Paxillin phosphorylation sites mapped by mass spectrometry

Donna J. Webb[1,*,‡,§], Melanie J. Schroeder[2,‡], Cynthia J. Brame[4], Leanna Whitmore[1], Jeffrey Shabanowitz[2], Donald F. Hunt[2,3] and A. Rick Horwitz[1]

[1]Department of Cell Biology, [2]Department of Chemistry, and [3]Department of Pathology, University of Virginia, 1300 Jefferson Park Avenue, Charlottesville, VA 22908, USA
[4]Department of Biology, Centenary College of Louisiana, 2911 Centenary Avenue, Shreveport, LA 71104, USA

*Present address: Department of Biological Sciences and Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN 37235, USA
‡These authors contributed equally to this work
§Author for correspondence
(e-mail: donna.webb@vanderbilt.edu)

Journal of Cell Science 118, 4925-4929
Published by The Company of Biologists 2005
doi:10.1242/jcs.02563

Paxillin is a 68 kDa protein that was originally identified as a substrate for the non-receptor tyrosine kinase Src (Glenney and Zokas, 1989; Turner et al., 1990). Paxillin localizes primarily to specialized sites of adhesion between cells and the extracellular matrix and functions as an adaptor molecule that recruits signaling and structural proteins to these sites through its multiple domains (Brown and Turner, 2004; Turner et al., 1990; Turner, 2000a). The N-terminal half of paxillin contains five short, leucine-rich peptide sequences (LDXLLXXL), which mediate interactions with other proteins (Brown et al., 1998a), and a proline-rich region that provides a binding site for the Src homology 3 (SH3) domains of Src family members (Weng et al., 1993). Phosphorylation of several tyrosine residues in this region creates binding sites for proteins containing SH2 domains (Bellis et al., 1995; Schaller and Parsons, 1995; Turner and Miller, 1994). The C-terminal half of paxillin contains four LIM domains that also facilitate protein-protein interactions (Turner and Miller, 1994). Phosphorylation of serine and threonine residues in this region potentiates the localization of paxillin to adhesions (Brown et al., 1998b). Thus, it appears that the localization of paxillin and its function as an adaptor molecule are both regulated by phosphorylation.

In this study, we used mass spectrometry to map tyrosine, serine and threonine phosphorylation sites in paxillin. Samples were prepared by transfecting HEK cells with either FLAG- or FLAG-GFP-tagged paxillin (10 ng to 3.5 μg per 100 mm dish) followed by immunoprecipitation of these FLAG-tagged molecules with FLAG-agarose (Sigma). Before cells were lysed, they were treated for 30 minutes with peroxovanadate (1 mM) and calyculin A (10 nM), a tyrosine and a serine/threonine phosphatase inhibitor, respectively. In the absence of phosphatase inhibitors, several peptides containing phosphoserine and phosphothreonine residues were observed at reduced levels. Under the same conditions, peptides phosphorylated on tyrosine often went undetected (Table 1).

Immunoprecipitated FLAG-tagged paxillin was digested with either tryspin, chymotrypsin, trypsin/chymotrypsin or Glu-C in an effort to generate peptides that provided complete coverage of the protein sequence. These peptides were analyzed by using high performance liquid chromatography (HPLC) interfaced to electrospray ionization on tandem mass spectrometers (LCQ-XP or LTQ-FT, Thermo Electron). Enrichment of phosphopeptides was performed using immobilized metal affinity chromatography (IMAC) (Ficarro et al., 2002). With these techniques, we obtained greater than 97% coverage of the serine, threonine and tyrosine residues in paxillin (Fig. 1). Ten of the phosphorylation sites that we identified had been described previously (tyrosines 31, 40, 88, 118 and 182, serines 85, 126, 130 and 188/190, and threonine 403) (Bellis et al., 1995; Bellis et al., 1997; Brown et al., 1998b; Huang et al., 2004; Schaller and Parsons, 1995; Schaller and Schaefer, 2001; Turner and Miller, 1994; Turner, 2000b; Woodrow et al., 2003). Phosphorylation of serine 85 and tyrosines 31 and 118 regulates cell migration (Huang et al., 2004; Petit et al., 2000). The functional significance of the other sites remains to be determined.

