A novel muscle LIM-only protein is generated from the *paxillin* gene locus in *Drosophila*

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Paxillin is a protein containing four LIM domains, and functions in integrin signaling. We report here that two transcripts are generated from the *paxillin* gene locus in *Drosophila*; one encodes a protein homolog of the vertebrate Paxillin (DPxn37), and the other a protein with only three LIM domains, partly encoded by its own specific exon (PDLP). At the myotendinous junctions of *Drosophila* embryos where integrins play important roles, both DPxn37 and PDLP are highly expressed with different patterns; DPxn37 is predominantly concentrated at the center of the junctions, whereas PDLP is highly enriched at neighboring sides of the junction centers, primarily expressed in the mesodermal myotubes. Northern blot analysis revealed that DPxn37 is ubiquitously expressed throughout the life cycle, whereas PDLP expression exhibits a biphasic pattern during development, largely concomitant with muscle generation and remodeling. Our results collectively reveal that a unique system exists in *Drosophila* for the generation of a novel type of LIM-only protein, highly expressed in the embryonic musculature, largely utilizing the Paxillin LIM domains.

INTRODUCTION

The LIM domain is a cysteine-rich sequence functioning as a protein-binding interface (Schmeickel and Beckerle, 1994), and is present in single or multiple copies in a variety of proteins that are generally involved in transcriptional regulation, cell signaling and control of cell fate (for review see Dawid *et al.*, 1998). LIM domains were grouped by sequence similarity into four classes (A–D), with some outliers (Dawid *et al.*, 1995), and LIM-containing proteins have been classified into three groups, based on

sequence relationships among LIM domains and on the overall structure of the proteins (Dawid *et al.*, 1998). Group 1 includes proteins bearing A and B class LIM domains in tandem, such as LHX (LIM homeodomain) proteins, LMOs (LIM-only proteins) and LIM kinases. Group 2 includes proteins composed largely of LIM domains of class C, such as CRIPs (cysteine-rich intestinal proteins) and CRPs (cysteine-rich proteins). Most of the proteins in group 3 contain class D LIM domains, and are more heterogeneous, containing different numbers of LIM domains located at the C-terminus, such as Paxillin, Zyxin and PINCH.

Paxillin contains four repeats of the LIM domain at its C-terminus and acts as an integrin-assembly scaffolding adaptor protein (for review see Turner, 2000). Paxillin can interact with a number of other integrin assembly proteins with scaffolding and/or catalytic signaling properties, including vinculin, talin, Fak, Pyk2, c-Src and Csk (for review see Turner, 1998), and also interacts with several ARFGAP proteins (Turner *et al.*, 1999; Di Cesare *et al.*, 2000; Kondo *et al.*, 2000; Mazaki *et al.*, 2001). Paxillin has been shown to play important roles in determining the actin-cytoskeletal organization, cell polarity and cell migration (Nakamura *et al.*, 2000; Petit *et al.*, 2000; Yano *et al.*, 2000). Moreover, Paxillin is highly tyrosine phosphorylated upon integrin activation (Burridge *et al.*, 1992) and represents the major phosphotyrosine-protein during vertebrate development (Turner, 1991).

To investigate the possible role of *paxillin* during development as well as cell migration, we have initiated a reverse genetic approach in *Drosophila melanogaster*, and report here a unique use of *paxillin* LIM domains in *Drosophila*.

