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Focal adhesion kinase: structure and signalling

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SUMMARY

Studies on the attachment and spreading of cells in culture have provided valuable insights into the mechanisms by which cells transmit information from the outside to the inside of the cell. This brief review considers recent information on the role of focal adhesion-associated protein tyrosine kinases in integrin-regulated cell signalling.

Key words: integrin, paxillin, focal adhesion kinase

INTRODUCTION

Cell adhesion and motility play a central role in a diverse array of cellular events, including cellular differentiation, development and cancer (Albelda and Buck, 1990; Hynes, 1992). An experimental entry into the study of the molecular events triggering cell adhesion comes from the analysis of cell attachment and spreading, a process that is driven by the formation of molecular structures called focal adhesions. Focal adhesions (also referred to as focal contacts) are points of close apposition between the cell membrane and the extracellular matrix (ECM), which is comprised of proteins such as collagen, fibronectin or vitronectin (Burridge et al., 1988; Luna and Hitt, 1992). The structural organization of focal adhesions is complex. Integrins, heterodimeric transmembrane receptors comprised of α and β subunits (Albelda and Buck, 1990; Hynes, 1992) bridge the cell membrane, the extracellular ligand-binding domains engaging the ECM on the outside of the cell and the short cytoplasmic tails interacting with the cytoplasmic cytoskeleton. Thus, integrins physically link the ECM to the cytoplasmic actin cytoskeletal network and may function to transmit signals from the extracellular matrix to the cytoplasm (Turner and Burridge, 1991; Schwartz, 1992). The actual linkage between integrin cytoplasmic tails and actin bundles or stress fibers appears to be mediated by an intricate structure comprised of focal adhesion-associated proteins. Considerable evidence suggests that at least two of these focal adhesion-associated proteins, talin and α-actinin, interact directly with the cytoplasmic domain of β integrin subunits (Tapley et al., 1989a; Otey et al., 1990). Both talin and α-actinin have also been shown to bind to the actin-binding protein vinculin, supporting the idea that protein-protein interactions are responsible in large part for the ordered structure of the focal adhesion (Burridge et al., 1988).

Several lines of evidence point to the importance of tyrosine phosphorylation in the formation and organization of focal adhesions. In cells transformed by the tyrosine kinase oncogene pp60^{src}, two focal adhesion-associated proteins, tensin and paxillin, are highly phosphorylated on tyrosine (Turner et al., 1990; Davis et al., 1991). In addition, in Src-transformed cells, other focal adhesion proteins, talin, vinculin and β$_1$ integrin subunits have been reported to be tyrosine phosphorylated, albeit at low stoichiometry (Sefton and Hunter, 1981; DeClue and Martin, 1987; Tapley et al., 1989b). Thus the dramatic alterations in cytoskeletal structure induced by Src transformation may be due in part to the tyrosine phosphorylation of focal adhesion-associated proteins. In normal cells, immunofluorescence analysis with antibodies to phosphotyrosine reveals prominent staining of focal adhesions, indicating the presence of significant levels of tyrosine phosphorylated proteins (Maher et al., 1985; Burridge et al., 1988). The attachment and spreading of rodent fibroblasts in culture leads to the increased tyrosine phosphorylation of both paxillin and tensin (Burridge et al., 1992; Bockholt and Burridge, 1993), while treatment of cells with inhibitors of protein tyrosine kinases blocks spreading of fibroblasts in culture (Burridge et al., 1992).