Several of the novel phosphorylation sites identified in this study reside in regulatory domains in paxillin. S273 is located in the LD4 domain of paxillin whereas S308 resides in the LD5 domain of paxillin. The LD4 motif serves as a binding site for a number of signaling molecules, including focal adhesion kinase (FAK), G-protein-coupled receptor kinase-interacting protein 1 (GIT1) and p95 paxillin-kinase linker (PKL), which is the chicken homolog of GIT2 (Brown et al., 1996; Thomas et al., 1999; Turner et al., 1999). In addition, T344 and S361 are found within the first LIM domain, T403 is in the second LIM domain, and S504 and T540 reside within the fourth LIM domain. Interestingly, serine and threonine phosphorylation of the LIM domains facilitates the localization of paxillin to adhesions (Brown et al., 1998b).

Several of the novel phosphorylation sites are potential targets for kinases that are implicated in the regulation of various cellular processes including migration and adhesion. Serines 91, 98, 108, and 382 and threonines 295 and 540 are predicted sites for protein kinase C (PKC), while serines 112, 173, 217, 259 and 501 are predicted substrates for protein kinase A (PKA) (Table 1). GSK3 is a predicted kinase for serines 85, 90, 94, 106, 108, 126 and 227, and serine 173 is a predicted site for Akt. Although earlier studies focused on the function of Akt in apoptosis, emerging evidence suggests that this protein kinase also plays a key role in cell migration (Brazil et al., 2002). Extracellular signal-regulated kinase (ERK), which has recently been shown to localize to adhesions and to regulate paxillin disassembly (Fincham et al., 2000; Webb et al., 2004), is predicted to phosphorylate several sites in paxillin (S106, S231 and S290). Whether these sites also play a role in the regulation of cell migration and adhesion dynamics remains to be determined. Many residues that are phosphorylated in chicken paxillin are conserved in human and mouse paxillin (hpax and mpax) and in another paxillin family member (hic-5) (Table 1).

A selection of paxillin peptides that contain more than one phosphorylated residue are shown in Table 2. All nine of these peptides contain phosphorylation sites separated by 0-8 residues. In several of these peptides, both the

Table 1. Summary of paxillin phosphorylation sites

FLAG-GFP-Pax lower-level expression (LTQ-FT)	Detected without inhibitors and without enrichment (trypsin)	Homologs/other family members	Putative kinases‡
Y31		mpax and hpax	Src/FAK§
Y40		mpax and hpax	Src¶
GlcNAc S74	Yes (ETD)	mpax and hpax	–
S83		mpax and hpax	–
S85	Yes	hpax	p38MAPK**, GSK3
Y88		mpax and hpax	Src¶
S89		mpax	–
S96		mpax and hpax	–
S98		mpax and hpax	PKC
S106	Yes	mpax and hpax	ERK, GSK3
S108	Yes	hic-5	PKC, GSK3
S112		-	PKA
Y118		mpax, hpax and hic-5	Src/FAK††,§
S119		mpax, hpax and hic-5	–
S126	Yes	mpax, hpax and hic-5	GSK3
S130	Yes	mpax and hpax	–
S137		mpax, hpax and hic-5	–
S164		–	–
S171	Yes	mpax, hpax and hic-5	–
S173	Yes	–	PKA, Akt, CaMII
Y182		mpax and hpax	EGFR, PDFGR
S187-190		–	–
S227/228		mpax and hpax	GSK3 (S227)
S231		mpax, hpax and hic-5	ERK
S239		–	–
S245		mpax and hpax	p34cdc2, Cdk5
S259		mpax, hpax and hic-5	PKA
S290	Yes	mpax, hpax and hic-5	ERK
S382		mpax, hpax and hic-5	PKC
S501	Yes	mpax, hpax and hic-5	PKA, CaMII

Additional sites detected

FLAG-Pax overexpression (LCQ-XP)*	Comments (multiple enzymes)	Homologs/other family members	Putative kinases‡
T29	Observed once	hpax	–
S94		–	GSK3
S143	Observed once	mpax and hpax	–
T199		hpax	–
S262		mpax, hpax and hic-5	–
S273		mpax, hpax and hic-5	–
T295		hic-5	PKC, Cdk5
S308		mpax and hpax	–

