hINADl/PATJ, a Homolog of Discs Lost, Interacts with Crumbs and Localizes to Tight Junctions in Human Epithelial Cells*

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dCrumbs is an apical organizer crucial for the maintenance of epithelial polarity in Drosophila (1). It is known that dCrumbs interacts with Discs lost (Dlt), a protein with four PDZ (PSD95/Discs Large/ZO-1) domains (2), and Stardust (Sdt), a protein of the MAGUK (membrane-associated guanylate kinase) family (3, 4). We have searched for potential homologs of Dlt in human epithelial cells and characterized one of them in intestinal epithelial cells. Human INAD-like (hINADl) contains 8 PDZ domains, is concentrated in tight junctions, and is also found at the apical plasma membrane. Overexpression of hINADl disrupted the tight junctions localization of ZO-1 and 3. We also identified a partial cDNA coding the transmembrane and cytoplasmic domains of a new human crumbs (CRB3) expressed in Caco-2 cells. This CRB3 was able to interact through its C-terminal end with the N-terminal domain of hINADl. Taken together, the data indicate that hINADl is likely to represent a Dlt homolog in mammalian epithelial cells and might be involved in regulating the integrity of tight junctions. We thus propose to rename hINADl PATJ for protein associated to tight junctions.

The polarized organization of epithelial cells is a fundamental process in animal development and the use of genetics in Drosophila has made a significant contribution to the understanding of some of the mechanisms involved in epithelial polarity (for recent reviews, see Refs. 1, 5, and 6). This approach has led to the identification of Armadillo (7), Discs Large (8), and Scribble (9), to name a few of the genes regulating epithelial cell shape and polarity. Among the protein complexes that have been identified as playing a major role in creating or maintaining epithelial cell polarity, dCrumbs has made a significant contribution to the understanding of some of the mechanisms involved in epithelial polarity (for recent reviews, see Refs. 1, 5, and 6). This approach has led to the identification of Armadillo (7), Discs Large (8), and Scribble (9), to name a few of the genes regulating epithelial cell shape and polarity. Among the protein complexes that have been identified as playing a major role in creating or maintaining epithelial cell polarity, dCrumbs has made a significant contribution to the understanding of some of the mechanisms involved in epithelial polarity. It is known that dCrumbs interacts with Discs lost (Dlt), a protein with four PDZ (PSD95/Discs Large/ZO-1) domains (2), and Stardust (Sdt), a protein of the MAGUK (membrane-associated guanylate kinase) family (3, 4). We have searched for potential homologs of Dlt in human epithelial cells and characterized one of them in intestinal epithelial cells. Human INAD-like (hINADl) contains 8 PDZ domains, is concentrated in tight junctions, and is also found at the apical plasma membrane. Overexpression of hINADl disrupted the tight junctions localization of ZO-1 and 3. We also identified a partial cDNA coding the transmembrane and cytoplasmic domains of a new human crumbs (CRB3) expressed in Caco-2 cells. This CRB3 was able to interact through its C-terminal end with the N-terminal domain of hINADl. Taken together, the data indicate that hINADl is likely to represent a Dlt homolog in mammalian epithelial cells and might be involved in regulating the integrity of tight junctions. We thus propose to rename hINADl PATJ for protein associated to tight junctions.

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lacked a transmembrane and cytoplasmic domain (18). No homolog of Dlt has yet been described and therefore the existence of a conserved complex in vertebrates is unknown.

To answer this question, we have searched for putative homologs of Dlt in human epithelial cells using the first PDZ domain of Dlt, which was suggested to bind the cytoplasmic tail of dCrumbs (2). We have identified two human proteins with a highly conserved PDZ domain, Mupp1 (19) and hINADl (20) and have characterized one of these, hINADl. hINADl was localized to the apical membrane and was highly concentrated at the level of the TJs both in Caco-2 and MDCK epithelial cells. Overexpression of hINADl in MDCK cells disrupted the localization of TJs markers (ZO-1 and ZO-3). In addition, hINADl co-immunoprecipitated with a new human Crumbs expressed in epithelial cells, CRB3, in which the extracellular domain was replaced by a vesicular stomatitis virus protein G (VSV-G) epitope (Crumbs-VSV-G). This interaction involved the N-terminal region of hINADl and the C-terminal region of the CRB3 cytoplasmic domain. Thus a complex similar to the one found in Drosophila can regulate the function of TJs in mammalian epithelial cells outside the retina where CRB1 is expressed.