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Fig. 1. Structure and expression of *Drosophila* *DPxn37* and *PDLP*. **(A)** Intron-exon organization of *DPxn37* and *PDLP*. Exons are indicated as thick bars, intronic and intergenic sequences as thin lines. Purple bars indicate the 5'- and 3'-untranslated regions. **(B)** A developmental northern blot analysis of poly(A)⁺ RNA from embryonic (numbered as hours of development at 25°C), larval, pupal or adult stages. RNAs were hybridized with ³²P-labeled *DPxn37* cDNA. Numbers to the left refer to the size of the RNA markers. **(C)** Amino acid alignment of the third LIM domains of *DPxn37* and *PDLP*. **(D)** Protein expression. S2 cells were transfected with empty vector, (myc)₆-tagged *DPxn37* and *PDLP* plasmids. Ten micrograms of cell protein from each transfection of S2 cells, or from animals of the embryonic, the first larval or the adult stage, were resolved by SDS-PAGE and immunostained sequentially with affinity-purified polyclonal antibodies against *DPxn37* (upper panel) and *PDLP* (lower panel). **(E and F)** Schematic representation of the *DPxn37* and human Paxillin-superfamily proteins (E), and *PDLP* and Lepidopteran *DALP* (F). Numbers on the right depict amino acid identity of pairwise sequence comparisons of each human Paxillin-superfamily protein with *DPxn37* (E), and of *PDLP* with *DALP* (F), regarding the entire coding region or the LIM1-LIM4 domains. The genetics computer program BLAST2 was used. Major tyrosine phosphorylation sites in human Paxillin and putative phosphorylation sites in *DPxn37* are also shown in (E).

RESULTS AND DISCUSSION

To identify a gene encoding the *Drosophila* Paxillin homolog, we used TBLASTN to search the expressed-sequence tag (EST) database (Berkley *Drosophila* Genome Project, BDGP), and detected *Drosophila* EST CK00121 by homology to the LIM domains of human *paxillin*. By using the EST cDNA as a probe, a cDNA 2 kb long was isolated from a λgt10 cDNA library. The corresponding mRNA consists of an open reading frame 1743 nt long, and the predicted 581 amino acid protein is most similar to human Paxillin (Figure 1A and E). During our analysis, BDGP mapped the gene to polytene band 37E, and it has hence been designated *DPxn37*. *DPxn37* protein shows 57% overall identity to human Paxillin and retains most of the protein interaction motifs,

including five LD motifs and four LIM domains (LIM1-LIM4) (Figure 1E). Three out of the four major tyrosine phosphorylation sites of human Paxillin (Nakamura *et al.*, 2000) are conserved at analogous positions; only the tyrosine residue that corresponds to Tyr31 is missing (Figure 1E). When expressed in mammalian cells, *DPxn37* localizes to focal adhesions and is tyrosine-phosphorylated during cell adhesion (Figure 4; data not shown). Thus, *DPxn37* is a *Drosophila* homolog of human Paxillin. Although human Paxillin consists of three isoforms generated by alternative splicing (Mazaki *et al.*, 1997), the submitted genomic sequence reveals that *Drosophila* only encodes a Paxillin homolog to the α isoform (<http://www.fruitfly.org>). There are several members of the Paxillin super-family in human,

Fig. 2. *Drosophila* *DPxn37* and *PDLP* gene expression in embryos. Embryos were hybridized with antisense *DPxn37* (A) or *PDLP* (B) probes to reveal their gene expressions. Different stages of embryos were used as indicated, and are viewed laterally unless otherwise indicated. Several frames are enlarged, showing *DPxn37* expression at the somatic muscle junction sites (indicated by arrowheads) and *PDLP* expression in the somatic muscles. Controls included stage 16 embryos hybridized with sense *DPxn37* (C) or *PDLP* (D) probes. In all frames, embryos are oriented with anterior to the left.

including Hic-5 (Zhang *et al.*, 2000) and Leupaxin (Lipsky *et al.*, 1998), which have several LIM motifs and four LIM domains with the same cysteine-rich consensus sequence. *DPxn37* is also highly homologous to these proteins (Figure 1E), but distinct homologs of Hic-5 and Leupaxin are not found in the euchromatic genomic sequences of *D. melanogaster*.