FLAG-Pax overexpression (LTQ-FT or ETD)*	Comments (trypsin)	Homologs/other family members	Putative kinases‡
Y76		mpax and hic-5	–
S90	ETD	mpax and hpax	GSK3
S91	ETD	mpax and hpax	PKC
S217/220		mpax and hpax	PKA (S217)
S275		mpax, hpax and hic-5	–
T344	ETD	mpax and hpax	–
S361	ETD	mpax and hpax	–
†Y377		mpax, hpax and hic-5	–
T403	ETD	mpax and hpax	–
Y436		mpax and hpax	–
S504	ETD	mpax and hpax	–
†T540	ETD	mpax and hpax	PKC

The table shows phosphorylation sites detected in chicken paxillin by mass spectrometry (the numbering is according to the chicken paxillin sequence).

*The FLAG-paxillin sequence (the FLAG sequence is not included):
MDDLDALLADLESTTSHISKRPVFLTEETPYSYPTGNHTYQEIAVPPPVPPPPSSEALNGTDPLDQWQPSVSRYGHQQPQSQSPIYSSSAKSSSASVPRDGLSSPSPR-ASEEEHVYSFPNKQKSAEPSPTMTSTSLGSNLSELDRLLLELNAVQHNPPSGFSADEVSRSPSLPNVTGPHYVIPESSSSAGGKAAPPTKEKPKRNGGRGIEDVRPS-VESLLDELESSVPSPVPAITVSQGEVSSPQRVNASQQQTRISASSATRELDELMASLSDFKFMAQGKAGGSSSPPSTTPKPGSQLDTMLGSLQSDLNKLGVATVAK-GVCGACKKPIAGQVVTAMGKTWHPEHFVCTHCQEEIGSRNFFERDWQPYCEKYYHNLFSPRCYYCNGPILDKVVTAFDRTWHPEHFFCAQCGVFFGPEGFHEK-DGKAYCRKDYFDMFAPKCGGCARAILENYISALNTLWHPECFVCRECFTPFINGSFFEHDGQPYCEVHYHERRGSLCSGCQKPITGRYITAMGKKFHPEHFVCAF-CLKQLNKGTFKEQNYKPYCQNCFLKLFC. Using multiple enzymes, 97% coverage of this sequence was detected by mass spectrometry.

†This phosphopeptide contained an amino acid that differed from the corresponding FLAG-GFP peptide sequence.

‡This column shows the kinases predicted to phosphorylate the indicated sites using NetPhos 2.0 and Scansite. Note that this is a partial list; we have only included potential kinases that have been implicated in cell migration. References are given for kinases that have been shown experimentally to phosphorylate the site.

LCQ-XP, dynamic range approximately 100, collisional-activated dissociation MS/MS spectra. LTQ-FT, dynamic range approximately 5000, collisional-activated dissociation MS/MS spectra. ETD, electron transfer dissociation. Peptide fragmentation by ETD enabled phosphorylation or glycosylation site identification.

For additional data regarding paxillin phosphorylation, see the Cell Migration Consortium web site (http://www.cellmigration.org).

§Schaller and Parsons, 1995; ¶Schaller and Schaefer, 2001; **Huang, et al., 2004; ††Bellis et al., 1995.