MATERIALS AND METHODS

Reagents and Antibodies—Protein A-Sepharose was from Amersham Biosciences. Polyclonal antibodies against ZO-1 and ZO-3 were a kind gift from Dr. K. Matter (London, UK), and polyclonal antibodies against villin were from Dr. D. Louvard (Paris, France). Monoclonal antibody against dipetidyl peptidase IV (DPP IV) was a gift from Dr. A. Quaroni (Bethesda, NY), and monoclonal antibody against Ag525 has been characterized previously (21). Polyclonal anti-placental alkaline phosphatase was from Accurate Chemical and Scientific Corp. (Westbury, NY). Monoclonal antibody against occludin was from Zymed Laboratories Inc. (San Francisco, CA), and 9E10 monoclonal antibody against Myc was from Santa Cruz (La Jolla, CA). A mouse monoclonal anti-VSV-G antibody P5D4 (Sigma) was used at a dilution of 1:500 and 1:300 for immunoprecipitation and immunofluorescence, respectively. Polyclonal antibodies against hINADl (fragments 10–130 and 800–1000) and Mupp1 (fragment 787–989) were produced by the injection of purified histidine-tagged fragments into rabbits (50 µg per boost; a boost every 3 weeks). His-tagged hINADl and Mupp1 were also coupled to CNBr-activated Sepharose beads to purify the respective polyclonal antibodies.

Constructs, Cell Culture, and Transfection—His-tagged hINADl and Mupp1 were obtained by subcloning the 10–130 or 800–1000 fragments of hINADl and the 787–989 fragment of Mupp1 in pQE-30 vector from Escherichia coli, according to the manufacturer's instructions. Caco-2 cells were obtained from Dr. A. Zweibaum (Villejuif, France) and grown as described (22) with neomycin (0.25 mg/ml) when transfected. Caco-2 and HeLa cells were transfected using FuGENE 6 (Roche Diagnostics). hINADl or hINADl is 84% for this PDZ domain.

Comparison of Dlt, hINADl, and Mupp1. A. alignment of Dlt, hINADl, and Mupp1 shows the localization of the PDZ domains and their correspondence. Amino acid numbers are indicated. B. alignment of the first PDZ domain of Dlt with the second domain of Mupp1 and hINADl. Identical amino acids are shaded in black, and conserved amino acids are shaded in grey. The homology between Dlt and Mupp1 or hINADl is 84% for this PDZ domain.

TCAGATGAGGCCTCTTCCGGC (reverse) for CRB3. PCR products were sequenced to verify the identity of the amplified products.

Immunofluorescence, Confocal Microscopy, and Immunogold Labeling of Cells and Tissues—Cells were grown on glass coverslips and processed as described (23). Confocal microscopy analysis was performed using a Zeiss confocal microscope (LSM 410 invert). For immunoelectron microscopy, ultrathin frozen sections of Caco-2 cells and human colon were obtained and processed as described (24).

Northern blots, Immunoblots, and Immunoprecipitations—Northern blots were performed as previously described (25) using human poly(A) RNA from CLONTECH, whereas total cellular RNA was extracted from Caco-2 cells using RNAzol (Bioprobe, Montreuil-sous-Bois, France). Immunoblots and immunoprecipitations were performed as previously described (26). For co-immunoprecipitation, cells were lysed in 20 mM TRIS, pH 7.5, 240 mM NaCl, 50 mM KCl, 4 mM EGTA, 4 mM EDTA, 0.2 mM dithiothreitol, and 1% Triton X-100 (buffer N) at 4 °C. Immunoprecipitates were washed three times with buffer N and analyzed by SDS-PAGE and Western blotting.

