Paxillin: Adapting to Change

MICHAEL C. BROWN AND CHRISTOPHER E. TURNER

Department of Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, New York

I. Introduction

It is well established that an organism's normal development and maintenance, as well as its capacity to recover from injury and infection, is dictated to a large degree by the ability of individual cells to sense and respond appropriately to changes in their immediate external environment. Thus a complex, interwoven array of intracellular signaling pathways is modulated by cell adhesion to the extracellular matrix, combined with engagement of soluble growth factors and cytokines with their cognizant receptors to control cell proliferation and survival as well as changes in cell shape and motility. Functional defects or imbalance in these pathways can result in developmental abnormalities, tissue degeneration, hypertrophy, cell transformation, and metastasis.

Cell adhesion signaling from the extracellular matrix is initiated primarily via engagement of members of the integrin family of transmembrane receptors (103), with their appropriate ligands: fibronectin, laminin, vitronectin, etc. Contributions from other matrix receptor types such as the syndecan family of proteoglycans provide an additional level of control and complexity (306). In contrast to many growth factor receptors such as receptor tyrosine kinases, integrins have no inherent enzymatic activity; instead, intracellular signaling is initiated via clustering of the receptors in the plane of the plasma membrane and the concurrent recruitment and activation of intracellular signaling molecules via association with

---

Brown, Michael C., and Christopher E. Turner. Paxillin: Adapting to Change. Physiol Rev 84: 1315–1339, 2004; 10.1152/physrev.00002.2004.—Molecular scaffold or adaptor proteins facilitate precise spatiotemporal regulation and integration of multiple signaling pathways to effect the optimal cellular response to changes in the immediate environment. Paxillin is a multidomain adaptor that recruits both structural and signaling molecules to focal adhesions, sites of integrin engagement with the extracellular matrix, where it performs a critical role in transducing adhesion and growth factor signals to elicit changes in cell migration and gene expression.
the cytoplasmic tails of the integrin α- and β-subunits (70). Structural proteins are similarly recruited to provide a physical link to the actin cytoskeleton (153). These macromolecular adhesion complexes are commonly referred to as focal complexes or focal adhesions (226). The fidelity of integrin and growth factor receptor signaling relies on several common features. In particular, multidomain adaptor or scaffold proteins are utilized to recruit the appropriate complement of signaling intermediates and effector proteins to discrete subcellular compartments. Not only does this promote efficient activation of a given pathway but also adaptor proteins provide an ideal platform for the controlled integration of multiple signaling pathways, thereby coordinating such diverse cellular responses as changes in gene expression and reorganization of the cytoskeleton.

First described over a decade ago, paxillin is one of the prototypical adaptor proteins involved in integrin signaling. In the intervening years, two new paxillin family members have emerged, and numerous new paxillin binding proteins have been identified. This review presents our current understanding of the role for the paxillin family in the integration of integrin and growth factor signaling (Fig. 1).

II. PAXILLIN SUPERFAMILY

A. Paxillin

Paxillin was initially characterized as a 68-kDa focal adhesion protein exhibiting a significant increase in tyrosine phosphorylation upon v-src expression (72). Shortly thereafter, it was purified to homogeneity from chicken gizzard smooth muscle tissue, an abundant source of cytoskeletal proteins, and identified as a novel binding partner for the focal adhesion and actin binding protein vinculin (279). It was named paxillin, derived from
the Latin *paxillus*, a stake or peg, consistent with its proposed function in linking actin filaments to integrin-rich cell adhesion sites.

Paxillin was cloned by /H9261gt11/ expression screening in 1994 from an avian cDNA library (281) and shown to encode for a 559-amino acid protein. This protein is organized into a series of protein binding modules (Fig. 2). The amino terminus contains five LD motifs and several SH2-binding domains while the carboxy terminus consists of four LIM domains (48, 124, 274). These domains will be discussed in further detail below. Paxillin encoding cDNAs have subsequently been isolated from human (221), mouse (81, 177), frog (193), zebrafish (43), fly (304, 311), slime mold (AAM09351) (73), and yeast (163). Interestingly in nematode, a paxillin that contains both the amino terminus and carboxy terminus has yet to be identified, although a protein with the four LIM domains well conserved (including exon splice boundaries) exists (NM065879 *Caenorhabditis elegans*, BQ611130 *Caenorhabditis briggsae*). Further work on nematode genomic content and organization will reveal whether the full-length coding sequence remains to be identified or if the unique physiology of worms mandates the expression of a paxillin lacking the amino-terminal protein interaction domains. Regardless, the evolutionary conservation of paxillin attests to the critical importance of this molecule.

In higher eukaryotes, three paxillin alternative splice isoforms have been identified. Paxillin /H9251/ is the principal, ubiquitously expressed isoform, whereas the /H9252/ and /H9253/ isoforms contain a 34- and 48-amino acid insertion, respectively, between amino acids 277/278 (Fig. 2). Although initial reports suggested the lack of a murine /H9253/ isoform (177), all three isoforms appear to also be expressed in mouse (308). It is not yet known whether other splice isoforms exist in lower organisms, although in zebrafish two proteins that differ by 5 kDa are detectable by Western immunoblotting (43). A fourth paxillin isoform denoted paxillin /H9254/ is the product of alternative translation initiation beginning at amino acid 132 (293; unpublished observations). Importantly, the consensus Kozak for this downstream start methionine is conserved across species. In total therefore, there is the potential in higher eukaryotes for the expression of six paxillin isoforms by a combination of alternative splicing and translation initiation.

Interestingly, a novel splice variant called PDLP is expressed in *Drosophila* that encodes a carboxy-terminal portion of paxillin including LIM domains 1 and 2 as well as the first zinc finger of LIM3 and a novel second zinc finger to generate a new LIM3 (304, 311). A Lepidopteran PDLP ortholog, death-associated LIM-only protein (DALP), originally proposed to be a Hic-5 ortholog, has been described (100).

Insofar as genomic organization is concerned, in humans the paxillin gene is comprised of 11 exons located on chromosome 12, mapped to 12q24.2 (221). In rat, paxillin is located on chromosome 12q24.2, whereas mouse paxillin is positioned on chromosome 5. Zebrafish paxillin is reported at linkage group 5, in *Drosophila* paxillin is on...
the long arm of chromosome 2 at 37D and chromosome III in *C. elegans*.

### B. Hic-5

The paxillin paralog hydrogen peroxide inducible clone-5 (Hic-5) was first identified in an analysis of mouse osteoblast transforming growth factor (TGF)-β and hydrogen peroxide-inducible cDNAs and encodes a 444-amino acid protein (247) (Fig. 2). Human Hic-5 was later cloned in an androgen receptor two-hybrid screen and named ARA55 for androgen receptor coactivator 55-kDa protein (63). A Hic-5 splice isoform that contains an additional 17 amino acids on the amino terminus (containing an LD motif) was originally identified in a screen for murine paxillin (266) and later in a differential display screen of senescent versus non-senescent human keratinocytes (328). Based on homology with paxillin, we suggest that the “full-length” Hic-5 splice isoform containing the amino-terminal LD1 “extension” be denoted Hic-5 α and the smaller form Hic-5 β. As yet, there is no evidence that Hic-5 orthologs exist in lower eukaryotes, although unpublished observations suggest Hic-5 is expressed in zebrafish (43). Interestingly, Hic-5 shares the same 11-exon genomic organization as paxillin, consistent with an evolutionary duplication event, and is located on chromosome 16p11 in humans (328) and chromosome 7 in mouse (266).

### C. Leupaxin

Leupaxin is a 386-amino acid 45-kDa family member that is predominantly expressed in leukocytes, as is reflected in the naming convention (150). Leupaxin is located on chromosome 11cen-11q12.3 and is encoded by 9 exons. It lacks its paralogs exons 4 and 5 and consequently has a significantly different structural composition and therefore likely unique regulation and function (Fig. 2). No evidence exists to support leupaxin expression in lower eukaryotes.

