Interaction between Erbin and a Catenin-related Protein in Epithelial Cells*

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Integrity of epithelial tissues relies on the proper apical-basolateral polarity of epithelial cells. Members of the LAP (LRR and PDZ) protein family such as LET-413 and Scribble are involved in maintaining epithelial cell polarity in Caenorhabditis elegans and Drosophila melanogaster, respectively. We previously described Erbin as a mammalian LET-413 homologue interacting with ERBB2/HER2, an epidermal growth factor receptor family member. Erbin and ERBB2/HER2 are located in the basolateral membranes of epithelial cells. We show here that Erbin interacts with p0071 (also called plakophilin-4), an armadillo repeat protein linked to the cytoskeleton. Erbin binds to p0071 in vitro and in vivo in a PDZ domain-dependent manner, and both proteins co-localized in desmosomes of epithelial cells. Using a dominant negative approach, we found that integrity of epithelial cell monolayer is impaired when interaction between Erbin and p0071 is disrupted. We propose that Erbin is connected by p0071 to cytoskeletal networks in an interaction crucial for epithelial homeostasis.

Asymmetric distributed multiprotein complexes in polarized epithelial cells contain cell adhesion molecules, signaling and structural proteins whose organization relies on protein-protein interactions. They are organized by protein modules that are found in one or more copies in proteins and interact with specific peptides of their binding partner (4). Among the protein modules, PDZ (PSD-95/DLG/ZO-1) domains bind to short peptides and are involved in receptor signaling and targeting. They are conserved from bacteria to mammals (5). PDZ domain proteins are scaffold proteins important for different developmental processes as evidenced by Shroom and Psd-95 knockouts in mice (6, 7). Recent studies in nonvertebrates have evidenced the role of PDZ protein complexes as crucial for tissue morphogenesis and proper development. Vulval development in Caenorhabditis elegans relies on the LIN-2-LIN-7-LIN-10 complex, whereas PAR-6 and PAR-3 proteins form a complex important for cell polarity in worms and flies. These protein complexes are conserved in mammals and are connected to signaling pathways driven by protein kinases (8–12). Multiple copies of PDZ domains in the same protein help scaffold an array of receptors, enzymes, and adaptors, thus creating a functional unit. This is well documented in the case of INAD, a five-PDZ domain protein, gathering a protein kinase C, calmodulin, phospholipase C, rhodopsin, and light-sensitive Ca2+ channel in a stable transducisome machinery crucial for signaling and stability of photoreceptors (13, 14). Complexity is increased by the possibility for a PDZ domain to bind peptide sequences found in different proteins and conversely for a protein to bind several PDZ domains. For example, PSD-95 binds to glutamate, Shaker K+ channels, and tyrosine kinase receptors as well as to neurotrophin at the postsynapse of neurons (15–18).

The LAP family is a novel PDZ domain protein family comprising LET-413 in C. elegans; Scribble in Drosophila melanogaster; and Erbin, Densin-180, hScribble, and Lano in mammals (19). LAP proteins are membrane-bound adaptor proteins and contain 16 amino-terminal leucine-rich repeats and one (LET-413, Densin-180, Erbin), four (Scribble), or no (Lano) PDZ domains. Genetic analyses have thoroughly demonstrated the importance of LAP proteins in worm and fly development and their role in cell polarity and epithelial morphogenesis. Loss of function of let-413 (homologue of Erbin and Densin-180) and scribble (orthologue of hScribble) is embryonic lethal and results in disorganization of the cytoskeleton and disruption of epithelial integrity with mislocalization of apical determinants (20, 21). Despite a genetic interaction existing between scribble and disc large or lethal giant larvae, two other tumor suppres-