When developmental northern blotting analysis was performed with poly(A)⁺ RNA preparations from the embryonic, larval, pupal or adult stages using the entire coding region of *DPxn37* cDNA as a probe, two different size transcripts were detected. One is ~2.4 kb long, corresponding to the full-length transcript for *DPxn37*, and expressed ubiquitously throughout the life cycle (Figure 1B). The other is only ~1.4 kb long and too short to encode the entire coding region of *DPxn37*. Moreover, unlike the long transcript, the short version shows a biphasic pattern of expression during development (Figure 1B). We were therefore interested in identifying the nature of this small sized transcript. To this end, we divided *DPxn37* cDNA into four pieces (#1, #2, #3 and #4 in Figure 1A), again performed northern blotting analysis, and found that probes #3 and #4 hybridized with both transcripts while probes #1 and #2 hybridized only with the long one (data not shown). Therefore, the short transcript appeared to contain only some of the 3'-portion of the *DPxn37* gene, and the corresponding cDNA was then identified as described in Methods. Sequencing analysis of the cDNA thus obtained revealed that the short transcript encodes a protein with three LIM domains and 197 amino acids long (Figure 1A). The first and second LIM domains are the same as those in *DPxn37*. The third LIM domain, however, is generated by alternative splicing and contains the NH₂-half of the *DPxn37* LIM3 domain and a novel amino acid sequence at its C-terminal

end, which is encoded by an extra exon located 0.3 kb 3'-downstream of the eighth exon of *DPxn37* (Figure 1A). Despite this alternative splicing, a cysteine-rich sequence of the third LIM domain is conserved in the newly formed LIM domain, except that the last histidine residue is replaced by cysteine (Figure 1C). We named this protein *paxillin*-derived LIM-only protein (*PDLP*). The mRNA for *PDLP* seems to start at ~0.1 kb 5'-upstream of exon 8 of *DPxn37*, and contains its own polyadenylation site located 0.64 kb 3'-downstream of *DPxn37* exon 8 (data not shown; also see Figure 1A). Such a transcript corresponding to *Drosophila PDLP* and the exon specific to *PDLP* could not be found in mice and humans (data not shown).

The expression of *DPxn37* and *PDLP* transcripts was then analyzed during embryogenesis (Figure 2). *DPxn37* transcript is detected at a very early stage, perhaps partly representing their maternal expression; and is found more or less throughout the body at later stages. From stage 13, up-regulated expression of *DPxn37* transcript was detected as a segmentary pattern along the somatic musculature. On the other hand, *PDLP* transcript is almost undetectable at the early stages, becoming detectable after stage 13. They are then also expressed in a segmentary pattern predominantly along the somatic musculature, although their pattern appears to be different from that of *DPxn37* (also see below). *PDLP* mRNA is also expressed in cephalic muscles.

We next generated two different preparations of polyclonal antibodies, each specifically recognizing *DPxn37* or *PDLP* (Figure 1D). Using these antibodies, the somatic musculature at stage 16 of embryogenesis was investigated (Figure 3). Phosphotyrosine is known to be highly enriched at the myotendinous junctions (Harden *et al.*, 1996), at least partly representing integrin-mediated focal adhesion events. We found that the

Fig. 3. Protein expression of DPxn37 and PDLP at embryonic myotendinous junctions. Stage 16 embryos were stained with antibodies against DPxn37, PDLP, phosphotyrosine (pY) and α PS2 integrin, as indicated. The horizontal section (A) and lateral surface views (B–D) are presented showing prominent expression of DPxn37 at the center of the myotendinous junctions, well colocalized with phosphotyrosine signals (A and B), and showing PDLP expression in the somatic muscles with particularly strong staining near the segmentary boundaries (C and D). The right column shows merged views of the left and middle images.

