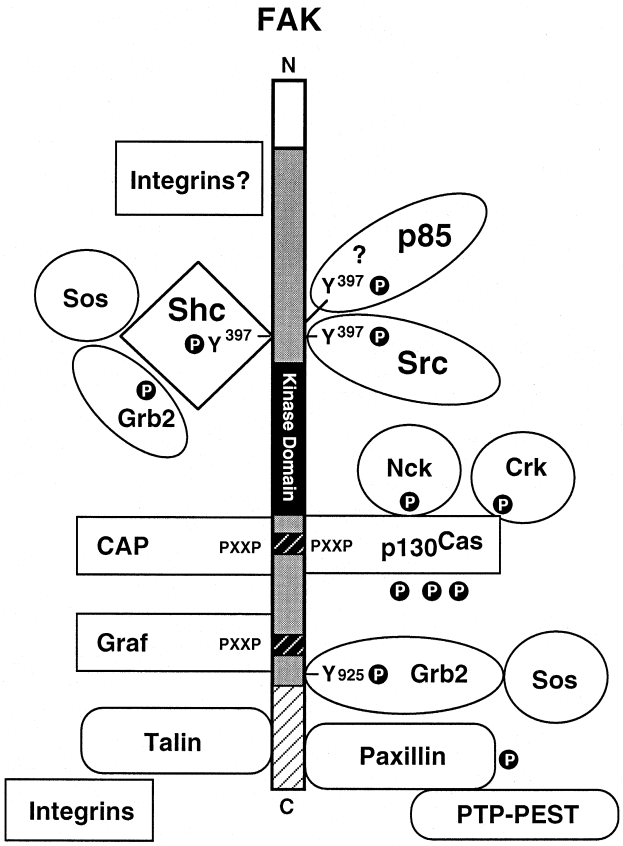

Fig. 3. Proteins associated with FAK. A variety of different signaling proteins have been shown to directly associate with FAK as indicated. It is possible that different combinations of proteins may affect the involvement of FAK in different signaling pathways. Namely, direct Grb2 or Shc binding to FAK and the indirect association of the SOS GDP-GTP exchange factor for Ras with Grb2 can link FAK to the activation of the Ras-ERK2/MAP kinase pathway. FAK is also linked to other signaling pathways through the association and phosphorylation of $p130^{Cas}$ which promotes Crk and Nck adaptor protein binding to $p130^{Cas}$. The direct binding of paxillin and its association with the protein tyrosine phosphatase PTP-PEST may be important for the dynamic regulation of integrin-stimulated cycles of phosphorylation and dephosphorylation. N-terminal FAK domain association with integrin cytoplasmic domains or talin binding to the FAK C-terminal domain may be important for the regulation of FAK by integrins. The p85 subunit of PI 3-kinase binding to FAK and FAK-associated PI 3-kinase activity may be important for FAK-mediated cell survival signals. The roles of CAP and Graf protein binding to FAK have not been fully determined.

rich motif in both FAK and Pyk2. Whereas p130^{Cas} binding to FAK has been linked to integrin-stimulated signaling and migration events (discussed in Sections 4.4 and 5.2), the role for Graf and other FAK-associated proteins such as CAP (Ribon et al., 1998) have not been determined.

In summary, since the SH2 and SH3 binding sites on FAK and Pyk2 are conserved, a reasonable assumption is that signaling through either Pyk2 or FAK will involve these common sites and may involve a common group of interacting proteins. As shown in Fig. 3, FAK can associate with a number of different signaling, adaptor, as well as structural proteins. It is FAK kinase activity and autophosphorylation at Tyr-397 which regulates the direct binding of signaling proteins such as Src-family PTKS, the p85 subunit of phosphatidylinositol 3-kinase (Chen et al., 1996), and the Shc adaptor protein (Schlaepfer et al., 1998). Src-family PTK phosphorylation of FAK at secondary sites regulates the direct binding of Grb2 at Tyr-925 and the FAK-mediated enhancement of Src-family PTK activity is dependent upon FAK kinase activity (Schlaepfer et al., 1997). As will be discussed later, a number of FAK-enhanced signaling events are dependent on FAK autophosphorylation at Tyr-397. However, FAK kinase activity may not be essential for all FAK functions. Overexpression of kinase-inactive FAK has been shown to promote Chinese hamster ovary cell migration (Cary et al., 1996) and kinase-inactive FAK can function to promote FcεRI receptor-stimulated mast cell secretion events (Hamawy et al., 1997). Since proteins such as paxillin, p130^{Cas}, and Graf bind to FAK in a phosphorylation-independent manner, it is quite possible that FAK may also function in a kinase-independent manner as an adaptor or linker protein in intracellular signal transduction pathways.

2.5. Emerging consensus of FAK substrate phosphorylation sites

Although the exact manner in which FAK may function as a scaffolding protein has not been determined, proteins such as paxillin and p130^{Cas} are also considered targets for FAK transphosphorylation. In many instances, cell stimulation leads to parallel increases in both FAK and paxillin tyrosine phosphorylation with the implication being that FAK activation may lead to increased paxillin phosphotyrosine (pTyr) levels. In vitro, FAK isolated from mammalian cell lysates can phosphorylate paxillin at two sites, Tyr-31 and Tyr-118 (Bellis et al., 1995; Schaller and Parsons, 1995). Significantly, this FAK-dependent phosphorylation of paxillin required the integrity of the autophosphorylation/Src SH2 binding site at Tyr-397 (see Table 2). Since Phe-397 FAK exhibits significant kinase activity in vitro (Schaller et al., 1994; Calalb et al., 1995) but does not associate with Src-family PTKs, and since paxillin is a good substrate for activated Src (Thomas et al., 1995), it is possible that phosphorylation of paxillin may be mediated in part by FAK-associated Src-family PTK activity.

Similar to paxillin, a number of studies have documented increased p130^{Cas} pTyr levels which parallel that of FAK after integrin stimulation of cells. Overexpression studies with Phe-397 FAK have shown that FAK may preferentially phosphorylate either Tyr-762 or Tyr-764 within the p130^{Cas} (YDYVHL) sequence (Tachibana et al., 1997) (Table 2). Significantly, this region has been shown to be important for Src SH2 binding to p130^{Cas} (Nakamoto et al., 1996). A model has been suggested that FAK may initiate integrin-mediated p130^{Cas} tyrosine phosphorylation (Tachibana et al., 1997), leading to the recruitment of Src-family PTKs to

Table 2
Emerging consensus of FAK substrate phosphorylation sites

FAK Substrate	Site	Motif	Observations/function
Autophosphorylation	Tyr-397	*ETDD*<u>*Y*</u>	Src-family PTK SH2 binding (YAEI)
Paxillin	Tyr-31	*EE*TP<u>*Y*</u>	dependent on FAK Tyr-397
Paxillin	Tyr-118	*EE*HV<u>*Y*</u>	dependent on FAK Tyr-397
Shc	Tyr-317	*DD*PS<u>*Y*</u>	phosphorylated by Phe-397 FAK
$p130^{Cas}$	Tyr-762	WMED<u>*Y*</u>	phosphorylated by Phe-397 FAK
$p130^{Cas}$	Tyr-764	*ED*Y*D*<u>*Y*</u>	phosphorylated by Phe-397 FAK
$p130^{Cas}$	Tyr-332[a]	*EQDE*<u>*Y*</u>	Crk/Nck SH2 binding site (YDTP)
$p130^{Cas}$	Tyr-460[a]	A*ED*V<u>*Y*</u>	Crk/Nck SH2 binding site (YDVP)
c-Src	Tyr-417[b]	*ED*N*E*<u>*Y*</u>	regulatory/kinase activation loop

[a] Putative phosphorylation sites within $p130^{Cas}$ 'substrate domain'.[b]Potential mechanism of FAK-enhanced c-Src PTK activation.

$p130^{Cas}$ and the further Src-family PTK phosphorylation of $p130^{Cas}$ within the substrate domain. However, in cells deficient for Src-family PTKs, $p130^{Cas}$ is not detectably phosphorylated even though FAK becomes activated and tyrosine phosphorylated after integrin stimulation (Vuori et al., 1996; Hamasaki et al., 1996; Sakai et al., 1997). These results suggest that it may be a complex of FAK and Src-family PTKs which leads to increased $p130^{Cas}$ tyrosine phosphorylation events.

Interestingly, stable expression of a fragment encompassing the SH3 and SH2 domains of c-Src (Src 1–298) in Src-knockout cells promoted the enhanced FAK-mediated binding to and phosphorylation of $p130^{Cas}$ (Schlaepfer et al., 1997). Under these conditions, Src 1–298 acted as an adaptor protein to facilitate a linkage between FAK and $p130^{Cas}$. In the Src 1–298 expressing cells, FAK phosphorylated $p130^{Cas}$ at a number of different sites; some of which facilitated the Nck adaptor protein binding to $p130^{Cas}$ (Schlaepfer et al., 1997) (Fig. 3 and Table 2). In comparing the known sites phosphorylated by FAK, a consensus motif can be assembled (Table 2). It is reasonable to consider that an optimal FAK phosphorylation recognition sequence may be its own autophosphorylation site (Glu–Thr–Asp–Asp–pTyr). Accordingly, a clustering of glutamate or aspartate acidic residues in the +4 to +1 positions precede the phosphorylated tyrosine residue in the FAK substrate proteins (Table 2). This FAK consensus is similar to that determined for other nonreceptor tyrosine kinases. However, there is no obvious selectivity for a hydrophobic residue immediately preceding the phosphorylated tyrosine site as was determined for Abl and Src-family PTKs (Songyang et al., 1995).

In some instances, such as the FAK-mediated phosphorylation of Shc at Tyr-317 (Schlaepfer et al., 1998), the reactions are facilitated by the direct Shc SH2 domain binding to the FAK autophosphorylation site at Tyr-397 (D. Schlaepfer, unpublished results). In addition, since FAK kinase activity is important for the FAK-enhanced elevation of c-Src PTK activity after integrin stimulation of cells (Schlaepfer and Hunter, 1997), it is interesting to speculate whether this may involve the transphosphorylation of c-Src at Tyr-417 in the kinase activation loop (Glu–Asp–Asn–Glu–pTyr). In this manner, since Src family PTKs can phosphorylate FAK within the kinase activation loop to promote maximal FAK kinase activity (Calalb et al.,

Table 3
Non-integrin-mediated stimuli which enhance FAK tyrosine phosphorylation levels

Stimulus	Cell type	Observations	References
Phorbol 12-myristate 13-acetate (PMA)	murine fibroblasts	dependent on PKC activation	(various)
Bovine serum	Swiss 3T3 fibroblasts	stress fiber and focal contact formation	(Chrzanowska-Wodnicka and Burridge, 1994)
Lysophosphatidic acid	Swiss 3T3 fibroblasts	independent of PKC or Ca^{2+} pathways	(Seufferlein and Rozengurt, 1995)
Activated Rho	Swiss 3T3 fibroblasts	dependent upon actin cytoskeleton	(Flinn and Ridley, 1996)
Bombesin	Swiss 3T3 fibroblasts	rapid response (seconds)	(Zachary et al., 1992)
	Swiss 3T3 fibroblasts	blocked by rho p21 inhibition	(Rankin et al., 1994)
	prostate carcinoma cells	stimulated FAK co-immunoprecipitation with β1, β3, and β5-integrins	(Aprikian et al., 1997)
	Swiss 3T3 fibroblasts	increased FAK kinase activity dependent upon intact cytoskeleton and cell adherence	(Rodriguez-Fernandez and Rozengurt, 1998)
Bradykinin	Swiss 3T3 fibroblasts	dependent upon PKC or Ca^{2+} pathways	(Leeb-Lundberg et al., 1994)
Sphingolipid metabolites	Swiss 3T3 fibroblasts	requirement of Rho signaling pathway	(Seufferlein and Rozengurt, 1995)
	Swiss 3T3 fibroblasts	not blocked by wortmannin	(Sasaki et al., 1996)
Anandamide	rat hippocampal slices	actions through inhibition of adenylyl cylase and PKA	(Derkinderen et al., 1996)
Cholecystokinin	pancreatic acinar cells	dependent on cytoskeleton and p21rho activity	(Garcia et al., 1997)
Angiotensin II	vascular smooth muscle cells	rapid response (seconds)	(Polte et al., 1994)
Platelet-activating factor	human endothelial cells	inhibited by calphostin C	(Soldi et al., 1996)
Muscarinic m3 receptors (carbachol)	transfected HEK cells	dependent on cellular integrin engagement and reduced by PKC inhibitors	(Slack, 1998)
$G\alpha_{12}$ and $G\alpha_{13}$	transfected HEK cells	dependent on cytoskeleton and $p21^{rho}$ activity	(Needham and Rozengurt, 1998)
Platelet-derived growth factor (PDGF)	Swiss 3T3 fibroblasts	maximal at low concentrations (1–5 ng/ml) and dependent on the actin cytoskeleton	(Rankin and Rozengurt, 1994)
	vascular smooth muscle cells	2.5 ng/ml stimulates paxillin tyrosine phosphorylation and cell migration	(Abedi et al., 1995)

(continued on next page)

Table 3 (*continued*)

Stimulus	Cell type	Observations	References
Platelet-derived growth factor (PDGF)	porcine aortic endothelial cells	inhibited by wortmannin and blocked by Phe-740/751 mutations in PDGF receptor	(Rankin et al., 1996)
	rat mesangial cells grown in 3D culture	stimulated collagen gel contraction which was not affected by MEK inhibitor	(Zent et al., 1998)
Hepatocyte growth factor (HGF)	oral squamous carcinoma cell lines	transient response inhibited by cell pretreatment with herbimycin A	(Matsumoto et al., 1994)
Macrophage colony-stimulating factor	human monocytes	stimulates Grb2 SH2 domain binding to FAK	(Kharbanda et al., 1995)
Insulin-like growth factor (IGF-1)	human neuroblastoma cells (SH-SY5Y)	IGF-1 promoted lamellipodia extension and paxillin tyrosine phosphorylation	(Leventhal et al., 1997)
Insulin	transfected Rat-1 fibroblasts	direct insulin receptor phosphorylation of FAK only in suspended cells	(Baron et al., 1998)
Vascular endothelial growth factor (VEGF)	endothelial cells (HUVECs)	dependent upon cytoskeleton and reduced in presence of PKC inhibitors	(Abedi and Zachary, 1997)
Steel factor	human erythroleukemic cell line (TF-1)	integrin-dependent and enhanced the rate of cell spreading on fibronectin	(Takahira et al., 1997)
Growth hormone	transfected Chinese hamster ovary cells	requires Pro-rich box 1 of GH receptor and stimulates FAK association with JAK2	(Zhu et al., 1998)
Hyaluronan mediated by RHAMM receptor	ras-transformed 10T1/2 fibroblasts	rapid, blocked by herbimycin A, and cells acquire motile phenotype	(Hall et al., 1994)
IgE receptor (FcεRI)	mast cell line RBL-2H3	requires cell adherence	(Hamawy et al., 1993)
	3B6 variant of RBL-2H3 mast cells	FAK Tyr-397 and kinase activity are not needed for enhanced histamine release	(Hamawy et al., 1997)
Amyloid β peptide	neuroblastoma cells	stimulates Fyn PTK association with FAK	(Zhang et al., 1996a)
		requires cytoskeleton and PKC activity	(Zhang et al., 1996b)
Cyclic stretching (1 Hz)	rat mesangial cells	rapid within 10 min	(Hamasaki et al., 1995)
	human umbilical endothelial cells	morphological cell changes blocked by herbimycin A and FAK antisense treatments	(Naruse et al., 1998)

Table 3 (*continued*)

Stimulus	Cell type	Observations	References
Hydrodynamic shear stress (12 dyn/cm^2)	bovine aortic endothelial cells	FAK Tyr-397 is important for stimulated signals to ERK2 and JNK1	(Li et al., 1997a,b)
Neural cell adhesion molecule clustering	transfected COS-7 fibroblasts	Src-family mediated association of FAK with the NCAM-140 receptor	(Beggs et al., 1997)
Glutamate	rat hippocampal slices	membrane depolarization-induced activation blocked by PKC inhibitors	(Siciliano et al., 1996)
K-252a inhibitor	neuroblastoma cell line SH-SY5Y	increased FAK kinase activity and independent of integrin-matrix interaction	(Maroney et al., 1995)
RANTES	human CD4$^+$ T cells	rapid activation of FAK kinase activity	(Bacon et al., 1996)
P-selectin	human peripheral blood lymphocytes	increased paxillin tyrosine phosphorylation with cell attachment to ligand	(Haller et al., 1997)
Prolactin	human breast carcinoma cells	maximal at 5 ng/ml and results in increased paxillin tyrosine phosphorylation	(Canbay et al., 1997)
Hydrogen peroxide	human glioblastoma cell line (T98G)	FAK antisense treatment led to higher levels of peroxide-induced cell death	(Sonoda et al., 1997)
Human papillomavirus type 18	human genital epithelial cells	increased FAK activity, paxillin tyrosine phosphorylation and fibronectin deposition	(McCormack et al., 1997)
Urokinase-type plasminogen activator receptor (uPAR)	bovine aortic endothelial cells	correlation between FAK and ERK2 activation	(Tang et al., 1998)

1995), the transient complex formed between FAK and c-Src after integrin stimulation of cells (Schlaepfer et al., 1994) may mutually lead to the maximal activation of both PTKs.