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formation in tissues relies on protein networks involved in targeting of proteins to apical or basolateral membranes and specifying the three-dimensional structure of the cell. Organized epithelial cells have an apical membrane in contact with fluids, intercellular lateral membranes, and a basal membrane connected to extracellular matrices. Apical and basolateral compartments are separated by tight and adherens junctions and contain different sets of proteins and lipids (1–3). These highly organized structures regulate exchanges between extra- and intracellular compartments in normal situations and may be affected in carcinoma and autoimmune diseases.
In mammals, the search for LAP interactors has been more successful. Densin-180 is a neuron-specific protein linked to calmodulin kinase II and α-actinin (25, 26). Scribble is targeted for degradation by an ubiquitin-ligase activity associated to E6 oncoprotein (27). Lano is associated to human Discs Large (hDLG), a potential tumor suppressor (28). We have characterized the interaction between ERBB2, a mammalian epidermal growth factor receptor family member, and Erbin, which is reminiscent of LET-23/LIN-2-LIN-7-LIN-10 complex interaction. In both cases, PDZ domain interactions are important for receptor localization or retention in epithelial cells (29, 30). The Erbin-ERBB2 interaction takes place at the basolateral side of epithelial cells. The Erbin PDZ domain interacts with ERBB2 and controls its basolateral localization in epithelial cells (29–31). Recently, it was demonstrated that Erbin directly binds to BPAgl (bullous pemphigoid antigen-1), a component of hemidesmosomes, electron dense structures in contact with the basal membrane associated with the cytoskeleton (32). BPAgl belongs to the plakin family and is involved in cytoskeletal organization (33). The integrin β1 subunit, a cell adhesion molecule involved in hemidesmosome assembly, also associates with Erbin through a protein interaction domain lying amino-terminal to the Erbin PDZ domain (32). It is unknown whether Erbin is present in hemidesmosomes. Although the functional relevance of these novel interactions remains to be appreciated, these data suggest that Erbin is linked to cytoskeleton-associated protein complexes in hemidesmosomes and participates to cell adhesion processes.

In this report, using the two-hybrid system in yeast, we identified p0071 (also called plakophilin-1), a member of the armadillo repeat family, as a new interactor for Erbin. Proteins of the p120-catenin family, including p0071, link cadherin adhesion molecules to the cytoskeleton (34, 35). Interaction is mediated by the p0071 carboxyl-terminal sequence and the Erbin PDZ domain. Erbin and p0071 specifically interact in cultured epithelial cells and tissues. By surface plasmon resonance (SPR)1 analysis, we measured a better affinity between p0071 and Erbin than between ERBB2 and Erbin. Erbin is found at the lateral membrane of epithelial cells and colocalizes with p0071 in desmosomes. Disruption of the p0071-Erbin interaction by a dominant negative approach provoked alteration of cell morphology, suggesting that connection of Erbin to cytoskeletal networks is important for epithelial integrity.

MATERIALS AND METHODS

Two-hybrid Procedure—To prepare the baits used in this paper, proteins or peptides were fused to the LexA-BD subunit using the pbTM116 vector, which carries Trp1. For library screening, an oligo(dT)-primed human cDNA breast library cloned in pACT2 vector (CLONTECH), which carries Leu2 as a selection marker, was screened by using the LexA-Erbin as a bait and the yeast strain L40 following the lithium-acetate protocol. Approximately 10^5 Trp ′ Leu ′ transformants were selected on plates with supplemented minimum medium that lacked tretoquin, leucine, and histidine in the primary screening and containing 10 mM 3-amino triazole and then tested for the β-galactosidase activity by the filter method in the secondary screening. After rescue, the DNA of selected clones was retransformed in L40 yeast containing LexA-Erbin or LexA fused to control proteins. Specific clones were positive for growth in histidine-deficient medium and β-galactosidase activity.

Protein Destimations—Cells were washed twice with cold PBS and lysed in lysis buffer (50 mM HEPES, pH 7.5, 10% glycerol, 150 mM NaCl, 1% Triton X-100, 1.5 mM MgCl2, 1 mM EGTA) supplemented with 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. After centrifugation at 16,000 × g for 20 min, lysate proteins were immunoprecipitated using the Bio-Rad protein G-agarose slurry. For immunoprecipitation, lysates were incubated with antibodies overnight at 4 °C. Protein A-agarose was added, and immune complexes bound to beads were recovered after 1 h, washed three times with HNTG buffer (50 mM HEPES, pH 7.5, 10% glycerol, 150 mM NaCl, 0.1% Triton X-100), boiled in 1× sample buffer, and separated by SDS-PAGE. Transfer and immunoblotting on nitrocellulose using a horseradish peroxidase-anti-rabbit or horseradish peroxidase-anti-mouse antibody/chemiluminescence method were performed as described (36).