Studies from our own laboratory have led to the identification of a major pp60^{src} substrate, of M_r 125,000 (pp125), which localizes to focal adhesions of normal adherent chicken embryo cells (Schaller et al., 1992). The isolation and characterization of cDNA clones encoding pp125 revealed that pp125 was a novel protein tyrosine kinase, which we designated focal adhesion kinase, or pp125^{FAK}. Clues to the function of pp125^{FAK} come from numerous studies showing that the tyrosine phosphorylation of pp125^{FAK} is increased as a consequence of either the engagement of integrins with the extracellular matrix, for example the attachment and spreading of embryo fibroblasts onto a fibronectin matrix (Guan et al., 1991; Burridge et al., 1992; Schaller et al., 1993) or the cross-linking of surface integrins with integrin-specific antibodies (Kornberg et al., 1991, 1992). In addition, activation of fibrinogen-dependent platelet aggregation also induces tyrosine phospho-

rylation of pp125^{FAK} in vivo and an increase in pp125^{FAK} tyrosine kinase activity in vitro (Lipfert et al., 1992). Thus, the increased tyrosine phosphorylation of pp125^{FAK} appears to be closely coupled with binding and activation of cell surface integrin receptors. In this brief review, we consider recent experimental data indicating that pp125^{FAK} plays a role in regulating cellular events leading to the assembly of focal adhesions. In addition, we speculate on the possible role of pp125^{FAK} in cellular signalling via pathways that modulate or control cellular gene expression.

THE BASIC FAKS: FUNCTIONAL DOMAINS OF pp125^{FAK}

To date, pp125^{FAK} homologues have been identified in mouse, human and *Xenopus* (Hanks et al., 1992; Andre and Becker-Andre, 1993; Whitney et al., 1993; M. Hens and D. DeFranone, personal communication). The structure of the pp125^{FAK} in each of these species is highly conserved and is distinct from all other known protein tyrosine kinases. The catalytic domain exhibits most of the structural hallmarks of a typical tyrosine kinase, however, in the case of pp125^{FAK} the catalytic domain is flanked by two non-catalytic domains that exhibit little sequence similarity to other proteins (or gene products) present in the existing data bases (Fig. 1). FAK is expressed in most cell lines and tissues examined to date (Hanks et al., 1992; Andre and Becker-Andre, 1993; Turner et al., 1993). In some cells the carboxyl-terminal domain of pp125^{FAK} is expressed autonomously as a 41,000 M_r protein called FRNK - FAK-related non-kinase; Schaller et al., 1993). In avian cells and tissues, FRNK is encoded by an alternatively processed 2.4 kb mRNA (Schaller et al., 1993). A similar sized mRNA has been detected in human tissues, but it remains to be determined if this mRNA encodes p41^{FRNK}. A notable feature of pp125^{FAK} structure is the absence of SH2 and SH3 domains, domains present in the Src family of kinases and other cytoplasmic protein tyrosine kinases, as well as many protein components of receptor-directed signalling pathways (reviewed by Pawson and Gish, 1992). In most SH2-containing proteins, the SH2 domains appear to direct protein-protein interactions, promoting stable interactions with unique phosphotyrosine-containing peptide sequence motifs (Pawson and Gish, 1992; Songyang et al., 1993). SH3 domains also appear to mediate protein-protein interactions, directing binding to proteins with proline-rich peptide sequence motifs (Ren et al., 1993). The lack of SH2 and SH3 domains in pp125^{FAK} suggests that FAK may play a role in cell signalling distinct from previously characterized non-receptor protein tyrosine kinases. In addition, as we will discuss below, it is likely that the non-catalytic domains of pp125^{FAK} participate in directing the protein-protein interactions that regulate and control pp125^{FAK} function.