3. FAK activation events

3.1. Non-integrin stimuli that enhance FAK or Pyk2 tyrosine phosphorylation

FAK colocalizes with integrin receptors at cell-substratum contact sites in fibroblasts, and its PTK activity is enhanced by cellular binding to extracellular matrix proteins. In consequence, FAK has been proposed to play a central role in integrin-stimulated signaling events. However, a number of other cellular stimuli that generate signals through either G-protein linked receptors, transmembrane growth factor receptors, or through unknown mechanisms can

Table 4
Non-integrin-mediated stimuli which enhance Pyk2 tyrosine phosphorylation levels

Stimulus	Cell Type	Observations	References
Calcium ionophores	various	inhibited by extracellular EGTA	(various)
Phorbol 12-myristate 13-acetate (PMA)	various	inhibited by intracellular calcium chelation, calphostin C, and cytochalasin D	(various)
KCl membrane depolarization	PC12 cells	rapid (seconds) and dependent upon extracellular calcium	(Lev et al., 1995)
	rat hippocampal slices	blocked by PKC inhibitors	(Siciliano et al., 1996)
Thrombin	human megkaryocytic cell line (CMK)	cells in suspension exhibited morphological changes after stimulation	(Avraham et al., 1995)
	human platelets	not dependent upon integrin GPIIb-IIIa but inhibited by cytochalasin D	(Raja et al., 1997)
Tumor necrosis factor α	PC12 and HL-60 cells	not dependent on calcium signals	(Tokiwa et al., 1996)
Osmolarity stress	HL-60 cells	dependent on calcium signals	(Tokiwa et al., 1996)
	smooth muscle cells	dependent upon cell adhesion costimuli	(Zheng et al., 1998)
Bradykinin	PC12 cells	not blocked by pertussis toxin	(Dikic et al., 1996)
Lysophosphatidic acid	PC12 cells	blocked by pertussis toxin but not dependent on extracellular calcium	(Dikic et al., 1996)
Angiotensin II and thapsigargin	rat liver epithelial cell lines (WB and GN4)	rapid (seconds) and correlated with JNK activation	(Yu et al., 1996; Li et al., 1997a,b)
T cell receptor activation	jurkat and H9 T cell lines	rapid within 1 min and promotes Fyn and Grb2 binding to Pyk2	(Ganju et al., 1997)
	mouse thymocytes	not blocked by extracellular EGTA and dependent upon Fyn PTK activity	(Qian et al., 1997)
Stem cell factor	CMK cells	mediated by a PKC-dependent pathway	(Hiregowdara et al., 1997)
Vascular endothelial growth factor (VEGF)	human Kaposi's sarcoma cells	rapid and transient activation	(Liu et al., 1997a)
Basic fibroblast growth factor (bFGF)	human bone marrow endothelial cells	rapid and transient activation	(Liu et al., 1997b)

Table 4 (*continued*)

Stimulus	Cell Type	Observations	References
Chemokine CCR5 ligands	DU6 $CD4^+$ T cells	rapid, transient and inhibited by pertussis toxin	(Davis et al., 1997)
		alternate-spliced Pyk2-H is activated by both antigen and chemokine stimuli	(Dikic and Schlessinger, 1998)
	transfected pre-B lymphoma cells	Pyk2-enhanced signaling to JNK and increased paxillin pTyr levels	(Ganju et al., 1998)
	human monocytes	cell adhesion and cytoskeletal integrity are required for maximal stimulation	(Li et al., 1998)
IgE receptor (FcεRI)	mast cell line RBL-2H3	dependent upon Syk PTK activation and dependent upon cell adhesion	(Okazaki et al., 1997)
PDGF	smooth muscle cells	slow kinetics and dependent on calcium signals	(Brinson et al., 1998)
Interleukin-2 (IL-2)	human peripheral blood lymphocytes	dependent on JAK PTK activation and involved in IL-2-induced cell growth	(Miyazaki et al., 1998)
Macrophage colony-stimulating Factor-1	human monocytic cell line THP1	association of p85 and PI-3 kinase activity with Pyk2	(Hatch et al., 1998)
Fluoroaluminate	mouse osteoblastic cells	stimulated association with Src PTKs	(Jeschke et al., 1998)
Muscarinic acetylcholine receptor	transfected HEK cells	carbachol-stimulated c-Src and Grb2 binding to Pyk2	(Felsch et al., 1998)

enhance the level of FAK tyrosine phosphorylation in cells (see Table 3). Some of these stimuli have been shown to affect FAK kinase activity directly whereas others may activate another PTK leading to the indirect elevation of FAK tyrosine phosphorylation. In many cases, such as those of G-protein-coupled agonists, the events leading to increased FAK tyrosine phosphorylation have been shown to be dependent upon the activity of the small GTP binding protein p21 Rho (Needham and Rozengurt, 1998). It is not known what pathways downstream of Rho are connected to FAK or whether the Rho effects are mediated through changes in the actin cytoskeleton. However, one consistent observation has emerged from these studies of non-integrin stimulated FAK tyrosine phosphorylation events. Namely, they are inhibited when cells are incubated in the presence of cytochalasin D, a toxin which binds to the barbed ends of actin filaments and prevents actin polymerization.

A variety of cellular stimuli can also affect the level of Pyk2 tyrosine phosphorylation in cells (Table 4). Many of these factors are the same as those shown to influence FAK tyrosine phosphorylation whereas others such as cell depolarization, osmolarity stress, or calcium ionophore treatment activate Pyk2 to a greater extent in cells which express both FAK and Pyk2 (Lev et al., 1995; Hiregowdara et al., 1997; Zheng et al., 1998). This difference between

FAK and Pyk2 has been hypothesized to be mediated by elevated intracellular calcium signals and thus Pyk2 is also known as CADTK, the calcium-dependent tyrosine kinase. However, it needs to be re-emphasized that these calcium-mediated signals can also stimulate changes in FAK tyrosine phosphorylation, albeit at lower levels than Pyk2 (Siciliano et al., 1996). Calcium signals leading to enhanced Pyk2 tyrosine phosphorylation can be generated by the influx of extracellular calcium through voltage-gated calcium channels (Lev et al., 1995; Tokiwa et al., 1996) or through calcium release from intracellular stores (Yu et al., 1996). Although the mechanistic connections between these calcium signals and enhanced Pyk2 tyrosine phosphorylation are not well defined, one possibility is that it may involve unique interactions of the Pyk2 N-terminal domain with putative calcium binding proteins.

3.2. FAK connections to β-integrin cytoplasmic domains

Increased tyrosine phosphorylation of intracellular proteins is one of the earliest responses stimulated by integrin receptor activation when cells contact matrix proteins such as fibronectin. Since transmembrane integrin receptors lack catalytic activity and null mutations in both the murine fibronectin (FN) and FAK genes result in similar early embryonic lethal phenotypes, it has been proposed that FAK activation is important for FN-mediated signal transduction events (for a review see Ilic et al., 1997). FAK colocalizes to sites of integrin-receptor clustering and attention has been focused on the linkage between integrin cytoplasmic domains and increased FAK tyrosine phosphorylation. Initial observations found that either direct clustering of β1 integrins could stimulate FAK tyrosine phosphorylation and that naturally occurring genetic mutations in residues of the β3 cytoplasmic domain disrupted the integrin linkage to increased FAK tyrosine phosphorylation events (for a review see (Schwartz et al., 1995). Our focus will be on β1 and β3 integrins, however evidence is emerging that α-integrin cytoplasmic domains also can affect the regulation of FAK tyrosine phosphorylation events as well (Leong et al., 1995).

Although integrins heterodimerize in α/β pairs, single subunit chimeras of the β1 or β3 cytoplasmic domain fused to the transmembrane-extracellular domain of non-integrin cell surface receptors can promote increased FAK tyrosine phosphorylation in mammalian cells (Akiyama et al., 1994; Lukashev et al., 1994). As shown in Fig. 4, the cytoplasmic domains of the β1 and β3 integrins share regions of sequence similarity and peptides derived from the juxtamembrane region of these cytoplasmic domains can bind to the N-terminal domain of FAK in vitro (Schaller et al., 1995). However, an alternate spliced version of the β3 tail region failed to stimulate FAK tyrosine phosphorylation (Akiyama et al., 1994) and the juxtamembrane region involved in FAK binding has been shown to be neither required nor sufficient to stimulate FAK tyrosine phosphorylation in intact cells (Tahiliani et al., 1997). It is the tail region of the β1 integrin cytoplasmic domain which is required for the recruitment of FAK to integrin clusters (Lewis and Schwartz, 1995) and an alternate spliced version of the β1 tail (termed β1B) does not stimulate FAK tyrosine phosphorylation and acts as a dominant-negative integrin to inhibit cell spreading and focal adhesion formation (Retta et al., 1998). Although evidence is emerging that phosphorylation of the conserved β1/β3 tyrosine residues is important for the regulation of integrin receptor ligand avidity (Blystone et al., 1997; Sakai et al., 1998), recent studies have shown that it may be a particular secondary structure and not

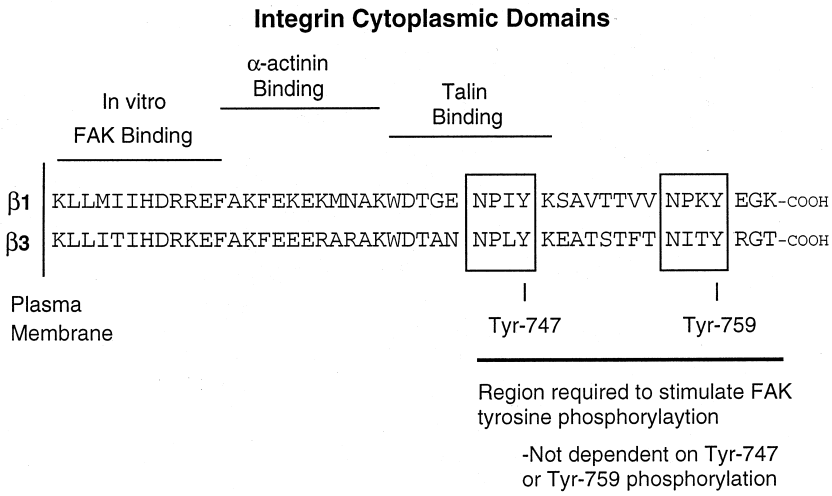

Fig. 4. β_1 and β_3 integrin cytoplasmic domain regions important for signaling events. Peptides derived from the juxtamembrane portion of the β-cytoplasmic tails can bind to the FAK N-terminal domain in vitro. The integrity of both C-terminal NXXY motifs, independently of their tyrosine phosphorylation, are important factors for integrin-mediated FAK association and activation. Other short stretches of the β-integrin cytoplasmic tails can bind proteins such as talin and α-actinin, and FAK interactions with these or other proteins may also facilitate the linkage between the integrin cytoplasmic domains and FAK activation.

the phosphorylation of the integrin cytoplasmic NXXY motifs (Fig. 4) which is important for β3-mediated stimulation of FAK tyrosine phosphorylation (Tahiliani et al., 1997; Schaffner-Reckinger et al., 1998).

Unfortunately, a clear model of the β-integrin connections to FAK has not emerged and events in addition to integrin activation may also be required to stimulate maximal FAK activation (Lyman et al., 1997). Significantly, the β3 and β1 integrin cytoplasmic tails also associate with other proteins such as talin and α-actinin (Fig. 4) and therefore integrin connections to FAK may be indirect and mediated by the binding of other integrin-associated proteins. In summary, these studies are consistent with some sort of direct regulation of FAK tyrosine phosphorylation by integrin receptor clustering, with the β subunit playing an important role. FAK activation at sites of integrin clustering may involve multiple protein interactions and be dependent on the summation of several low affinity binding events facilitated through high local protein concentrations after integrin receptor clustering.

3.3. Multiple mechanisms of FAK activation

As discussed above, both integrin and non-integrin stimuli can lead to increases in cellular FAK tyrosine phosphorylation levels. However, it should be emphasized that since FAK associates with and is a substrate for other PTKs, caution needs to be taken in making a direct correlation between FAK tyrosine phosphorylation levels and FAK kinase activation. This problem is most prevalent in serum-starved adherent fibroblasts where FAK can exist in a state

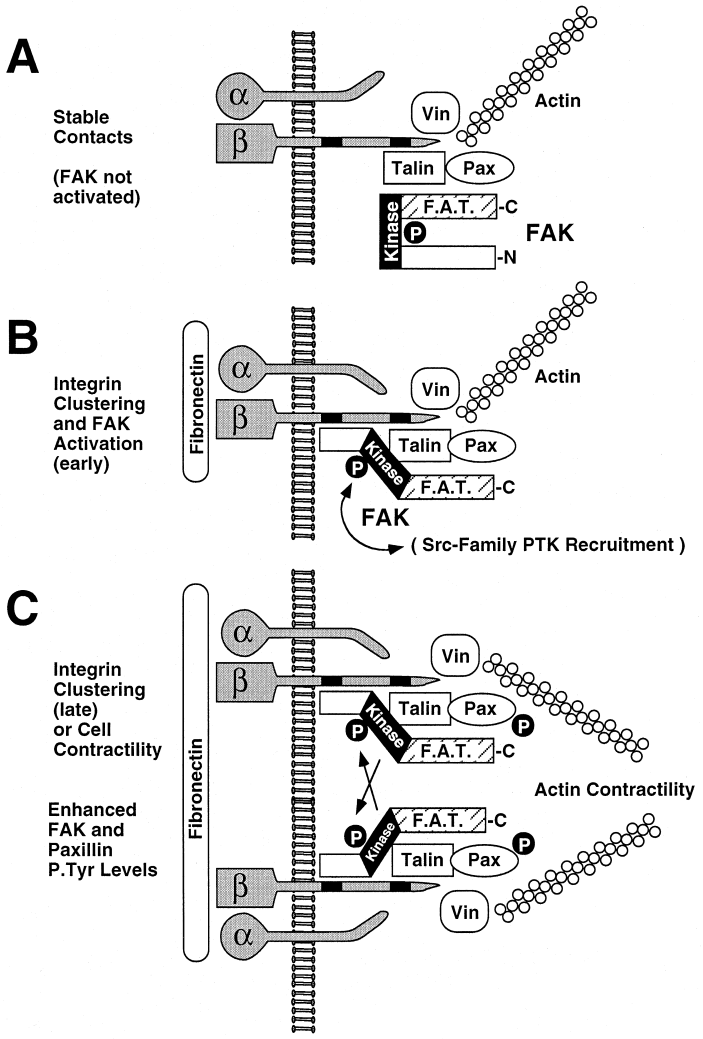

Fig. 5. Models of FAK activation at focal contact sites. A) In serum-starved adherent fibroblasts, FAK is localized at vinculin (Vin)-containing focal contact sites at the junction between the actin cytoskeleton and integrin receptors. Though FAK is tyrosine phosphorylated under these conditions, it is not associated with Src-family PTKs and exhibits only low in vitro kinase activity (probably due to an intramolecular steric interaction). B) Upon replating of the cells on extracellular matrix proteins such as fibronectin, FAK becomes activated either through conformational changes or as a result of clustering mediated by integrin clustering. FAK autophosphorylation at Tyr-397 under these conditions promotes the transient association of Src-family PTKs with FAK and the formation of a signaling complex. C) Intracellular actin contractility events can promote further integrin receptor clustering and FAK tyrosine phosphorylation. These events may promote the recruitment of additional proteins to focal contacts and lead to the transphosphorylation of proteins such as paxillin (Pax). In all cases, FAK activation is dependent upon the integrity of the actin cytoskeleton.

where the Tyr-397/Src SH2 binding site is highly tyrosine-phosphorylated, but under these conditions, Src-family PTKs are not significantly associated with FAK and FAK exhibits only low levels of in vitro kinase activity (Schlaepfer et al., 1998). This situation is depicted in a model presented in Fig. 5A where FAK is localized to focal contacts, tyrosine phosphorylated, but present in these integrin-containing structures in an inactive conformation. Since there are precedents for the negative regulation of kinases through intramolecular steric interactions, one speculative model is that FAK may be held in an inactive conformation though interactions between the N- and C-terminal domains. Support for this model comes from studies whereby either truncations (Schlaepfer and Hunter, 1996), insertions of alternate spliced sequences (Burgaya et al., 1997), or chimeric membrane-spanning fusion proteins of FAK (Chan et al., 1994) can generate FAK proteins with enhanced in vitro kinase activities.