Cell Culture—COS-1, human embryonic kidney (HEK) 293, HeLa, and Madin-Darby canine kidney cells were grown in Dulbecco’s modiﬁed Eagle’s medium containing 100 units/ml 1 penicillin and 100 μg/ml streptomycin sulfate, supplemented with 10% fetal calf serum. Caco-2 cells were maintained in Dulbecco’s modiﬁed Eagle’s medium supplemented with 20% fetal calf serum and 1% nonessential amino acids. All cell transfections were made using Fugene 6 reagent according to manufacturer’s recommendations (Roche Molecular Biochemicals).

DNA Constructs—The human Erbin cDNA was used as a template to create different constructs. Different expression vectors of GST, Myc-tagged, LexA-BD, and GAL4 activation domain fusion proteins (29). The RKS-Myc (36) and GFP-C1 (CLONTECH) vectors were used to express proteins fused to the amino-terminal Myc or GFP epitopes. The pGEX-Tag vector was used to produce all GST fusion proteins. Site-directed mutagenesis was performed using the QuikChange kit (Stratagene). All constructs were sequenced by Genome Express, SA (Grenoble, France). p0071 headless (aa 509–1193), arm repeats (aa 509–989), and tail (aa 990–1193) domains were cloned by RT-PCR from HeLa cell RNA. PCR products were ligated into the PCR2.1TOPO vector (Invitrogen) and fully sequenced. Constructs were then subcloned into pCDNA4T-OmyeHis vector (Invitrogen) or pEGFP vector (CLONTECH) for eucaryotic expression or into pGAD424 for two-hybrid analyses.

Antibodies—Monoclonal anti-Myc 9E10 and polyclonal anti-GFP antibodies are from Oncogene Research Products and CLONTECH, respectively. Goat anti-rabbit and anti-mouse IgG coupled to horseradish peroxidase were purchased from Jackson Laboratories and Dako, respectively. Anti-Erbin polyclonal antibody has been previously described (29). Monoclonal anti-Erbin antibodies were produced by injecting a soluble GST-Erbin (941–1371) fusion protein to BALB/c mice. Several clones of immunized mice were fused with the nonsecreting myeloma X63Ag8.653 as previously described (37). Culture supernatants were tested by immunoprecipitation on Erbin-containing lysates. Positive clones were tested by double limiting dilution.

Monoclonal anti-p0071 antibodies were obtained by immunization of mice with the purified recombinant tail domain expressed in pSET and E. coli BL21DE3 cells. Clones 5A4-3 and 6D1-10 will be described in detail elsewhere.2 Immunolocalization and Cell Surface Labeling—For immunostaining procedures, MCF-7 cells and Caco-2 cells grown on coverslips for 20 days after confluence were double labeled as described below with an affinity-purified rabbit polyclonal antibody against Erbin (diluted 1:100) (29) and a mouse monoclonal antibody against p0071 (5A4). Images were obtained using a confocal microscope (Zeiss LSM2). Frozen sections (0.5–1.0 μm) of human colon were treated as described (29).

Immunoelectron Microscopy—Immediately after removal, biopsies of human colon (kindly provided by Dr. Monges, Institut Paoli-Calmettes, Marseille) were fixed at room temperature in 8% paraformaldehyde in PBS. Tissues were then infiltrated with 2.5% sucrose in PBS and frozen in liquid nitrogen. Ultrathin cryosections were prepared using a cryo-ultramicrotome and collected on Formvar-coated grids. Sections were successively treated for 30 min with PBS, for 20 min with 0.5 μM NH4Cl in PBS, and for 30 min with PBS supplemented with 10% goat serum. They were then incubated overnight at 4 °C with anti-Erbin and anti-p0071 (5A4) antibodies diluted 1:20 or 1:5 in 5% goat serum in PBS, respectively. The grids were rinsed in PBS, and primary antibodies were revealed by incubation for 1 h with either 15-nm conjugated goat anti-rabbit or anti-mouse IgG, respectively, diluted 1:25 in 5% goat serum in PBS. After extensive washing with double-distilled water, sections were rapidly fixed with 2% glutaraldehyde, stained for 6 min with 2% uranyl acetate, rinsed, and treated with 0.3% lead citrate.