THE 'INS AND OUTS' OF THE FOCAL ADHESION: SEQUENCES THAT TARGET pp125^{FAK} TO FOCAL ADHESIONS

Little information is available as to how focal adhesion-associated proteins are directed to the existing or newly formed focal adhesions. The first clues as to how pp125^{FAK} is targeted to focal adhesions came from the analysis of a series of deletion mutations within the amino- and carboxyl-terminal non-catalytic domains (Hildebrand et al., 1993). Deletion of sequences between residues 853 and 1012 greatly diminished the translocation of retrovirally expressed FAK protein to the focal adhesions of chicken embryo cells grown in culture. In contrast, deletion of sequences within the amino-terminal non-catalytic domain or small deletions within a region of the C-terminal domain proximal to the kinase domain had no effect on the efficient localization of pp125^{FAK} to focal adhesions. These data indicate that residues 853 to 1012 comprise a targeting sequence (termed the 'focal adhesion targeting' or 'FAT' sequence) necessary for the efficient localization of pp125^{FAK} to focal adhesions (Fig. 2). Further evidence for the importance of the FAT sequence comes from studies analyzing hybrid proteins comprised of unmyristylated, cytosolic pp60^{src} fused to a polypeptide containing residues 853 to 1052 of pp125^{FAK}. Immunofluorescence staining of chicken embryo cells infected with a retrovirus encoding the Src-FAT fusion protein showed efficient localization of Src-FAT protein to focal contacts, providing additional evidence that FAT sequences direct the translocation of pp125^{FAK} to focal adhesions.

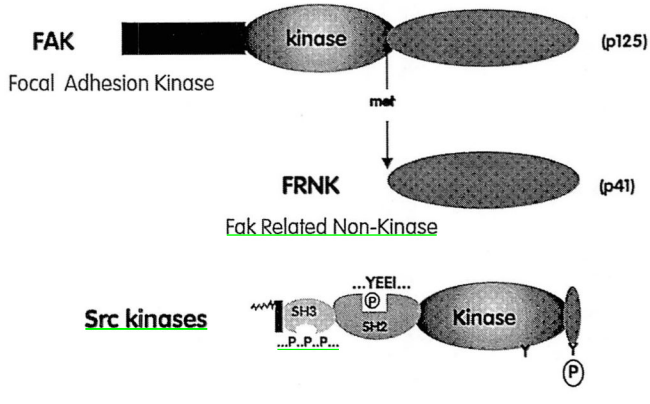

Fig. 1. A comparison of the structure of pp125^{FAK} and the Src family kinase, pp60^{src}. See text for detail.

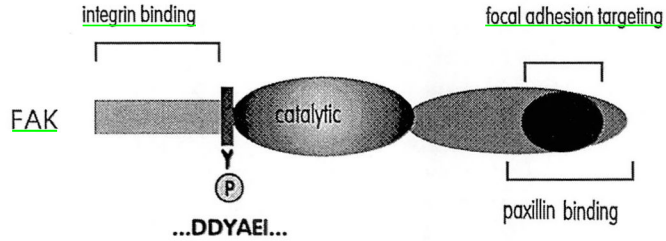

Fig. 2. Functional domains of the focal adhesion kinase, pp125^{FAK}. Genetic and biochemical studies described in the text have led to the identification of domains within pp125^{FAK}. Integrin binding is localized to the amino terminal domain, whereas interactions with the focal adhesion-associated protein, paxillin, and targeting to the focal adhesion is mediated by sequences present in the carboxyl-terminal domain.

MORE THAN FAT: THE CARBOXYL-TERMINAL NON-CATALYTIC DOMAIN DIRECTS THE BINDING OF pp125^{FAK} TO THE FOCAL ADHESION PROTEIN PAXILLIN

Recent evidence indicates that within the cell there is a direct interaction between pp125^{FAK} and the focal adhesion-associated protein paxillin. Immunoprecipitation of pp125^{FAK} from extracts of cells expressing wild-type pp125^{FAK} demonstrates the efficient co-immunoprecipitation of pp125^{FAK} and paxillin (M. Schaller, J. Hildebrand and J. T. Parsons, unpublished observations). The stable association of these two proteins was not observed when cells expressing FAK mutants lacking the FAT sequence or mutants lacking the C-terminal 11 residues of pp125^{FAK} were subjected to a similar analysis. Parallel in vitro experiments using glutathione S-transferase fused to FAK peptides containing sequences present in residues 687 to 1052 confirmed that paxillin could efficiently bind to sequences present in the carboxyl-terminal non-catalytic domain of pp125^{FAK} (J. Hildebrand, M. Schaller and J. T. Parsons, unpublished observations). Furthermore, the binding to paxillin appears to be direct, since isotopically labelled GST-FAK bound to paxillin immobilized on a filter matrix (a 'south-western' blot). A careful analysis of a series of GST fusion proteins containing deletions of residues within the carboxyl-terminal domains shows that paxillin binding was functionally distinct from sequences necessary for focal adhesion targeting, although sequences required for paxillin binding appear to overlap, in part, the sequences required for focal adhesion targeting (Fig. 2). These results provide evidence for a role for the carboxyl-terminal non-catalytic domain of pp125^{FAK} in both the localization of pp125^{FAK} to focal adhesions as well as directing the binding of pp125^{FAK} to a potential cellular substrate.