DPxn37 protein is abundant at the junctions, and colocalized well with phosphotyrosine. These areas highly positive for DPxn37 may include the epidermal tendon cells, which can be identified by staining with tendon cell markers such as Kakapo and α tubulin (Gregory and Brown, 1998; Strumpf and Volk, 1998; data not shown). The possible expression, however, of DPxn37 at the very ends of the myotube edges cannot be precluded. In sharp contrast, the PDLP protein overlapped only slightly with phosphotyrosine, and was almost undetectable at the center of the junctions, but was highly expressed on both sides of the junctions. These areas appeared to correspond primarily to the mesodermal myotubes, as assessed by staining of the myotube edges with α PS2 integrins (Brower *et al.*, 1984; Bogaert *et al.*, 1987; Figure 3). Both DPxn37 and PDLP signals were also detected at low levels in the cell body of the myotubes. With the late stage embryos, we also examined several other tissues and found that the PDLP protein is highly expressed in the cephalic muscles (data not shown), consistent with the mRNA expression.

We next examined the possible subcellular localization of DPxn37 and PDLP (Figure 4). DPxn37, when expressed in vertebrate fibroblasts, localized to focal adhesions, and colocalized well with endogenous Paxillin. On the other hand, PDLP localized along actin bundles, and only marginally overlapped with the DPxn37-containing focal adhesions, formed at the edges of actin bundles. Similar colocalization with actin bundles has been

observed with two *Drosophila* LIM-only proteins, Mlp60A and Mlp84B, composed of one and five LIM domains, respectively (Stronach *et al.*, 1996, 1999). These proteins are abundant in several musculatures at the late stages of embryogenesis and implicated in myogenesis. In vertebrates, there is also a CRP family of LIM-only proteins with the ability to localize to actin bundles, which are implicated in muscle differentiation (Sadler *et al.*, 1992; Arber *et al.*, 1994; Crawford *et al.*, 1994). Therefore, unlike DPxn37, PDLP exhibits a property similar to those shared among muscle LIM proteins, although its biological significance remains to be elucidated (Arber *et al.*, 1994; Stronach *et al.*, 1996). It should be noted, however, that the cysteine-rich consensus sequence of these muscle LIM proteins and CRPs, which is CX2CX17HX2CX2CX2CX17/18CX2C (Stronach *et al.*, 1996), is different from that of the LIM1 and LIM2 domains, which is CX2CX19HX2CX2CX17CX2D, and the LIM3 domain (see Figure 1C) of PDLP. Moreover, the LIM3 domain of vertebrate Paxillin has been implicated in the focal adhesion localization (Brown *et al.*, 1996), and identification of sequences involved in the different subcellular localizations may be important for understanding possible functional differences between DPxn37 and PDLP. A fraction of PDLP, but not DPxn37, was also found in cell nuclei (Figure 4), again as observed with other *Drosophila* muscle LIM proteins as well as CRPs (Arber *et al.*, 1994; Stronach *et al.*, 1996). However, we did not observe PDLP to be concentrated in cell nuclei in the

Fig. 4. Association of PDLP with actin bundles. EGFP- or DsRed-tagged DPxn37, and EGFP-tagged PDLP were ectopically expressed in NIH 3T3 fibroblasts, as indicated, and visualized by detecting the autofluorescence from the tags, together with endogenous Paxillin (**A**) and F-actin (**B**). The focus was adjusted near the bottom of cells, to detect intracellular proteins at 0.5–2.0 μm above the surface of the glass chamber plates. The right column represents the merging of the left images. Bar, 10 μm . Note that PDLP only marginally overlapped with DPxn37 at the edges of actin stress fibers. HA- or Myc-tagged PDLP showed essentially the same subcellular localization as that of EGFP-PDLP (data not shown).

embryos (data not shown). The biological relevance and significance of the nuclear localization of these LIM-only proteins also remains to be elucidated, as discussed previously (Arber et al., 1994; Stronach et al., 1996).