RESULTS

Characterization of hINADl in Epithelial Cells—To find potential functional human homologs of Dlt we searched for proteins with a PDZ domain resembling the first PDZ domain of Dlt, which is involved in Drosophila Crumbs interaction (2). We found two human proteins, hINADl (20 and Mupp1 (19), with a PDZ domain (PDZZ2) that is highly conserved (84% similarity) (Fig. 1). Both proteins only possess PDZ domains (8 and 13, respectively), suggesting that they act, similar to Dlt, as scaffold proteins with no enzymatic activities. The first five PDZ domains of hINADl and Mupp1 are aligned, whereas the PDZ domains 6, 7, and 8 of hINADl resemble PDZ domains 7, 8, and 10 of Mupp1, respectively. The tissue distribution of Mupp1 in human has been previously reported (19), and a 8.5-kb mRNA is present in heart, brain, placenta, liver, skeletal muscle, kidney, and pancreas. Moreover two other transcripts of 35 and 4 kb are also present in some of these tissues. We performed a similar analysis of hINADl distribution in human tissues and found that a major transcript of 7 kb was expressed in the bladder, testis, ovary, small intestine, colon, heart, skeletal muscle, and pancreas (Fig. 2). A smaller transcript of 4.1 kb was also detected in testis. In Caco-2 cells at least 3 transcripts (7, 4.1, and 3 kb) were detected, indicating that indeed several forms of hINADl were expressed in cells derived from human colon.

Because nothing is known about the cellular role or distribution of hINADl, we produced antibodies against the bridge region (amino acids 800–1000) between PDZ domains 5 and 6, which is the least conserved region of this protein, and against the N-terminal domain (amino acids 10–130) (Fig. 1). Antibodies were affinity-purified using injected protein fragments and
tested by Western blotting on total lysates from epithelial cell lines and human colon. Using antibodies against the 800–1000 domain, hINADl offered a complex pattern with several species both in HeLa and Caco-2 cells and also in human colon (Fig. 3, A and C). The main species were 230, 200, and 135 kDa (some of them were doublets; 230 and 135 kDa, in particular), and the 75-kDa species was detected mostly in Caco-2 cells (Fig. 3C). The antibodies against the N-terminal region recognized the high molecular mass bands (230, 200, and 135 kDa in particular) (Fig. 3B), suggesting that the 75-kDa species has a different site for initiation of translation. All of these different molecular masses most likely represent isoforms of hINADl because we discovered in the cDNA data libraries at least six isoforms either with alternative splicing or with different initiation sites for translation (not shown). To support this hypothesis some of these transcripts are tissue-specific (27), and we detected at least three transcripts in Caco-2 cells by Northern blotting and many alternative spliced forms by RT-PCR (not shown). We cannot exclude, however, that some of the bands are natural or experimental degradation products. This hypothesis is not supported by the fact that when we expressed hINADl with the prediction that a potential functional homolog of Dlt should be membrane-associated because Dlt itself is found predominantly in the membrane fraction derived from Drosophila S2 cells. All isoforms of hINADl were found associated with the membrane fraction of Caco-2 and human colon cells (Fig. 3C) with the exception of the 75-kDa form, which was also found in the cytosol.

hINADl Associates with Tight Junctions—Purified antibodies were used to localize hINADl in Caco-2 cells (Fig. 4A) and in MDCK cells (Fig. 4B). When Caco-2 cells were grown in subconfluent cultures we observed that hINADl was recruited early at cell-cell contacts indicating that it might associate with cell-cell junctions (not shown). In confluent cells, hINADl colocalized with occludin, a marker of TJs in epithelial cells (28), and with DPPIV, a marker of the apical membrane of intestinal cells (29). No co-localization was observed with a basolateral marker, Ag525 (21). This distribution was also observed in MDCK cells with the antibody against the first 10–130 residues, confirming that the endogenous protein recognized was hINADl (Fig. 4B). The same pattern of labeling was seen on sections of human colon indicating that hINADl was associated with the apical plasma membrane and the tight junction complex in vivo (not shown). To better evaluate the relationship between hINADl and TJs, we performed immunolocalization experiments with gold particles on ultrathin frozen sections of Caco-2 cells and human colon (Fig. 4C). In both cell types, hINADl was clearly associated with tight junctions, often with
particles) double-labeled with anti-occludin antibodies (15-nm gold particles on ultrathin frozen section of Caco-2 cells. Bar
concentrated at the level of the TJs.

arrowheads point to the TJs.