1 The abbreviations used are: SPR, surface plasmon resonance; PBS, phosphate-buffered saline; HEK, human embryonic kidney; GST, glutathione S-transferase; aa, amino acids; GFP, green fluorescent protein; RU, resonance unit.

2 M. Hatzfeld, manuscript in preparation.
uranyl acetate and 1.8% methylcellulose in double-distilled water on ice. Sections were air-dried and examined under a Zeiss electron microscope.

**Surface Plasmon Resonance Analysis**—Biotinylated (Biotin) peptides were synthesized by NeoSystem, SA. Peptide sequences were as follows: Biotin-PTAEPEYGLDVPY (ERBB2), Biotin-TVLPPPYPHRHNTVV (ERBB4), and Biotin-SYRAEQYFGPSDSWV (p0071).

SPR studies were performed using an upgraded BIAcore apparatus (BIAcore AB, Uppsala, Sweden) and streptavidin-coupled SA sensor microscope. Sections were air-dried and examined under a Zeiss electron microscope.

RESULTS

**p0071, an Armadillo Repeat Protein, Is a New Partner for Erbin**—To identify new ligands for Erbin, we did a two-hybrid screen of a human breast cDNA library using full-length Erbin as a bait. Two overlapping clones of 543 and 126 aa, respectively, strongly interacted with Erbin but not with control Lamin (data not shown) and are identical to the carboxy-terminal region of p0071. p0071 is a member of the armadillo family and contains 10 armadillo repeats (38). The p0071 clones bound to constructs encompassing the Erbin PDZ domain (i.e., Erbin, Erbin-(853–1371), and Erbin PDZ domain). Truncation of this region (ErbinΔPDZ) abrogated the interaction (Fig. 1A).

In the next experiments, we used biochemical assays to confirm the p0071-Erbin interaction. We expressed a Myc-tagged version of Erbin and ErbinΔPDZ and performed a pull-down assay with GST-p0071. This fusion protein encompassed the 126 last residues of p0071 fused to the GST protein. Erbin, but not ErbinΔPDZ, was precipitated by GST-p0071, confirming that the PDZ domain was sufficient for this interaction (Fig. 1B). We also expressed a Myc-tagged version of p0071 in COS cells and performed a GST pull-down assay with recombinant proteins produced in Escherichia coli. GST-Erbin PDZ domain precipitated p0071, whereas no interaction was found with GST alone (data not shown) and GST fused to Densin-180, AF6, and LIN-2 PDZ domains (Fig. 1C).

The largest clone pulled out of the library encoded the seven last armadillo repeats and the carboxyl terminus of p0071, while the shortest one only encoded the last 126 residues of the protein. The peptide sequence of this latter form, shown in Fig. 2A, contains a carboxyl-terminal SWV motif matching with a class I PDZ domain binding site (STXV) (39). We precipitated proteins extracted from a Caco-2 cell lysate by GST-p0071 encompassing the last 126 amino acids of p0071. Erbin was efficiently precipitated by a GST-p0071, but not by GST alone (Fig. 2B). The original paper describing the cloning of p0071 mentioned that the SWV motif of p0071 was not located at the very carboxy-terminal end of the protein (38). However, the p0071 sequence stops with a SWV motif in the two clones pulled out of our two-hybrid screening. Furthermore, we found in databases numerous expressed sequence tags coding for the p0071 carboxy-terminal sequence that end with the SWV motif. None encoded the longer splice variant described originally. We now know that this discrepancy is due to a sequencing error in the original report describing p0071 cDNA.3