HOW DO INTEGRINS SIGNAL FAK: THE AMINO-TERMINAL NON-CATALYTIC DOMAIN DIRECTS THE BINDING OF pp125^{FAK} TO THE CYTOPLASMIC DOMAINS OF INTEGRINS

The cell adhesion-dependent activation of pp125^{FAK} tyrosine phosphorylation suggests that integrins may directly regulate, in some fashion, the activation of pp125^{FAK} kinase activity. Previous experiments by Otey et al. (1990) showed that the interactions of focal adhesion proteins and integrin cytoplasmic domains can be analyzed in vitro. The focal adhesion-associated protein, α-actinin, binds in vitro to synthetic peptides mimicking the 47 amino acid cytoplasmic domain of the β_1 integrins. A similar experimental approach reveals that pp125^{FAK} also binds efficiently to peptides mimicking the complete cytoplasmic domain of β_1 and β_3 integrins (M. Schaller, C. Otey and J. T. Parsons, unpublished observations). Further, analysis of pp125^{FAK} binding to a set of four overlapping peptides comprising the total cytoplasmic domain sequence of β_1 shows that pp125^{FAK} interacts preferentially with a peptide sequence representative of the first 13 residues adjacent to the transmembrane domain of β_1 (Fig. 3). Binding of pp125^{FAK} to peptide-containing beads can be blocked by preincubation with excess soluble peptide and pp125^{FAK} does not bind to beads containing a 'scrambled' sequence peptide. To determine where in pp125^{FAK} the integrin peptide-binding sequences resides, individual domains of pp125^{FAK} were expressed in *Escherichia coli* and used in the in vitro binding assay. Significant binding activity was observed with peptides derived from the amino-terminal non-catalytic domain, whereas no binding activity was observed with peptides derived from the carboxyl-terminal region of pp125^{FAK}. These data argue convincingly that pp125^{FAK} is capable of directly binding to integrin cytoplasmic domain sequences in vitro. Interestingly a comparison of the sequences of individual β cytoplasmic domains shows a a high degree of sequence conservation within sequences corresponding to the β_1 pp125^{FAK}-binding regions (Fig. 3). Whether such sequences direct the binding of pp125^{FAK} and integrins in vivo, whether pp125^{FAK} interacts with different β integrins via a conserved sequence motif, and how such interactions regulate pp125^{FAK} activity are issues under current investigation.

WHERE'S THE PHOSPHOTYROSINE? THE MAJOR AUTOPHOSPHORYLATION SITE OF pp125^{FAK} IS TYR^{397}, A HIGH AFFINITY BINDING SITE FOR pp60^{src} AND p59^{fyn}

In Src-transformed cells the tyrosine phosphorylation of pp125^{FAK} is increased several fold, an observation that lead to its original identification as a Src-substrate (Kanner et al., 1990). In these cells the majority (>80%) of pp125^{FAK} is stably associated with pp60^{src} (Cobb et al., 1994). Genetic experi-