A

B

C

DPP IV

Caco-2

Colon

ap

TJ

Oc

merge

N-ter

a

b

c

Fig. 4. Localization of hINADl in Caco-2 cells. A, confocal Z sections of Caco-2 cell monolayers double-labeled with the affinity-purified polyclonal antibody against hINADl (shown in red in a, b, and c) and a mouse monoclonal antibody against Ag525 (α), a basolateral antigen (shown in green); a mouse monoclonal antibody against DPPIV (β), an apical hydrolase (shown in green); a mouse monoclonal antibody against occludin (γ), a marker of the TJs (shown in green). hINADl is found at the level of the tight junctions (arrowheads) and at the apical membrane (arrows). Bar, 10 μm. B, confocal X-Y sections of MDCK cell monolayer double-labeled with the affinity-purified polyclonal antibody against the N-terminal of hINADl (shown in red) and a mouse monoclonal antibody against occludin (Oc) (shown in green). Dots seen with the anti-hINADl are microvilli on the apical membrane. Arrowheads point to the TJs. Bar, 10 μm. C, double immunostaining of hINADl (6 nm gold particles; arrowhead) and DPPIV (15-nm gold particles; arrow) on ultrathin frozen section of Caco-2 cells. Bar, 100 nm. hINADl is found at the level of the TJs. Immunostaining of hINADl (6-nm gold particles) double-labeled with anti-occludin antibodies (15-nm gold particles; arrow) on ultrathin frozen sections of human colon. Bar, 100 nm. hINADl (arrowheads) is found on the apical membrane (ap) and is concentrated at the level of the TJs.

the apical side, and was also localized beneath the apical plasma membrane and in the microvilli. Double staining using a monoclonal antibody against occludin confirmed that the two proteins were indeed located in close proximity, confirming that hINADl was associated with TJs (Fig. 4C).

To determine whether it was indeed hINADl that was enriched in the TJs and not a protein cross-reacting with the antibodies, a full-length construct coding for hINADl and tagged with a Myc epitope was transiently expressed in Caco-2 cells and localized using the monoclonal anti-Myc antibody. When expressed at moderate levels, this construct concentrated at the TJs where it co-localized with ZO-1 (Fig. 5B), confirming that hINADl is part of the TJs complex. We next evaluated the impact of overexpression of Myc-hINADl on the integrity of TJs in epithelial cells. For this, we transiently transfected MDCKII cells reaching confluence with Myc-hINADl or with a form in which the first five PDZ domains were deleted (F2 construct) (Fig. 5A). In cells overexpressing Myc-hINADl the labeling of ZO-1 and ZO-3 was disrupted leading to partial or total loss of peripheral staining (Fig. 6). Polarity of apical (BC44) or basolateral (BC11) markers and of γ-catenin was not altered (not shown). These data indicate that cell polarity and adhesion were not affected by these levels of overexpression. No disruption of the ZO-1 and ZO-3 staining was observed with the F2 construct under the same conditions (not shown). Thus, association of at least two molecules of the TJs was impaired, but the overall structure of epithelial cell architecture was preserved.

hINADl Can Interact with Drosophila Crumbs—We next tested whether hINADl is a potential homolog of Dlt in mammalian epithelial cells by investigating its capacity to interact with the Drosophila Crumbs. We designed a construct in which the extracellular domain of Drosophila Crumbs was replaced by a VSV-G epitope leaving only the stalk region, the transmembrane, and the cytoplasmic domains (see Fig. 7A). When this dCrumbs-VSV-G was expressed in HeLa cells, it was transported to the plasma membrane (not shown) and thus could be used to study potential interactions with hINADl. dCrumbs-VSV-G was immunoprecipitated from two independent stable clones using the P5D4 antibody against the VSV-G epitope. Immunoprecipitates were then probed for the presence of hINADl, while untransfected HeLa cells were used as a control (Fig. 7B). hINADl co-immunoprecipitated specifically with dCrumbs-VSV-G, indicating that it could interact like Dlt does with Crumbs in Drosophila (2, 14). Because the interaction between dCrumbs and Dlt involves the last amino acids of dCrumbs cytoplasmic tail, we also expressed a truncated mutant of dCrumbs-VSV-G (Fig. 7A). This SF105 mutant lacks the last 23 residues and acts as a null allele in Drosophila (30). As expected, dCrumbs-VSV-G SF105 could not precipitate hINADl, confirming that the interaction between the two proteins involves the distal part of the dCrumbs cytoplasmic domain. The full-length Myc-tagged hINADl was also co-expressed as a control and was precipitated with anti-VSV-G antibodies, confirming that it is hINADl indeed that interacts with dCrumbs and not a protein recognized by cross-reacting antibodies (Fig. 7B).

