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Minireview

The paxillin LD motifs

David A. Tumbarello, Michael C. Brown, Christopher E. Turner*

Department of Cell and Developmental Biology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210, USA

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Abstract Adapter/scaffold proteins, through their multidomain structure, perform a fundamental role in facilitating signal transduction within cells. Paxillin is a focal adhesion adapter protein implicated in growth factor- as well as integrin-mediated signaling pathways. The amino-terminus of paxillin contains five leucine-rich sequences termed LD motifs. These paxillin LD motifs are highly conserved between species as well as within the paxillin superfamily. They mediate interactions with several structural and regulatory proteins important for coordinating changes in the actin cytoskeleton associated with cell motility and cell adhesion as well as in the regulation of gene expression.

Key words: Actin cytoskeleton; Cell adhesion; Focal adhesion; Integrin signaling

1. Introduction

Cell homeostasis is regulated through the integration of extracellular signals impacting both growth factor- and adhesion- or integrin-mediated receptor pathways. Engagement of these receptors with their cognate ligands initiates multiple convergent intracellular signaling cascades that give rise to a variety of cellular responses including changes in gene expression, cell proliferation, modulation of cell shape and changes in cell motility [1–3]. Efficient signal transduction, specification and amplification is effected through the utilization of selective scaffold or adapter proteins which facilitate the assembly of multi-protein signaling complexes. Although the concept of scaffold proteins was first invoked in the context of growth factor signaling through the mitogen-activated protein kinase cascade [4], examples of similar multi-domain proteins have emerged in the context of integrin-mediated signaling events. One protein that has been suggested to function in such an adapter/scaffold capacity is the focal adhesion protein paxillin (Fig. 1) [5]. This review will focus on a discussion of a novel protein-binding domain, the LD motif, identified in the N-terminus of paxillin, and detail its role in facilitating paxillin's adapter function in the context of cell–extracellular matrix-mediated signaling events.

*Corresponding author. Fax: (1)-315-464 8535.
E-mail address: turnerce@upstate.edu (C.E. Turner).

2. The paxillin superfamily and LD motif structure

Paxillin is a 559 amino acid protein with an apparent molecular weight of 68 kDa. It is comprised of an N-terminal 313 amino acid domain that at first glance exhibits no obvious sub-domain structure but contains several tyrosine and serine phosphorylation sites important in integrin-mediated signaling associated with cell adhesion, motility and growth factor signaling [5]. The C-terminus is made up of four double zinc finger LIM domains, which serve as binding sites for the tyrosine phosphatase PTP-PEST, tubulin and several uncharacterized serine/threonine kinases, and also contains the focal adhesion targeting motif (Fig. 2) [5–8]. Paxillin was originally identified in a screen for proteins tyrosine-phosphorylated by the pp60v-src oncogene in Rous sarcoma virus-transformed fibroblasts [9,10]. Subsequently it was characterized as a focal adhesion protein with the capacity to bind directly to the structural actin-binding protein, vinculin [10,11], as well as the non-receptor tyrosine kinase FAK [12,13]. FAK represents an important transducer of integrin and growth factor signaling being associated with regulation of cell proliferation, cell motility and apoptosis [14]. The paxillin N-terminus contains the binding sites for both vinculin and FAK [12,15]. Deletion mutagenesis studies delineated the specific amino acid sequences within paxillin that mediate these interactions [15]. Interestingly, both vinculin and FAK were found to bind to overlapping sequences, each of which contains a short leucine-rich sequence with the general consensus LDXLLXXL. In view of the conserved leucine and aspartic acid residues (found in all the LD motifs except LD 3 where the LD is substituted by a VE) they have been named LD motifs [15,16]. This sequence is repeated five times within the paxillin amino-terminus (Fig. 2). Furthermore, the LD motifs are highly conserved throughout the paxillin superfamily members, Hic-5, PaxB and Leupaxin, as well as being conserved between species as diverse as *Drosophila*, *Xenopus*, and humans (Figs. 2 and 3) [16–20]. Indeed the highest level of conservation between all of these proteins is found within the LD motifs and the LIM domains suggesting evolutionary conservation of important structural domains. The diversity of the paxillin family has recently been extended to include a truncated protein comprising just the LIM domains of paxillin, called DALP (death-associated LIM-only protein), perhaps representing a natural antagonist to the function of the full length protein (Fig. 2) [21].