### III. PAXILLIN STRUCTURE

The multidomain structure of paxillin and the lack of identifiable enzymatic motifs first suggested it was an adaptor protein (281). Within the amino terminus are five leucine-rich regions, termed paxillin LD motifs, that function in protein recognition (23). The carboxy terminus is comprised of four lin-11, isl-1, mec-3 (LIM) domains that also mediate protein-protein interactions (48). Dispersed throughout the molecule are many serine/threonine and tyrosine phosphorylation sites that will be discussed in more detail below. In addition, several potential proline-rich SH3-binding motifs are present within the paxillin amino terminus, consistent with a described Src SH3-paxillin association (302). The paxillin amino terminus is particularly proline and glycine rich, which in addition to the existence of a multitude of phosphoisoforms, is reflected by its aberrant electrophoretic mobility of 68–75 kDa versus a calculated molecular weight of 62 kDa.

#### A. Paxillin LD Motifs

Thus far the most extensively characterized domains within paxillin are the LD motifs. These protein recognition domains were first identified during a biochemical microdissection of paxillin initiated to identify the binding sites for the proteins vinculin and FAK (24, 281). Truncation and deletion analyses delineated two binding sites for FAK and one for vinculin. Visual sequence gazing revealed the binding sites to share a leucine-rich motif that was found to be repeated four times within the amino terminus of paxillin. Based on this, a “consensus” LDXYXXL paxillin LD-motif was proposed as an evolutionarily conserved peptide docking site for FAK and vinculin (23, 24, 274). Tong et al. (269) later proposed a fifth “degenerate” LD (LD3) that was originally disregarded due to lack of the conserved “LD” start but has since been incorporated into the nomenclature (Fig. 2).

The paxillin LD1 motif is conserved across species and paralogs and is encoded by exons 1 and 2. Notably, paxillin 5 and Hic-5 β lack the LD1 motif. The paxillin LD2 motif is encoded by exon 4 and is conserved across species and in Hic-5 but is absent in leupaxin, which has evolved a second unique LD motif that is encoded by exon 2. Paxillin LD3 motif is present within the highly divergent exon 5 and is conserved only among orthologs. The paxillin LD4 motif is encoded by exon 6 and is conserved across species and paralogs. Interestingly, the coding sequence for this LD motif is the most highly evolutionarily conserved DNA sequence of the paxillin family outside of the LIM domains. The paxillin LD5 motif is encoded by exon 7 and is conserved across species and paralogs.

The individual LD motifs provide specific protein interaction interfaces (23, 24) (Fig. 3). Within paxillin, LD1 mediates interactions with actopaxin (183), the integrin-linked kinase (ILK) (184), vinculin (278), and the papillomavirus protein E6 (268, 288). LD2 binds to vinculin and FAK/PYK2 (24, 278). LD4 binds to actopaxin (183), FAK/PYK2 (24, 265, 278), the Arf-GAPs p95PKL/GIT2/GIT1 (278), and perhaps PAK3 (90), clathrin (278), and FAPB1 (307). Thus far no binding partners have been identified for LD5 or the degenerate LD3 motif. Importantly, the capacity of Hic-5 and leupaxin to interact with these LD-binding proteins has been confirmed (64, 79, 150, 186, 266, 278).

Upon their identification, the leucine-rich paxillin LD motifs were modeled and suggested to fold as amphip-
pathic α-helices with the leucines providing a hydrophobic interface with its binding partners (23, 228, 274), a prediction subsequently confirmed experimentally (7, 91, 152). The individual paxillin (and Hic-5) LD motifs are flanked by proline- and glycine-rich segments (average composition of 25%), which may also contribute to the global folding, presentation, regulation, and function of the individual paxillin protein binding domains. This is reflected in the β- and γ-paxillin isoforms exhibiting reduced affinity for the LD4 binding proteins FAK and GIT (175, 176). Also interspersed between the LD motifs are potential SH3-binding domains and numerous phosphorylation sites, including SH2 binding domains, that may also regulate the activities and context of the LD binding partners, thereby imparting an additional means of temporal-spatial regulation of the adaptor functions of paxillin. Hic-5 and leupaxin exhibit significant differences from paxillin in the length and composition of the LD “spacing” regions and consequently likely exhibit unique regulatory and functional capacities. Indeed, Hic-5 binds to PYK2 more efficiently than to FAK (174, 194), and Hic-5 binds to GIT1 more efficiently than does paxillin to GIT1 (188).

Before our identification of LD motifs as discrete domains mediating binding to vinculin and FAK, the binding sites for paxillin on the vinculin tail (305) and the FAK focal adhesion targeting (FAT) domain (95) were identified through truncation and deletion mutagenesis. Subsequently, a FAk point mutagenesis study further localized the site of paxillin binding and allowed for a comparison to the known site of paxillin binding to vinculin (305). This led to the description of paxillin-binding subdomains (PBS) as an evolutionarily conserved paxillin binding motif (257). Thus a novel protein interaction pair, LD-PBS, was discovered. These data allowed for the creation of an algorithm to search and identify the PBS sequences within the paxillin LD binding partners PKL, ILK, and actopaxin and other potential candidates (23, 183, 184, 274, 278). The general utility of LD-PBS associations has emerged with the discovery of an interaction between gelsolin and PYK2 (294) as well as between the papillomavirus E6 protein and the ubiquitin ligase E6-AP (14, 47), the Rap-GAP E6TP1(67), and the Ca²⁺-binding protein ERC-55/E6BP (38), in addition to paxillin (211).

The crystal structures of the vinculin tail and the FAK FAT domain, which contain the PBS, have been solved (7, 10, 91, 114). FAk FAT solution structures have also been reported (66, 152). The vinculin tail and FAk FAT domain share a parallel up-down-up-down four-helix bundle. This general structural organization is shared by α-catenin, apolipoprotein E, and the p130Cas family of proteins (7, 10, 91, 114), although only vinculin and FAk bind paxillin. Interestingly, structural predictions suggest that the PBS-
containing PKL carboxy terminus also folds in a similar manner (7). In contrast, this structural organization is unlikely in the case of the ILK and actopaxin PBS domains (7, 183). Such differences are likely to dictate the optimal binding parameters for each protein and contribute to selection of the “appropriate” paxillin binding partner(s) in response to a particular extracellular cue.

B. Paxillin LIM Domains

LIM domains are double-zinc finger motifs first identified in the lin-11, isl-1, and mec-3 homeodomain proteins. The four LIM domains of paxillin are present in tandem on the carboxy terminus, thus designating paxillin a group 3 LIM family protein (48). The structures of several LIM domains have been solved and reveal that LIM domains are arranged such that each individual zinc finger is comprised of two antiparallel β-sheets that are separated by a tight turn. In addition, the two zinc fingers pack together due to hydrophobic interactions, and each LIM domain ends with a short α-helix (48, 203, 289). Interestingly, although it is now generally accepted that the primary function for LIM domains is in mediating protein-protein interactions (235), the CRIP and CRP structures are nearly identical to the DNA-binding domains of GATA-1 and the steroid hormone receptor family (203).

The capacity of LIM domains to mediate discrete subcellular localization to the actin cytoskeleton and focal adhesions was first demonstrated for muscle LIM protein (MLP) (6) and paxillin (24) and has subsequently been found to be a common link between many group 3 LIM domain proteins including the zyxin (190), PINCH (144), and FHL families of proteins (40). Although it is not believed that tandem LIM domains physically interact, there is strong evidence for cooperativity between adjacent LIM domains (6, 24, 190).