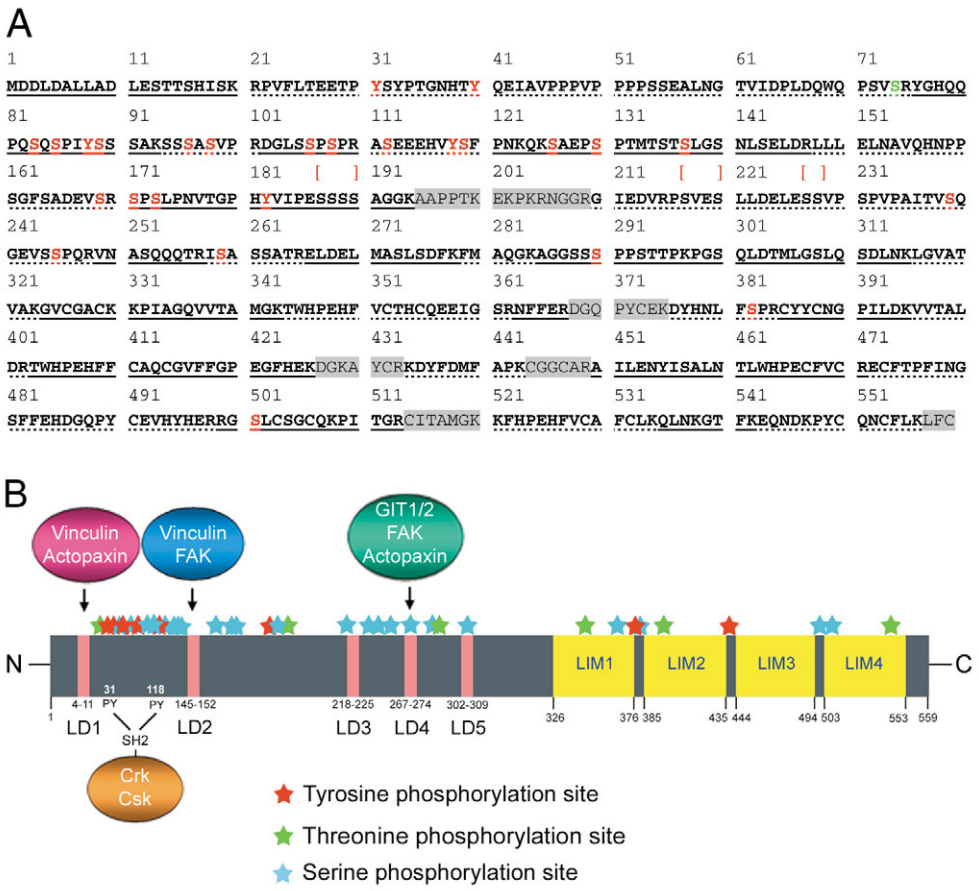

Fig. 1. Phosphorylation sites detected in chicken paxillin. (A) Serine, threonine and tyrosine coverage of the FLAG-GFP-paxillin sequence (tag not shown) generated with trypsin. Peptides were analyzed by nHLPC-μESI MS/MS (LTQ-FTMS). Detected tryptic peptides are bold and alternate between solid and dashed underlines. Residues not covered are shaded in gray. Observed phosphorylation sites are red. Red brackets above residues indicate ambiguity in specific phosphorylation site assignment. Observed GlcNAc site is green. Coverage of the Ser, Thr and Tyr sites is 92%. (B) A schematic of paxillin is shown. The paxillin phosphorylation sites are represented by stars, which show their positions relative to the paxillin domains. The binding sites within paxillin for some adhesion/migration molecules are also shown.

phosphorylation sites and the intervening residues are conserved in chicken, mouse and human paxillin. We suggest that these sequences function as multiply phosphorylated, regulatory or recognition motifs. As shown in Table 2, two peptides contain adjacent phosphorylated tyrosine and serine residues (Y118/S119 and Y88/S89). Although the simultaneous phosphorylation of adjacent tyrosine and serine residues has not been previously described, it seems likely that this combination serves a regulatory function within the molecule. Phosphorylation of an adjacent serine residue could block protein-protein interactions that recognize the phosphotyrosine site and therefore act as an on-off switch for the formation of such complexes. A similar phenomenon is known to exist in histones, where proteins that recognize methylated lysine residues are blocked by phosphorylation of adjacent serine or threonine residues (Fischle et al., 2003).

Of additional interest is the observation that 50% of the paxillin molecules are modified with an O-linked *N*-acetylglucosamine (GlcNAc) moiety on serine 74. This type of modification is reversible and thought to have a modulatory function similar to that of phosphorylation (Hart, 1997). GlcNAc modifications on proteins can occur at the same sites as those that are phosphorylated, but the modification is not restricted to these residues (Comer and Hart, 2000). In the present study, we did not detect phosphorylation of serine 74.

Although the phosphorylation map presented here is extensive, it may not be complete. For example, we detected phosphorylation on all three of the predicted ERK sites, but not on two potential PKC sites (S19 and T511) and one PKA site (T516). This raises the possibility that other phosphorylation sites exist. Additional phosphorylation sites could be generated under different growth conditions or with other cell types. It is also possible that some sites are phosphorylated at very low levels, owing to high spatial and temporal regulation. In this context, some sites were only observed in a single experiment (T29 and S143). Finally, although we have not assigned relative abundances of the phosphopeptides detected in the present work, those detected without inhibitors or IMAC are among the most abundant. The least abundant phosphopeptides are those that are only detected in the presence of phosphatase inhibitors and require enrichment via IMAC prior to analysis by mass spectrometry.