How might integrin engagement with matrix proteins such as fibronectin regulate FAK PTK activity? By analogy to growth factor and cytokine receptors, ligand-induced integrin clustering may also activate associated PTKs such as FAK (Fig. 5B). Integrin clustering or integrin cytoplasmic domain conformation and alterations may promote the binding of the N-terminal domain of FAK to the tail of a β integrin or another ligand, thus exposing Tyr-397 (or pTyr-397). These FAK conformational changes could lead to the unmasking of the FAK active site and/or allowing the catalytic domain to adopt an active conformation. Integrin-dependent oligomerization of FAK would then permit transphosphorylation at Tyr-397, and this in turn would recruit Src-family PTKs, resulting in the phosphorylation of Tyr-576 and Tyr-577 in the FAK activation loop and full catalytic FAK activation. Experimental conditions used to initiate this type of FAK activation are those which involve the plating of adherent cell types onto matrix-coated dishes. FAK activation under these conditions is transient (10 to 80 min) and occurs during the initial stages of rapid cell spreading on the matrix-coated dishes. It is believed that this type of assay may mimic the integrin-initiated signaling events that may occur at the leading edge of a migrating cell. Again, it should be emphasized that it is difficult to distinguish enhanced FAK kinase activity from FAK-associated Src-family PTK activity under these conditions. However, recent studies have shown that the Src-specific PTK inhibitor, PP1, has minimal effects on FAK in vitro kinase activity (Rodriguez-Fernandez and Rozengurt, 1998) and may be useful in deciphering FAK-specific phosphorylation events.

Another point of emphasis should be placed upon the fact that transient integrin-stimulated FAK activation depends upon the integrity of the actin cytoskeleton and is inhibited in the presence of cytochalasin D. This connection to a cytoskeletal scaffold for FAK activation is most likely the key to understanding how FAK can become activated through p21 Rho-mediated cell contractility events (Chrzanowska-Wodnicka and Burridge, 1996) and may also represent a later stage (Fig. 5C) of replating-mediated FAK activation when actin stress fibers are formed and paxillin tyrosine phosphorylation is increased (Schlaepfer et al., 1998). This type of conformational FAK activation would be dependent upon actin contractility combined with cellular adhesion to a rigid substrate as in the case of a mature focal contact site. Further integrin clustering under these circumstances would be driven by forces within the cell. It is also possible that many of the non-integrin stimuli (Table 3) that affect FAK tyrosine phosphorylation levels may engage FAK activation in this manner. In summary, it is likely

that a combination of cellular localization, aggregation, and conformational changes act to regulate FAK activity and tyrosine phosphorylation events.

3.4. Integrin-mediated activation of Pyk2 and Src-family PTKs

Although initial reports suggested that Pyk2 was not activated by FN stimulation of rat fibroblasts (Sasaki et al., 1995), subsequent studies have shown that Pyk2 tyrosine phosphorylation is enhanced by integrin stimulation of B cells (Astier et al., 1997a), megakaryocytes (Li et al., 1996a), T lymphocytes (Ma et al., 1997), osteoclasts (Duong et al., 1998) and after exogenous expression in chicken embryo fibroblasts (Schaller and Sasaki, 1997). In addition, Pyk2 expression is elevated in the FAK-null fibroblasts and the FN-stimulated regulation of Pyk2 tyrosine phosphorylation in the FAK-null cells is very similar to that of FAK in normal fibroblasts (Sieg et al., 1998). Is Pyk2 kinase activity, like FAK, also regulated by integrin clustering? Again, direct analyses of Pyk2-associated in vitro kinase activity are complicated by the integrin-stimulated association of Src-family PTKs with Pyk2 (Duong et al., 1998; Sieg et al., 1998). However, in Src-deficient osteoclast-like cells, Pyk2 is not detectably tyrosine phosphorylated after β3-mediated integrin stimulation (Duong et al., 1998). In FAK-null fibroblasts, FN stimulation in the presence of the PP1 inhibitor of Src-family PTKs or after overexpression of $p50^{csk}$ (a negative regulator of Src-family PTKs), resulted in dramatically lower levels of FN-stimulated Pyk2 tyrosine phosphorylation (Sieg et al., 1998). As a comparison, inhibition of Src-family PTK activity in normal fibroblasts had only minor effects on FN-stimulated increases in FAK tyrosine phosphorylation (Schlaepfer et al., 1998; Sieg et al., 1998).

These results would suggest that elevated Pyk2 tyrosine phosphorylation may be downstream of integrin-stimulated Src-family PTK activation events and this may be due to the inefficient localization of Pyk2 to sites of integrin clustering. This model is consistent with increased Pyk2 tyrosine phosphorylation being dependent upon Fyn PTK activity after T cell receptor stimulation (Qian et al., 1997) and increased Pyk2 tyrosine phosphorylation being dependent upon Syk PTK activity after FcεRI receptor stimulation (Okazaki et al., 1997). Interestingly, whereas the tyrosine phosphorylation of wild type Pyk2 expressed in CHO cells was not regulated by FN plating, chimeric Pyk2 with the addition of FAK C-terminal domain exhibited enhanced tyrosine phosphorylation and localization to focal contacts sites upon FN stimulation of CHO cells (Zheng et al., 1998). These results reinforce the conclusion that FAK or forced Pyk2 localization to sites of integrin receptor clustering is an important factor in facilitating the direct activation of these PTKs.

Consistent with their importance for mediating integrin-stimulated signaling events in the FAK^- cells, Src-family PTKs can be independently activated with respect to Pyk2 and FAK after FN stimulation of cells (Schlaepfer et al., 1998; Wary et al., 1998). In unstimulated fibroblasts, Src-family PTKs such as c-Src and Fyn are held in an inactive state through an intramolecular association of the SH2 domain with a phosphorylated tyrosine residue in the C-terminal tail of c-Src/Fyn (site of $p50^{csk}$ phosphorylation) and by an interaction of the SH3 domain with the N-terminal lobe of the catalytic domain (Xu et al., 1997). Two possible initial Src-family PTK activation mechanisms are dephosphorylation of the $p50^{csk}$ phosphorylation site or the binding of the SH2 and/or SH3 domains to alternative ligands.

Src-family PTK activity is enhanced during the initial stages of adherent cell spreading on FN (Kaplan et al., 1995) and in cells deficient for $p50^{csk}$, c-Src is highly activated and constitutively localized to sites resembling focal contacts (Howell and Cooper, 1994). Dephosphorylation of pTyr-527 in the C-terminal tail of c-Src is an early event in integrin-stimulated c-Src activation (Kaplan et al., 1995) and in the FAK-null fibroblasts, $p50^{csk}$ overexpression inhibited FN-stimulated Src-family PTK activity (Sieg et al., 1998). Moreover, overexpressed Csk localizes to focal contact sites and inhibits cell spreading (Bergman et al., 1995). The identity of the integrin-stimulated PTP(s) that act on c-Src pTyr-527 are not known. However, the transmembrane protein-tyrosine phosphatase, PTPα, has been shown to promote the activation of both c-Src (den Hertog et al., 1993) and Fyn (Bhandari et al., 1998). In addition, PTPα-null fibroblasts exhibit decreased Src PTK kinase activity and reduced levels of FN-stimulated cell spreading and migration (J. Sap, personal communication).

When dephosphorylated in the C-terminal tail, the Src-family PTK SH2 domains would be free to bind other ligands such as FAK. Overexpression of FAK enhances the level of FN-stimulated Src-family PTK activity and this depends upon FAK Tyr-397 and FAK kinase activity (Schlaepfer and Hunter, 1997). Conformational c-Src activation is likely to involve the binding of the c-Src SH2 domain to FAK, via the FAK autophosphorylation site at pTyr-397. Overexpression of Efs/Sin, a $p130^{Cas}$-related adaptor protein and Src-family PTK interacting protein, also has been shown to enhance c-Src activity through both SH2 and SH3 domain-mediated interactions (Alexandropoulos and Baltimore, 1996). Another proposed connection between integrins and Src-family PTK activation events may involve the transmembrane protein, caveolin-1 (Wary et al., 1998). In this model, caveolin-1 is proposed to form a specific link between the transmembrane region of α integrins and Fyn (not c-Src) and upon α-integrin clustering, Fyn is activated through unknown mechanisms. This model is controversial for the fact that previous studies have shown that caveolin-1 association inhibits the activity of a number of different signaling proteins, including Src-famiiy PTKs (Li et al., 1996b). In summary, there may be multiple mechanisms linking integrins with Src-family PTK activation events and in the next section, we will focus on how these PTK activation events are connected to downstream signaling pathways.

4. Connections to downstream signaling pathways

4.1. FAK and Src-family PTKs link integrins to the activation of ERK2/mitogen-activated protein (MAP) kinase

In 1991, it was first observed that plating of fibroblasts onto FN-coated dishes rapidly (within 10 min) stimulated the tyrosine phosphorylation of a ~120 kDa protein (Guan et al., 1991) and this protein turned out to be FAK. In 1994, a number of labs reported that FN-stimulation of fibroblasts promoted signaling to and the activation of the ERK2/MAP kinase cascade (for a review see Schwartz et al., 1995). This observation now has been extended to a number of different cell types, however the role of FAK in these signaling events endures as a debated issue (for a review see Aplin et al., 1998). One of the first issues raised was that antibody-mediated clustering α_6 integrins led to increases in FAK tyrosine phosphorylation

without detectable ERK2 activation and that a cytoplasmic tail-less α_1 integrin chimera could promote ERK2 activation in the absence of detectable FAK tyrosine phosphorylation events (Wary et al., 1996). It has been subsequently shown that α_6 integrin stimulation can indeed facilitate ERK2 activation (Wei et al., 1998) and as will be discussed below, results obtained from FAK knockout fibroblasts show that there is more than one pathway leading to ERK2 activation and that FAK per se is not essential for FN-stimulated signaling to ERK2.

A second debatable issue raised is the time course of FN-stimulated FAK and ERK2 activation in fibroblasts allowed to spread on FN-coated dishes. One study using NIH3T3 fibroblasts showed that maximal FN-stimulated ERK2 activation occurred within 10 min which preceded cell spreading and detectable increases in FAK tyrosine phosphorylation (Lin et al., 1997). However, other studies using human foreskin fibroblasts and bead clustering experiments with the $\alpha_5\beta_1$ FN receptor, showed that FAK was rapidly recruited to aggregated integrin receptor complexes prior to the accumulation of other signaling proteins such as the Src-family PTKs (Miyamoto et al., 1995a). In this latter study, maximal ERK2 activation was observed ~30 min after stimulation of cells with FN-coated beads. Also, in a number of other cell lines stimulated by the replating method, increased FAK tyrosine phosphorylation occurred in parallel with FN-stimulated ERK2 activation (maximal between 20 and 40 min) and was correlated with the initiation of cell spreading (Zhu and Assoian, 1995; Schlaepfer et al., 1998; Short et al., 1998).

Given that stimulated tyrosine kinase activation events are important for integrin receptor signal generation, there is no disagreement as to whether initial integrin receptor clustering can generate signals to targets such as ERK2. The question is at what point does maximal integrin signaling occur, is FAK involved, and does integrin-stimulated ERK2 activation affect a particular biological response? Clearly these events may differ between cell types, however it has been shown that fibroblasts allowed to spread on FN-coated plates can generate signals leading to enhanced FAK tyrosine phosphorylation and extended ERK2 activation for up to 1–2 h (Zhu and Assoian, 1995; Schlaepfer et al., 1998). Although these ERK2 signals on their own are not sufficient to promote DNA synthesis (Zhu and Assoian, 1995), it has been shown that cell spreading on and remodeling of a FN matrix enhances cell growth (Sechler and Schwarzbauer, 1998; Sottile et al., 1998). Antibody-mediated clustering of integrins (Wary et al., 1996) or cell binding to fragments of FN which do not stimulate spreading, generate transient signals to ERK2 (maximal at 10 min) (Lin et al., 1997). This transient level of integrin activation is not sufficient to promote cell survival signals (Chen et al., 1997; Sottile et al., 1998). In adherent cell types, it is likely that there is a greater and extended level of integrin receptor activation in a spreading cell pulling and moving on the FN matrix substratum as opposed to a rounded cell with a small patch of membrane containing clustered integrin receptors.

It should be emphasized that one distinguishing feature between integrin receptor- and growth factor receptor-stimulated ERK2 activation events is that the integrin signals are dependent upon the integrity of the actin cytoskeleton whereas growth factor receptor PTKs can signal to ERK2 in the presence of cytochalasin D (Miyamoto et al., 1995a; Schlaepfer et al., 1998; Short et al., 1998). Integrin-stimulated ERK2 activation and cell spreading is connected to the coordinated engagement and rearrangement of the cellular actin cytoskeleton. Signaling models which propose that the transmembrane integrin receptor domains are

connected to caveolin-1-mediated clustering events within the plasma membrane do not fully address the critical role of the cytoskeleton in integrin-stimulated ERK2 activation events (Wary et al., 1998). In contrast, it is more likely that multiple inputs from the activation of tyrosine kinases, small GTP binding proteins (Renshaw et al., 1996; Clark et al., 1998), serine-threonine kinases such as protein kinase C (Schlaepfer et al., 1998), and lipid kinases such as phosphatidylinositol 3-kinase (King et al., 1997) act in a coordinated manner to regulate the extent and duration of integrin-stimulated ERK2 activation.

Our focus will be on the multiple connections linking the FAK and Src-family PTKs with the activation of ERK2. Maximal FAK kinase activity, ERK2 activation, and cell spreading occurred in the 20 to 60 min period following initial FN stimulation of mouse fibroblasts (Schlaepfer et al., 1998). As discussed in the preceding sections, maximal FN-stimulated FAK kinase activity is correlated with the binding of Src-family PTKs to FAK. Both activated FAK (Schlaepfer et al., 1998; Sharma, 1998) and c-Src (Kaplan et al., 1995) selectively partition to the 1% triton-insoluble fraction of cell lysates after FN stimulation of cells. This may explain why other studies have failed to visualize initial FAK tyrosine phosphorylation events after integrin stimulation of cells (Wary et al., 1996; Lin et al., 1997). Notably, the phosphorylation of FAK residues in addition to FAK Tyr-397, were enhanced in the Triton-insoluble cell fraction after FN stimulation (Schlaepfer et al., 1998) and one of these was the Grb2 binding site on FAK at Tyr-925. Grb2 binding to FAK and its association with the SOS GDP-GTP exchange factor for Ras is a direct link to the activation of the ERK2/MAP kinase pathway (Schlaepfer et al., 1994; Chen et al., 1998).

As shown in the signaling model (Fig. 6), Src-family PTK binding to FAK at FAK Tyr-397 after integrin-stimulation is required for the subsequent Src-mediated phosphorylation of FAK Tyr-925 (Schlaepfer and Hunter, 1996). However, Grb2 binding to FAK is not essential to promote integrin signaling to ERK2, since overexpression of either wild type or the Grb2 binding site mutant (Phe-925) of FAK can both enhance FN-stimulated activation of ERK2 (Schlaepfer and Hunter, 1997). In contrast, overexpression of the c-Src binding site mutant (Phe-397) of FAK inhibits FN-stimulated signaling to ERK2 in human 293T cells (Schlaepfer and Hunter, 1997). In addition, Phe-397 FAK expression inhibits integrin-mediated shear stress-stimulated signaling to the JNK/MAP kinase in bovine aortic endothelial cells (Li et al., 1997a,b) implying that FAK interactions with Src family PTKs are important for the initiation of signaling events. However, mutation of the FAK Tyr-397 also disrupts the binding of other SH2 domain-containing proteins that are involved in signaling events. For instance, the Phe-397 FAK mutation disrupts the binding of the p85 subunit of PI 3-kinase (Chen et al., 1996), and inhibition of PI 3-kinase activity reduces the level of integrin-stimulated signaling to ERK2 in COS-7 fibroblasts (King et al., 1997). In addition, the Shc adaptor protein SH2 domain also binds to the Tyr-397 site in FAK (Schlaepfer and Hunter, 1997; Schlaepfer et al., 1998) (see Fig. 6).