The presence of a canonical PDZ binding site in p0071 suggested that this sequence (SWV) is involved in the interaction with the Erbin PDZ domain. To confirm this hypothesis, we fused the p0071 wild-type carboxy-terminal peptide sequence or mutant forms to LexA-BD used to screen a human breast cDNA library by two-hybrid analysis in yeast. Two overlapping positive clones encompassing the p0071 sequence were rechallenged with LexA-BD Erbin or truncated Erbin or Lamin (negative control). Only baits containing the Erbin PDZ domain were reactive in β-galactosidase activity and grew on His plates with the clones pulled out of the library. Lamin did not interact with p0071 (not shown). B. Myc-tagged Erbin (Erbin) or Myc-tagged ErbinΔPDZ (ErbinΔPDZ) were transiently expressed in COS cells and pulled down with GST or GST-p0071 fusion proteins. After Western blot, proteins were detected with anti-Myc antibody. P0071 bound to Erbin but not to ErbinΔPDZ. C, Myc-p0071HL was transiently expressed in COS cells and pulled down with the mentioned GST-PDZ domain fusion proteins. After washing, bound proteins were probed with anti-Myc antibody. One-tenth of the lysate used for the precipitation was run as control (total lysate). Comparable amounts of GST fusion proteins were present in the lanes (data not shown).

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3 M. Hatzfeld, personal communication.
cell lysis and immunoprecipitation of proteins with anti-Myc antibody, proteins were resolved by SDS-PAGE and transferred to a membrane. Probing the membrane with anti-Erbin antibody showed that p0071HL and p0071CT, but not p0071Arm, coimmunoprecipitated with Erbin (Fig. 2E). We thus confirmed by precipitation assays with purified fusion proteins, two-hybrid analysis in yeast, and coimmunoprecipitation experiments in COS cells that Erbin and p0071 associate through a PDZ domain interaction.

Use of Surface Plasmon Resonance Analysis to Measure the Erbin-p0071 Interaction—To gain insight into the respective affinities between Erbin and its ligands (i.e. ERBB2 and p0071), we used surface plasmon resonance analysis. Biotinylated peptides and recombinant GST fusion proteins fused to PDZ domains were tested in the in vitro binding assays. As expected, we found that GST alone (data not shown) or GST−GRB2 did not interact with ERBB2, ERBB4 (not shown), and p0071 peptides (Fig. 3). ERBB2 interacted with the Erbin PDZ domain with a low affinity ($K_d = 1400$ nM). Interestingly, we measured an affinity of $1200$ nM between LET-23 and LIN-7 in a similar SPR analysis (data not shown). The affinity between ERBB2 and Erbin is rather low, although both proteins coimmunoprecipitate in cell and tissue extracts (29, 31). We believe that a weak interaction between ERBB2 and the Erbin PDZ domain (and perhaps between LET-23 and LIN-7) allows a more dynamic regulation of this interaction to occur in vivo. We measured a better affinity between p0071 and Erbin ($K_d = 88$ nM). This affinity is within the same range as those observed for other PDZ domain–peptide interactions (39).

Erbin and p0071 Proteins Interact in Vivo—We next examined the in vivo interaction between Erbin and p0071 by coimmunoprecipitation with endogenous proteins. We produced mouse monoclonal anti-Erbin antibodies using a GST-Erbin-(1241–1370) as immunogen (see "Materials and Methods"). Monoclonal R24 and R99 mAb immunoprecipitated Erbin from a Caco-2 cell lysate (Fig. 4A). Caco-2 cell extracts were used to examine the in vivo interaction between Erbin and p0071. When we used an anti-p0071 monoclonal antibody to reveal proteins bound to immunoprecipitated Erbin, a 130-kDa protein corresponding to p0071 was detected (Fig. 4B). A similar result was obtained when we used a mouse brain extract as a source of Erbin and p0071 (Fig. 4C). We thus showed that Erbin and p0071 interact in epithelial cells and in mouse brain. Taken together, we have established that Erbin binds to p0071 in vitro and in vivo.

Erbin and p0071 Proteins Colocalize in Epithelial Cells and in Tissues—LAP proteins are membrane-associated proteins targeted to the basolateral membrane of polarized epithelial cells (20, 21, 28, 29). We first expressed Myc-p0071 in MCF-7 cells, where Erbin is endogenously present, and performed double immunostaining experiments with anti-Erbin and anti-Myc antibodies. Erbin and p0071 colocalized at cell-cell con-
tacts in Myc-p0071-positive cells (Fig. 5A). In polarized Caco-2 cells, where endogenous p0071 and Erbin can be detected, Erbin partially colocalized with p0071 (Fig. 5B). p0071 was localized both intracellularly and to specific areas on the lateral membrane, probably enriched in desmosomes (38). In these areas, a colocalization with Erbin was detected (Fig. 5B). To evaluate the colocalization in the desmosomes, we performed double immunolabeling and an immunoelectron microscopy analysis with secondary antibodies coupled to gold particles of different sizes (Fig. 6). Clearly, Erbin was present in desmosomes where it was in close vicinity with p0071, indicating that the two proteins interact in these structures.