Materials and Methods

Sample preparation

HEK cells were transfected with FLAG-tagged paxillin or FLAG-GFP-paxillin (10 ng to 3.5 μg per 100 mm dish) using lipofectamine. After 36-48 hours, cells were incubated with 1 mM peroxovanadate and 10 nM calyculin A for 30 minutes and extracted with 25 mM Tris, 100 mM NaCl, 0.5% NP-40, pH 7.4. The lysates were precleared twice with mouse IgG-agarose for 1 hour at 4°C and immunoprecipitated with FLAG-agarose (Sigma) for 2 hours at 4°C. Samples were washed twice with 25 mM Tris, 100 mM NaCl, pH 7.4 and FLAG-tagged paxillin was eluted by incubation of the beads with 0.2 mg/ml FLAG peptide in 25 mM Tris for 30 minutes at 4°C or left on beads.

Sample analysis

Mapping of the phosphopeptide sites was performed as described in detail elsewhere (Schroeder et al., 2005). Briefly, FLAG-eluted samples were reduced and alkylated with dithiothreitol and iodoacetamide,

Table 2. Paxillin peptides that contain more than one phosphorylated residue

Site	Position	Homologs/other family members	Peptides
31Y/40Y	28–42	Chicken	ETPYSYPTGNHTYQE
		Human	ETPYSYPTGNHTYQE
		Mouse	EPPYSYPTGNHTYQE
		Hic-5	ERP----------
31Y/40Y/74S	21-75	Chicken	RPVFLTEETPYSYPTGNHTYQEIAVPPPVPPPPSSEALNGTVIDPLDQWQPSV**S**R
		Human	RPVFLSEETPYSYPTGNHTYQEIAVPPPVPPPPSSEALNGTILDPLDQWQPSG**S**R
		Mouse	RPVFLSEEPPYSYPTGNHTYQEIAVPPPVPPPPSSEALNGTVLDPLDQWQPSG**S**R
		Hic-5	--LGAPKERP----------PETLTPPPP----------
83S/85S/88Y/89S	76–93	Chicken	YGHQQPQSQSPIYSSSAK and YGHQQPQSQSPIYSSSAK
		Human	FIHQQPQSSSPVYGSSAK and FIHQQPQSSSPVYGSSAK
		Mouse	YAHQQPPSPLPVYSSSAK and YAHQQPPSPLPVYSSSAK
		Hic-5	YGHQ---------- and YGHQ----------
98S/106S/108S	94–110	Chicken	SSSASVPRDGLSSPSPR
		Human	TSSASNPQDSVGSPCSR
		Mouse	NSSASNTQDGVGSLCSR
		Hic-5	------PQTGSGESSGT
118Y/119S	111–123	Chicken	ASEEEHVYSFPNK
		Human	VGEEEHVYSFPNK
		Mouse	AGEEEHVYSFPNK
		Hic-5	TGDKDHLYSTVCK
126S/130S/137S	124–147	Chicken	QKSAEPSPTMTSTSLGSNLSELDR
		Human	QKSAEPSPTVMSTSLGSNLSELDR
		Mouse	QKSAEPSPTVMSSSLGSNLSELDR
		Hic-5	PRSPKPVAPVSSSSGVLGNGLCEL
171S/187S-190S	171–194	Chicken	[SPS]LPNVTGPHYVIPE[SSSS]AGGK
173S/ 187S-190S	171–194	Chicken	[SPS]LPNVTGPHYVIPE[SSSS]AGGK
171S/182Y		Human	SPP LPGALSPLYGVPE TNSP LGGK
173S/182Y		Mouse	SPP LPGALSPLYGIPE NNTP LGGK
		Hic-5	--Q FPS--SKMAEGEE KEDQ SEDK
228S/231S/245S	210–248	Chicken	GIEDVRPSVESLLDELESSVPSPVPAITVSQGEVSSPQR
		Human	GLEDVRPSVESLLDELESSVPSPVPAITVNQGEMSSPQR
		Mouse	GLEDVRPSVESLLDELESSVPSPVPAITVNQGEMSSPQR
		Hic-5	----------PPSPFPA----------
290S/295T/308S	285–315	Chicken	AGGSSSPPSTTPKPGSQLDTMLGSLQSDLNK
		Human	TG-SSSPPGGPPKPGSQLDSMLGSLQSDLNK
		Mouse	TG-SSSPPGGLSKPGSQLDSMLGSLQSDLNK
		Hic-5	EG-CPSPPGQTSK-GS-LDTMLGLLQSDLSR