It is the tyrosine phosphorylation of the Shc adaptor protein which is a major integrin-stimulated signaling pathway to ERK2. Phosphorylation of Shc at Tyr-239 or Tyr-317 creates multiple Grb2 SH2 domain binding sites on Shc (van der Geer et al., 1996). FAK overexpression enhances Shc tyrosine phosphorylation and Grb2 binding to Shc after FN stimulation of 293T cells (Schlaepfer and Hunter, 1997). However, although FAK can bind to and directly phosphorylate Shc at Tyr-317, thereby promoting Grb2 binding and low-level

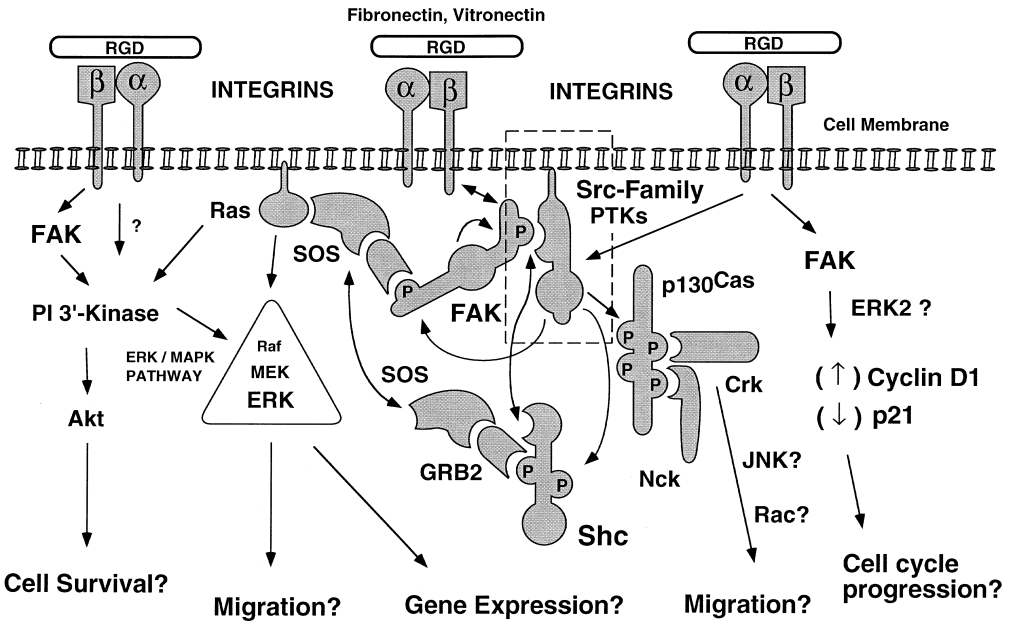

Fig. 6. Integrin-stimulated signaling events involving FAK. Integrin receptor engagement with ligands such as fibronectin can stimulate FAK autophosphorylation at Tyr-397 which creates a c-Src SH2 domain binding motif. The recruitment and subsequent activation of Src-family PTKs is an important event in FAK-mediated signaling and this FAK/Src complex is linked to multiple signaling pathways. Src-mediated phosphorylation of FAK at Tyr-925 creates a binding site for the SH2 domain of the Grb2 adaptor protein. Integrin activation of both FAK and Src-family PTKs can promote Shc tyrosine phosphorylation and Grb2 binding to Shc at Tyr-317. Grb2 binding to these signaling complexes can potentiate the translocation of the GDP/GTP exchange protein SOS to the plasma membrane leading to enhanced GTP exchange on Ras. The activation of the ERK/MAP kinase cascade is one target for the actions of GTP-bound Ras. Integrin-stimulated Ras may also activate PI 3′-kinase which may provide cell survival signals through the activation of targets such as the Akt serine-threonine kinase. PI 3′-kinase may also facilitate the coupling of Ras to the Raf-1 kinase leading to the enhanced activation of ERK/MAP kinase pathway. Src-family association with FAK at sites of integrin clustering can also potentiate the association and tyrosine phosphorylation of $p130^{Cas}$. Crk and Nck adaptor protein binding to tyrosine-phosphorylated $p130^{Cas}$ may lead to enhanced cell migration through the activation of pathways potentially involving the Rac GTPase or the JNK MAP kinase cascade. FAK may also link integrin activation to the regulation of cell cycle progression by influencing the levels of cyclin D1 and p21 inhibitor expression.

signaling to ERK2 (Schlaepfer et al., 1998), maximal integrin-stimulated signaling to ERK2 also requires the activation of Src-family PTKs (Schlaepfer et al., 1997). In Src-deficient fibroblasts, FN-stimulated Shc tyrosine phosphorylation and ERK2 activity are reduced compared to the same cells re-expressing mouse c-Src (Schlaepfer et al., 1997) and compared to normal fibroblasts (D. Schlaepfer, unpublished results). More dramatic reductions in FN-stimulated Shc tyrosine phosphorylation and ERK2 activation have been reported to occur in Fyn-deficient fibroblasts (Wary et al., 1998).

Significantly, it has been shown that Phe-317 Shc overexpression can block FN-stimulated and Fyn-mediated ERK2 activation in fibroblasts (Wary et al., 1998). In contrast, c-Src can phosphorylate both wild type (WT) and Phe-317 Shc to promote Grb2 binding (Schlaepfer et

al., 1998; van der Geer et al., 1996). In addition, Phe-317 Shc has been shown to be tyrosine phosphorylated after FN-stimulation in human 293T cells and its expression did not block ERK2 activation in these cells (Schlaepfer et al., 1998). This contrasts with studies which have detected only the FN-stimulated tyrosine phosphorylation of WT and not Phe-317 Shc in 293T cells (Wary et al., 1998). Since Src-family PTKs such as c-Src and Fyn have similar substrate specificities (Songyang et al., 1995), it is very difficult to reconcile these observations. Although these findings call in question whether one particular Grb2 binding site on Shc is essential for FN-stimulated signaling to ERK2, overexpression of SH3 domain-inactivated mutant of Grb2 that is unable to bind targets such as the SOS GDP-GTP exchange protein for Ras, inhibited both FN-stimulated and FAK-enhanced ERK2 activation (Schlaepfer et al., 1998). These latter results are consistent with a model whereby multiple Grb2-dependent interactions with FAK, Shc, and perhaps other yet-to-be-determined phosphorylated targets represent parallel signaling pathways upstream of Ras that cooperate to promote maximal FN-stimulated ERK2 activation (Fig. 6). One of these other FN-stimulated ERK2 signaling pathways may involve the protein-tyrosine phosphatase SHP-2 which associates with Grb2 and the tyrosine-phosphorylated membrane glycoprotein SHPS-1 (Tsuda et al., 1998).

4.2. Fibronectin-stimulated signaling to ERK2 in the absence of FAK

Although the involvement of FAK-related PTKs in matrix-mediated ERK2 activation events may be evolutionarily conserved down to trophozoites (Perez et al., 1996), accumulating evidence suggests that FAK is not essential for FN-stimulated ERK2 activation events. This was first observed upon overexpression of dominant-negative constructs such as FRNK which can reduce FAK tyrosine phosphorylation levels (Gilmore and Romer, 1996; Richardson et al., 1997a) but which do not block FN-stimulated ERK2 activation events (Lin et al., 1997; Wary et al., 1998). Although interpretations of results using dominant-negative constructs can be problematic, FAK-independent integrin-stimulated signaling events are likely to occur in hematopoietic cells (Gao et al., 1997; Hunter and Shimizu, 1997; Meng and Lowell, 1998) where FAK protein expression is quite low. Importantly, FAK-deficient (FAK^-) fibroblasts have been established and not surprisingly, the expression of Pyk2 is elevated in the FAK^- cells (Ueki et al., 1998). However unlike FAK, Pyk2 exhibits a perinuclear distribution and does not strongly colocalize with the elevated number of focal contact sites of the FAK^- cells (Sieg et al., 1998). Significantly, increased Pyk2 pTyr levels paralleled the time course of Grb2 binding to Shc and the activation of ERK2 after FN stimulation of the FAK^- cells (Sieg et al., 1998). Interestingly, Pyk2 autophosphorylation activity was not enhanced by FN stimulation of the FAK^- cells, instead, FN stimulation promoted the association of Pyk2 with active Src-family PTKs (Sieg et al., 1998). Overexpression of both wild type (WT) and kinase-inactive (Ala-457) but not the autophosphorylation site mutant (Phe-402) of Pyk2 enhanced endogenous FN-stimulated c-Src in vitro kinase activity in the FAK^- cells, but only WT Pyk2 overexpression enhanced the FN-stimulated activation of cotransfected ERK2 (Sieg et al., 1998).

These results support the hypothesis that in the absence of FAK, Pyk2 functions in the FN-stimulated signaling events leading to ERK2 activation (see Fig. 9). The Pyk2 overexpression results suggest that an SH2-mediated interaction of Src-family PTKs with Pyk2 at Tyr-402 may

lead to the Pyk2 kinase-independent enhancement of Src-family PTK activity in the FAK^- cells. In addition, these results support the conclusion that Pyk2 kinase activity and the phosphorylation of Pyk2 Tyr-402 are important components of the FN-stimulated signaling cascade to ERK2 in the FAK^- cells. The fact that the FN-stimulated regulation of Pyk2 tyrosine phosphorylation in the FAK^- cells (but not kinase activity) is very similar to FAK in normal fibroblasts suggests that increased Pyk2 expression and tyrosine phosphorylation in the FAK^- cells is a compensatory event for the loss of FAK. Interestingly, cytochalasin D treatment of the FAK^- cells inhibits both increased Pyk2 tyrosine phosphorylation and ERK2 activation (D. Schlaepfer, unpublished results) whereas cytochalasin D has been shown to stimulate Src-family PTK activity (Lock et al., 1998). This would suggest that Src-family PTKs cannot function singularly to promote FN-stimulated ERK2 activation. Instead, it is more likely that phosphorylated Pyk2 or the actin cytoskeleton are both functioning in combination with Src-family PTKs to facilitate integrin signaling to ERK2.

4.3. Negative regulators of integrin signaling events

In addition to the elevated expression of Pyk2 in the FAK^- cells, the level of FN-stimulated Src-family PTK activity was higher in the FAK^- compared to normal FAK^+ fibroblasts (Sieg et al., 1998). This was due in part to the elevated dephosphorylation of the murine c-Src Tyr-529 regulatory residue which is the site of $p50^{csk}$ phosphorylation. Significantly, overexpression of $p50^{csk}$ inhibited FN-stimulated Src-family PTK activity, Pyk2 tyrosine phosphorylation, Grb2 binding to Shc, and ERK2 activation in the FAK^- cells (Sieg et al., 1998). These results suggest that the integrin-stimulated increase in Pyk2 tyrosine phosphorylation in the FAK^- cells is indirect and occurred through the activation of Src-family PTKs. However, the molecular mechanism connecting the FN receptor integrins with c-Src and Fyn PTK activation in the FAK^- cells is at present not known.

In normal fibroblasts, $p50^{csk}$ overexpression did not block FN-stimulated increased FAK tyrosine phosphorylation and $p50^{csk}$ only partially reduced the amount of FN-stimulated Grb2 binding to Shc and signaling to ERK2 (Sieg et al., 1998). These results are consistent with the direct activation of FAK after integrin stimulation of cells and also support the hypothesis for the existence of Src-independent integrin-stimulated signaling pathways to ERK2 in normal fibroblasts (Schlaepfer et al., 1998). Therefore, the molecular mechanisms of integrin signaling to ERK2 should not be envisioned as a linear pathway (Wary et al., 1998), but instead these events can be considered as a networked interaction of a number of different signaling proteins. In this network, the positive elements (PTKs) are balanced by the activity of the negative regulators, $p50^{csk}$ and protein-tyrosine phosphatases (PTPs). One such PTP that acts as a negative regulator is PTP1B which binds to $p130^{Cas}$ (Liu et al., 1998). Overexpression of PTP1B resulted in lower levels of FN-stimulated FAK and $p130^{Cas}$ tyrosine phosphorylation and PTP1B inhibited FN but not growth factor-stimulated ERK2 activation (Liu et al., 1998). Other potential negative regulators of FN-stimulated signaling events include the FAK-associated PTP, PTEN (Tamura et al., 1998) and the $p130^{Cas}$ and paxillin-associated PTP, PTP-PEST (Garton et al., 1997; Shen et al., 1998). Interestingly, overexpression of a catalytically inactive form of SHP-2 inhibited FN-stimulated ERK2 activation implying that

this phosphatase may have a positive role in promoting integrin-stimulated signaling events (Tsuda et al., 1998).

4.4. FAK and Pyk2 signals through $p130^{Cas}$ tyrosine phosphorylation

In contrast to the pathways leading to FN-stimulated ERK2 activation, less is known about what signals are generated by phosphorylation of the $p130^{Cas}$ adaptor protein. In a number of different cell types, $p130^{Cas}$ or related adaptor proteins (Ishino et al., 1997; Law et al., 1998; Ohashi et al., 1998) have been shown to be substrates for either FAK (Tachibana et al., 1997), Pyk2 (Manie et al., 1997b), Src-family (Manie et al., 1997a; Sakai et al., 1997), or activated Abl (Law et al., 1996; Salgia et al., 1996b) PTKs. These tyrosine phosphorylation events have been shown to promote the SH2-dependent binding of either the Crk (Vuori et al., 1996), Crkl (de Jong et al., 1997) or Nck (Schlaepfer et al., 1997) SH3/SH2 domain-containing adaptor proteins to $p130^{Cas}$ (Fig. 6). Although both Crk and Nck have been shown to bind to the SOS GDP-GTP exchange factor that activates Ras and the ERK2/MAP kinase pathway, integrin-stimulated $p130^{Cas}$ tyrosine phosphorylation may not be tightly linked to ERK2 activation. In Src-deficient fibroblasts, where $p130^{Cas}$ is not appreciably tyrosine phosphorylated after integrin stimulation, stable expression of a kinase-inactive Src fragment (Src 1–298) in the Src-deficient cell background lead to strong FAK-mediated $p130^{Cas}$ tyrosine phosphorylation, Nck binding to $p130^{Cas}$, but relatively small increases in integrin-stimulated ERK2 activation compared to Src^- cells (Schlaepfer et al., 1997).

Recent results have suggested that the Nck adaptor protein may connect to the JNK/MAP kinase cascade through interactions with SH3 domain-associated protein-serine/threonine kinases such as PAK or NIK (Bokoch et al., 1996; Su et al., 1997). In addition, it has been shown that Crk can connect to the JNK/MAP kinase cascade through an interaction with the C3G guanine nucleotide exchange protein (Tanaka et al., 1997). Thus, the ability of integrins to facilitate JNK activation (Miyamoto et al., 1995b; Mainiero et al., 1997) may be mediated in part by either Nck or Crk adaptor binding to tyrosine phosphorylated $p130^{Cas}$.

4.5. Targets of integrin-stimulated MAP kinase activation events

The role of JNK/MAP kinase activation downstream of integrins is not known. For the ERK/MAP kinase pathway, signals can feed back to decrease integrin receptor ligand binding affinity and this may provide a negative feedback loop in the regulation of integrin-stimulated signaling events (Hughes et al., 1997). In addition, integrin-activated ERK2 can phosphorylate and regulate the activity of cytoplasmic proteins such as phospholipase A_2 (Clark and Hynes, 1996) and myosin light chain kinase (Klemke et al., 1997). Since FN-stimulation can promote the activation and nuclear translocation of ERK2 (Chen et al., 1994), ERK or JNK could promote transcription factor phosphorylation events and account for some of the known gene induction responses to integrin signals (Huhtala et al., 1995; Wary et al., 1996; Mainiero et al., 1997).

It has also been proposed that integrin-stimulated ERK2 activation is important for the cell adhesion-dependent regulation of cell cycle progression (Wary et al., 1996; Wary et al., 1998). One target for ERK2 activity is the regulation of cyclin-dependent kinase activity (for a review see Bottazzi and Assoian, 1997). Consistent with a role of FAK in the regulation of integrin

signaling to ERK2, a C-terminal domain FAK truncation mutant (ΔC13) can block cell cycle progression at the G1 phase through the inhibition of cyclin D1 expression and by promoting the increased expression of the p21 inhibitor of cyclin-dependent kinases (Zhao et al., 1998). This FAK mutant was not localized to focal contacts and its inhibitory activity was dependent on the integrity of the FAK Tyr-397 phosphorylation site. It has been hypothesized that this inhibitory FAK mutant competes with endogenous FAK in cells for the binding of signaling molecules such as the Src-family PTKs (Zhao et al., 1998). Other studies also support a role for FAK in the regulation of cell cycle progression as the treatment of cells with fragments of FN suppressed FAK tyrosine phosphorylation events and resulted in the delay of the G1 to S transition (Sechler and Schwarzbauer, 1998).

4.6. Role for FAK and p130^{Cas} in Src-mediated cell transformation events

The integrin stimulated increases in FAK and p130^{Cas} tyrosine phosphorylation events occur transiently in normal cells and are subject to many regulatory factors. In Src-transformed cells, both FAK and p130^{Cas} tyrosine phosphorylation levels are significantly elevated even in cells deprived of integrin-generated adhesion signals. Moreover, FAK was originally identified using a monoclonal antibody developed against a v-Src-associated protein (Kanner et al., 1990), and p130^{Cas} was originally characterized as a v-Crk-associated protein (Sakai et al., 1994). In v-Src transformed cells the Grb2 binding site on FAK at Tyr-925 is constitutively phosphorylated and the SOS GDP-GTP exchange protein for Ras can be detected in association with FAK isolated from v-Src transformed cells (Schlaepfer et al., 1994). Fibroblasts isolated from the p130^{Cas} knockout mouse and transfected with an activated Src allele show a reduced ability to grow in soft agar compared to p130^{Cas} re-expressing cells in combination with activated Src (Honda et al., 1998). In addition, the p130^{Cas}-deficient cells show resistance to Src-induced morphological changes. These findings support the hypothesis that the same FAK, Src, and p130^{Cas} signaling pathways which are transiently activated by integrin stimulation in normal cells may push cells toward a transformed phenotype when constitutively activated in Src-transformed cells (for a review see Brugge, 1998).

4.7. FAK signals important for cell survival

To grow in culture, many normal cells require both adhesion to ECM proteins and stimulation by serum or growth factors. One mechanism by which integrin receptors enhance cell survival is by counteracting signals for programmed cell death. When deprived of contact with ECM proteins, epithelial and endothelial cells rapidly lose viability and undergo 'anoikis' or apoptosis (for a review see Meredith and Schwartz, 1997). Integrin stimulation of both the Rho (Schwartz et al., 1996) and Ras/PI 3-kinase (Khwaja et al., 1997) signaling pathways can prevent this suspension-mediated cell death. In addition, it has been shown that endothelial cell spreading but not simple attachment to FN generates important cell survival signals (Chen et al., 1997). As we discussed in this review, FAK is activated during the process of FN-stimulated cell spreading in many cell types. Antisense (Xu et al., 1996) or antibody-mediated (Hungerford et al., 1996) inhibition of FAK has been shown to result in increased apoptosis of cultured cells.