		pp125^{FAK} binding
SP1	KLLMIIH**D**RR**E**FA	+++
SP2	FAKFEKEKMNAKW	–
SP3	KWDTGENPIYKSA	–
SP4	AVTT----VV-NPKYEGK	–
β1	KLLMIIH**D**RR**E**FAKFEKEKMNAKWDTGENPIYKSAVTT----VV-NPKYEGK	+++
β2	KALIHLS**D**LR**E**YRRFEKEKLKSQWNN-DNPIFKSATTT----VM-NPKFAES	NT
β3	KLLITIH**D**RK**E**FAKFEEERARAKWDTANNPIYKEATST----FT-NITYRGT	+++
β5	KLLVTIH**D**RR**E**FAKFQSERSRARYEMASNPIYRKPISTHTVDFTFNKSYNGTVD	NT
β6	KLLVSFH**D**RK**E**VAKFEAERSKAKWQTGTNPLYRGSTST----FK-NVTYKHREKQKVDLSTDC	NT
β7	RLSVEIY**D**RR**E**YSRFEKEQQQLNWKQDSNPIYKSAITTTI-----NPRFQEADSPTL	NT

Fig. 3. Comparison of the sequences of the cytoplasmic domains of β integrins. SP1-4 denote the sequences of four short peptides that together comprise the complete sequence of β_1 cytoplasmic domain. (+++) denotes significant binding of pp125^{FAK}, (–) denotes little detectable binding; NT, not tested.

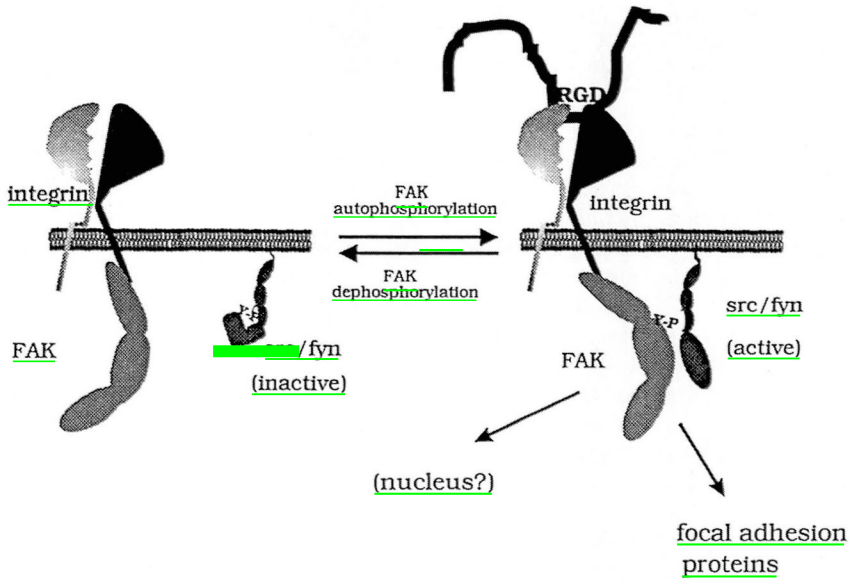

Fig. 4. Model for the integrin-dependent activation of pp125^{FAK} and Src family kinases. See text for discussion.

ments using retroviruses expressing mutants of Src, as well as in vitro analysis of pp60^{src}-pp125^{FAK} complex formation, clearly indicate that the assembly of stable FAK-Src complexes requires both the SH2 domain of pp60^{src} and the autophosphorylation site of pp125^{FAK}. Peptide mapping experiments, coupled with site-directed mutagenesis of potential phosphorylation sites have identified the major site of pp125^{FAK} autophosphorylation as Tyr^{397} (Fig. 2) (Schaller et al., 1994). Mutation of Tyr^{397} to Phe efficiently blocks pp60^{src}-pp125^{FAK} interactions in vivo and in vitro. Several features of the Tyr^{397} autophosphorylation site are of interest. The position of Tyr^{397} within pp125^{FAK} distinguishes it from other receptor and non-receptor tyrosine kinases. In most instances tyrosine kinase autophosphorylation occurs at a highly conserved tyrosine within the catalytic domain (equivalent to Tyr^{576} in pp125^{FAK}; Tyr^{416} in pp60^{src}), within a kinase insert domain, which is a nonconserved insert found within the catalytic domains of some receptor protein tyrosine kinases (but not in pp125^{FAK}) or distal to the catalytic domain at sites near the C terminus (a region found in many growth factor protein tyrosine kinases). Tyr^{397} resides immediately amino-terminal to the catalytic domain, in relative proximity to the ATP-binding site. In addition Tyr^{397} is embedded in the sequence DDYAEI, a sequence very similar to the consensus of a high-affinity Src SH2-binding peptide, YEEI (Songyang et al., 1993). These observations pose the possibility that in normal cells, integrin engagement may trigger autophosphorylation of pp125^{FAK}, which may, in turn, direct the translocation and concomitant activation of Src or other Src-like tyrosine kinases. Experimental support for such a model comes from the identification of pp125^{FAK}-p59^{fyn} complexes in extracts of normal adherent cultures of chicken embryo cells (Cobb et al., 1994).