Functionally, LIM3 (with LIM2 cooperating) mediates the localization of paxillin (24) and Hic-5 (64) to focal adhesions with serine/threonine phosphorylation of LIM2/3 regulating paxillin localization to focal adhesions and consequently cell adhesion to fibronectin (25) (Figs. 3 and 4). These sites of phosphorylation are largely conserved in paxillin orthologs. The localization of leupaxin has not been extensively studied, although it colocalizes with cortical actin in lymphoid cells attached to intracellular adhesion molecule (ICAM)-I (150), podosomes in osteoclasts (79), and focal adhesions in fibroblastic cells (D. E. Staunton, personal communication). The insect paxillin splice isomoph (PDLP/DALP) that is comprised solely of LIM domains localizes to myotendonous junctions and along actin stress fibers (311), whereas the LIM domains of the yeast ortholog Pxl mediate targeting to the bud neck (163), thus offering evidence for the ancestral conservation of LIM function in subcellular compartmentalization. The identity of the paxillin focal adhesion targeting molecule has proven elusive, as it has for these other LIM domain containing proteins. It will be interesting to determine whether any relationship exists between the protein(s) that target each of the diverse LIM family proteins to their respective cytoskeletal compartments.

In addition to the identification of the LIM domains as mediating paxillin subcellular targeting, tubulin was identified as a LIM2 and LIM3 binding partner (94) and PTP-PEST as a binding partner for tandem LIM3 and LIM4 of paxillin, LIM4 in Hic-5 (41, 186), and leupaxin (79) (Fig. 3). The ability of Hic-5 and leupaxin to bind tubulin is unknown. Paxillin LIM2 also associates with and precipitates a kinase that can phosphorylate T396/401 (human),

**Paxillin Phosphorylation and Function**

![Diagram of Paxillin Phosphorylation and Function](https://example.com/paxillin_phosphorylation.png)

FIG. 4. Paxillin phosphorylation and function. Paxillin is phosphorylated on multiple tyrosine (PY), serine (PS) and threonine (PT) residues in response to cell adhesion, and/or exposure to a various soluble growth factors and cytokines (see text for details). Tyrosine phosphorylation generates docking sites for SH2 domain containing proteins such as Crk (binds PY31,118) to facilitate downstream signaling. In contrast, phosphorylation of serine and threonine residues is more likely to influence paxillin conformation and thereby allosterically affect its ability to interact with specific binding partners.
and LIM3 binds to a detergent-insoluble kinase that can phosphorylate LIM3 on S455/479 (25). The identity of these kinases as well as the capacity of Hic-5 and leupaxin LIM domains to bind to and be phosphorylated by serine/threonine kinases remains to be determined. In addition to these cytoplasmic LIM associations, Hic-5 LIM domains have been shown to be capable of binding to DNA (187), and the capacity of paxillin and Hic-5 to interact with the nuclear matrix (315) perhaps through LIM3 (121) has been described. Furthermore, both paxillin and Hic-5 can interact with the androgen and glucocorticoid receptors through LIM4 (208). The significance of these interactions and the function of paxillin and Hic-5 in gene expression is discussed below.

C. Other Paxillin Binding Partners

In addition to the LD and LIM binding partners described above, paxillin binding to many other molecules has been reported, although in many cases the sites of interaction are not clearly defined (Table 1). A direct association of paxillin with β1-integrin (231, 259), α4- and α9-subunit cytoplasmic tails has been reported (153, 155, 156, 322). The α4- and α9-integrin binding site has been localized to a region of paxillin amino-terminal and partially overlapping LD4 (154). Interestingly, mutations in the α9-integrin cytoplasmic tail that ablate paxillin and Hic-5 binding to the α-tail only partially disrupt binding to leupaxin (155). The association between paxillin and the α4-tail is inhibited by cAMP-dependent protein kinase (PKA) phosphorylation of α4 S988, thereby triggering α4-mediated motility (74, 86).

Paxillin can also interact with talin (223), poly(A)-binding protein 1 (307), ASAP1/2 (128, 195), the nerve growth factor (NGF) receptor TrkA (178), the antiapoptosis protein bcl-2 (254, 255), the focal adhesion protein TES (69), RACK (42), the serine/threonine phosphatase PP2A (110), the tyrosine phosphatase PTP-μ (206), Crk and Crkl (19, 225, 232), the tyrosine kinases Abl (143) and p210BCR/ABL (225), the protooncogene Cbl (224), and LIM kinase (62). Paxillin and Hic-5 also bind to the syndecan binding protein syndesmos (52), Hsp27 (118), Hsp72 (172), and the ring-finger ubiquitination component Rnf5 (54). Other studies have reported the coprecipitation of paxillin in complexes that contain γ-sarcoglycan (320), the tyrosine phosphatases SHP-1/2 (318), phospholipase D (113), STAT transcription factors, and translation elongation factor-1α (318). Characterization of the molecular basis of these associations so that discrete perturbation of paxillin binding can be effected will allow for the determination of the physiological consequences in forming these interactions.

IV. PAXILLIN PHOSPHORYLATION

A. Tyrosine Phosphorylation

A report characterizing the dramatic changes in paxillin tyrosine phosphorylation that occur during the complex morphogenetic events accompanying embryonic development (a process involving major changes in integrin and growth factor signaling) provided our first glimpse of the potential role for paxillin in responding to diverse extracellular cues (275). Although adhesion-associated integrin ligation is now classically associated with induction of paxillin tyrosine phosphorylation (31) via activation of FAK and Src kinases (15, 232), it is clear that a wide variety of agents that signal through a range of transmembrane receptor families trigger paxillin tyrosine phosphorylation. Paxillin is now considered to be at the crossroads of cell adhesion and growth factor modulation of intracellular signal transduction pathways (Fig. 1) (218, 230, 277). A point of commonality between these disparate extracellular stimuli is that they provoke significant changes in the organization of the cytoskeleton and/or the state of cellular proliferation.

Among receptor tyrosine kinase ligands, epidermal growth factor (EGF) (263), growth hormone (219), hepatocyte growth factor (93), insulin-like growth factor I (IGF-I) (32, 35, 141), MCP-1 (314), NGF (178), platelet-derived growth factor (210), SCF (238), steel factor (258), and vascular endothelial growth factor (1) each stimulates paxillin tyrosine phosphorylation. TGF-β and activin A, which stimulate receptor serine/threonine kinase activity, also indirectly trigger paxillin tyrosine phosphorylation (212). Similarly, agonists of many 7-pass transmembrane serpentine family receptors induce paxillin tyrosine phosphorylation including acetylcholine (298), epineph-

---

**TABLE 1. Additional paxillin binding partners**

<table>
<thead>
<tr>
<th>Binding Region</th>
<th>Amino terminus</th>
<th>Carboxy terminus</th>
<th>Unknown</th>
<th>Direct?</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL</td>
<td>+</td>
<td></td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>BCL-2</td>
<td></td>
<td>+</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>ERK</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>EF-1α</td>
<td></td>
<td></td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>HSP72</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>β1-Integrin</td>
<td></td>
<td>+</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>MEK</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Merlin/NF2</td>
<td></td>
<td>+</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Phospholipase D</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>P2A</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>PTP-μ</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>RNF5</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>α-Sarcoglycan</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>SHP-1/2</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Syndesmos</td>
<td></td>
<td>+</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>TrkA</td>
<td></td>
<td>+</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>


rime (330), angiotensin II, thrombin (283), bombesin, vasopressin, endothelin (326), bradykinin (140), cholecystokinin (68, 324), CSF-1 (318), lysophosphatidic acid (LPA), and other sphingosine metabolites (218, 240, 241). Binding of ephrins to their cognate receptors also stimulates paxillin tyrosine phosphorylation and plays a key role in regulating tissue remodeling and cell migration during embryonic development (34, 291).