Expression of the activated CD2-FAK chimera in epithelial cells confers resistance to anoikis while also promoting anchorage-independent cell growth in soft agar (Frisch et al., 1996). Significantly, both FAK kinase activity and the Tyr-397 autophosphorylation site were required for FAK-mediated survival signals, which implicates that signaling may occur through Src-family PTKs or other SH2-containing adaptor proteins such as the p85 subunit of PI 3-kinase (Chen et al., 1996). In addition, it has been shown that integrin and FAK-mediated survival signals suppress a p53-regulated apoptotic pathway (Ilic et al., 1998). In the absence of p53 or in the presence of dominant-negative p53 constructs, cells can survive even if they lack integrin-generated survival signals or even if they lack FAK (Ilic et al., 1998). A model emerging from these results is that p53 monitors survival signals from integrins/FAK in anchorage dependent cells and that during the processes of cell transformation, mutations in the p53 tumor suppressor gene will allow for anchorage-independent cell survival.

4.8. Early caspase cleavage of FAK during apoptosis

Since FAK has been shown to be involved in the processes of promoting cell survival, it is not surprising that cell stimuli such as Fas ligand and Apo-2L (Wen et al., 1997), UV irradiation (Widmann et al., 1998), or growth factor deprivation (Levkau et al., 1998) can promote the cleavage and degradation of FAK during the early stages of cell apoptosis. Interestingly, during c-Myc-induced apoptotic events, proteolysis of FAK is suppressed by FN stimulation of cells suggesting that FAK activation can prevent its own degradation (Crouch et al., 1996). The cleavage of FAK coincides with the loss of FAK from focal contacts, cell rounding, and the redistribution of FAK to characteristic apoptotic membrane protrusions (Levkau et al., 1998). In human endothelial cells, FAK is cleaved into two fragments of ~90 and ~35 kDa. The site of FAK cleavage was mapped to Asp-772 in the C-terminal domain of FAK (Gervais et al., 1998) and this site (Asp-Gin-Thr-Asp*) is recognized by the caspase-3 protease in vitro (Fig. 7). The significance of FAK cleavage at Asp-772 is that the remaining 90 kDa fragment of FAK containing the kinase domain would not contain the residues important for focal contact localization in cells. Overexpression of the predicted caspase-generated C-terminal FAK fragment (773–1053) promoted the dephosphorylation of endogenous FAK (Gervais et al., 1998) similar to the observed effects of FRNK overexpression on FAK pTyr levels in chicken embryo fibroblasts (Richardson and Parsons, 1996).

Although this Asp-772 caspase-3 cleavage site is conserved in human, chicken, and frog FAK cDNA sequences, it is not conserved in murine or rat FAK (Fig. 7). In NIH3T3 murine fibroblasts, staurosporine treatment led to the generation of a ~75 kDa FAK fragment and the caspase site of FAK cleavage was mapped to Asp-704 (Gervais et al., 1998). It is of interest that these two sites of caspase cleavage lie on either side of the first proline-rich domain in FAK which is the site of SH3 domain protein binding interactions. Not only does caspase cleavage of FAK separate the kinase domain from the C-terminal focal adhesion targeting sequences, these C-terminal FAK fragments may act to advance the pace of the cell death process. Exogenous expression of the FAK FAT domain (residues 841–1053) into primary fibroblasts promotes apoptosis in a p53-dependent manner which is suppressible by Bcl2 but not CrmA (Ilic et al., 1998). This fragment of FAK has been termed the 'killer' FAT domain

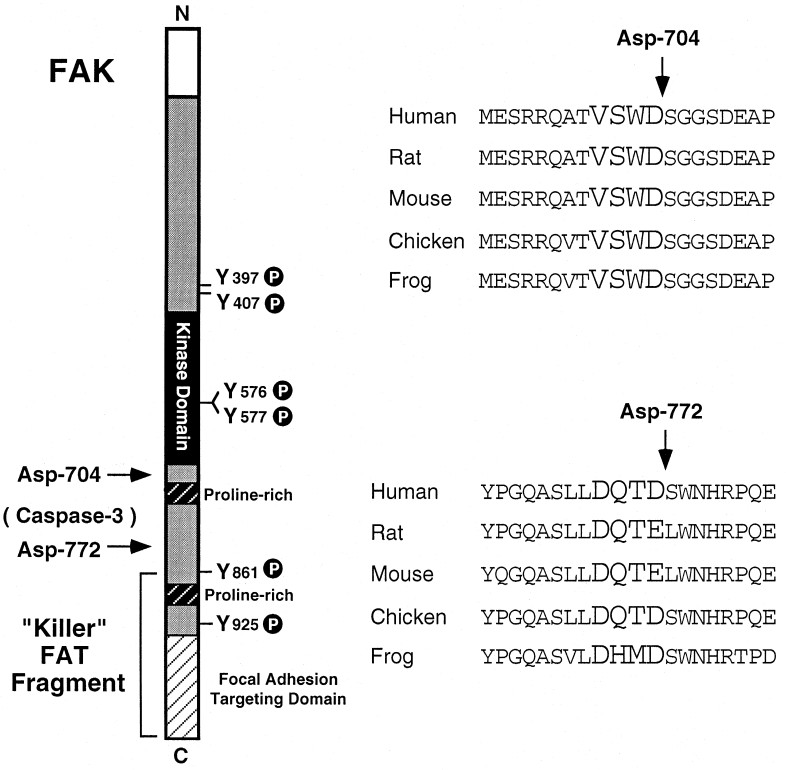

Fig. 7. Caspase cleavage of FAK. During the initial events of cell apoptosis, caspase-3 has been shown to cleave FAK at Asp-772 and Asp-704 of human and mouse FAK, respectively. The sequences recognized by caspase-3 are conserved over a broad range of species. Cleavage at these sites leads to the generation of a large N-terminal fragment comprising the kinase domain (~90 kDa for human FAK, ~75 kDa in the case of mouse FAK) and a smaller C-terminal fragment (~35 kDa or 50 kDa, respectively) that contains the focal adhesion targeting sequence and one or both proline-rich SH3-domain binding sites. Exogenous expression of the Asp-772 C-terminal FAK fragment can promote cell apoptosis and has been termed the 'killer' FAT fragment. This activity is dependent upon the integrity of the FAT domain.

(Fig. 7). Importantly, the pro-apoptotic activity of this FAK fragment is dependent upon its localization to focal contacts and the longer FRNK fragment of FAK (residues 691–1053) does not promote cell death (Ilic et al., 1998). These results would indicate that FAK residues such as the first proline-rich domain (712–718) may prevent the killer FAT-mediated cell death through the balanced activation of some survival pathway. Interestingly, it has been noted that murine NIH3T3 fibroblasts are relatively resistant to apoptosis (Frisch et al., 1996) and caspase cleavage of murine FAK at Asp-704 would not generate the killer FAT fragment of FAK. However, it remains to be determined whether exogenous expression of the FAK Asp-772 cleavage fragment will push NIH3T3 cells faster toward cell death or whether the larger Asp-704 fragment will delay these events.

5. Linkage to the cell migration machinery

5.1. FAK functions to enhance cell spreading and migration

Cell migration relies upon forces generated by the cell and the regulation of the adhesive strength between the integrin and cell substratum interface (for reviews see Lauffenburger and Horwitz, 1996; Sheetz et al., 1998). In contrast to recent progress in the understanding of intracellular signaling pathways involved in cell proliferation and survival, less is known about potential signal transduction pathways leading to cell migration. The mechanisms regulating integrin-stimulated cell migration are very complex and the activation of tyrosine kinases play an important role in these events (Klemke et al., 1994; Romer et al., 1994). Emerging evidence supports the role of both FAK and Src-family PTKs in these processes. Src-deficient fibroblasts exhibit slower kinetics of FN-initiated cell spreading (Kaplan et al., 1995) and FAK-deficient fibroblasts exhibit an enhanced number of cell substratum contacts and reduced rates of cell migration (Ilic et al., 1995b). Interestingly, vinculin knockout fibroblasts exhibit no apparent defects in focal adhesion formation, however these cells displayed elevated levels of FN-stimulated FAK tyrosine phosphorylation and increased rates of cell migration (Xu et al., 1998). In the epidermal-dermal junction of repairing burn wounds, FAK expression is elevated as detected by immunohistochemical staining (Gates et al., 1994). These sites correspond to the basal edge of only those keratinocytes that are actively migrating and rapidly proliferating in repairing burn wounds.

In cultured cells, FAK overexpression can increase FN-stimulated cell motility and this activity depends upon the integrity of the FAK Tyr-397 autophosphorylation site (Cary et al., 1996). In addition, overexpression of FRNK can inhibit endogenous FAK tyrosine phosphorylation, cell spreading on FN-coated dishes (Richardson et al., 1997a) and inhibit endothelial cell migration in cultured cell wound healing assays (Gilmore and Romer, 1996). Significantly, the FRNK-mediated inhibitory effects can be rescued by coexpression of active Src-family PTKs in chicken embryo fibroblasts (Richardson et al., 1997a). These results would suggest that FAK-recruitment of Src-family PTK to sites of integrin clustering is an important component of FAK-mediated cell migration events. This model is supported by results showing that FAK-mediated recruitment of Src to focal adhesions controls the turnover of these structures during cell migration (Fincham and Frame, 1998). Although there is much evidence in support of the importance of a FAK/Src complex in promoting cell migration, to date there is no consensus on what downstream signaling events from this complex are required for the initiation of cell migration. In the next section, we will review a number of different proposed models of FAK/Src function in promoting cell migration events.

5.2. FAK/Src complex connections to increased cell motility

One hypothesis is that FAK-mediated Src recruitment to focal contacts promotes yet-to-be-identified tyrosine phosphorylation events that led to the degradation of FAK and the remodeling of cell substratum contact sites (Fincham and Frame, 1998). This model is based upon the assumption that FAK is involved in the formation of focal contact structures and that its degradation allows for the turnover of these structures. Other work has focused on the

role of paxillin tyrosine phosphorylation in FAK/Src-mediated cell spreading events (Richardson et al., 1997a). Since paxillin has been shown to associate with PTP-PEST (Shen et al., 1998), it is possible that this connection may somehow regulate the cycles of phosphorylation and dephosphorylation important for the cell migration process. Another potential component linking the FAK/Src complex to cell migration is the activation of the ERK2/MAP kinase cascade. It has been shown that blockage of the ERK2 pathway at the level of the MEK kinase inhibits FN-stimulated Rat-1 fibroblast migration (Anand-Apte et al., 1997). In other cells, ERK2 inhibition also reduces cell motility and it has been shown that direct ERK2 phosphorylation of myosin light chain kinase promotes the enhanced phosphorylation of myosin light chains, an initial event necessary for contractility-mediated cell migration (Klemke et al., 1997). In agreement with the importance of ERK2 in cell migration, it has been shown that FAK and Ras were required for ERK2 activation and matrix metalloproteinase secretion in the processes of FN-stimulated ovarian cancer cell migration (Shibata et al., 1998). The increased secretion and activity of metalloproteinases is another point at which the adhesive strength between the integrins and cell substratum interface could be modulated to promote increased cell migration. These models connecting FAK and ERK2 to either the regulation of cytoskeletal architecture or gene expression events fit with the required role of Ras activity for endothelial cell migration (Fox et al., 1994).

However not all migratory signals may go through the Ras-ERK2 cascade. Recent evidence suggests that Src-associated signaling events may be able to promote epithelial cell scattering through the activation of signaling pathways distinct from those activated by Ras (Boyer et al., 1997). Namely, it has been proposed that Src can promote epithelial cell dispersion in the absence of gene expression through phosphorylation events that affect the organization of the cortical cytoskeleton (Boyer et al., 1997). Although, it is quite possible that mechanistic differences exist between epithelial cell scattering and integrin-stimulated cell migration of fibroblasts or endothelial cells, the FAK or Src-mediated phosphorylation of a target such as $p130^{Cas}$ may connect to a novel non-ERK2-dependent signaling pathway involved in promoting cell migration. This model is supported by the fact that the first proline-rich domain of FAK (binding site of $p130^{Cas}$) is important for FAK overexpression-mediated CHO cell migration and exogenous expression of the SH3 domain of $p130^{Cas}$ can inhibit FAK-mediated cell motility (Cary et al., 1998). Significantly, Crk adaptor protein binding to phosphorylated $p130^{Cas}$ has been shown to promote cell migration in a Rac-dependent and Ras-independent manner (Klemke et al., 1998). Although not completely defined, downstream components of this Rac-mediated migration pathway may involve PI-3-kinase (Keely et al., 1997; Shaw et al., 1997) and/or cytoskeletal-associated targets such as gelsolin (Azuma et al., 1998; Chellaiah et al., 1998).

5.3. Phenotype of the FAK knockout

The strongest evidence linking FAK with the cell migration machinery comes from the analyses of the FAK-deficient fibroblasts. Null mutations in either the murine FN (George et al., 1993) or FAK (Ilic et al., 1995a) genes lead to similar embryonic lethal phenotypes as a result of defective developmental gastrulation events. As shown in Fig. 8, FAK^- fibroblasts exhibit a rounded morphology and an elevated number of small vinculin-positive sites of cell-

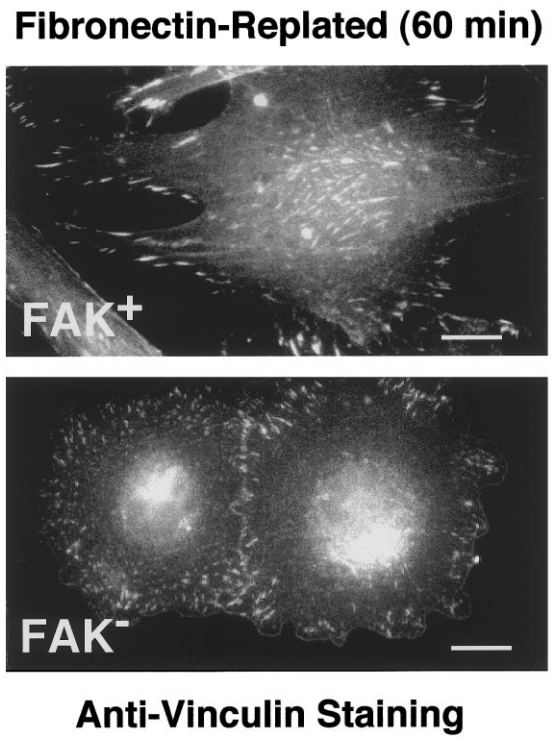

Fig. 8. Focal contact formation in FAK$^+$ and FAK$^-$ fibroblasts. FAK-expressing (FAK$^+$) and FAK-deficient (FAK$^-$) primary mouse embryo fibroblasts were allowed to adhere to a FN-coated surface for 60 minutes. Whereas the FAK$^+$ cells spread over the surface and acquired an elongated phenotype, the FAK$^-$ cells maintained a rounded cell morphology. Despite the different appearance, both cell types formed numerous focal contact sites which were visualized by indirect immunofluorescent staining of vinculin. The vinculin distribution was concentrated in the cell periphery and these spots seem to be more abundant, but smaller in the FAK$^-$ fibroblasts compared to the FAK$^+$ cells. The scale bar is ~10 μm.

substratum contact compared to normal FAK-containing (FAK$^+$) fibroblasts. Since the FAK$^-$ fibroblasts exhibit reduced rates of cell motility (Ilic et al., 1995a), it is likely that FAK-mediated tyrosine phosphorylation events are involved in the turnover of these contact sites during the initial stages of cell spreading and migration. In addition to the FAK knockout results, experiments with chimeric mice made from β_1 integrin-null stem cells have shown that β_1-initiated signals in the context of a developing mouse are also important for cell migration events (Hirsch et al., 1996). One interpretation of these combined results is that FN may stimulate cell migration through β_1 integrin-mediated signaling events involving FAK.

Similar to models of FAK function in signal transduction pathways, it is tempting to link increased FAK tyrosine phosphorylation with increased cell migration. Indeed, studies have shown that the PTEN tyrosine phosphatase is a negative regulator of FAK tyrosine phosphorylation and cell migration (Tamura et al., 1998). However, fibroblasts containing a N-terminally truncated form of the SHP2 tyrosine phosphatase (Yu et al., 1998) or fibroblasts deficient in the PTP-PEST (M. Tremblay, personal communication) contain elevated tyrosine phosphorylation levels of FAK, paxillin, and p130^{Cas} and these fibroblasts also exhibit

decreased rates of cell migration in vitro. In addition, there are striking similarities between the phenotypes of the SHP2 mutant and FAK knockout cells. They both exhibit a rounded morphology, increased focal contact formation, and a disorganized cytoskeletal architecture (Yu et al., 1998). In order to reconcile these observations, it may be necessary to view the phosphorylation of FAK, $p130^{Cas}$, and paxillin within the context of a dynamic process. Namely, that increased tyrosine phosphorylation per se may not be the critical factor for cell migration events, but instead, repeated cycles of phosphorylation and dephosphorylation may be essential for efficient coordinated cell movement. It is also possible that the inactive phosphatase blockage of cell migration occurs at a different stage of the cell migration process and that elevated FAK tyrosine phosphorylation events are unable to overcome this impediment.