Using structural modeling programs, the LD motifs of paxillin have been predicted to form an amphipathic, α-helical structure with each of the leucine residues being positioned

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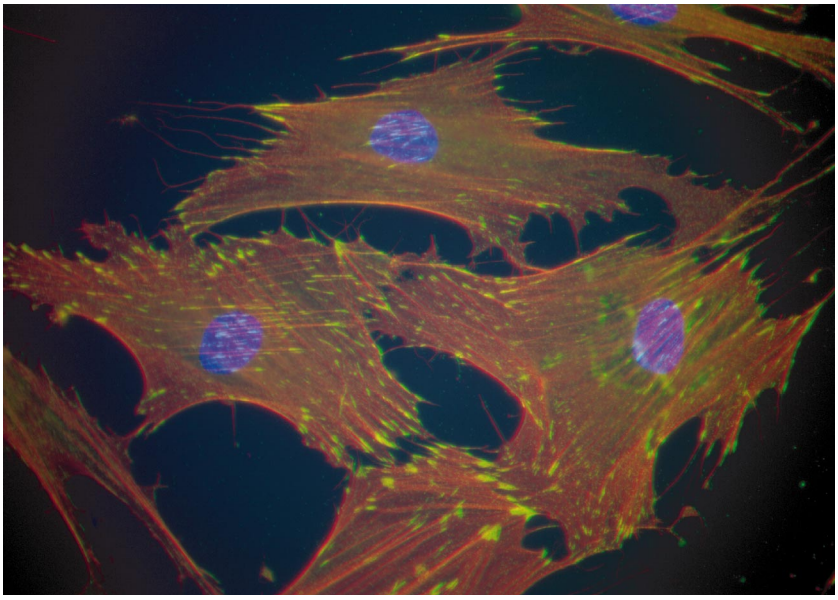

Fig. 1. Paxillin subcellular localization in CHO K1 cells. Paxillin (green) localizes to focal adhesions at the ends of actin stress fibers (red). Nuclei are labeled in blue.

on one face of the helix thereby forming a hydrophobic protein-binding interface (Fig. 2B) [16,22]. Subsequent crystallographic or nuclear magnetic resonance studies will be required to confirm this prediction. The paxillin LD motifs are evenly distributed throughout the N-terminus. One intriguing possibility raised by this arrangement is that the LD motifs may function together in the recruitment of multiple signaling partners into close proximity with each other to facilitate signal propagation. Specificity may be imparted through the recruitment of different combinations of proteins reflecting the individual signaling requirements arising as a result of different external stimuli.

To determine whether the LD motifs could function as specific protein-binding motifs, each was expressed as a separate GST-LD fusion protein and used in 'pull-down' assays. From these experiments several observations are noteworthy. First they confirmed that the LD motifs were indeed responsible for the vinculin and FAK interactions with paxillin. Second, they demonstrated the specificity of the interactions and third that this specificity varied depending on the particular binding partner involved. For instance, vinculin can bind to LDs 1, 2 and 4 while FAK binding is restricted to LDs 2 and 4 [15,23]. This combination of redundancy and specificity has been reinforced by the subsequent identification and characterization of additional LD motif-binding partners including paxillin kinase linker (PKL) which binds only to LD 4 [23],