Signaling through immunomodulators such as tumor necrosis factor-α (65) or formyl-methionyl-leucyl-phenylalanine (76), IgE (85), T-cell receptor (55) or complement receptor ligation (76), Fc-mediated phagocytosis (77), and reactive oxygen intermediate exposure (75) as well as viral or bacterial infection (3, 288) and exposure to their toxins including Pasteurella multocida toxin, cytotoxic necrotizing factor 1, dermonecrotic toxin, and lipopolysaccharide (136, 137) can also induce paxillin tyrosine phosphorylation. In each of these scenarios, profound alterations in the cytoskeleton are effected that are essential to activate and regulate host defenses. Finally, many different modes of cellular stress such as membrane depolarization (123, 260), tissue injury (89), hypertonicity (168), stretch (253, 317), shear stress (83), increased cell density (13), β-amylase exposures (17), and treatment with the phorbol ester phorbol 12-myristate 13-acetate (PMA) (140) have also been reported to induce paxillin tyrosine phosphorylation.

The second messenger pathways utilized to elicit these changes in paxillin tyrosine phosphorylation are beyond the scope of this review; however, as a matter of course, cell adhesion, an intact dynamic actin cytoskeleton, phosphatidylinositol 3-kinase (P3K) activity, and RhoA stimulation are generally common elements. For the most part the sites of phosphorylation induced by the stimuli listed above are not known; however, the initial characterization of adhesion-induced phosphorylation by FAK and Src revealed tyrosine residues 31 and 118 as the primary targets (15, 232). The advent of paxillin phosphorylation state-specific antibodies has greatly facilitated the identification of additional functional paxillin phosphorylation sites Y40, Y88, and Y181 (182, 233), with all but Y181 competent Src substrates (233).

A major consequence of paxillin tyrosine phosphorylation is the generation of functional SH2-binding domains that consequently operate as inducible, regulatable protein interaction domains (Fig. 4). The most extensively characterized SH2 association is with the adaptor proteins Crk/CrkL, which binds to pY31 and pY118 (19, 225, 232, 273). Other SH2 interactions that have been described are the association of p85 P3K with pY31, pY40, and pY118 and p120RasGAP with pY31 and pY118 (273). Binding to the Src-inactivating tyrosine kinases Csk via Y31 and Y118 (220, 273) and Chk (78) have also been reported. The role of paxillin tyrosine phosphorylation in modulating cellular motility has been an area of active investigation and is discussed below.

As for tyrosine phosphorylation of other paxillin family members, Hic-5 tyrosine phosphorylation through PYK2 (174) but not FAK (64) has been described following adhesion, platelet activation (194), hyperosmotic stress, v-src expression, and stimulation with LPA (174). Phosphorylation may contribute to Src inactivation via recruitment of CSK (266) and likely occurs on tyrosine-60 (Y43 Hic-5 β), which corresponds to paxillin tyrosine-118 based on exon 3 coding alignment. Leupaxin tyrosine phosphorylation has been reported (150). This likely occurs on Y20, a site encoded on exon 2, that presumably corresponds to paxillin Y31.

B. Serine/Threonine Phosphorylation

In addition to tyrosine phosphorylation, changes in paxillin serine/threonine phosphorylation are well documented (Fig. 4). After cellular activation by integrin ligation, serine residues 188/190 are phosphorylated (16, 53). Similarly, LIM2 T396/401 and LIM3 S455/479 are phosphorylated after adhesion and stimulation with angiotensin II (26). Growth factors such as activin A (212), EGF (58, 264), heregulin (286), and interleukin (IL)-3 (221), as well as the tumor promoter PMA (26, 58), muscle contraction (202), and virus infection (288) each induces serine/threonine phosphorylation of paxillin. The onset of mitosis also stimulates paxillin serine/threonine phosphorylation (313), which may function to regulate various paxillin-protein associations leading to focal adhesion and cytoskeletal disassembly associated with cell rounding before cytokinesis. For the most part, the respective kinases have not been identified. However, protein kinase C and mitogen-activated protein (MAP) kinase activities have been implicated in some instances (53, 264). Of note, the MAP kinase JNK has been reported to directly phosphorylate paxillin β T178 (102) and the p38 MAP kinase to target S85 (101) to regulate cell migration and neurite outgrowth, respectively. Interestingly, a Y88F mutation, adjacent to the S85 site, attenuated the paxillin electrophoretic mobility that is widely associated with paxillin serine/threonine phosphorylation (233). Further studies examining the potential functional interrelationship between sites of paxillin phosphorylation will be necessary. ERK1/2 (107, 158) and the p21-activated kinase PAK3 (90) have been reported to directly phosphorylate paxillin as well. The advent of phosphorylation state-specific antibodies for the serine/threonine paxillin phosphorylation sites will certainly facilitate further investigation.

C. Dephosphorylation

Cellular responses to extracellular cues are not limited to increases in paxillin phosphorylation. ACTH (290),
EGF (108), ephrin (180), insulin (129), IGF-I (80), and nitric oxide (122) can initiate tyrosine dephosphorylation of paxillin. In vivo, myometrial remodeling at the onset of labor is associated with a drop in paxillin tyrosine phosphorylation (165) as is Heymann nephritis (270) and ethacrynic acid-induced cell retraction (192). In addition, deadhesive signals result in rapid paxillin tyrosine dephosphorylation (31), perhaps through activation of PKA (88, 99). Cell adhesion to matricellular proteins such as thrombospondin, tenascin, and SPARC can also trigger paxillin tyrosine dephosphorylation in a PKA- or protein kinase G-dependent manner. A role for PKA activation mediating paxillin tyrosine dephosphorylation is intriguing. As RhoA activation is often a critical component of paxillin phosphorylation, it is interesting to speculate that PKA activation targets RhoA for phosphorylation and inactivation, which leads to “passive” loss of paxillin phosphorylation by severing the stimulatory signaling pathway (57, 146).

Along the same vein, with respect to insulin, PTP1D phosphatase may dephosphorylate and thereby activate Csk leading to inactivation of Src and consequent “passive” decrease in paxillin tyrosine phosphorylation (196, 267). Conversely, IGF-I induces an association of paxillin with the phosphatase SHP-2 (170); similarly, the tyrosine phosphatase PTP-PEST is a paxillin binding partner (41, 243). PTP-PEST may directly target paxillin for dephosphorylation (242); alternatively, it may result in a “passive” decrease through paxillin’s recruitment of PTP-PEST to target Src and FAK for inactivation (41). Regardless, mutagenesis of the LIM domains that mediate PTP-PEST binding decreases cell adhesion and motility on fibronectin, perhaps indicating a role for paxillin binding to PTP-PEST in focal adhesion dynamics (27, 41). Finally, paxillin also interacts with the serine/threonine phosphatase PP2A, and although it is not known if paxillin is a physiological protein phosphatase 2A substrate, perturbation of this association and consequent increase in paxillin LIM3 serine phosphorylation enhances cell metastasis (110, 323).

V. PAXILLIN FUNCTION

A principal function for paxillin is in the integration and dissemination of signals from integrins and growth factor receptors to effect efficient cellular migration. Motility is a complex multistep process that requires the coordination of membrane trafficking and the reorganization of the actin and tubulin cytoskeleton networks to realize net cellular movement (reviewed in Refs. 12, 98, 252, 300, 301). The activities of several p21 GTPase families are critical to this process (84, 209), and paxillin is an important mediator of signal cross-talk between these families through its phosphorylation and multipotent associations (276, 282, 285) (Figs. 4 and 5).