**Dominant Negative p0071 Affects Epithelial Integrity in HeLa Cells**—To gain insight on the functional relevance of the Erbin-p0071 interaction, we constructed a potentially dominant-negative mutant of p0071 susceptible to disrupt the interaction with Erbin. We expressed GFP-p0071CT and GFP-p0071CTΔ4 (a protein lacking the PDZ domain binding site) and GFP-PICK1 (a control protein) in HEK 293 cells and showed that these proteins are equally expressed (Fig. 7A). We then expressed the GFP fusion proteins in HeLa cells, and we performed immunostaining with anti-Erbin antibody to stain the epithelial membranes. GFP-p0071CT induced a profound effect on the morphology of cells with disruption of cell-cell

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**FIG. 3.** SPR analysis of binding of Erbin to p0071 and ERBB2. A, direct association of Erbin with p0071 and ERBB2. 1 μM GST-Erbin was injected at a flow rate of 20 μl/min on either ERBB2-coated surface (300 RU) or p0071-coated surface (57 RU). As a control, the irrelevant GST-GRB2 was injected in a same way. Binding of GRB2 to ERBB2 or p0071 peptides was not significant. B, determination of equilibrium dissociation constants \( K_D \). Injections of GST-fusion proteins were performed at a flow rate of 30 μl/min until equilibrium state was reached. The Erbin fusion protein was injected on ERBB2-coated surface (59 RU) and p0071-coated surface (57 RU) at concentrations ranging from 50 to 250 nM and 500 nM to 8 \( \mu \)M, respectively. Responses at the equilibrium (\( Req \)) were plotted against (\( Req/c \)) each GST fusion protein concentration. Equilibrium dissociation constant \( K_D \) value was calculated from the slope of this linear regression, \( Req = -K_D Req/cm + R_{max} \). One representative experiment of two is shown. C, comparison of the equilibrium dissociation constants (\( K_D \)).

**FIG. 5.** Partial colocalization of Erbin and p0071 in epithelial cells. A and B, confocal XY sections of MCF-7 (A) and Caco-2 (B) cells double-labeled for Erbin and p0071 (recognized by anti-Myc antibody in A and by anti-p0071 antibody in B). In B, the right panel shows the merge of the two confocal sections (Erbin in red and p0071 in green). Erbin and p0071 colocalize at the lateral membrane (arrowheads). Scale bar, 10 μm.
contacts (Fig. 7, B and C). This effect was specific, since GFP-PICK1 and GFP-p0071CTΔ4, which are unable to bind to Erbin, failed to produce this phenotype (Fig. 7C). We evaluated the percentage of cells giving an altered phenotype with the different GFP constructs by counting the cells in three different experiments. For GFP-PICK1 and GFP-p0071CTΔ4, we obtained 7.5 ± 3.5 and 16.5 ± 6.4%, respectively, of altered morphologies, whereas GFP-p0071CT gave 61 ± 19.8% of altered cells. Furthermore, many patches of living green cells expressing GFP-p0071CTΔ4 were obtained, whereas GFP-p0071CT-expressing cells were rare and presented aberrant morphologies. We conclude that p0071 interacts with Erbin through its carboxyl terminus and forms protein complexes important for epithelial integrity.

**DISCUSSION**

Identification of protein networks associated with LAP proteins will help to decipher their functions in combination with genetic studies in animals. LET-413 and Scribble are basolateral and septate junction-associated proteins involved in the localization of apical proteins and epithelial integrity, although molecular mechanisms underlying these functions remain unknown (20, 21). In nonvertebrates, loss of function of let-413 and scribble genes provokes cytoskeletal defects and lethality. It is likely that LET-413 and Scribble, as well as their mammalian homologues may be connected, directly or not, to cytoskeleton-associated proteins. Likewise, Erbin was recently shown to interact with BPAG1, a plakin associated with intermediate filaments in epithelial cells (32).