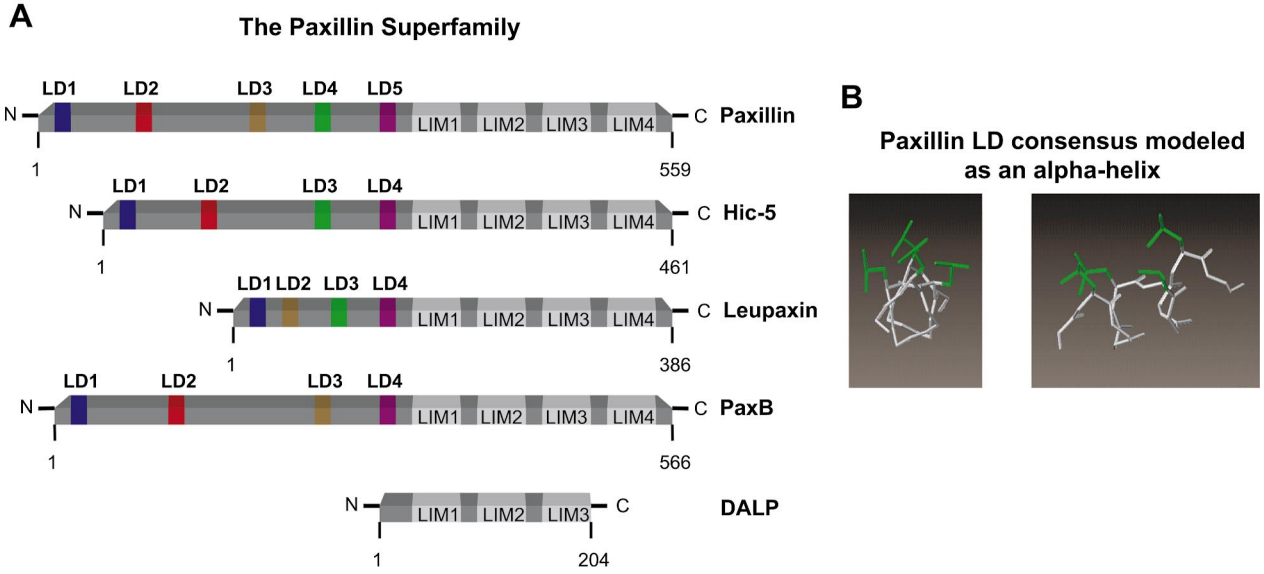

Fig. 2. A: Paxillin superfamily members. Colors of LD motifs represent corresponding LD motifs conserved between family members. B: Paxillin LD consensus (LDXLLXXL) modeled as an α-helix. Using Insight II 970 (MS1) molecular modeling software, the paxillin LD consensus was modeled as an amphipathic helix. The conserved non-polar leucine residues are depicted in green and are arranged on one face of the α-helix, presenting a hydrophobic-binding surface.

Fig. 3. Amino acid sequence alignment of the amino-terminus of paxillin from different species. Multi-alignment of amino acids 1–322 of human paxillin against avian, *Xenopus*, and *Drosophila* forms of paxillin. LD motifs are color coded in accordance with Fig. 1A.

integrin-linked kinase (ILK) which binds to LD 1 and the actin-binding protein actopaxin which binds both LD 1 and 4 [24,25]. Importantly, the specificity of these interactions is maintained when the LD motifs are presented in the context of the intact paxillin protein. The FAK-related tyrosine kinase PYK2 also binds paxillin. Although the binding parameters are likely to be the same, which LD motifs are utilized for this interaction remains to be determined. No binding partners have thus far been identified for the paxillin LD 3 and LD 5 motifs.

Consistent with the high degree of conservation of sequence between the LD motifs of different paxillin family members, these proteins, as far as has been tested, interact with the same complement of proteins. For instance Hic-5 binds vinculin, FAK, PKL, and actopaxin [18,23,24,26] while the hematopoietic cell-specific form of paxillin, leupaxin, binds PYK2 [17].

3. The paxillin-binding subdomain (PBS)

The binding module through which proteins interact with paxillin LD motifs was first identified as a result of delineation of the paxillin-binding sites on vinculin and FAK and was named the paxillin-binding subdomain (PBS) [11,27]. Related functional sequences have now been identified in the other LD-binding proteins actopaxin, ILK, and PKL [24,25]. Details of the structural and molecular interaction between the PBS and the LD motif are unknown to date. It is evident, however, from an alignment of these sequences that the PBS domains are poorly conserved at the amino acid level when compared with the LD motifs. Interestingly though, secondary structure predictions, using the Hierarchical Neural Network, suggest that each of these PBS sequences forms an α-helical structure flanked by regions of random coil. The heterogeneity between the individual PBS domains may form the basis for their selective binding to particular LD motifs, alternatively such specificity may be provided by the flanking sequences of both the PBS and the LD motifs.

4. Paxillin LD motifs and cell signaling

How does paxillin, through interaction with its LD motif-binding partners, contribute to cell function and signaling? First, mutagenesis of individual paxillin LD motifs or corresponding PBS domains indicates that the interaction with paxillin is essential for normal subcellular localization to focal adhesions of vinculin, actopaxin, ILK and PKL [15,24,25]. Results from Tachibana et al. [27] suggest that this is also the case for FAK targeting although this remains controversial [28].