A. Paxillin Phosphorylation and Migration

The first critical demonstration of a role for paxillin tyrosine phosphorylation in cell migration was in a study of NBTII bladder tumor cells in which cell adhesion, spreading, and motility was associated with induced paxillin tyrosine phosphorylation and consequent association with CrkII (205). Introduction of a nonphosphorylatable paxillin tyrosine phosphorylation mutant Y31F/Y118F blocked migration, which could be reversed by overexpression of wild-type paxillin or CrkII (205). The functional coupling of paxillin to CrkII was also found to be important in the process of epithelial-mesenchymal transition (138). In this MDCK model system, overexpression of CrkII induced the localization of paxillin to focal adhesions resulting in the stimulation of lamellipodia and cell scattering. Expression of a paxillin Y31F/Y118F mutant that no longer bound CrkII blocked the effect. Interestingly, expression of a paxillin LD4 mutant molecule no longer competent to bind PKL, functioned similarly (138). Furthermore, a recent study of ephrin B1-stimulated cell migration in a variety of cell types including renal vascular endothelial cells demonstrated a requirement for paxillin tyrosine Y31/118 phosphorylation and the LD4 motif. In this example, the LD4 motif was required for Nck-mediated recruitment to the activated receptor (291). A model is emerging in which growth factor and integrin-mediated phosphorylation of paxillin stimulates the formation of a paxillin-Crk complex at focal adhesions where Crk-DOCK180 can then locally activate Rac to produce lamellipodial extension and enhance migration (Figs. 4 and 5).

Notably, in a study of migration on collagen using Cos7, NMuMG mammary epithelial, and MM1 hepatoma cells, no motility defect was identified upon expression of a non-tyrosine phosphorylatable paxillin mutant (316). In this study, a role for CrkII binding to p130Cas rather than paxillin was concluded. Although the specific paxillin mutants and conditions between the two studies were not identical, it suggests cell type specificity in signaling cell motility. In a subsequent study, these investigators determined that tyrosine phosphorylated paxillin associated with the SH2 domain of p120RasGAP, thereby competing with and displacing p190RhoGAP from p120RasGAP (273). The consequence of releasing p190RhoGAP was the localized suppression of RhoA in focal adhesions that potentially facilitates Rac-mediated lamellipodial extension, focal adhesion turnover, and thereby stimulates cell motility.

Roles for the MAP kinase family in the regulation of cell motility are well established. For instance, in germline deletion analyses of MEKK1 (325) and JNK (116), a significant impairment in cellular motility was observed. A role for paxillin in JNK activation has been identified (106), and recently JNK phosphorylation of
paxillin T178 has been implicated as critical for cell motility with expression of a paxillin T178A mutant blocking cell migration (102). Intriguingly, expression of a c-Jun molecule with alanine substitutions for the two serines targeted by JNK similarly blocks cell motility (116). Future studies will be required to determine the precise mechanism by which paxillin influences JNK signaling and motility. In addition to a role for paxillin in the JNK MAP kinase signaling pathway, paxillin is essential for adhesion-mediated activation of ERK (81). One mechanism identified, in response to cell adhesion and scatter factor treatment, was the stimulation of Src phosphorylation of paxillin Y118 to create an Erk binding site on paxillin that results in Erk recruitment to focal adhesions (107). Paxillin then binds to Raf and MEK to activate Erk in these dynamic focal adhesions. Subsequently, Erk phosphorylation of paxillin then increases the association of FAK with paxillin to potentiate cell spreading and motility (158).

A second mechanism involves the paxillin-binding protein GIT1 functioning as a MEK scaffold to facilitate ERK activation in focal adhesions (319).

B. p21-GTPase Regulation and Migration

The process of directed migration can be broken down into several discrete steps, namely, protrusion/polarization, attachment/adhesion assembly, traction/translocation, and adhesion disassembly/retraction (12, 98, 252, 300, 301). After cellular stimulation with a chemoattractant growth factor, the cell extends a protrusion resulting from the polymerization of a dense network of actin toward the chemoattractant in the form of filopodial projections and thin lamellipodial sheets. This occurs in a Cdc42- and Rac p21GTPase-dependent manner, respectively (84). How are these GTPases activated?

Recent studies have implicated heterotrimeric G protein βγ-subunits liberated from activated receptors (145). They bind to the Ser/Thr kinase PAK that stimulates the
GEF function of the PAK interacting exchange factor PIX to generate active Cdc42 at the leading edge. This activated Cdc42 can then induce PAK kinase activity to regulate actomyosin function (20) as well as stimulate the function of WASP to activate Arp2/3 actin nucleation and branching to trigger polarized protrusion (215, 256). Paxillin and Nck are critical for the appropriate localization and function of PAK and PIX; thus it will be of interest to further examine the role for these interactions in protrusion (20, 28, 171). The demonstration that PIX binds to the actin and paxillin-binding protein actopaxin/parvin, which regulates cell adhesion and spreading, provides an additional paxillin connection to the initial events of protrusion (183, 217, 278).

The mechanism by which paxillin contributes to PAK function is likely through binding of the Arf-GAP PKL to paxillin LD4 (28, 303). Activation of the PAK scaffold function, perhaps by G protein paxillin LD4 (28, 303). Activation of the PAK scaffold function is likely through binding of the Arf-GAP PKL to the actomyosin connection to the initial events of protrusion (20, 28, 171). The demonstration that PIX binds to the actin and paxillin-binding protein actopaxin/parvin, which regulates cell adhesion and spreading, provides an additional paxillin connection to the initial events of protrusion (183, 217, 278).

The coordination of protrusion stabilization and forward extension is a fine balance of many components (139, 300). After generation of a productive protrusion, the cell attaches and anchors the membrane with focal complexes. Importantly, paxillin is among the first proteins to localize to these sites (139). Following lamellipodial extension these complexes remodel with paxillin loss from these (now) Rho focal adhesions correlating temporally with the formation of new paxillin-containing peripheral Cdc42/Rac focal complexes at the leading edge of the new protrusion (139). Perturbation of paxillin by germline knockout or introduction of paxillin lacking LD4 causes an inhibition of this focal adhesion turnover, loss of polarization, and therefore a loss of directional motility (81, 299, 303). Interestingly, a similar phenotype is observed upon overexpression of kinase dead PAK (125, 239). In addition to a role for PAK in focal adhesion turnover, microtubule targeting of focal adhesions promotes their turnover and potentiates protrusion. The role for paxillin binding to tubulin (94) in this process, possibly via influencing GEFH1 activity (133), is currently an area of active investigation.

In addition to the requisite primary initiation of polarized actin assembly for protrusion and lamellipodial extension, a global reorientation of the cell to face the chemotactic agent must occur for efficient directional motility in many cell types. This is thought to function in part by orienting the microtubule cytoskeleton towards the lamellipodium and facilitating membrane cycling (181). One measure of cellular polarization is the reorientation of the Golgi towards the leading edge. This process is independent of actin polymerization, but dependent on integrin ligation and Cdc42 activation (60, 199). Interestingly, expression of a paxillin molecule that lacks LD4 results in a profound inhibition of the capacity to reorient the Golgi in a scratch wound assay (Fig. 6), the generation of multiple randomly oriented protrusions, and loss of directed cell migration (303). Thus paxillin plays a significant role in cell polarization.

Cdc42 and Arf1 activities are essential for integrity of the Golgi and microtubule organizing center (MTOC) (59). Importantly, Arf1-dependent paxillin shuttling between the Golgi and focal adhesions has been described (191), with the ArfGAPs, GIT2short, and ASAP2/PAPα implicated in this process (128, 175). Other critical mediators of Golgi reorientation/polarization are Src (120) and p120RasGAP (135), which are paxillin binding partners (112, 302). p120RasGAP is essential for polarization likely through the temporal-spatial regulation of Rho activities (135). Paxillin, via pY31/118-p120RasGAP binding and resulting dissociation of p190RhoGAP from p120RasGAP, has been proposed as a mechanism to regulate p190RhoGAP activity (112). Through this exchange paxillin may contribute both to the inhibition of Rho function at the leading edge to promote lamellipodial extension and activation of Cdc42 in the context of cell polarity. Roles for PYK2 or FAK binding to the PKL/GIT and ASAP families of ArfGAPs and the PSGAP/GRAF family of RhoGAPs in influencing Golgi/polarization are currently unknown, although FAK regulation of diaphanous and FAK phosphorylation by cdk5 have been implicated in microtubule stabilization and maintenance of the leading edge (197, 198, 309).