5.4. Pyk2 does not fully function to enhance FAK$^-$ cell migration

As discussed above and shown in a summary model (Fig. 9), Pyk2 expression is elevated in the FAK$^-$ fibroblasts and Pyk2 functions in a compensatory manner in combination with Src-family PTKs to facilitate FN-stimulated ERK2 activation in the absence of FAK. Interestingly, whereas FAK primarily colocalizes with integrins at focal contacts, Pyk2 exhibited a punctate peri-nuclear distribution in the FAK$^-$ cells and only weakly costained with vinculin-containing focal contacts in the FAK$^-$ cell periphery (Sieg et al., 1998). These Pyk2 localization results are in agreement with the perinuclear distribution of Pyk2 in rat fibroblasts (Sasaki et al., 1995; Matsuya et al., 1998) and in rat smooth muscle cells (Zheng et al., 1998). With respect to signaling events necessary for cell migration, FN-stimulation of the FAK$^-$ cells promoted ERK2 activation to a level that was slightly less than that measured in the FN-stimulated FAK$^+$ cells (Sieg et al., 1998). Although it is possible that this level of ERK2 activity is below

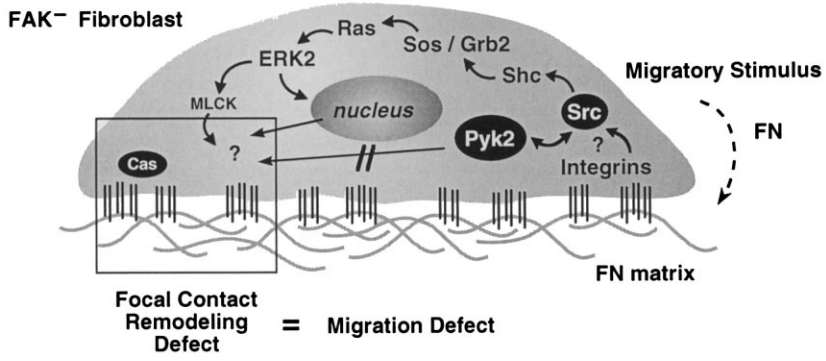

Fig. 9. Model of FN-stimulated signaling in the FAK$^-$ fibroblasts. In FAK$^-$ cells, Pyk2 expression is elevated, however Pyk2 exhibits a predominant perinuclear distribution and does not strongly localize to focal contact sites. FN stimulation of FAK$^-$ cells indirectly stimulates Pyk2 tyrosine phosphorylation through the activation of Src-family PTKs. Although the connections between integrins and Src-family PTKs in the FAK$^-$ cells have not been defined, Src-family PTK activity regulates FN-stimulated Shc tyrosine phosphorylation, Grb2 binding to Shc, and ERK2 activation in the FAK$^-$ cells. The FAK$^-$ cells exhibit an elevated number of focal contact sites and the FAK$^-$ cell migration defect may involve the inability to remodel these sites effectively in response to migratory stimuli.

some threshold for cell migration, both Pyk2 and FAK overexpression potentiated FN-stimulated ERK2 activity in the FAK^- cells whereas only FAK but not Pyk2 effectively promoted FAK^- cell migration (Sieg et al., 1998). These results suggest that the defect in FAK^- cell migration does not involve signaling linkages to ERK2.

The inability of the FAK^- cells to efficiently migrate also may not involve linkages to $p130^{Cas}$ since Pyk2 is associated with $p130^{Cas}$ in the FAK^- cells (Ueki et al., 1998), $p130^{Cas}$ is tyrosine phosphorylated after FN stimulation of the FAK^- cells (Vuori et al., 1996), and a fraction of $p130^{Cas}$ has been localized to FAK^- focal contact sites (Nakamoto et al., 1997). Since studies have shown that only a small fraction of overexpressed Pyk2 localizes to focal contact sites in chicken embryo fibroblasts (Schaller and Sasaki, 1997), the inability of Pyk2 to effectively promote FAK^- cell migration may be related to its observed perinuclear distribution in the FAK^- cells. Thus, the ability of FAK but not Pyk2 to effectively stimulate FAK^- cell migration to FN may not be related to differences in signaling pathways activated, but instead it may involve the preferential localization of FAK-associated proteins to sites of FN receptor clustering leading to the dynamic regulation of cell substratum contact sites.

5.5. FAK regulation of cell morphology

To date, many of the experimental results in support of a role for FAK in cell migration or in the regulation of focal contact turnover events have been based upon either overexpression analyses or conclusions extrapolated through the use of dominant-negative inhibitors. In contrast, the FAK^- fibroblasts can be considered as an excellent physiological model system to test the essential role of FAK in the regulation of fibroblast cell shape and migration events. As shown in Fig. 10, stable FAK re-expression in the FAK^- cells led to dramatic changes in cell morphology at both low and high cell densities. The FAK reexpressing fibroblasts reassume a spread morphology identical to that observed in normal FAK^+ cells in subconfluent densities and the FAK re-expressing cells form the characteristic fibrillar pattern of primary fibroblasts at confluent densities (Fig. 10). In addition to the cell shape changes, the FAK re-expressing cells exhibit elevated FN-stimulated migration rates compared to FAK^- cells and these migration rates are equivalent to that measured for normal fibroblasts (D. Sieg, unpublished results). FAK also exhibits a dynamic distribution pattern in the re-expressing cells showing costaining with actin at the leading edges in lamellipodial extensions in actively spreading cells and costaining with vinculin-containing focal contact sites at the ends of actin stress fiber extensions in stably-spread cells (C.R. Hauck, unpublished results). These results support the conclusion that FAK is directly functioning to promote migration and cell shape changes within fibroblasts.

What functional sites on FAK are important for these events? Transient expression of various FAK mutants in the FAK^- cells have shown that kinase activity, SH2 binding at Tyr-397, and SH3-mediated binding interactions at the first proline-rich motif (Pro-712/713) all are important for maximal FAK-enhanced cell migration events (D. Sieg, unpublished results). FAK autophosphorylation at Tyr-397 and the recruitment of SH2 domain containing proteins such as Src-family PTKs may be the first of several sequential events important for FAK-mediated cell migration. Secondary events such as the Src-mediated phosphorylation of proteins associated with FAK via SH3 domain-mediated binding interactions at the first FAK

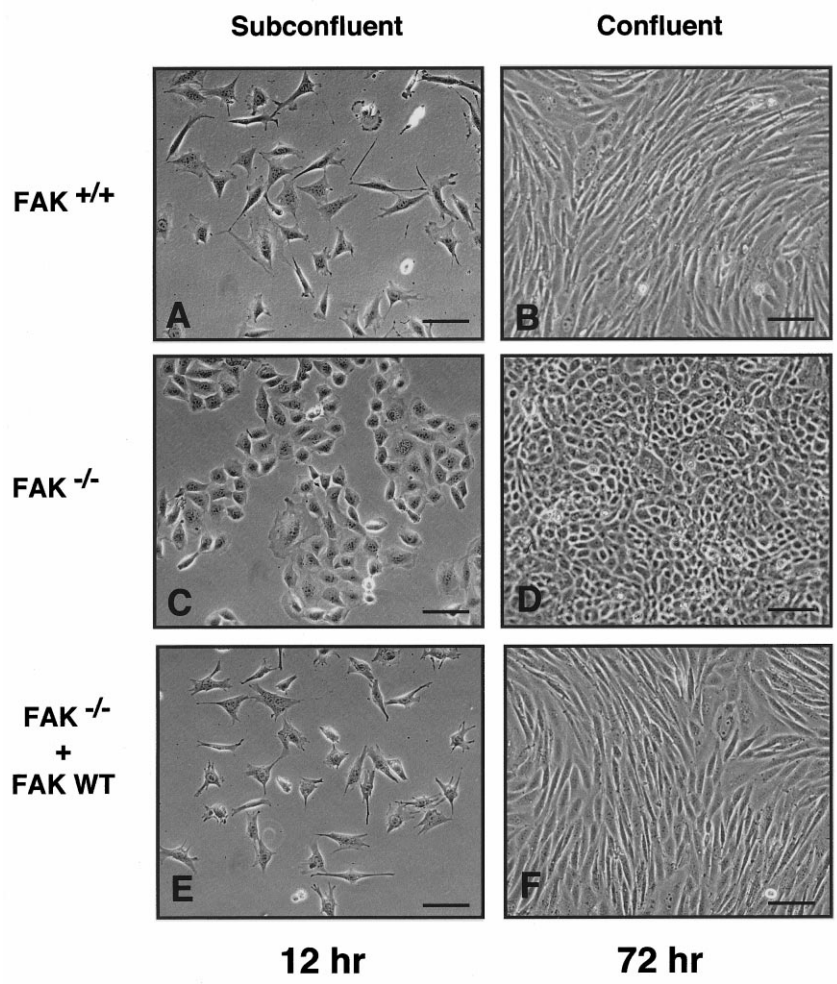

Fig. 10. Morphological reversion of FAK^- fibroblast cell shape by stable FAK re-expression. Normal mouse embryo fibroblasts ($FAK^{+/+}$, A and B), FAK-deficient cells ($FAK^{-/-}$, C and D), or FAK-deficient cells stably re-expressing wild type FAK ($FAK^{-/-}$ + FAK WT, E and F) were plated onto FN-coated dishes and phase contrast pictures of the cells were taken after 12 h (A, C and E) or 72 h (13, D and F). The FAK^- fibroblasts exhibit a round morphology at low and high cell densities (C and D). FAK re-expressing fibroblasts appear morphologically indistinguishable from normal primary mouse embryo fibroblasts at low cell densities (A and E) and exhibit a characteristic fibrillar pattern at confluent cell densities (B and F). The scale bars are ~200 μm.

proline-rich motif may initiate specific signals promoting cell migration. Therefore, FAK autophosphorylation creates a template for the binding of these or other signaling molecules, and through unknown mechanisms, promotes the turnover of focal contacts thereby allowing cell migration to proceed. As shown in the summary model (Fig. 11), FAK localization and recruitment of Src-family PTKs to sites of integrin clustering functions to promote maximal FN-stimulated signals to ERK2 through multiple Grb2-mediated pathways upstream of Ras. Integrin-mediated ERK2 activation can enhance actin-myosin contractility events which generates intracellular forces important for cell migration. In addition, the dynamic regulation

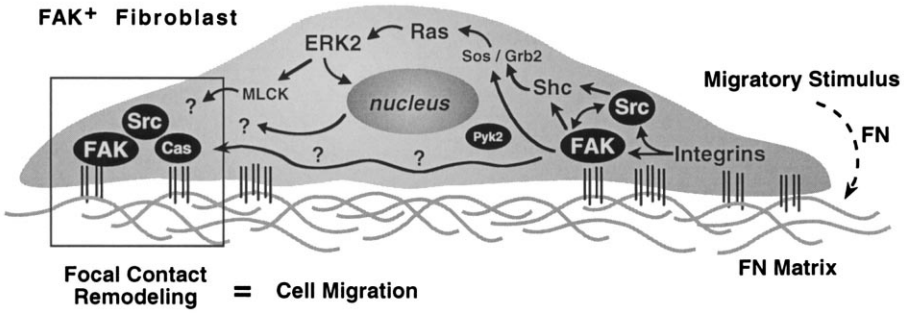

Fig. 11. Model of FN-stimulated signaling and cell migration in the FAK $^{+}$ fibroblasts. In FAK $^{+}$ fibroblasts, FAK localizes to sites of integrin clustering and can promote intracellular signal transduction events. The combined action of FAK and Src-family PTKs facilitates integrin signaling to the Ras-ERK2 cascade through multiple Grb2 binding interactions with FAK and Shc. Targets for activated ERK2 in the FAK $^{+}$ cells include myosin light chain kinase (MLCK) and transcription factors in the nucleus that can affect gene transcription events. The FAK-mediated recruitment of signaling proteins such as p130^{Cas} (Cas) to sites of integrin clustering and the dynamic regulation FAK-associated tyrosine phosphorylation events also promotes the remodeling of cell substratum contacts sites (through unknown mechanisms) leading to enhanced cell migration.

of tyrosine phosphorylation events at sites of integrin clustering also serves to modify the linkages between integrin receptors and the actin cytoskeleton. In this way, FAK can be envisioned to function at the junction of both FN-stimulated signaling and cell migration events.

5.6. FAK overexpression in metastatic human tumors

In addition to its critical role in embryonic development and in fibroblast cell motility events, FAK has been found to be overexpressed in a number of different human tumor samples. Whereas in proliferative benign tumors there was no observed increase in FAK mRNA levels (1/8) compared to normal tissue samples, a very high correlation was observed between increased FAK mRNA levels in colon carcinomas of primary invasive (17/20) or metastatic origin (15/15) (Weiner et al., 1993). The findings were extended to the elevation of FAK protein levels in colon and breast tumors that were in the process of becoming invasive or had already demonstrated their capacity for invasion and metastasis (Owens et al., 1995). High levels of FAK expression have also been found in preinvasive and invasive oral cancers (Kornberg, 1998). In addition, a direct correlation has been made between increased FAK expression and elevated cell motility in 6 different cell lines established from a primary human malignant melanoma (Akasaka et al., 1995). It is possible that FAK overexpression may be a common pathway for a variety of epithelial and mesenchymal tumor types to gain an invasive potential. In addition, these observations indicate that increased FAK expression levels in premalignant conditions may be useful as a marker for early cellular events leading to tumor metastasis and raise the possibility that FAK might be a rational therapeutic target for the disruption of the invasive process.

6. Final remarks

In this review, we have focused on the connections between integrins and FAK-mediated signaling events. One of the best analogies to describe the role of FAK in cells is that it functions as a 'scaffold' for the assembly of signaling complexes through the coordinated recruitment of other signaling and adaptor proteins. One important but yet undetermined aspect of FAK function is whether the proteins associated with FAK in promoting cell migration events are the same or different from those associated with FAK in delivering signals important for cell survival. It is also becoming clear that FAK can become activated by stimuli other than integrin receptor clustering events. This puts FAK in a position to modulate or contribute to signaling cascades activated by both integrins and other cell surface receptors. Future efforts to determine and decipher the interactions between FAK and other signaling proteins will continue to enhance our knowledge on the regulatory mechanisms of cell growth, shape, and migration.

Acknowledgements

We thank our colleagues who shared results prior to publication. David Schlaepfer is supported by grants from the National Cancer Institute (R29 CA75240), American Cancer Society (RPG-98-109-01-TBE), and the American Heart Association (9750682N). David Sieg is supported by a NRSA postdoctoral training grant (H1-07195-21) and Christof Hauck is supported by a fellowship from the Deutsche Forschungsgemeinschaft (HA-2856/1-1).

References

Abedi, H., Zachary, I., 1997 J. Biol. Chem. 272, 15442–15451.
Abedi, H., Dawes, K.E., Zachary, I., 1995 J. Biol. Chem. 270, 11367–11376.
Akasaka, T., van Leeuwen, R.L., Yoshinaga, I.G., Mihm, M.C., Jr., Byers, H.R., 1995 J. Invest. Dermatol. 105, 104–108.
Akiyama, S.K., Yamada, S.E., Yamada, K.M., LaFlamme, S.E., 1994 J. Biol. Chem. 269, 15961–15964.
Alexandropoulos, K., Baltimore, D., 1996 Genes Dev. 10, 1341–1355.
Anand-Apte, B., Zetter, B.R., Viswanathan, A., Qiu, R.G., Chen, J., Ruggieri, R., Symons, M., 1997 J. Biol. Chem. 272, 30688–30692.
Aplin, A.E., Howe, A., Alahari, S.K., Juliano, R.L., 1998 Pharmacol. Rev. 50, 197–263.
Aprikian, A.G., Tremblay, L., Han, K., Chevalier, S., 1997 Int. J. Cancer 72, 498–504.
Astier, A., Avraham, H., Manie, S.N., Groopman, J., Canty, T., Avraham, S., Freedman, A.S., 1997a J. Biol. Chem. 272, 228–232.
Astier, A., Manie, S.N., Avraham, H., Hirai, H., Law, S.F., Zhang, Y., Golemis, E.A., Fu, Y., Druker, B.J., Haghayeghi, N., Freedman, A.S., Avraham, S., 1997b J. Biol. Chem. 272, 19719–19724.
Avraham, S., London, R., Fu, Y., Ota, S., Hiregowdara, D., Li, J., Jiang, S., Pasztor, L.M., White, R.A., Groopman, J.E., Avraham, H., 1995 J. Biol. Chem. 270, 27742–27751.
Azuma, T., Witke, K., Stossel, T.P., Hartwig, J.H., Kwiatkowski, D.J., 1998 EMBO J. 17, 1362–1370.
Bacon, K.B., Szabo, M.C., Yssel, H., Bolen, J.B., Schall, T.J., 1996 J. Exp. Med. 184, 873–882.
Baron, V., Calleja, V., Ferrari, P., Alengrin, F., Van Obberghen, E., 1998 J. Biol. Chem. 273, 7162–7168.
Beggs, H.E., Baragona, S.C., Hemperly, J.J., Maness, P.F., 1997 J. Biol. Chem. 272, 8310–8319.