SPECULATIONS, SPECULATIONS: A MODEL FOR pp125^{FAK} SIGNALLING

One important function of the integrins is to translate extracellular cues into cytoplasmic signals, a function that is presumably important for the biological activities of integrins. On the basis of the data summarized above we are led to speculate that integrin engagement with the extracellular matrix may result in either the direct clustering of pp125^{FAK}, allosteric changes in pp125^{FAK} or the stimulation of a regulator protein(s) that triggers pp125^{FAK} activation. A direct consequence of such an activation step is the autophosphorylation of pp125^{FAK} and generation of a high affinity binding site for Src and Src-family kinases. In normal cells the enzymatic activity of pp60^{src} and pp59^{fyn} is repressed through the action of a negative regulatory phosphorylation site at the C terminus of these kinases (Fig. 4). Phosphorylation of a highly conserved tyrosine within this region by a regulatory protein tyrosine kinase (Csk) is critical for down-regulation of catalytic activity (reviewed by Cooper and Howell, 1993). Current models for Src regulation suggest that the tyrosine phosphorylated C-terminal sequence binds in an intramolecular interaction to its own SH2 domain (Cantley et al., 1991; Cooper and Howell, 1993). The amino acid sequence flanking this C-terminal tyrosine does not resemble the consensus high affinity binding site and while a tyrosine phosphorylated C-terminal peptide can bind to the SH2 domain of pp60^{src}, it does so poorly (Songyang et al., 1993). In vitro, pp60^{src} can be enzymatically activated by incubation with a synthetic phosphopeptide containing the consensus, high affinity, Src SH2-binding site, presumably by binding more efficiently to the SH2 domain than the regulatory C-terminal peptide (Liu et al., 1993). It is intriguing to speculate that autophosphorylation of Tyr^{397} of pp125^{FAK} may create a high affinity binding site for pp60^{src} and pp59^{fyn} and that these kinases may bind to pp125^{FAK} resulting in the displacement of their C-termini from their SH2 domains. Thus, binding to pp125^{FAK} may be a mechanism by which pp60^{src} and pp59^{fyn} are enzymatically activated in addition to a mechanism for the recruitment of these kinases to a highly localized site within the cell.

What might be the consequences of the activation of pp125^{FAK} or the translocation-dependent activation of Src or Fyn? The adhesion-dependent increase in tyrosine phosphorylation of paxillin and tensin suggests that either or both of these

focal adhesion proteins may be direct substrates for pp125^{FAK} or the pp125^{FAK} -Src/Fyn complex. The association of pp125^{FAK} and paxillin is interesting in this context and is consistent with the idea that pp125^{FAK} may play a direct role in bringing paxillin into the tyrosine kinase complex. It is interesting to speculate that activation of both pp125^{FAK} and Src/Fyn may be necessary for catalyzing the formation of focal adhesion assembly and for initiating signals that may direct the activation of other cellular signalling pathways. For example it is well established that cell adhesion and spreading can trigger the expression of cellular genes (Damsky and Werb, 1992). The association of pp125^{FAK} with either Src or Fyn may be sufficient to activate cellular signalling pathways that in turn lead to the activation of cellular genes. What these pathways are and how they function to regulate adhesion-dependent phenomena remain to be elucidated.