A cysteine-rich consensus sequence of PDLP is highly homologous to that of Lepidopteran DALP (death-associated LIM-only protein), which also consists of only three LIM domains and is implicated in the programmed cell death of intersegmental muscles of adult moth *Manduca sexta* (Hu et al., 1999; see Figure 1F). We have not yet described the function of PDLP in adult insects. Overexpression of DALP has been shown to inhibit *in vitro* myotube formation of mouse C2C12 myoblasts, and hence DALP was proposed to function as a homolog of one of the Paxillin-superfamily members, Hic-5 (Hu et al., 1999). However, overexpression of PDLP did not cause such an inhibitory effect under conditions where overexpressed Hic-5 acted as an inhibitor (data not shown). Rather than being involved in the determination of the final fate of mesodermal cells in *Drosophila* embryogenesis (Bate, 1993), the timing of PDLP expression appears to be concomitant with late events in the myogenesis, cell migration and attachment, and cytoskeletal rearrangements, where integrins play important roles, again similar to those assigned for *Mlp60A* and *Mlp84B* (Stronach et al., 1996).

The myotendinous junctions of the *Drosophila* embryo are an ideal system for studies of primitive yet fundamental functions and diversities of integrins, where the tendon cells express $\alpha\text{PS1}\beta\text{PS}$ integrin and the muscle cells express $\alpha\text{PS2}\beta\text{PS}$ integrin (for review see Brown et al., 2000). They also provide model systems for the analysis of the inductive interactions between cells of distinct fates, as well as the musculature development in higher organisms (for review see Volk, 1999). The study

described here revealed that *Drosophila* has a unique system expressing two types of LIM proteins from the *paxillin* gene locus, which show different patterns of expression at the myotendinous junctions during embryogenesis; the biological significance of which is thus worth analyzing further.

During our analysis, a paper describing the identification and properties of the *Drosophila paxillin* gene and its protein product has appeared (Wheeler and Hynes, 2001), in which PDLP was not identified.

METHODS

Cloning of DPxn37 and PDLP cDNAs. Using a 0.3 kb *Xho*I fragment of CK00121 (DDBJ/EMBL/GenBank accession No. AA142016) as a probe, an eye imaginal disc cDNA library in $\lambda\text{gt}10$ (a gift from A. Cowman) was screened using standard protocols. Among 16 independent clones thus obtained from approximately one million screened, the longest clone being 2.0 kb in size, DPAX10 (DDBJ/EMBL/GenBank accession No. AB048194) was analyzed for nucleotide sequence of the DPxn37 transcript.

cDNA for PDLP (DDBJ/EMBL/GenBank accession No. AB056465) was isolated as follows using poly(A)⁺ RNA of the late pupae, by a method of 5'- and 3'-rapid amplification of cDNA ends (RACE), which was carried out according to the manufacturer's instructions (SMART RACETM cDNA Amplification Kit, Clontech). 5'-RACE was first carried out using the primer 5'-GTTCA-GCGCCGAGATGTAGTTCTC, synthesized based on the nucleotide sequence of DPxn37 exon 8 (nt 1590–1567 of DPxn37). Two different sized cDNAs, 1.5 and 0.7 kb long, were then obtained and cloned into pCR2.1-TOPO (Invitrogen). Sequence

analysis revealed that the long cDNA corresponded to the *DPxn37* transcript, and the short one contained an exon at its 5'-end that was not present in the *DPxn37* transcript. 3'-RACE was next carried out using the primer 5'-TTCAGTCGAG-GCAAGCACATCGAC, which corresponded to the 5'-end nucleotide sequence of the short cDNA, and cDNA 1.4 kb in size was obtained, which corresponds to the *PDLP* transcript, and subjected to sequence analysis after being cloned into pCR2.1-TOPO. The *PDLP* cDNA thus obtained contains a 207-bp 5'-untranslated region, and a 593-bp 3'-untranslated region.

Gene expression. Poly(A)⁺ RNAs from flies in various stages of development were prepared by Micro Poly(A) Pure™ mRNA purification kit (Ambion), and 4 µg were used per lane. Northern blotting was performed by standard methods, using ³²P-labeled DPAX10 cDNA as a probe.