Interestingly, several of the paxillin LD motif-binding partners function in the context of actin cytoskeleton dynamics. For instance, both vinculin and actopaxin bind to F-actin [24,29]. In the case of actopaxin, over-expression of a PBS mutant defective for paxillin binding results in impaired cell adhesion and cell spreading on collagen suggesting that the ability of paxillin to recruit actopaxin to the plasma membrane may be a critical step in an actopaxin-dependent nucleation and assembly of actin filaments at these sites of cell adhesion [24]. PKL is a member of a larger family of ARF GAP proteins including GIT-1/2 [30,31]. PKL links paxillin, through its LD 4 motif [23], to a complex of proteins comprising the Rac exchange factor, PIX/COOL [32,33] linked to the p21 activated serine/threonine kinase, PAK and the SH2-SH3 adapter protein Nck. PAK is an important mediator of cytoskeletal reorganization associated with cell motility and neurite outgrowth [34,35]. The importance of the PKL-paxillin interaction in facilitating PAK's function is suggested by the abnormal cell morphology and reduced directed motility of cells over-expressing either a paxillin LD 4 motif deletion mutant or a PKL mutant lacking its PBS domain [36]. Additionally, the ability of paxillin to recruit GIT proteins to focal adhesions may facilitate the disassembly of these structures, thereby further enhancing cell motility [37]. The association of PKL with the paxillin LD 4 motif has also been shown to be necessary for PAK activation by the T-cell receptor [38].

As mentioned earlier, FAK is a critical regulator of cytoskeletal organization and focal adhesion turnover [39]. The binding of FAK to paxillin is critical for efficient FAK/Src-dependent tyrosine phosphorylation of paxillin [40] which in turn is necessary for cell motility in certain cell types [41] and facilitates normal cell adhesion to fibronectin [42].

The serine/threonine kinase ILK plays a prominent role in integrin- and growth factor-mediated signaling events. It is involved in cell survival, cell growth control/transformation, fibronectin matrix assembly and epithelial–mesenchymal transition [43–45]. The significance of the ILK–paxillin interaction in controlling these events through the ability of paxillin to target ILK to focal adhesions [25] represents an important

area for future study. Recent evidence for a direct interaction between ILK and actopaxin [46,47,55] provides an additional level of complexity.

Other than paxillin, Hic-5 has been the most extensively studied paxillin superfamily member [18,48]. Hic-5 and paxillin share many of the same binding partners, but differ in their ability to serve as substrates for tyrosine kinases. These features combined with their frequent co-expression raise the intriguing possibility that Hic-5 may at times complement and at other times antagonize paxillin function. For instance, the LD 3 motif of Hic-5, which corresponds to the paxillin LD 4 motif, is required for Hic-5-mediated inhibition of cell spreading, possibly by sequestering FAK from paxillin [49].

Paxillin and its LD motifs also represent an important target for transforming DNA viruses such as papillomavirus, which is the cause of a variety of dysregulated growth conditions ranging from benign warts to malignant cervical cancers. Over-expression in fibroblasts of the BE6 oncoprotein produced by bovine papillomavirus leads to cell transformation and associated disruption of the actin cytoskeleton. This is likely the result of a direct interaction between BE6 and the paxillin LD motifs which in turn disrupts paxillin binding to vinculin and FAK [50–52]. The paxillin–BE6 interaction is necessary for the transforming capabilities of the E6 oncoprotein and the pathogenicity of the papillomavirus [51]. Since BE6 binds preferentially to the LD 1 motif of paxillin [50] the paxillin interactions with actopaxin and ILK may also be compromised, although this awaits formal testing. Paxillin's interaction with BE6 has been shown, through mutational analysis, to be mediated via a minimal LXXLL sequence within its LD motif termed the 'charged leucine motif' [53,54]. This modified BE6–paxillin LD motif association may impart a unique selective advantage for the BE6 protein over the various physiologic LD motif-binding proteins.

5. Conclusions

There is now substantial evidence that the LD motifs within the paxillin superfamily represent a discrete protein-binding module. Their importance in facilitating integrin signaling through interactions with a growing list of binding partners is clear. Less certain is the more general utility of the LD motif beyond the paxillin superfamily. A database search for LD motif-containing proteins yields a limited number of potential candidates, as does a similar search for proteins containing PBS motifs [16]. Although this list represents an interesting cross-section of structural and signaling proteins, the ability of any of these molecules to utilize their LD or PBS motifs in productive interactions with other proteins remains to be determined.

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