As yet no human Crumbs expressed in epithelial cells has been discovered, so we searched human Expressed Sequence Tag (EST) databases with the cytoplastic domain of TJs of Drosophila Crumbs and found three different human Crumbs (Fig. 8A). One was CRB1 (18), whereas the other two are new gene products and were named, respectively, CRB2 and CRB3. They all share the conserved motif around tyrosine 10 (starting from the membrane) and the four last amino acids, ERLI, essential for dCrumbs function in Drosophila (14). Using specific primers for each human Crumbs, we performed RT-PCR with total RNA from Caco-2 cells (Fig. 8B) and found that CRB3 was expressed in these cells and could be the endogenous partner of hINADl in colon epithelial cells. We therefore expressed a chimera containing the epitope VSV-G as an extracellular domain, the transmembrane domain of dCrumbs, and CRB3 cy-
toplamic domain. This construct was able to co-immunoprecipitate Myc-hINADl when co-expressed in COS-7 cells although a construct deleted of the last four amino acids, ERLI, did not (Fig. 8C). Thus CRB3 can interact with hINADl in cellulo. To better define the domain of hINADl that interacts with CRB3 we next co-expressed two constructs of Myc-hINADl deleted either of the first N-terminal 125 amino acids (ΔN-ter) or the PDZ2 domain (ΔPDZ2). To our surprise VSV-G-CRB3 was still able to interact with ΔPDZ2 but not with ΔN-ter, demonstrating that the interaction between hINADl and CRB3 was not mediated by an hINADl PDZ domain as was suggested for dCrumbs and Dlt (2).

DISCUSSION

hINADl was cloned by homology to Drosophila INAD (20), where INAD is a key protein in photo-transduction in Drosophila and consists of five PDZ domains (31). Its similarity to hINADl, however, is not as strong as the similarity found between hINADl and Dlt. PDZ domains 1–4 of Dlt can be aligned on PDZ domains 2–5 of hINADl with identity percentages ranging from 51 to 63%. PDZ domains 1–2 of INAD, on the other hand, exhibit 35 and 46% identity with PDZ domains 6–7 of hINADl. It is thus unlikely that hINADl is an ortholog of INAD but is rather a mammalian protein combining the Dlt four PDZ domains and a C-terminal region with at least three PDZ domains, which performs another function. This is supported by the existence of a connecting domain that is specific to hINADl. The similar organization of Mupp1, which is divided into two groups of five and eight PDZ domains, suggests that the two proteins may have evolved from the same ancestor. Although known partners of Mupp1 have been described (32, 33, 34), no protein binding to hINADl has yet been found. Uncovering the molecular network associated with hINADl will help us to understand its role in epithelial organization and physiology. We also propose to change its name to PATJ (protein associated to tight junctions) to avoid confusion with Drosophila INAD.

![Diagram of PDZ domains and hINADl mutants](image)

**Fig. 5. Expression of hINADl mutants in Caco-2 cells.** A, scheme of the different hINADl mutants used in this work. The shaded boxes represent PDZ domains, and the dotted boxes represent the Myc epitope. B, hINADl-Myc (a–c) was transiently expressed in confluent Caco-2 cells. Double immunofluorescence labeling was performed using the mouse anti-Myc antibody (shown in green; a and c) and rabbit anti-ZO-1 (shown in red; b and c), and Z sections were performed using confocal microscopy. Arrowheads indicate TJs where hINADl-Myc is localized. Bar, 10 μm.

![Images of VSV-G-CRB3 and hINADl-Myc expression](image)