In situ hybridization using digoxigenin-labeled antisense RNA probes and alkaline phosphatase substrate was carried out as described (Ishimaru *et al.*, 1999). The RNA probes correspond to nt 1–876 of the DPAX10 cDNA, and nt 709–1012 of the *PDLP* cDNA, which corresponds to the COOH-half of the LIM3 domain of *PDLP* and the 3'-untranslated region.

Antibodies and protein expression. DNA fragments encoding the N-terminal region of *DPxn37* (amino acids 2–346) and the *PDLP*-specific region (amino acids 168–197) were cloned into pGEX-2T and pGEX-2TK (Amersham Pharmacia), respectively, using PCR-coupled engineered restriction sites, and expressed in bacteria as fusion proteins with glutathione *S*-transferase (GST) sequence. Each fusion protein was purified to near homogeneity using glutathione–Sephadex beads (Amersham Pharmacia), and was immunized into rabbits. Resulting sera were first subjected to ammonium sulfate precipitation (30–50%). After being resolved in phosphate-buffered saline, each preparation of serum was pre-absorbed using GST protein coupled to NHS-activated Sepharose beads (Amersham Pharmacia Biotech) and then affinity-purified using the GST fusion protein used for the immunizations, coupled to NHS-activated Sepharose beads.

S2 cells, maintained at 27°C with 10% fetal calf serum (FCS)/HyQ CCM3 medium (Hyclone), were transfected by Cellfectin (Gibco-BRL) with pUAST-(myc)₆-*DPxn37* or pUAST-*PDLP* plasmids, constructed by using PCR-coupled engineered restriction sites, together with pWA-GAL4. At 48 h after transfection, cell proteins were subjected to immunoblotting analysis, coupled with SDS-PAGE, as described (Mazaki *et al.*, 1997).

Whole mount staining of embryos was performed by standard procedures using affinity-purified rabbit polyclonal anti-*DPxn37* or anti-*PDLP* antibodies, anti-α PS2 integrin antibody (CF.2C7, a gift from D.L. Brower) or mouse monoclonal anti-phosphotyrosine antibody 4G10 (UBI). In brief, embryos were collected, dechorinated, fixed with a mixture of 4% paraformaldehyde solution and heptane, and devitellinized with a methanol–heptane mixture. Embryos were then washed and permeabilized with PBS-S (PBS containing 0.2% saponin), and blocked with 5% FCS in PBS-S for 30 min before incubation with antibodies. Secondary antibodies used were Cy3-conjugated donkey anti-rabbit IgG and Cy5-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch). Stained embryos were imaged by using a confocal laser scanning microscope (model 510; Carl Zeiss).

Protein subcellular localization. NIH 3T3 cells transfected with EGFP- or DsRed-tagged *DPxn37* and EGFP-tagged *PDLP* plasmids were fixed and subjected to immunofluorescence characterization,

as described previously (Nakamura *et al.*, 2000). These tagged constructs were made by ligating each entire region of *DPxn37* and *PDLP* cDNA in-frame with the C-terminal end of each tag protein cDNA and cloned into pBabePuro vectors using PCR-coupled engineered restriction sites, similar to the case of human *paxillin* (Nakamura *et al.*, 2000). Endogenous Paxillin was detected using polyclonal rabbit Ab199-217 (Mazaki *et al.*, 1997), which does not cross-react with *DPxn37* or *PDLP*, coupled with Cy3-conjugated donkey anti-rabbit IgG. F-actin was visualized by biotinylated-phalloidin and Cy5-conjugated streptavidin (Molecular Probes). Confocal images were acquired using a confocal laser scanning microscope (model 510; Carl Zeiss).