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REFERENCES

Albelda, S. M. and Buck, C. A. (1990). Integrins and other cell adhesion molecules. *FASEB J.* **4**, 2868-2880.

Andre, E. and Becker-Andre, M. (1993). Expression of an N-terminally truncated form of human focal adhesion kinase in brain. *Biochem. Biophys. Res. Commun.* **190**, 140-146.

Bockholt, S. M. and Burridge, K. (1993). Cell spreading on extracellular-matrix proteins induces tyrosine phosphorylation of tensin. *J. Biol. Chem.* **268**, 14565-14567.

Burridge, K., Fath, K., Kelly, T., Nuckolls, G. and Turner, C. (1988). Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu. Rev. Cell Biol.* **4**, 487-525.

Burridge, K., Turner, C. E. and Romer, L. H. (1992). Tyrosine phosphorylation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. *J. Cell Biol.* **119**, 893-903.

Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. and Soltoff, S. (1991). Oncogenes and signal transduction. *Cell* **64**, 281-302.

Cobb, B. S., Schaller, M. D., Horng-Leu, T. and Parsons, J. T. (1994). Stable association of pp60^{src} and pp59^{fyn} with the focal adhesion-associated protein tyrosine kinase, pp125^{FAK}. *Mol. Cell. Biol.* **14**, 147-155.

Cooper, J. A. and Howell, B. (1993). The when and how of Src regulation. *Cell* **73**, 1051-1054.

Damsky, C. H. and Werb, Z. (1992). Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. *Curr. Opin. Cell. Biol.* **4**, 772-781.

Davis, S., Lu, M. L., Lo, S. H., Lin, S., Butler, J. A., Druker, B. J., Roberts, T. M., An, H., Chen, Q. and Chen, B. (1991). Presence of an SH2 domain in the actin-binding protein tensin. *Science* **252**, 712-715.

DeClue, J. E. and Martin, G. S. (1987). Phosphorylation of talin at tyrosine in Rous sarcoma virus-transformed cells. *Mol. Cell. Biol.* **7**, 371-378.

Guan, J.-L., Trevithick, J. E. and Hynes, R. O. (1991). Fibronectin/integrin interaction induces tyrosine phosphorylation of a 120-kDa protein. *Cell Regul.* **2**, 951-964.

Hanks, S. K., Calalb, M. B., Harper, M. C. and Patel, S. K. (1992). Focal adhesion protein tyrosine kinase phosphorylated in response to cell spreading on fibronectin. *Proc. Nat. Acad. Sci. USA* **89**, 8487-8489.

Hildebrand, J. D., Schaller, M. D. and Parsons, J. T. (1993). Identification of sequences required for the efficient localization of the focal adhesion kinase, pp125^{FAK}, to cellular focal adhesions. *J. Cell Biol.* **123**, 993-1005.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

Kanner, S. B., Reynolds, A. B., Vines, J. T. and Parsons, R. R. (1990). Monoclonal antibodies to tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases. *Proc. Nat. Acad. Sci. USA* **87**, 3328-3332.

Kornberg, L. J., Earp, H. S., Turner, C. E., Prockop, C. and Juliano, R. L. (1991). Signal transduction by integrins: increased protein tyrosine phosphorylation caused by clustering of β1 integrins. *Proc. Nat. Acad. Sci. USA* **88**, 8392-8396.

Kornberg, L., Earp, H. S., Parsons, J. T., Schaller, M. and Juliano, R. L. (1992). Cell adhesion or integrin clustering increases phosphorylation of a focal adhesion-associated tyrosine kinase. *J. Biol. Chem.* **267**, 23439-23442.

Lipfert, L., Haimovich, B., Schaller, M. D., Cobb, B. S., Parsons, J. T. and Brugge, S. (1992). Integrin-dependent phosphorylation and activation of the protein tyrosine kinase pp125^{FAK} in platelets. *J. Cell Biol.* **119**, 905-912.