Bellis, S.L., Miller, J.T., Turner, C.E., 1995 J. Biol. Chem. 270, 17437–17441.
Bergman, M., Joukov, V., Virtanen, I., Alitalo, K., 1995 Mol. Cell. Biol. 15, 711–722.
Bhandari, V., Lim, K.L., Pallen, C.J., 1998 J. Biol. Chem. 273, 8691–8698.
Blystone, S.D., Williams, M.P., Slater, S.E., Brown, E.J., 1997 J. Biol. Chem. 272, 28757–28761.
Bokoch, G.M., Wang, Y., Bohl, B.P., Sells, M.A., Quilliam, L.A., Knaus, U.G., 1996 J. Biol. Chem. 271, 25746–25749.
Bottazzi, M.E., Assoian, R.K., 1997 Trends Cell Biol. 7, 348–352.
Boyer, B., Roche, S., Denoyelle, M., Thiery, J.P., 1997 EMBO J. 16, 5904–5913.
Brinson, A.E., Harding, T., Diliberto, P.A., He, Y., Li, X., Hunter, D., Herman, B., Earp, H.S., Graves, L.M., 1998 J. Biol. Chem. 273, 1711–1718.
Brown, M.C., Perrotta, J.A., Turner, C.E., 1996 J. Cell Biol. 135, 1109–1123.
Brugge, J.S., 1998 Nature Gen. 19, 309–311.
Burgaya, F., Girault, J.-A., 1996 Mol. Brain. Res. 37, 63–73.
Burgaya, F., Toutant, M., Stdier, J.-M., Costa, A., Le Bert, M., Gelman, M., Girault, J.-A., 1997 J. Biol. Chem. 272, 28720–28725.
Burridge, K., Chrzanowska-Wodnicka, M., 1996 Ann. Rev. Cell Dev. Biol. 12, 463–519.
Burridge, K., Chrzanowska-Wodnicka, M., Zhong, C., 1997 Trends Cell Biol. 7, 342–347.
Calalb, M., Polte, T., Hanks, S.K., 1995 Mol. Cell. Biol. 15, 954–963.
Calalb, M.B., Zhang, X., Polte, T.R., Hanks, S.K., 1996 Biochem. Biophys. Res. Comm. 228, 662–668.
Canbay, E., Norman, M., Kilic, E., Goffin, V., Zachary, I., 1997 Biochem. J. 324, 231–236.
Cary, L.A., Chang, J.F., Guan, J.-L., 1996 J. Cell Sci. 108, 1787–1794.
Cary, L.A., Han, D.C., Polte, T.R., Hanks, S.K., Guan, J.-L., 1998 J. Cell Biol. 140, 211–221.
Chan, P.Y., Kanner, S.B., Whitney, G., Aruffo, A., 1994 J. Biol. Chem. 269, 20567–20574.
Chellaiah, M., Fitzgerald, C., Alvarez, U., Hruska, K., 1998 J. Biol. Chem. 273, 11908–11916.
Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., Ingber, D.E., 1997 Science 276, 1425–1428.
Chen, H.C., Appeddu, P.A., Isoda, H., Guan, J.L., 1996 J. Biol. Chem. 271, 26329–26334.
Chen, H.C., Appeddu, P.A., Parsons, J.T., Hildebrand, J.D., Schaller, M.D., Guan, J.L., 1995 J. Biol. Chem. 270, 16995–16999.
Chen, H.C., Chan, P.C., Tang, M.J., Cheng, C.H., Chang, T.J., 1998 J. Biol. Chem. 273, 25777–25782.
Chen, Q., Kinch, M.S., Lin, T.H., Burridge, K., Juliano, R.L., 1994 J. Biol. Chem. 269, 26602–26605.
Chrzanowska-Wodnicka, M., Burridge, K., 1994 J. Cell Sci. 107, 3643–3654.
Chrzanowska-Wodnicka, M., Burridge, K., 1996 J. Cell Biol. 133, 1403–1415.
Clark, E.A., Hynes, R.O., 1996 J. Biol. Chem. 271, 14814–14818.
Clark, E.A., King, W.G., Brugge, J.S., Symons, M., Hynes, R.O., 1998 J. Cell Biol. 142, 573–586.
Crouch, D.H., Fincham, V.J., Frame, M.C., 1996 Oncogene 12, 2689–2696.
Davis, C.B., Dikic, I., Unutmaz, D., Hill, C.M., Arthos, J., Siani, M.A., Thompson, D.A., Schlessinger, J., Littman, D.R., 1997 J. Exp. Med. 186, 1793–1798.
de Jong, R., van Wijk, A., Haataja, L., Heisterkamp, N., Groffen, J., 1997 J. Biol. Chem. 272, 32649–32655.
den Hertog, J., Pals, C.E.G.M., Peppelenbosch, M.P., Tertoolen, L.G.J., de Last, S.W., Kruijer, W., 1993 EMBO J. 12, 3789–3798.
Derkinderen, P., Toutant, M., Burgaya, F., Le Bert, M., Siciliano, J.C., de Franciscis, V., Gelman, M., Girault, J.-A., 1996 Science 273, 1719–1722.
Dikic, I., Schlessinger, J., 1998 J. Biol. Chem. 273, 14301–14308.
Dikic, I., Tokiwa, G., Lev, S., Courtneidge, S.A., Schlessinger, J., 1996 Nature 383, 547–550.
Duong, L.T., Lakkakorpi, P.T., Nakamura, I., Machwate, M., Nagy, R.M., Rodan, G.A., 1998 J. Clin. Invest. 102, 881–892.
Eide, B.L., Turck, C.W., Escobedo, J.A., 1995 Mol. Cell. Biol. 15, 2819–2827.
Felsch, J.S., Cachero, T.G., Peralta, E.G., 1998 Proc. Natl. Acad. Sci. USA 95, 5051–5056.
Fincham, V.J., Frame, M.C., 1998 EMBO J. 17, 81–92.
Flinn, H.M., Ridley, A.J., 1996 J. Cell Sci. 109, 1133–1141.
Fox, P.L., Sa, G., Dobrowolski, S.F., Stacey, D.W., 1994 Oncogene 9, 3519–3526.
Frisch, S.M., Vuori, K., Ruoslahti, E., Chan-Hui, P.Y., 1996 J. Cell. Biol. 134, 793–799.
Ganju, R.K., Dutt, P., Wu, L., Newman, W., Avraham, H., Avraham, S., Groopman, J.E., 1998 Blood 91, 791–797.

Ganju, R.K., Hatch, W.C., Avraham, H., Ona, M.A., Druker, B., Avraham, S., Groopman, J.E., 1997 J. Exp. Med. 185, 1055–1063.
Gao, J., Zoller, K.E., Ginsberg, M.H., Brugge, J.S., Shattil, S.J., 1997 EMBO J. 16, 6414–6425.
Garcia, L.J., Rosado, J.A., Gonzalez, A., Jensen, R.T., 1997 Biochem. J. 327, 461–472.
Garton, A.J., Burnham, M.R., Bouton, A.H., Tonks, N.K., 1997 Oncogene 15, 877–885.
Gates, R.E., King, L.E.J., Hanks, S.K., Nanney, L.B., 1994 Cell Growth Diff. 5, 891–899.
George, E.L., Georges-Labouesse, E.N., Patel-King, R.S., Rayburn, H., Hynes, R.O., 1993 Development 119, 1079–1091.
Gervais, F.G., Thomberry, N.A., Ruffolo, S.C., Nicholson, D.W., Roy, S., 1998 J. Biol. Chem. 273, 17102–17108.
Giancotti, F.G., 1997 Curr. Opin. Cell Biol. 9, 691–700.
Gilmore, A.P., Romer, L.H., 1996 Mol. Biol. Cell 7, 1209–1224.
Guan, J.L., 1997 Int. J. Biochem. Cell Biol. 29, 1085–1096.
Guan, J.-L., Trevithick, J.E., Hynes, R.O., 1991 Cell Regul. 2, 951–964.
Hall, C.L., Wang, C., Lange, L.A., Turley, E.A., 1994 J. Cell Biol. 126, 575–588.
Haller, H., Kunzendorf, U., Sacherer, K., Lindschau, C., Walz, G., Distler, A., Luft, F.C., 1997 J. Immunol. 158, 1061–1067.
Hamasaki, K., Mimura, T., Furuya, H., Morino, N., Yamazaki, T., Komuro, I., Yazaki, Y., Nojima, Y., 1995 Biochem. Biophys. Res. Commun. 212, 544–549.
Hamasaki, S., Mimura, T., Morino, N., Furuya, H., Nakamoto, T., Aizawa, S., Morimoto, C., Yazaki, Y., Hirai, H., Nojima, Y., 1996 Biochem. Biophys. Res. Comm. 222, 338–343.
Hamawy, M.M., Mergenhagen, S.E., Siraganian, R.P., 1993 J. Biol. Chem. 268, 6851–6854.
Hamawy, M.M., Swieter, M., Mergenhagen, S.E., Siraganian, R.P., 1997 J. Biol. Chem. 272, 30498–30503.
Hanks, S.K., Polte, T.R., 1997 Bioessays 19, 137–145.
Hanks, S.K., Calalb, M.B., Harper, M.C., Patel, S.K., 1992 Proc. Natl. Acad. Sci. USA 89, 8487–8491.
Harte, M.T., Hildebrand, J.D., Burnham, M.R., Bouton, A.H., Parsons, J.T., 1996 J Biol. Chem. 271, 13649–13655.
Hatch, W.C., Ganju, R.K., Hiregowdara, D., Avraham, S., Groopman, J.E., 1998 Blood 91, 3967–3973.
Hens, M.D., DeSimone, D.W., 1995 Dev. Biol. 170, 274–288.
Hildebrand, J.D., Schaller, M.D., Parsons, J.T., 1993 J. Cell Biol. 123, 993–1005.
Hildebrand, J.D., Schaller, M.D., Parsons, J.T., 1995 Mol. Biol. Cell 6, 637–647.
Hildebrand, J.D., Taylor, J.M., Parsons, J.T., 1996 Mol. Cell. Biol. 16, 3169–3178.
Hiregowdara, D., Avraham, H., Fu, Y., London, R., Avraham, S., 1997 J. Biol. Chem. 272, 10804–10810.
Hirsch, E., Iglesias, A., Potocnik, A.J., Hartmann, U., Fassler, R., 1996 Nature 380, 171–175.
Honda, H., Oda, H., Nakamoto, T., Honda, Z., Sakai, R., Suzuki, T., Saito, T., Nakamura, K., Nakao, K., Ishikawa, T., Katsuki, M., Yazaki, Y., Hirai, H., 1998 Nat. Genet. 19, 361–365.
Howell, B., Cooper, J.A., 1994 Mol. Cell. Biol. 14, 5402–5411.
Hughes, P.E., Renshaw, M.W., Pfaff, M., Forsyth, J., Keivens, V.M., Schwartz, M.A., Ginsberg, M.H., 1997 Cell 88, 521–530.
Huhtala, P., Humphries, M.J., McCarthy, J.B., Tremble, P.M., Werb, Z., Damsky, C.H., 1995 J. Cell Biol. 129, 867–879.
Hungerford, J.E., Compton, M.T., Matter, M.L., Hoffstrom, B.G., Otey, C.A., 1996 J. Cell Biol. 135, 1383–1390.
Hunter, A.J., Shimizu, Y., 1997 J. Immunol. 159, 4806–4814.
Ilic, D., Almeida, E.A., Schlaepfer, D.D., Dazin, P., Aizawa, S., Damsky, C.H., 1998. J. Cell Biol., 143, 547–560.
Ilic, D., Damsky, C.H., Yamamoto, T., 1997 J. Cell Sci. 110, 401–407.
Ilic, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., Yamamoto, T., Aizawa, S., 1995a Nature 377, 539–544.
Ilic, D., Furuta, Y., Suda, T., Atsumi, T., Fujimoto, J., Ikawa, Y., Yamamoto, T., Aizawa, S., 1995b Biochem. Biophys. Res. Commun. 209, 300–309.
Ishino, M., Ohba, T., Inazawa, J., Sasaki, H., Ariyama, Y., Sasaki, T., 1997 Oncogene 15, 1741–1745.
Jeschke, M., Standke, G.J.R., Scaronuscarona, M., 1998 J. Biol. Chem. 273, 11354–11361.
Kanner, S.B., Aruffo, A., Chan, P.Y., 1994 Proc. Natl. Acad. Sci. USA 91, 10484–10487.
Kanner, S.B., Reynolds, A.B., Vines, R.R., Parsons, J.T., 1990 Proc. Natl. Acad. Sci. USA 87, 3328–3332.
Kaplan, K.B., Swedlow, J.R., Morgan, D.O., Varmus, H.E., 1995 Genes Dev. 9, 1505–1517.

Keely, P.J., Westwick, J.K., Whitehead, I.P., Der, C.J., Parise, L.V., 1997 Nature 390, 632–636.
Kharbanda, S., Saleem, A., Yuan, Z., Emoto, Y., Prasad, K.S.V., Kufe, D., 1995 Proc. Natl. Acad. Sci. USA 92, 6132–6136.
Khwaja, A., Rodriguez-Viciana, P., Wennstrom, S., Warne, P.H., Downward, J., 1997 Cell 16, 2783–2793.
King, W.G., Mattaliano, M.D., Chan, T.O., Tsichlis, P.N., Brugge, J.S., 1997 Mol. Cell. Biol. 17, 4406–4418.
Klemke, R.L., Cai, S., Giannini, A.L., Gallagher, P.J., de Lanerolle, P., Cheresh, D.A., 1997 J. Cell Biol. 137, 481–492.
Klemke, R.L., Leng, J., Molander, R., Brooks, P.C., Vuori, K., Cheresh, D.A., 1998 J. Cell Biol. 140, 961–972.
Klemke, R.L., Yebra, M., Bayna, E.M., Cheresh, D.A., 1994 J. Cell Biol. 127, 859–866.
Kornberg, L.J., 1998 Head Neck 20, 634–639.
Lauffenburger, D.A., Horwitz, A.F., 1996 Cell 84, 359–369.
Law, D.A., Nannizzi-Alaimo, L., Phillips, D.R., 1996 J. Biol. Chem. 271, 10811–10815.
Law, S.F., Zhang, Y.Z., Klein-Szanto, A.J.P., Golemis, E.A., 1998 Mol. Cell. Biol. 18, 3540–3551.
Leeb-Lundberg, L.M.F., Song, X.H., Mathis, S.A., 1994 J. Biol. Chem. 269, 24328–24334.
Leong, L., Hughes, P.E., Schwartz, M.A., Ginsberg, M.H., Shattil, S.J., 1995 J. Cell Sci. 108, 3817–3825.
Lev, S., Moreno, H., Martinez, R., Canoll, P., Peles, E., Musacchio, J.M., Plowman, G.D., Rudy, B., Schlessinger, J., 1995 Nature 376, 737–745.
Leventhal, P.S., Shelden, E.A., Kim, B., Feldman, E.L., 1997 J. Biol. Chem. 272, 5214–5218.
Levkau, B., Herren, B., Koyama, H., Ross, R., Raines, E.W., 1998 J. Exp. Med. 187, 579–586.
Lewis, J.M., Schwartz, M.A., 1995 Mol. Bio. Cell 6, 151–160.
Li, X., Earp, H.S., 1997 J. Biol. Chem. 272, 14341–14348.
Li, J., Avraham, H., Rogers, R.A., Raja, S., Avraham, S., 1996a Blood 88, 417–428.
Li, S., Couet, J., Lisanti, M.P., 1996b J. Biol. Chem. 271, 29182–29190.
Li, S., Kim, M., Hu, Y.-L., Jalali, S., Schlaepfer, D.D., Hunter, T., Chien, S., Shyy, J.Y.-J., 1997a J. Biol. Chem. 272, 30455–30462.
Li, X., Hunter, D., Morris, J., Haskill, S.J., Earp, H.S., 1998 J. Biol. Chem. 273, 9361–9364.
Li, X., Yu, H., Graves, L.M., Earp, H.S., 1997b J. Biol. Chem. 272, 14996–15002.
Lin, T.H., Aplin, A.E., Shen, Y., Chen, Q., Schaller, M., Romer, L., Aukhil, I., Juliano, R.L., 1997 J. Cell Biol. 136, 1385–1395.
Lipsky, B.P., Beals, C.R., Staunton, D.E., 1998 J. Biol. Chem. 273, 11709–11713.
Liu, F., Sells, M.A., Chernoff, J., 1998 Curr. Biol. 8, 173–176.
Liu, Z.-Y., Ganju, R.K., Wang, J.-F., Ona, M.A., Hatch, W.C., Zheng, T., Avraham, S., Gill, P., Groopman, J.E., 1997a J. Clin. Invest. 99, 1798–1804.
Liu, Z.Y., Ganju, R.K., Wang, J.F., Schweitzer, K., Weksler, B., Avraham, S., Groopman, J.E., 1997b Blood 90, 2253–2259.
Lock, P., Abram, C.L., Gibson, T., Courtneidge, S.A., 1998 Embo. J. 17, 4346–4357.
Lukashev, M.E., Sheppard, D., Pytela, R., 1994 J. Biol. Chem. 269, 18311–18314.
Lyman, S., Gilmore, A., Burridge, K., Gidwitz, S., White, G.C., 1997 J. Biol. Chem. 272, 22538–22547.
Ma, E.A., Lou, O., Berg, N.N., Ostergaard, H.L., 1997 Eur. J. Immunol. 27, 329–335.
Mainiero, F., Murgia, C., Wary, K.K., Curatola, A.M., Pepe, A., Blumemberg, M., Westwick, J.K., Der, C.J., Giancotti, F.G., 1997 EMBO J. 16, 2365–2375.
Manie, S.E., Astier, A., Hagayeghi, N., Canty, T., Druker, B.J., Hirai, H., Freedman, A.S., 1997a J. Biol. Chem. 272, 15636–15641.
Manie, S.E., Beck, A.R.P., Astier, A., Law, S.F., Canty, T., Hirai, H., Druker, B.J., Avraham, H., Haghayeghi, N., Sattler, M., Salgia, R., Griffin, J.D., Golemis, E.A., Freedman, A.S., 1997b J. Biol. Chem. 272, 4230–4236.
Maroney, A.C., Lipfert, L., Forbes, M.E., Glicksman, M.A., Neff, N.T., Siman, R., Dionne, C.A., 1995 J. Neurochem. 64, 540–549.
Matsumoto, K., Matsumoto, K., Nakamura, T., Kramer, R.H., 1994 J. Biol. Chem. 269, 31807–31813.
Matsuya, M., Sasaki, H., Aoto, H., Mitaka, T., Nagura, K., Ohba, T., Ishino, M., Takahashi, S., Suzuki, R., Sasaki, T., 1998 J. Biol. Chem. 273, 1003–1014.
McCormack, S.J., Brazinski, S.E., Moore, J.L., Jr., Werness, B.A., Goldstein, D.J., 1997 Oncogene 15, 265–274.
Meng, F., Lowell, C.A., 1998 Embo J. 17, 4391–4403.
Meredith, J.E., Schwartz, M.A., 1997 Trends Cell Biol. 7, 146–150.