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REFERENCES

- Arber, S., Halder, G. and Caroni, P. (1994) Muscle LIM protein, a novel essential regulator of myogenesis, promotes myogenic differentiation. *Cell*, **79**, 221–231.
- Bate, M. (1993) The mesoderm and its derivatives. In Bate, M. and Martinez Arias, A. (eds), *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1013–1090.
- Bogaert, T., Brown, N. and Wilcox, M. (1987) The *Drosophila* PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments. *Cell*, **51**, 929–940.
- Brower, D.L., Wilcox, M., Piovant, M., Smith, R.J. and Reger, L.A. (1984) Related cell-surface antigens expressed with positional specificity in *Drosophila* imaginal discs. *Proc. Natl Acad. Sci. USA*, **81**, 7485–7489.
- 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, N.H., Gregory, S.L. and Martin-Bermudo, M.D. (2000) Integrins as mediators of morphogenesis in *Drosophila*. *Dev. Biol.*, **223**, 1–16.
- Burridge, K., Turner, C.E. and Romer, L.H. (1992) Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. *J. Cell Biol.*, **119**, 893–903.
- Crawford, A.W., Pino, J.D. and Beckerle, M.C. (1994) Biochemical and molecular characterization of the chicken cysteine-rich protein, a developmentally regulated LIM-domain protein that is associated with the actin cytoskeleton. *J. Cell Biol.*, **124**, 117–127.
- Dawid, I.B., Toyama, R. and Taira, M. (1995) LIM domain proteins. *C. R. Acad. Sci. III*, **318**, 295–306.
- Dawid, I.B., Breen, J.J. and Toyama, R. (1998) LIM domains: multiple roles as adaptors and functional modifiers in protein interactions. *Trends Genet.*, **14**, 156–162.