Liu, X., Brodeur, S. R., Gish, G., Zhou, S., Cantley, L. C., Laudano, A. P. and Pawson, T. (1992). Regulation of c-src tyrosine kinase activity by the Src SH2 domain. *Oncogene* **8**, 1119-1126.

Luna, E. J. and Hitt, A. L. (1992). Cytoskeleton-plasma membrane interactions. *Science* **258**, 955.

Maher, P. A., Pasquale, E. B., Wang, J. Y. and Singer, S. J. (1985). Phosphotyrosine containing proteins are concentrated in focal adhesions and intercellular junctions in normal cells. *Proc. Nat. Acad. Sci. USA* **82**, 6576-6580.

Otey, C. A., Pavalko, F. M. and Burridge, K. (1990). An interaction between α-actinin and the β1 integrin subunit in vitro. *J. Cell Biol.* **111**, 721-729.

Pawson, T. and Gish, G. D. (1992). SH2 and SH3 domains: from structure to function. *Cell* **71**, 359-362.

Ren, R., Mayer, B. J., Cicchetti, P. and Baltimore, D. (1993). Identification of a ten-amino acid proline-rich SH3 binding site. *Science* **259**, 1157-1161.

Schaller, M. D., Borgman, C. A., Cobb, B. C., Reynolds, A. B. and Parsons, J. T. (1992). pp125^{FAK}, a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc. Nat. Acad. Sci. USA* **89**, 5192-5196.

Schaller, M. D., Borgman, C. A. and Parsons, J. T. (1993). Autonomous expression of a noncatalytic domain of the focal adhesion-associated protein tyrosine kinase pp125^{FAK}. *Mol. Cell. Biol.* **13**, 785-791.

Schaller, M. D., Hildebrand, J. D., Shannon, J. D., Fox, J. W., Vines, R. R. and Parsons, J. T. (1994). Autophosphorylation of the focal adhesion kinase, pp125^{FAK}, directs SH2-dependent binding of pp60^{src}. *Mol. Cell. Biol.* **14**, 1680-1688.

Schwartz, M. (1992). Transmembrane signalling by integrins. *Trends Cell Biol.* **2**, 304-308.

Sefton, B. and Hunter, T. (1981). Vinculin: A cytoskeletal target of the transforming protein of Rous sarcoma virus. *Cell* **24**, 165-174.

Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratnofsky, S., Lechleider, R. J., Neel, B. G., Birge, R. B., Fajardo, J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B. and Cantley, L. C. (1993). SH2 domains recognize specific phosphopeptide sequences. *Cell* **72**, 767-778.

Tapley, P., Horwitz, A., Buck, C., Burridge, K., Duggan, K., Hirst, R. and Rohrschneider, L. (1989a). Analysis of the avian fibronectin receptor (integrin) as a direct substrate for pp60v-src. *Oncogene* **4**, 325-333.

Tapley, P., Horwitz, A., Buck, C., Duggan, K. and Rohrschneider, L. (1989b). Integrins isolated from rous sarcoma virus-transformed chicken embryo fibroblasts. *Oncogene* **4**, 325-333.

Turner, C. E. and Burridge, K. (1991). Transmembrane molecular assemblies in cell-extracellular matrix interactions. *Curr. Opin. Cell Biol.* **3**, 849-853.

Turner, C., Glenney, J. R. and Burridge, K. (1990). Paxillin: a new vinculin-binding protein present in focal adhesions. *J. Cell Biol.* **111**, 1059-1068.

Turner, C. E., Schaller, M. D. and Parsons, J. T. (1993). Tyrosine phosphorylation of the focal adhesion kinase pp125^{FAK} during development: relation to paxillin. *J. Cell Sci.* **105**, 637-645.

Whitney, G. S., Chan, P.-Y., Blake, J., Cosar, W. L., Neubauer, M. G., Aruffo, A., Kanner, S. (1993). Human T and B lymphocytes express a structurally conserved focal adhesion kinase, pp125^{FAK}. *DNA Cell Biol.* **9**, 823-830.