The lipid microenvironment also plays an important role in cell polarization, with activation of PI3K by βγ-subunits and/or Ras (145) at the leading edge being important in the localized generation of phosphoinositides that can activate the Cdc42 and Rac GEF activities of Vav and PIX (87, 161, 321). In this regard paxillin, tyrosine phosphorylated in response to integrin engagement with the extracellular matrix, can function to bind p85 PI3K (273), thereby positioning it as a potential adaptor for 3'phosphoinositide function. Paxillin also binds to talin, an integrin-binding protein that recruits phosphatidylinositol 4-phosphate 5-kinase (148) to produce localized...
phosphatidylinositol bisphosphate, which inhibits actin capping proteins in addition to stimulating Arp2/3 binding to vinculin (50, 51), another paxillin-binding protein (277). However, a role for paxillin adaptor function in these processes has not been established. Whereas polarization of lipid is important, it similarly has been found that sequestration of lipid phosphatases, including the 3′-phosphoinositide phosphatase PTEN, away from the lamellipodia is important for maintaining polarity (145). Interestingly, an ILK-actopaxin complex regulates PTEN partitioning to the membrane (9) likely in a paxillin-dependent manner (183–185). 3′-Phosphoinositides also regulate the activities of ArfGAPs like PKL and GIT1 (292), which may be important in the regulation of their Arf6 GAP activity and consequently the delivery of Rac and integrins to the leading edge of motile cells (55a, 207). These proteins are also likely important in recycling of membrane proteins (169), with GIT1 in Rab11 recycling endosomal and PKL in EEA1 early endosomal compartments (200).

Finally, retraction/tail release, which pulls the rear of the cell forward, is essential for efficient directional motility. This occurs through a combination of myosin IIB contractility (8, 127, 271), microtubule targeting of attachment sites, and calpain-mediated proteolysis of adhesion proteins including paxillin (18, 157, 312). Paxillin knock-out or overexpression of paxillin lacking LD4 causes the formation of long retraction fibers, further implicating paxillin in the regulation of this process (303). The MAP kinase Erk is involved in the regulation of cell migration at several levels. Its activity is required for calpain activation, in part through the formation of a ternary complex with FAK (33, 44). Erk also phosphorylates and activates calpain (71) as well as myosin light-chain kinase to promote contractility (22). Importantly Erk, which is in part regulated via PAK activation (251), binds paxillin and localizes to focal adhesions (107). Furthermore, Erk activation is defective in paxillin null cells as well as in Chinese hamster ovary cells expressing paxillin lacking LD4 (81; unpublished observations), suggesting that paxillin directly impacts on Erks’ contribution to cell migration. This is consistent with the recent report detailing the importance of Erk activation in focal adhesion turnover (299).

C. Integrin-Actin Linkages and Muscle Contraction

Physical linkage between the extracellular matrix, via integrin to the actin-based cytoskeleton, is critical for the transduction of force generated during contraction of skeletal, smooth, and cardiac muscle. The molecular organization of these structures is remarkably similar to that of focal adhesion architecture within fibroblasts and other more motile cells. Indeed, both the structural and regulatory proteins including paxillin, FAK, vinculin, etc. are highly enriched in the dense plaques of smooth muscle tissue (280), the intercalated discs of cardiac muscle, and the myocardin junctions of skeletal muscle (280).

Detailed studies in tracheal smooth muscle tissue have questioned the general perception that such physical linkages are robust, stable points of contact and suggest instead that these are quite dynamic structures that serve as important signal transduction centers. As with focal adhesion signaling, paxillin phosphorylation appears to play a critical role. Not only is paxillin tyrosine phosphorylation on residues Y31 and 118 stimulated during acetylcholine-induced muscle contraction, but overexpression...
of a paxillin Y31F/118F mutant in isolated muscle fibers inhibits tension development (261), suggesting phosphorylation of paxillin may be important in regulating actin filament dynamics. This may occur via p21 Rho family GTPase signaling since, as seen in other cell types, phosphorylation of paxillin on Y31/118 stimulates CrkII binding thereby potentially linking paxillin to Rac activation via a Crk-Cas-DOCK180 signaling pathway (29, 126). Interestingly, paxillin is actively recruited from the cytosol in response to acetylcholine stimulation, and this dynamic translocation is essential for tension development (261, 262). Paxillin recruitment to the membrane is necessary for a similar recruitment of vinculin, a paxillin and actin binding protein. Again, a clear parallel can be drawn with the focal adhesion and cytoskeletal reorganization that occurs in nonmuscle cells responding to mechanical stretching of the extracellular matrix caused either by the cell itself or external forces. In response to increased tension, both paxillin (229) and vinculin (11, 213) translocate from the cytosol and become enriched in focal adhesions, which in turn likely contributes to cytoskeletal stabilization and the activation of intracellular signaling cascades (37).

As noted earlier, paxillin interacts directly with the actin binding actopaxin family members as well as ILK (183, 184). Both of these proteins have emerged as evolutionarily conserved mediators of integrin-actin linkages in muscle tissue. Together with the LIM-only adaptor protein PINCH and possibly Nck2, ILK and actopaxin are required for normal muscle development and integrin-actin attachment in organisms as diverse as flies and worms (147, 164, 327). Although a role for paxillin in mammalian muscle cytoarchitecture is clear, the contribution played by this protein in lower organisms remains to be determined. It should be noted, however, that paxillin and many of its binding partners, such as PKL, vinculin, and FAK, are highly conserved between mammals and flies (104, 304), while the existence of a full-length worm ortholog of paxillin remains unresolved (54, 147).

Paxillin family members may also play a pivotal role in muscle differentiation. Forced overexpression of paxillin in cultured quail myoblasts blocks differentiation, instead promoting continued cell proliferation and signaling through the MAP kinase pathway (227). Phosphorylation of paxillin on amino acids Y31/118 and S188/190, which have each been shown to be regulated via integrin signaling, is necessary to maintain the undifferentiated phenotype (227). The role for Hic-5 is less clear. Forced expression Hic-5 in C2C12 myoblasts inhibits their fusion and conversion to myotubes, instead inducing apoptosis suggesting a negative role in muscle differentiation (100). In contrast, Shibanuma et al. (244), also using C2C12 cells, reported a proactive role for Hic-5 in early myogenic differentiation. Hic-5 is also expressed in embryonic cardiac muscle (30), although its function in the early development of this tissue is unclear.

D. Gene Expression

Historically, LIM domains have been shown to function in protein-protein interactions rather than DNA binding (48). However, a growing body of evidence detailing the capacity of LIM domain containing proteins to undergo regulated nucleocytoplasmic shuttling has refocused some attention towards the potential nuclear function for these proteins (297) (Fig. 7). The first demonstration of this level of regulation was in an analysis of zyxin (189, 190) and later confirmed for its paralogs LPP (204), trip6 (296), and Ajuba (119). Similar to that described for the zyxin family, expression of the paxillin or Hic-5 LIM domains as GFP-fusions results in a protein that localizes both to focal adhesions and the nucleus (266). For the zyxin family, a nuclear export sequence (NES) mediates the regulated shuttling. Interestingly, the paxillin family LID motifs resemble NES sequences, thus they may act as de facto NES sequences in addition to their protein recognition function.