Selected examples of multiply phosphorylated paxillin peptides and alignment with paxillin homologs and other family members (hic-5) are shown. Not all occurrences of multiply phosphorylated examples are shown. Observed phosphorylation sites in chicken paxillin are shown in red. The corresponding sites in paxillin homologs and hic-5 are also shown in red although these sites were not observed in this study. Red brackets indicate ambiguity in specific phosphorylation site assignments. An observed GlcNAc site in chicken paxillin and the corresponding site in paxillin homologs are shown in green.

respectively, as described previously (Schroeder et al., 2004). Generally, 10% of the eluted protein was digested with 500 ng of desired enzyme(s) in 100 mM ammonium bicarbonate, pH 8.5 for 8-12 hours at room temperature. Peptides from an aliquot corresponding to 10% of the solution digest (1% of the original IP) were separated with either a 1 or 2 hour gradient as described elsewhere (Schroeder et al., 2004). Analysis of FLAG-eluted samples was carried out using an LCQ-XP under conventional MS/MS mode (Schroeder et al., 2004). Reduction and alkylation steps were omitted for on-bead digestion (500 ng trypsin only, shaking at room temperature for 6 hours) and analysis was with an LTQ-FTMS. The on-bead digestion protocol did not reduce peptide coverage, but an intra-peptide disulfide bond was common among peptides containing two cysteines. Enrichment of phosphopeptides was performed according to (Ficarro et al., 2002) using 10-20× more sample except 250 mM ascorbic acid was used for phosphopeptide elution. ETD spectra were recorded on an in-lab modified LTQ mass spectrometer described in (Syka et al., 2004) with fluoranthene as the electron transfer reagent.

This work was supported by NIH The Cell Migration Consortium (U54 GM064346) and grant GM37537 (D.F.H.).

References

Bellis, S. L., Miller, J. T. and Turner, C. E. (1995). Characterization of tyrosine phosphorylation of paxillin *in vitro* by focal adhesion kinase. *J. Biol. Chem.* **270**, 17437-17441.

Bellis, S. L., Perrotta, J. A., Curtis, M. S. and Turner, C. E. (1997). Adhesion of fibroblasts to fibronectin stimulates both serine and tyrosine phosphorylation of paxillin. *Biochem. J.* **325**, 375-381.

Brazil, D. P., Park, J. and Hemmings, B. A. (2002). PKB binding proteins. Getting in on the Akt. *Cell* **111**, 293-303.

Brown, M. C. and Turner, C. E. (2004). Paxillin: adapting to change. *Physiol. Rev.* **84**, 1315-1339.

Brown, M. C., Perrotta, J. A. and Turner, C. E. (1996). Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding. *J. Cell Biol.* **135**, 1109-1123.

Brown, M. C., Curtis, M. S. and Turner, C. E. (1998a). Paxillin LD motifs may define a new family of protein recognition domains. *Nat. Struct. Biol.* **5**, 677-678.

Brown, M. C., Perrotta, J. A. and Turner, C. E. (1998b). Serine and threonine phosphorylation of the paxillin LIM domains regulates paxillin focal adhesion localization and cell adhesion to fibronectin. *Mol. Biol. Cell* **9**, 1803-1816.

Comer, F. I. and Hart, G. W. (2000). O-Glycosylation of nuclear and cytosolic proteins. Dynamic interplay between O-GlcNAc and O-phosphate. *J. Biol. Chem.* **275**, 29179-29182.

Ficarro, S. B., McCleland, M. L., Stukenberg, P. T., Burke, D. J., Ross, M. M., Shabanowitz, J., Hunt, D. F. and White, F. M. (2002). Phosphoproteome analysis by mass spectrometry and its application to *Saccharomyces cerevisiae*. *Nat. Biotechnol.* **20**, 301-305.

Fincham, V., James, M., Frame, M. and Winder, S. (2000). Active ERK/MAP kinase is targeted to newly forming cell-matrix adhesions by integrin engagement and v-Src. *EMBO J.* **19**, 2911-2923.