Miyamoto, S., Akiyama, S.K., Yamada, K.M., 1995a Science 267, 883–885.
Miyamoto, S., Teramoto, H., Coso, O.A., Gutkind, J.S., Burbelo, P.D., Akiyama, S.K., Yamada, K.M., 1995b J. Cell Biol. 131, 791–805.
Miyamoto, S., Teremoto, H., Gutkind, J.S., Yamada, K.M., 1996 J. Cell Biol. 135, 1633–1642.
Miyazaki, T., Takaoka, A., Nogueira, L., Dikic, I., Fujii, H., Tsujino, S., Mitani, Y., Maeda, M., Schlessinger, J., Taniguchi, T., 1998 Genes Dev. 12, 770–775.
Nakamoto, T., Sakai, R., Honda, H., Ogawa, S., Ueno, H., Suzuki, T., Aizawa, S., Yazaki, Y., Hirai, H., 1997 Mol. Cell. Biol. 17, 3884–3897.
Nakamoto, T., Sakai, R., Ozawa, K., Yazaki, Y., Hirai, H., 1996 J. Biol. Chem. 271, 8959–8965.
Naruse, K., Yamada, T., Sai, X.R., Hamaguchi, M., Sokabe, M., 1998 Oncogene 17, 455–463.
Needham, L.K., Rozengurt, E., 1998 J. Biol. Chem. 273, 14626–14632.
Ohashi, Y., Tachibana, K., Kamiguchi, K., Fujita, H., Morimoto, C., 1998 J. Biol. Chem. 273, 6446–6451.
Ohba, T., Ishino, M., Aoto, H., Sasaki, T., 1998 Biochem. J. 330, 1249–1254.
Okazaki, H., Zhang, J., Hamawy, M.M., Siraganian, R.P., 1997 J. Biol. Chem. 272, 32443–32447.
Owens, L.V., Xu, L.H., Craven, R.J., Dent, G.A., Weiner, T.M., Kornberg, L., Liu, E.T., Cance, W.G., 1995 Cancer Res. 55, 2752–2755.
Parsons, J.T., 1997 Curr. Opin. Cell Biol. 8, 146–152.
Parsons, J.T., Parsons, S.J., 1997 Curr. Opin. Cell Biol. 9, 187–192.
Perez, E., Munoz, M.L., Ortega, A., 1996 Exp. Parasitol. 82, 164–170.
Polte, T.R., Hanks, S.K., 1995 Proc. Natl. Acad. Sci. USA 92, 10678–10682.
Polte, T.R., Hanks, S.K., 1997 J. Biol. Chem. 272, 5501–5509.
Polte, T.R., Naftilan, A.J., Hanks, S.K., 1994 J. Cell. Biochem. 55, 106–119.
Qian, D., Lev, S., van Oers, N.S.C., Dikic, I., Schlessinger, J., Weiss, A., 1997 J. Exp. Med. 185, 1253–1259.
Raja, S., Avraham, S., Avraham, H., 1997 J. Biol. Chem. 272, 10941–10947.
Rankin, S., Rozengurt, E., 1994 J. Biol. Chem. 269, 704–710.
Rankin, S., Hooshmand-Rad, R., Claesson-Welsh, L., Rozengurt, E., 1996 J. Biol. Chem. 271, 7829–7834.
Rankin, S., Morii, N., Narumiya, S., Rozengurt, E., 1994 FEBS Lett. 354, 315–319.
Renshaw, M.W., Toksoz, D., Schwartz, M.A., 1996 J. Biol. Chem. 271, 21691–21694.
Retta, S.F., Balzac, F., Ferraris, P., Belkin, A.M., Fassler, R., Humphries, M.J., De Leo, G., Silengo, L., Tarone, G., 1998 Mol. Biol. Cell 9, 715–731.
Ribon, V., Herrera, R., Kay, B.K., Saltiel, A.R., 1998 J. Biol. Chem. 273, 4073–4080.
Richardson, A., Parsons, J.T., 1996 Nature 380, 538–540.
Richardson, A., Malik, R.K., Hildebrand, J.D., Parsons, J.T., 1997a Mol. Cell Biol. 17, 6906–6914.
Richardson, A., Shannon, J.D., Adams, R.B., Schaller, M.D., Parsons, J.T., 1997b Biochem. J. 324, 141–149.
Rodriguez-Fernandez, J.L., Rozengurt, E., 1998 J. Biol. Chem. 273, 19321–19328.
Romer, L.H., McLean, N., Turner, C.E., Burridge, K., 1994 Mol. Bio. Cell 5, 349–361.
Sakai, R., Iwamatsu, A., Hirano, N., Ogawa, S., Tanaka, T., Mano, H., Yazaki, Y., Hirai, H., 1994 EMBO J. 13, 3748–3756.
Sakai, R., Nakamoto, T., Ozawa, K., Aizawa, S., Hirai, H., 1997 Oncogene 14, 1419–1426.
Sakai, T., Zhang, Q., Fassler, R., Mosher, D.F., 1998 J. Cell Biol. 141, 527–538.
Salgia, R., Avraham, S., Pisick, E., Li, J.-L., Raja, S., Greenfield, E.A., Sattler, M., Avraham, H., Griffin, J.D., 1996a J. Biol. Chem. 271, 31222–31226.
Salgia, R., Pisick, E., Sattler, M., Li, J.-L., Uemura, N., Wong, W.-K., Burky, S.A., Hirai, H., Chen, L.B., Griffin, J.D., 1996b J. Biol. Chem. 271, 25198–25203.
Sasaki, H., Nagura, K., Ishino, M., Tobioka, H., Kotani, K., Sasaki, T., 1995 J. Biol. Chem. 270, 21206–21219.
Sasaki, T., Hazeki, K., Hazeki, O., Ui, M., Katada, T., 1996 Biochem. J. 315, 1035–1040.
Sastry, S., Horwitz, A.F., 1996 Dev. Biol. 180, 455–467.
Schaffner-Reckinger, E., Gouon, V., Melchior, C., Plancon, S., Kieffer, N., 1998 J. Biol. Chem. 273, 12623–12632.
Schaller, M.D., Parsons, J.T., 1995 Mol. Cell. Biol. 15, 2635–2645.
Schaller, M.D., Sasaki, T., 1997 J. Biol. Chem. 272, 25319–25325.
Schaller, M.D., Borgman, C.A., Parsons, J.T., 1993 Mol. Cell. Biol. 13, 785–791.

Schaller, M.D., Borgman, C.A., Cobb, B.S., Vines, R.R., Reynolds, A.B., Parsons, J.T., 1992 Proc. Natl. Acad. Sci. USA 89, 5192–5196.
Schaller, M.D., Hildebrand, J.D., Shannon, J.D., Fox, J.W., Vines, R.R., Parsons, J.T., 1994 Mol. Cell. Biol. 14, 1680–1688.
Schaller, M.D., Otey, C.A., Hildebrand, J.D., Parsons, J.T., 1995 J. Cell Biol. 130, 1181–1187.
Schlaepfer, D.D., Hunter, T., 1996 Mol. Cell. Biol. 16, 5623–5633.
Schlaepfer, D.D., Hunter, T., 1997 J. Biol. Chem. 272, 13189–13195.
Schlaepfer, D.D., Hunter, T., 1998 Trends Cell Biol. 8, 151–157.
Schlaepfer, D.D., Broome, M.A., Hunter, T., 1997 Mol. Cell. Biol. 17, 1702–1713.
Schlaepfer, D.D., Hanks, S.K., Hunter, T., van der Geer, P., 1994 Nature 372, 786–791.
Schlaepfer, D.D., Jones, K.C., Hunter, T., 1998 Mol. Cell. Biol. 18, 2571–2585.
Schneller, M., Vuori, K., Ruoslahti, E., 1997 EMBO J. 16, 5600–5607.
Schwartz, M.A., Schaller, M.D., Ginsberg, M.H., 1995 Ann. Rev. Cell Dev. Biol. 11, 549–599.
Schwartz, M.A., Toksoz, D., Khosravi-Far, R., 1996 EMBO J. 15, 6525–6530.
Sechler, J.L., Schwarzbauer, J.E., 1998 J Biol. Chem. 273, 25533–25536.
Seufferlein, T., Rozengurt, E., 1995 J. Biol. Chem. 270, 24343–24351.
Sharma, S.V., 1998 Oncogene 17, 271–281.
Shaw, L.M., Rabinovitz, I., Wang, H.H., Toker, A., Mercurio, A.M., 1997 Cell 91, 949–960.
Sheetz, M.P., Felsenfeld, D.P., Galbraith, C.G., 1998 Trends Cell Biol. 8, 51–54.
Shen, Y., Schneider, G., Cloutier, J.F., Veillette, A., Schaller, M.D., 1998 J. Biol. Chem. 273, 6474–6481.
Shibata, K., Kikkawa, F., Nawa, A., Thant, A.A., Naruse, K., Mizutani, S., Hamaguchi, M., 1998 Cancer Res. 58, 900–903.
Short, S.M., Talbott, G.A., Juliano, R.L., 1998 Mol. Biol. Cell 9, 1969–1980.
Siciliano, J.C., Toutant, M., Derkinderen, P., Sasaki, T., Girault, J.A., 1996 J. Biol. Chem. 271, 28942–28946.
Sieg, D.J., Ilic, D., Jones, K.C., Damsky, C.H., Hunter, T., Schlaepfer, D.D., 1998. EMBO J., 17, 5933–5947.
Slack, B.E., 1998 Proc. Natl. Acad. Sci. USA 95, 7281–7286.
Soldi, R., Sanavio, F., Aglietta, M., Primo, L., Defilippi, P., Marchisio, P.C., Bussolino, F., 1996 Oncogene 13, 515–525.
Songyang, Z., Carraway, K.L., Eck, M.J., Harrison, S.C., Feldman, R.A., Mohammadi, M., Schlessinger, J., Hubbard, S.R., Smith, D.P., Eng, C., Lorenzo, M.J., Ponder, B.A.J., Mayer, B.J., Cantley, L.C., 1995 Nature 373, 536–539.
Sonoda, Y., Kasahara, T., Yokota-Aizu, E., Ueno, M., Watanabe, S., 1997 Biochem. Biophys. Res. Commun. 241, 769–774.
Sottile, J., Hocking, D.C., Swiatek, P.J., 1998 J. Cell Sci. 111, 2933–2943.
Su, Y.-C., Han, J., Xu, S., Cobb, M., Skolnik, E.Y., 1997 EMBO J. 16, 1279–1290.
Tachibana, K., Sato, T., D'Avirro, N., Morimoto, C., 1995 J. Exp. Med. 182, 1089–1100.
Tachibana, K., Urano, T., Fujita, H., Ohashi, Y., Kamiguchi, K., Iwata, S., Hirai, H., Morimoto, C., 1997 J. Biol. Chem. 272, 29083–29090.
Tahiliani, P.D., Singh, L., Auer, K.L., LaFlamme, S.E., 1997 J. Biol. Chem. 272, 7892–7898.
Takahira, H., Gotoh, A., Ritchie, A., Broxmeyer, H.E., 1997 Blood 89, 1574–1584.
Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R., Yamada, K.M., 1998 Science 280, 1614–1617.
Tanaka, S., Ouchi, T., Hanafusa, H., 1997 Proc. Natl. Acad. Sci. USA 94, 2356–2361.
Tang, H., Kerins, D.M., Hao, Q., Inagami, T., Vaughan, D.E., 1998 J. Biol. Chem. 273, 18268–18272.
Thomas, S.M., Soriano, P., Imamoto, A., 1995 Nature 376, 267–271.
Tokiwa, G., Dikic, I., Lev, S., Schlessinger, J., 1996 Science 273, 792–794.
Tsuda, M., Matozaki, T., Fukunaga, K., Fujioka, Y., Imamoto, A., Noguchi, T., Takada, T., Yamao, T., Takeda, H., Ochi, F., Yamamoto, T., Kasuga, M., 1998 J. Biol. Chem. 273, 13223–13229.
Ueki, K., Mimura, T., Nakamoto, T., Sasaki, T., Aizawa, S., Hirai, H., Yano, S., Naruse, T., Nojima, Y., 1998 FEBS Lett. 432, 197–201.
van der Geer, P., Wiley, S., Gish, G.D., Pawson, T., 1996 Curr. Biol. 6, 1435–1444.
Vuori, K., Hirai, H., Aizawa, S., Ruoslahti, E., 1996 Mol. Cell. Biol. 16, 2606–2613.
Wary, K.K., Mainiero, F., Isakoff, S.J., Marcantonio, E.E., Giancotti, F.G., 1996 Cell 87, 733–743.
Wary, K.K., Mariotti, A., Zurzolo, C., Giancotti, F.G., 1998 Cell 94, 625–634.

Wei, J., Shaw, L.M., Mercurio, A.M., 1998 J. Biol. Chem. 273, 5903–5907.
Weiner, T.M., Liu, E.T., Craven, R.J., Cance, W.G., 1993 Lancet 342, 1024–1025.
Wen, L.P., Fahrni, J.A., Troie, S., Guan, J.L., Orth, K., Rosen, G.D., 1997 J. Biol. Chem. 272, 26056–26061.
Whitney, G.S., Chan, P.Y., Blake, J., Cosand, W.L., Neubauer, M.G., Aruffo, A., Kanner, S.B., 1993 DNA Cell. Biol. 12, 823–830.
Widmann, C., Gibson, S., Johnson, G.L., 1998 J. Biol. Chem. 273, 7141–7147.
Wood, C.K., Turner, C.E., Jackson, P., Critchley, D.R., 1994 J. Cell. Sci. 107, 709–717.
Xiong, W.C., Macklem, M., Parsons, J.T., 1998 J. Cell. Sci. 111, 1981–1991.
Xu, L.H., Owens, L.V., Sturge, G.C., Yang, X., Liu, E.T., Craven, R.J., Cance, W.G., 1996 Cell Growth Diff. 7, 413–418.
Xu, W., Baribault, H., Adamson, E.D., 1998 Development 125, 327–337.
Xu, W., Harrison, S.C., Eck, M.J., 1997 Nature 385, 595–602.
Yu, D.H., Qu, C.K., Henegariu, O., Lu, X., Feng, G.S., 1998 J. Biol. Chem. 273, 21125–21131.
Yu, H., Li, X., Marchetto, G.S., Dy, R., Hunter, D., Calvo, B., Dawson, T., Wilm, M., Anderegg, R., Graves, L., Earp, H.S., 1996 J. Biol. Chem. 271, 29993–29998.
Zachary, I., Sinnett-Smith, J., Rozengurt, E., 1992 J. Biol. Chem. 267, 19031–19034.
Zent, R., Ailenberg, M., Silverman, M., 1998 Exp. Cell. Res. 240, 134–143.
Zhang, C., Qiu, H.E., Krafft, G.A., Klein, W.L., 1996a Neurosci. Lett. 211, 187–190.
Zhang, C., Qiu, H.E., Krafft, G.A., Klein, W.L., 1996b FEBS Lett. 386, 185–188.
Zhao, J.-H., Reiske, H., Guan, J.-L., 1998. J. Cell Biol. 143, 1997–2008.
Zheng, C., Xing, Z., Bian, Z.C., Guo, C., Akbay, A., Warner, L., Guan, J.-L., 1998 J. Biol. Chem. 273, 2384–2389.
Zhu, T., Goh, E.L., Lobie, P.E., 1998 J. Biol. Chem. 273, 10682–10689.
Zhu, X., Assoian, R.K., 1995 Mol. Biol. Cell 6, 273–282.