The function of NES sequences can be negated by treatment with the nuclear export inhibitor leptomycin B. Such treatment of fibroblasts causes a retention of paxillin in the nucleus (307), providing evidence that paxillin normally cycles between the nucleus and cytoplasm. The capacity to sequester Hic-5 in the nucleus following leptomycin B inhibition of nuclear export machinery has also been shown (4, 245). For Hic-5, a redox-sensitive mechanism for regulating localization to the nucleus has been identified (245), providing the first compelling evidence for the normophysiological function of nucleocytoplasmic shuttling of group 3 LIM proteins. This switch mechanism may be regulated in part through Hic-5 dimerization (245). A potential role for PYK2 in the nuclear localization of paxillin and Hic-5 has also been reported (4). The localization of paxillin and Hic-5 to the nucleus in normally growing cells would provide additional assurance for a relevant role for these proteins in this compartment, and in fact, ~10% of cellular paxillin and Hic-5 can be recovered in nuclear matrix fractions (121, 315). Hic-5 association with the nuclear matrix is mediated through the LIM domains (315), whereas for paxillin, the amino terminus is required in addition to the LIM domains (121).

Once in the nucleus what is the function for paxillin family members? One possibility is that the nucleus provides an additional means of compartmentalizing and thereby restricting access to paxillin binding sites and in turn allowing for assembly and modification of a unique paxillin scaffold. Second, paxillin may function to assist in the translocation from the nucleus to focal adhesions for proteins such as Abl (142, 143) and STAT3 (250). How-
ever, the capacity of both Hic-5 and paxillin to bind steroid receptors provides a more direct path to nuclear function. They contribute to the transactivation of androgen, glucocorticoid, and progesterone receptors, but not the estrogen receptor (63, 121, 315). The level of transactivation is approximately fivefold stimulation, with paxillin and Hic-5 capable of additive transactivation. The mechanism in which this is accomplished differs between the two molecules. While both paxillin and Hic-5 bind to steroid receptors through their carboxy-terminal LIM domains (121, 315), the paxillin transactivation domain is confined to the carboxy terminus (121), whereas this activity resides solely within the amino terminus of Hic-5 (315). In addition, Hic-5 binding to steroid receptors may be ligand dependent (63) while paxillin binding does not require ligand-bound steroid receptor (121). The transactivating potential of Hic-5 can be suppressed by PYK2 phosphorylation of Y43 (295), providing further evidence for an important role as a coregulator. Evidence that Hic-5 is critical for androgen signaling was provided upon the identification of a Hic-5 mutant, A413T, that blocks androgen receptor function in a dominant negative fashion.

FIG. 7. Cytoplasmic-nuclear shuttling of paxillin family members. Paxillin nucleocytoplasmic translocation may regulate its function as well as influence a diverse number of cellular signaling pathways. Although it has been established that in addition to paxillin and other LIM family proteins a number of proteins including PKC, Pyk2 and Abl, phospholipase C, and actin transit the nucleus, the role(s) ascribed has not been fully elucidated. Segregation of paxillin family members within the nucleus may allow for the assembly of a unique signaling apparatus that may function within the nucleus or be specifically translocated to the cytosol and plasma membrane. Thus nucleocytoplasmic shuttling may function to temporally regulate paxillin access to focal adhesions. A further intriguing possibility is that transit through the nucleus results in a modification event that is important for its localization to focal adhesions. In considering these possibilities, it is appealing to equate paxillin with the yeast MAPK scaffold protein Ste5. Ste5 nucleates the assembly of a MEKK/ERK/PAK/Gα/β/Cdc42GEF/Cdc42 signaling complex at sites of polarized growth in response to pheromone (56), analogous to the scaffold formed in response to G protein-coupled activation of directional motility in metazoan cells (145, 310). Ste5 as well as the Cdc42GEF cdc24p cycle through the nucleus where Ste5 undergoes an as yet undefined modification to make it competent to localize to the site of polarized growth (167). The discovery of paxillin directly binding to steroid receptor transcription factors provided a direct link to function. In fact, paxillin and Hic-5 have been shown to function as transactivators for androgen and glucocorticoid receptors. Much work has been performed characterizing the mechanisms and identifying genes regulated by Hic-5 that include collagenase, fibronectin, the transcription factor c-fos, and cell cycle regulator p21CIP1/WAF1 (246, 248). In turn, these changes in gene expression likely impact on cell migration, invasion, survival, and proliferation. Interestingly, Hic-5 also regulates the mRNA splicing pattern of fibronectin. Paxillin has been demonstrated to bind to the poly(A) binding protein-1 (307) and speculated to regulate the splicing and movement of specific mRNAs into the cytosol and to the leading edge to facilitate their translation. Furthermore, paxillin has been identified in complex with the translation factor EF-1α, a protein that localizes to the leading edge of motile cells to support efficient protein production at these dynamic sites (151). In total, these observations position the paxillin family in a fully integrated role of responding to extracellular cues through immediate changes in the existing adhesion and signaling machinery, as well as to regulating gene expression and the global profile of protein expression within the cell. AR, androgen receptor; GCR, glucocorticoid receptor; PABP, poly(A) binding proteins; Pax, paxillin.
ion (208). This mutation is within LIM4 where an androgen receptor-binding FXXLF motif, which is conserved among paxillin family members, also resides (92). RNA interference-mediated silencing of Hic-5 β has provided further support for the importance of this molecule in normal androgen signaling (208).

Insofar as target genes are concerned, several reports have implicated Hic-5 in regulating c-fos expression (244, 245, 248). Hic-5 regulation of the cdk inhibitor p21WAF1/CIP1 promoter through Sp1 binding sites has also been established (246). The coregulator function was localized to LIM4 and suggested to involve Hic-5 binding to smad3 and CBP/p300. Because an androgen receptor-Sp1 complex regulates p21WAF1/CIP1 expression (159), further studies will be required to determine the precise mechanism of Hic-5 action. Nonetheless, these studies provide persuasive evidence for an important role of focal adhesion proteins in gene expression and merit considerable attention as relates to function of paxillin family members in cell, tissue, and organism development and function.

VI. DEVELOPMENT AND DISEASE

A. Paxillin Family Member Expression

At the organismal level, paxillin exhibits complex developmental regulation. In early murine gastrulation paxillin mRNA is expressed only in extraembryonic tissue, whereas Hic-5 and leupaxin are expressed in embryonic tissue (81). Later in midgastrulation, paxillin is expressed in mesodermal and endodermal tissue. Structures that are positive include the endocardium, dorsal aorta, and notochord (81). Although highly motile neural crest cells express paxillin (81), little neural expression is detectable, consistent with previous studies of protein expression in chick (275). Similar expression patterns have been reported in zebrafish (43) and Drosophila (304, 311). The α-paxillin isoform is first expressed followed by upregulation of the β-isoform in midgestation (177). Interestingly, during renal development in mouse, paxillin is expressed at high levels, whereas postpartum, paxillin is downregulated and a 43- to 47-kDa immunoreactive band, potentially Hic-5 but more likely paxillin δ, is upregulated and maintained (255). The expression and tyrosine phosphorylation profile of paxillin changes during the process of embryogenesis, closely mirroring the expression and activation profile of FAK (275, 284).

In analyses of mRNA expression profiles of adult human tissues, paxillin is expressed abundantly in most tissues with the exception of brain (176, 221). Hic-5 also demonstrates widespread expression (247), although it is largely absent from leukocyte cell-rich tissues like spleen and thymus (328), whereas leupaxin is restricted to leukocyte populations (150). The genomic promoter elements of paxillin, Hic-5, and leupaxin have not been characterized; however, similar to Hic-5 (247), paxillin mRNA is induced by TGF-β as determined using a retrovirus-mediated gene trap screen (2). Hic-5 mRNA expression is induced by retinoic acid, with Hic-5 then potentiating retinoic acid-dependent differentiation processes (249).