Here, we have identified a direct interaction between Erbin and p0071, a cell-cell contact-associated protein. The PDZ domain of Erbin binds specifically and directly to the very carboxyl-terminal residues of p0071 (DSWV motif). We demonstrated the in vivo interaction between Erbin and p0071 in pull-down and co-immunoprecipitation experiments. We measured a better affinity between Erbin and p0071 than between Erbin and ERBB2 by SPR analysis using 15-mer biotinylated peptides. In epithelial cells, different pools of Erbin may thus bind to p0071 and ERBB2 to participate in multiple biological functions including receptor localization or cell signaling (29, 35). Catenin-related proteins such as p0071 are armadillo repeat proteins presumably bound to actin or intermediate filaments and link adherens junctions and/or desmosomes to the cytoskeleton (34, 41). We found that Erbin indeed colocalized with p0071 in desmosomes of human colon cells and was also found all along the lateral membrane of epithelial cells. Desmocollins and desmogleins, members of the cadherin superfamily, are the major desmosomal glycoproteins. We did not detect an interaction between Erbin and Desmoglein-1 in Caco-2 cells by coimmunoprecipitation (not shown), but a more exhaustive study is needed to address the question of whether Erbin interacts with one of these cell adhesion molecules. Nevertheless, p0071 is not restricted to these compartments in epithelial cells (38).

Adherens junctions but not desmosomes are present in worms and flies, and a plakophilin/catenin-like protein 38% identical to p0071 is found in *C. elegans* data bases (accession number CAB60320). Interestingly, a DSWV motif terminates its peptide sequence, suggesting that interactions between armadillo repeat proteins and PDZ domains are conserved throughout evolution. We obtained no obvious phenotype using an RNA interference strategy in *C. elegans* to abolish the
A relationship between LAP proteins and tumor suppressors (hDLG, p0071) suggest their involvement in pathology, the multiple protein interactions required for stable epithelial cell anchorage and polarity is frequently observed in cancers. Disruption of these structures leads to abnormal cellular morphologies (Fig. 7). Erbin is probably only one component of protein complexes involved in these processes, since other PDZ domain proteins, including Papin, bind to p0071. Papin, a novel PDZ domain protein, interacts with p0071 in the insoluble fraction of cell extracts, whereas we recover the p0071-Erbin interaction in a 1% Triton X-100 extraction buffer (43). Other studies have shown that protein complexes containing p0071 or ds-catenin/NPRAP, a p0071-like protein, are extractable by a Triton-based buffer (44, 45). Different pools of p0071 may thus be involved in distinct subcellular compartments. LAP proteins also function in epithelial tissues (29, 31). Interestingly, in neurons, the p0071 armadillo repeat domain binds to the hydrophilic loop of presenilin 1 (PSEN1), a protein involved in neurodegenerative disorders (45). Since Erbin is expressed in the brain, a tripartite PSEN1-P0071-Erbin complex may be involved in neuronal functions still to be identified.

The presence of Erbin in desmosomes (this study) and in hemidesmosomes, as suggested by others (32), leads us to propose that Erbin plays a role in the establishment and the maintenance of cell-cell and cell-basement membrane adhesion. Disruption of these structures required for stable epithelial cell anchorage and polarity is frequently observed in cancers. Although the role of Erbin and other LAP proteins has to be evaluated in pathology, the multiple protein interactions found with well known or putative oncoproteins (E6, ERBB2) or tumor suppressors (hDLG, p0071) suggest their involvement in cancers (27–29). A relationship between LAP proteins and tumorigenic processes exists in Drosophila, since scribble behaves as a tumor suppressor gene (22). Furthermore, due to its participation in desmosomal and hemidesmosomal protein networks, Erbin may also be implicated in skin diseases such as bullous pemphigoid and junctional epidermolysis bullosa (33, 46). Identification of protein networks associated to LAP proteins should help to understand their roles in cellular and developmental processes as well as in human diseases.

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