Fischle, W., Wang, Y. and Allis, C. D. (2003). Binary switches and modification cassettes in histone biology and beyond. *Nature* **425**, 475-479.

Glenney, J. R., Jr and Zokas, L. (1989). Novel tyrosine kinase substrates from Rous sarcoma virus-transformed cells are present in the membrane skeleton. *J. Cell Biol.* **108**, 2401-2408.

Hart, G. W. (1997). Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins. *Annu. Rev. Biochem.* **66**, 315-335.

Huang, C., Borchers, C. H., Schaller, M. D. and Jacobson, K. (2004). Phosphorylation of paxillin by p38MAPK is involved in the neurite extension of PC-12 cells. *J. Cell Biol.* **164**, 593-602.

Petit, V., Boyer, B., Lentz, D., Turner, C. E., Thiery, J. P. and Valles, A. M. (2000). Phosphorylation of tyrosine residues 31 and 118 on paxillin regulates cell migration through an aassociation with CRK in NBT-II cells. *J. Cell Biol.* **148**, 957-969.

Schaller, M. D. and Parsons, J. T. (1995). pp125FAK-dependent tyrosine phosphorylation of paxillin creates a high-affinity binding site for Crk. *Mol. Cell. Biol.* **15**, 2635-2645.

Schaller, M. D. and Schaefer, E. M. (2001). Multiple stimuli induce tyrosine phosphorylation of the Crk-binding sites of paxillin. *Biochem. J.* **360**, 57-66.

Schroeder, M. J., Shabanowitz, J., Schwartz, J. C., Hunt, D. F. and Coon, J. J. (2004). A neutral loss activation method for improved phosphopeptide sequence analysis by quadrupole ion trap mass spectrometry. *Anal. Chem.* **76**, 3590-3598.

Schroeder, M. J., Webb, D. J., Shabanowitz, J., Horwitz, A. F. and Hunt, D. F. (2005). Methods for the detection of paxillin post-translational modifications and interacting proteins by mass spectrometry. *J. Proteome Res.* (in press).

Syka, J. E., Coon, J. J., Schroeder, M. J., Shabanowitz, J. and Hunt, D. F. (2004). Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc. Natl. Acad. Sci. USA* **101**, 9528-9533.

Thomas, J. W., Cooley, M. A., Broome, J. M., Salgia, R., Griffin, J. D., Lombardo, C. R. and Schaller, M. D. (1999). The role of focal adhesion kinase binding in the regulation of tyrosine phosphorylation of paxillin. *J. Biol. Chem.* **274**, 36684-36692.

Turner, C. E. (2000a). Paxillin and focal adhesion signalling. *Nat. Cell Biol.* **2**, E231-E236.

Turner, C. E. (2000b). Paxillin interactions. *J. Cell Sci.* **113**, 4139-4140.

Turner, C. E. and Miller, J. T. (1994). Primary sequence of paxillin contains putative SH2 and SH3 domain binding motifs and multiple LIM domains: identification of a vinculin and pp125Fak-binding region. *J. Cell Sci.* **107**, 1583-1591.

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

Turner, C. E., Brown, M. C., Perrotta, J. A., Riedy, M. C., Nikolopoulos, S. N., McDonald, A. R., Bagrodia, S., Thomas, S. and Leventhal, P. S. (1999). Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankryin repeat, ARF-GAP protein: a role in cytoskeletal remodeling. *J. Cell Biol.* **145**, 851-863.

Webb, D. J., Donais, K., Whitmore, L. A., Thomas, S. M., Turner, C. E., Parsons, J. T. and Horwitz, A. F. (2004). FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nat. Cell Biol.* **6**, 154-161.

Weng, Z., Taylor, J. A., Turner, C. E., Brugge, J. S. and Seidel-Dugan, C. (1993). Detection of Src homology 3-binding proteins, including paxillin, in normal and v-Src-transformed Balb/c 3T3 cells. *J. Biol. Chem.* **268**, 14956-14963.

Woodrow, M. A., Woods, D., Cherwinski, H. M., Stokoe, D. and McMahon, M. (2003). Ras-induced serine phosphorylation of the focal adhesion protein paxillin is mediated by the Raf-MEK-ERK pathway. *Exp. Cell Res.* **287**, 325-338.

Journal of Cell Science