**Fig. 6. Overexpression of hINADl-Myc in MDCK cells.** hINADl-Myc was transiently expressed in MDCK cells, and the cells were double-labeled with the anti-Myc (shown in green) and the anti-ZO-1 or anti-ZO-3 antibodies (shown in red). A, X-Y confocal sections show that cells overexpressing hINADl-Myc have a disrupted distribution of ZO-3 (arrows) when compared with non-expressing cells. An arrowhead indicates remaining spots of labeling at the level of TJs. B, Z confocal sections show that cells overexpressing hINADl-Myc have a disrupted distribution of ZO-1 and ZO-3 (arrows) when compared with non-expressing cells. Arrowheads indicate TJs. Bar, 10 μm.
PATJ Is a Multi-PDZ Protein Associated with Tight Junctions—dCrumbs is a transmembrane protein necessary for the correct positioning and assembly of the ZA in Drosophila ectodermal cells (13, 12). dCrumbs localizes to the subapical region (or marginal zone) of epithelial cells just above the ZA and interacts with a multi-PDZ protein called Dlt (2) and a MAGUK protein named Stardust (Sdt) (3, 4). One hypothesis is that by recruiting Dlt and Sdt, dCrumbs builds a cortical network of protein-protein interactions contacting the E-cadherin/armadillo complex found in the ZA (14). Proteins localized basolaterally to this structure, like Scribble, are also involved in positioning components of the ZA (9). Most of the genes and proteins involved in the control of cell polarity in Drosophila have been described now in vertebrates (for review, see Ref. 15), indicating that similar mechanisms are operating in mammalian epithelial cells. It must be pointed out, however, that in mammals the ZA is topped by the zonula occludens, which forms the TJs (35) whereas, in invertebrates, SJs seal membranes below the ZA. There was, however, an uncertainty about the existence in vertebrate epithelial cells of a protein complex homolog to the Drosophila dCrumbs complex. Recently, a Crumbs-like gene (CRB1) was described in human as being responsible for some type of retinitis pigmentosa when mutated (18), and the cytoplasmic domain of this CRB1 was able to rescue crb mutants in Drosophila (36). It is not expressed in epithelial cells, however, and so far no other dCrumbs homologs have been described. Dlt and stardust have not been studied in mammals either, and while searching for potential homologs of Dlt, we have found that PATJ exhibits several features that indicate it is a good candidate.

PATJ is a multi-PDZ protein that contains, like Dlt, no other obvious domain, and its PDZ domain 2 is highly homologous to Dlt PDZ domain 1. PDZ domain 1 of Dlt binds directly to the cytoplasmic tail of dCrumbs (2), strongly indicating that its mammalian counterpart, which shares 84% similarity, could also bind human Crumbs. This potential interaction was confirmed by the ability of dCrumbs and CRB3, a new human Crumbs, to pull-down PATJ in a co-immunoprecipitation as-
PATJ Is a Potential Regulator of TJs Formation and Integrity—PATJ is not only membrane-associated but is also localized primarily to TJs, the spatial equivalent of the subapical zone where Dlt is concentrated in Drosophila (14). PATJ interacts with CRB3 through its N-terminal region because its deletion abolishes the binding of PATJ to human CRBs. Thus the molecular basis of the binding of PATJ to CRB3 is the only human CRB that also binds to multiple partners and is likely to build a cortical network at the level of the TJs. PATJ is a new protein associated with the TJs, and it makes this structure even more complicated because many other proteins with PDZ domains have been shown to be closely associated with it. In particular, there is a complex that shows the same localization both in Drosophila and in mammals, namely the PAR-3, PAR-6, and aPKC complex. This complex is localized to the apical and subapical region in Drosophila (16) and to TJs in mammals (17) and was shown to be involved in the organization of TJs and in epithelial cell polarization (38, 39). No interaction, however, has been found yet between this complex and dCrums in Drosophila. Understanding how the two protein complexes are regulated and connected will be a major goal in the future. The other members of the Crumbs complex in epithelial cells remain to be characterized, but the fact that we could identify three human Crumbs with different cytoplasmic tails in EST databases and that among them CRB3 is expressed in Caco-2 cells points to a conservation of the whole complex. CRB3 has a highly conserved cytoplasmic domain when compared with dCrums (54% identity) and more importantly shares all the amino acids (Y10, E16, and ERLI) that were demonstrated to be essential (54% identity) and more importantly shares all the amino acids (Y10, E16, and ERLI) that were demonstrated to be essential for the function of dCrums in vivo (14). In addition, it is quite interesting to note that CRB3 is the only human CRB that also has a PXXP motif that could bind to an SH3 (Src homology) or a WW domain and thus be engaged in signal transduction cascades. So far we have not been able to produce specific antibodies against CRB3 that could help to determine its subcellular localization to ensure that it is co-localized with PATJ in epithelial cells. The cloning of a full-length cDNA tagged with an epitope should help us to localize this protein. The study of the precise role of each protein in the Crumbs complex will help to clarify the respective functions of Crumbs, Dlt, and Std homologs.

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