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- Di Cesare, A., Paris, S., Albertinazzi, C., Dariozzi, S., Andersen, J., Mann, M., Longhi, R. and de Curtis, I. (2000) p95-APP1 links membrane transport to Rac-mediated reorganization of actin. *Nature Cell Biol.*, **2**, 521–530.
- Gregory, S.L. and Brown, N.H. (1998) *kakapo*, a gene required for adhesion between and within cell layers in *Drosophila*, encodes a large cytoskeletal linker protein related to plectin and dystrophin. *J. Cell Biol.*, **143**, 1271–1282.
- Harden, N., Lee, J., Loh, H.Y., Ong, Y.M., Tan, I., Leung, T., Manser, E. and Lim, L. (1996) A *Drosophila* homolog of the Rac- and Cdc42-activated serine/threonine kinase PAK is a potential focal adhesion and focal complex protein that colocalizes with dynamic actin structures. *Mol. Cell Biol.*, **16**, 1896–1908.
- Hu, Y., Cascone, P.J., Cheng, L., Sun, D., Nambu, J.R. and Schwartz, L.M. (1999) *Lepidopteran* DALP, and its mammalian ortholog HIC-5, function as negative regulators of muscle differentiation. *Proc. Natl Acad. Sci. USA*, **96**, 10218–10223.
- Ishimaru, S., Williams, R., Clark, E., Hanafusa, H. and Gaul, U. (1999) Activation of the *Drosophila* C3G leads to cell fate changes and overproliferation during development, mediated by the RAS-MAPK pathway and RAP1. *EMBO J.*, **18**, 145–155.
- Kondo, A., Hashimoto, S., Yano, H., Nagayama, K., Mazaki, Y. and Sabe, H. (2000) A new paxillin-binding protein, PAG3/Pap α /KIAA0400, bearing an ADP-ribosylation factor GTPase-activating protein activity, is involved in paxillin recruitment to focal adhesions and cell migration. *Mol. Biol. Cell*, **11**, 1315–1327.
- Lipsky, B.P., Beals, C.R. and Staunton, D.E. (1998) Leupaxin is a novel LIM domain protein that forms a complex with PYK2. *J. Biol. Chem.*, **273**, 11709–11713.
- Mazaki, Y., Hashimoto, S. and Sabe, H. (1997) Monocyte cells and cancer cells express novel paxillin isoforms with different binding properties to focal adhesion proteins. *J. Biol. Chem.*, **272**, 7437–7444.
- Mazaki, Y. et al. (2001) An ADP-ribosylation factor GTPase-activating protein Git2-short/KIAA0148 is involved in subcellular localization of paxillin and actin cytoskeletal organization. *Mol. Biol. Cell*, **12**, 645–662.
- Nakamura, K., Yano, H., Uchida, H., Hashimoto, S., Schaefer, E. and Sabe, H. (2000) Tyrosine phosphorylation of paxillin α is involved in temporospatial regulation of paxillin-containing focal adhesion formation and F-actin organization in motile cells. *J. Biol. Chem.*, **275**, 27155–27164.
- 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 association with CRK in NBT-II cells. *J. Cell Biol.*, **148**, 957–970.
- Sadler, I., Crawford, A.W., Michelsen, J.W. and Beckerle, M.C. (1992) Zyxin and cCRP: two interactive LIM domain proteins associated with the cytoskeleton. *J. Cell Biol.*, **119**, 1573–1587.
- Schmeickel, K.L. and Beckerle, M.C. (1994) The LIM domain is a modular protein-binding interface. *Cell*, **79**, 211–219.
- Stronach, B.E., Siegrist, S.E. and Beckerle, M.C. (1996) Two muscle-specific LIM proteins in *Drosophila*. *J. Cell Biol.*, **134**, 1179–1195.
- Stronach, B.E., Renfranz, P.J., Lilly, B. and Beckerle, M.C. (1999) Muscle LIM proteins are associated with muscle sarcomeres and require dMEF2 for their expression during *Drosophila* myogenesis. *Mol. Biol. Cell*, **10**, 2329–2342.
- Strumpf, D. and Volk, T. (1998) Kakapo, a novel cytoskeletal-associated protein is essential for the restricted localization of the neuregulin-like factor, vein, at the muscle–tendon junction site. *J. Cell Biol.*, **143**, 1259–1270.
- Turner, C.E. (1991) Paxillin is a major phosphotyrosine-containing protein during embryonic development. *J. Cell Biol.*, **115**, 201–207.
- Turner, C.E. (1998) Paxillin. *Int. J. Biochem. Cell Biol.*, **30**, 955–959.
- Turner, C.E. (2000) Paxillin and focal adhesion signalling. *Nature Cell Biol.*, **2**, E231–E236.
- Turner, C.E., Brown, M.C., Perrotta, J.A., Riedy, M.C., Nikolopoulos, S., McDonald, A.R., Bagrodia, S., Thomas, S. and Leventhal, P.S. (1999) Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF-GAP protein: a role in cytoskeletal remodeling. *J. Cell Biol.*, **145**, 851–863.
- Volk, T. (1999) Singling out *Drosophila* tendon cells: a dialogue between two distinct cell types. *Trends Genet.*, **15**, 448–453.
- Wheeler, G.N. and Hynes, R.O. (2001) The cloning, genomic organization and expression of the focal contact protein paxillin in *Drosophila*. *Gene*, **262**, 291–299.
- Yano, H., Uchida, H., Iwasaki, T., Mukai, M., Akedo, H., Nakamura, K., Hashimoto, S. and Sabe, H. (2000) Paxillin α and Crk-associated substrate exert opposing effects on cell migration and contact inhibition of growth through tyrosine phosphorylation. *Proc. Natl Acad. Sci. USA*, **97**, 9076–9081.
- Zhang, J., Zhang, L.X., Meltzer, P.S., Barrett, J.C. and Trent, J.M. (2000) Molecular cloning of human Hic-5, a potential regulator involved in signal transduction and cellular senescence. *Mol. Carcinog.*, **27**, 177–183.

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