Expression of paxillin family members in hematopoietic cells shows a tremendous amount of regulation. The paxillin γ-isoform appears to be restricted to cells of the myeloid lineage, with differentiation of promonocytic cells leading to an upregulation of both the β- and γ-isoforms (176). Leupaxin is similarly upregulated in differentiating lymphocytes (150), while Hic-5 is specifically upregulated during the transition of megakaryocytes to platelets with a concomitant downregulation of paxillin (82).

B. Potential Roles in Disease

Hic-5 is upregulated during cellular senescence and downregulated during Ras transformation or spontaneous immortalization (247). Forced expression of Hic-5 in immortalized human fibroblasts induces senescence and blocks colony formation (248) in a FAK-dependent manner (109). These data suggest the exciting possibility that Hic-5 has tumor-suppressor functions. However, although it has been noted that Hic-5 expression is largely absent from immortalized and/or transformed cell lines (328), Hic-5 is widely expressed in prostate cancer lines and clinical samples (63) as well as in epithelial carcinomas (328) and in many breast cancer cell lines (160). The known roles for Hic-5 in scaffolding and steroid receptor coactivation positions this protein as a potential major mediator of carcinogenesis in these tissues.

The critical importance of paxillin was confirmed upon generation of a mouse knockout of the paxillin gene (81). The resulting embryonic lethal nature, despite the expression of Hic-5 and leupaxin, revealed the lack of compensation by these paralogs and thus the specific requirement for paxillin. Although no definitive human diseases thus far have been linked to the expression of a mutant paxillin, chromosome 12q24 duplications (the location of paxillin and PKL) have been reported which result in severe musculoskeletal, cardiovascular, and central nervous system malformation syndromes (162, 179, 214). In addition, amplification of 12q24 was found in many bladder tumors in a comparative genomic hybridization and cytogenetic analysis screen (130), whereas chromosome 12q24 was identified as containing tumor suppressors, in a micro cell-mediated chromosome transfer screen in prostate cancer cells (105). Interestingly, in this respect paxillin binds to the antiapoptosis protein bcl-2 (254, 255). Induction of apoptosis targets paxillin for caspase cleavage (36, 173) with amino acid residues D102
and D301 identified as major caspase cleavage sites (36). Importantly, expression of a paxillin molecule lacking these cleavage sites protected cells from apoptosis offering compelling support for an important role for paxillin in survival. Another direct link to cancer was identified with the finding that paxillin binding to the NF2 tumor suppressor (Merlin/Schwannomin) may mediate pathogenesis of neurofibromatosis type 2 in humans with exon 2 mutations (61). A role for paxillin in papillomavirus-mediated cell transformation associated with cervical cancer is postulated to be mediated in part through an association of paxillin with the E6 protein (47).

Additional roles for paxillin in cancer have been reasoned primarily based on the propensity of paxillin to be phosphorylated by integrin and growth factor receptor ligation (228, 230, 277) and the known roles for these transmembrane receptors in tumorigenesis and invasion (97, 201, 236). Studies in which paxillin function in normal and cancer cells is directly manipulated followed by examination of the consequences on cell function have identified clear roles for paxillin as addressed above. Profiling of the expression and activities of paxillin family members in various cancers is further illuminating the potential role for these proteins in cancer and disease.

Paxillin is transcriptionally upregulated by heregulin treatment of breast cancer cells, and the increased expression of paxillin directly correlates with HER2/3 receptor expression (228, 230, 277) and the known roles for these transmembrane receptors in tumorigenesis and invasion (97, 201, 236). Studies in which paxillin function in normal and cancer cells is directly manipulated followed by examination of the consequences on cell function have identified clear roles for paxillin as addressed above. Profiling of the expression and activities of paxillin family members in various cancers is further illuminating the potential role for these proteins in cancer and disease.

VII. FUTURE DIRECTIONS

With the identification of so many specific paxillin binding partners, several of which bind to overlapping domains, determining how and when these proteins associate, and what the consequence is for other binding partners will be critical to understanding how the cell effectively utilizes the paxillin adaptor function to respond to changes in the external environment and thereby derives the appropriate functional outcome. Several lines of evidence point to the LD-PBS association as one that is a potentially important point of such regulation. For instance, the paxillin LD-binding domain(s) on FAK contains Y925, a site that when phosphorylated functions as a Grb2 SH2 domain-binding site (7, 234). Importantly, a paxillin LD4-FAK association would likely sterically antagonize the capacity to target Y925 for phosphorylation and subsequent binding to Grb2 (96), thereby potentially regulating FAK-mediated activation of the Ras-MAPK pathway. With respect to vinculin binding, the face of the vinculin H2/5 helix, which corresponds to H1/4 of FAK and could potentially function as a second paxillin LD binding site, is blocked. Evidence exists, however, that this region may be regulatable by acidic phospholipids and/or other vinculin binding partners (10, 96, 114). Whether access is controlled and the proper hydrophobic interface is available for paxillin LD interactions under physiological conditions to perhaps stabilize this association and provide a platform for actin assembly remains to be determined. Similarly, the capacity of PKL to associate with the LD4 motif of paxillin is regulated, requiring a stepwise activation cascade involving the p21-activated kinase FAK transmitting a signal through the PKL binding partner PIX to elaborate, by an uncertain mechanism, the PBS domain to facilitate an interaction with paxillin LD4 (28, 329). Such modulation in paxillin binding partners combined with phosphorylation of paxillin itself to impart structural changes within the LD motifs will presumably be important in determining whether FAK, PKL, or actopaxin, for instance, associates with the LD4 motif and in so doing determine whether the downstream response is primarily of a structural readout, e.g., LD-actopaxin-actin, or primarily signaling via LD-FAK or LD-PKL-PIX-PAK. Clearly, further studies will shed more light on this exciting arena of dynamic reciprocity between paxillin and its LD binding partners.

As noted, paxillin is a remarkably frequent target of phosphorylation in response to cellular stimulation (230). Interestingly, Y118 may be the preferred target following phosphorylation (149) to identify the interrelationships between the many proteins required for normal function of signaling networks will surely illuminate our understanding of the many roles for paxillin.
adhesion, whereas Y31 is favored following growth factor stimulation (233). This potential point of bifurcation is likely important for programming the cells’ response to particular stimuli through the specific temporally-spatially regulated recruitment of SH2-domain proteins. How else does paxillin phosphorylation influence paxillin and thus cellular function? With respect to MAP kinase phosphorylation of paxillin, further understanding the role for paxillin in the activation and dissemination of MAP kinase family signaling pathways will certainly be another area receiving considerable attention in the coming years. Paxillin binds to MEK and Erk to potentially mediate their reassociation proteins. MEK1 binds to PIX/PAK, FAK, and paxillin and activates Raf and MEK (251), FAK binds and localizes to focal adhesions (107), PAK interacts with paxillin and activates Raf and MEK to potentially mediate their reassociation proteins. MEK1 binds to PIX/PAK, FAK, and paxillin and activates Raf and MEK (251), FAK binds and localizes to focal adhesions (107), PAXILLIN ADAPTING TO CHANGE 1331

REFERENCES


PAXILLIN ADAPTING TO CHANGE 1333


Lankaitis CM, Webb D, and focal adhesion Horwitz AF. Differential dynamics of alpha 5 integrin, paxillin, and alpha-actinin during...


263. Tapia JA, Camello C, Jensen RT, and Garcia LJ. EGF stimulates tyrosine phosphorylation of focal adhesion kinase (p155FAK) and paxillin in rat pancreatic acini by phospholipase C-independent process that depends on phosphatidylinositol 3-kinase, the small GTP-binding protein, p21rko, and the integrity of the actin cytoskeleton. Biochim Biophys Acta 1448: 486–499, 1999.


