A neural plakophilin-related *armadillo* repeat protein (NPRAP)/β-catenin interacts with one of Alzheimer disease-related gene products, presenilin 1. We have previously reported the interaction of NPRAP/β-catenin with synaptic scaffolding molecule, which is involved in the assembly of synaptic components. NPRAP/β-catenin also interacts with E-cadherin and β-catenin and is implicated in the organization of cell-cell junctions. p0071, a ubiquitous isoform of NPRAP/β-catenin, is localized at desmosomes in HeLa and A431 cells and at adherens junctions in Madin-Darby bovine kidney cells. We have identified here a novel protein interacting with NPRAP/β-catenin and p0071 and named this protein plakophilin-related *armadillo* repeat protein-interacting PSD-95/Dlg-AZO-1 (PDZ) protein (PAPIN). PAPIN has six PDZ domains and binds to NPRAP/β-catenin and p0071 via the second PDZ domain. PAPIN and p0071 are ubiquitously expressed in various tissues and are localized at cell-cell junctions in normal rat kidney cells and bronchial epithelial cells. PAPIN may be a scaffolding protein connecting components of epithelial junctions with p0071.

The *armadillo* repeat is a repeated motif of about 40 amino acids originally identified in the *Drosophila* segment polarity gene, *armadillo* (reviewed in Ref. 1). The list of proteins containing this repeat includes β-catenin, plakoglobin, adenomatous polyposis coli gene product, a regulatory protein for small G protein named smg GDP dissociation stimulator, and smg GDP-dissociation stimulator-associating protein (reviewed in Refs. 1 and 2). Among them, p120^ctn^ and its related proteins form a family. The p120^ctn^ family is composed of p120^ctn^, B6P/plakophilin 1, plakophilin 2, *armadillo*, repeat gene deleted in velo-cardiofacial syndrome, p0071, and neural plakophilin-related *armadillo* repeat protein (NPRAP)^3/δ-catenin (3–10).

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Maki Deguchi, Toshihiko Iizuka, Yutaka Hata, Watari Nishimura, Kazuyo Hira, Ikuko Yao, Hiroshi Kawabe, and Yoshimi Takai

*From the †Takai Biotimer Project, Exploratory Research for Advanced Technology, Japan Science and Technology Corporation, c/o JCR Pharmaceuticals Co. Ltd., Kobe 651-2241, Japan, ‡Department of Medical Biochemistry, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan, and the **Department of Molecular Biology and Biochemistry, Osaka University Graduate School of Medicine/Faculty of Medicine, Suita 565-0871, Japan

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RESULTS

To identify a NPRAP/ß-catenin-interacting protein, we performed a yeast two-hybrid screening using various bait constructs of NPRAP/ß-catenin. From $1 \times 10^4$ transformants, only one positive clone, pPrey 5001, was obtained. The sequence analysis of pPrey 5001 revealed that it contained three PDZ domains. To obtain a full-length clone, we screened a randomly primed rat brain cDNA library using the insert from pPrey 5001 as a probe. No single clone contained both of a putative initiation methionine and a termination codon, but from the analysis of several overlapping clones, we tentatively identified a protein composed of 2766 amino acids (Fig. 1A). We have named this protein PAPIN. PAPIN has four PDZ domains in the N-terminal region and two PDZ domains in the C-terminal region. The middle region between the fourth and fifth PDZ domains did not show any significant homology to known proteins (Fig. 1B, a). pPrey 5001 contained the amino acids 231–811 covering the second to fourth PDZ domains (Fig. 1A, underlined). A clone, p5001–52, had an insert of 52 amino acids between the second and third PDZ domains (Fig. 1B, b). Another clone, p5001–61, also had an insert and a termination codon after the fourth PDZ domain (Fig. 1B, c).

pPrey 5001 interacted only with pBTM116 ß-catenin-1 and not with pBTM116 ß-catenin-3 or -4, which did not contain the C-terminal region of NPRAP/ß-catenin (data not shown) (9, 10). The PDZ domains of PAPIN were speculated to bind the C-terminal PDZ-binding motif of NPRAP/ß-catenin. Actually, the GST fusion protein containing the first and second PDZ domains of PAPIN interacted with the native NPRAP/ß-catenin from rat crude synaptosomes (Fig. 2A). Furthermore, we confirmed that PAPIN was enriched in the synaptic plasma membrane and postsynaptic density fractions as well as NPRAP/ß-catenin in the rat brain subcellular fractions (Fig. 2B).

Next, we performed Northern blot analysis using various rat tissues. The messages with 12 kilobase pair were detected in heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (Fig. 3A). The tissue distribution of the messages of PAPIN was similar to that of p0071 (Fig. 3B), but not that of NPRAP/ß-catenin, which was specifically expressed in brain as previously reported (data not shown and Ref. 10). In the hybridization with the probe of ß-actin, all the lanes gave similar signals (data not shown).

p0071 has a PDZ-binding motif similar to that of NPRAP/ß-catenin, although the motif is not the C terminus of p0071 (Fig. 4A), suggesting that p0071 may also interact with PAPIN. To confirm this possibility, we tested whether PAPIN interacted with the PDZ-binding motif of p0071 in COS cells. For this purpose, we prepared various constructs of p0071 (Fig. 4B). p0071–1 contained the full-length of p0071 (Fig. 4B, a). p0071–2 lacked the C terminus but contained the PDZ-binding motif (Fig. 4B, b). p0071–3 lacked the PDZ-binding motif (Fig. 4B, c). First, we overexpressed PAPIN with either p0071–1 or -3 in COS cells and immunoprecipitated p0071. PAPIN was coimmunoprecipitated with p0071–1 but not with p0071–3 (Fig. 4C). We also examined the interaction of GST-PAPIN and various Myc-tagged constructs of p0071. The products of pCneo Myc p0071–1 and -2 interacted with the GST fusion protein containing the first and second PDZ domains of PAPIN, whereas that of pCneo Myc p0071–3 did not (Fig. 4D).

These results indicate that PAPIN interacts with the PDZ-binding motif of p0071.

To determine which PDZ domain of PAPIN is involved in the interaction with p0071, we prepared various Myc-tagged constructs of PAPIN (Fig. 5A). The products of pCneo Myc PAPIN-2 with the first and second PDZ domains and pCneo Myc PAPIN-9 with the second and third PDZ domains bound to the GST fusion protein containing the C terminus of p0071 (Fig. 5B, b and e). The products of pCneo Myc PAPIN-1 with the first PDZ domain and pCneo Myc Myc PAPIN-11 with the third and fourth PDZ domains did not bind (Fig. 5B, a and f). These data suggest that the second PDZ domain of PAPIN is the p0071-binding domain.

Finally, we compared the localizations of p0071 and PAPIN
in vivo. The anti-PAPIN antibody recognized a protein with a molecular mass of about 300 kDa in NRK cells (Fig. 6A). In the immunocytostaining of NRK cells, both PAPIN and p0071 were localized at cell-cell contacts (Fig. 6B). Because the message of PAPIN was detected remarkably in the lung, we examined the localization of PAPIN in rat lung. PAPIN and p0071 were detected similarly in bronchial epithelial cells (Fig. 7, A and B). Both proteins were localized at cell-cell contacts, although the signals were also detected at the apical side of bronchial epithelial cells.

DISCUSSION

In this paper, we have identified a novel multiple PDZ domain-containing protein, PAPIN, through the yeast two-hybrid...
FIG. 2. Interaction of PAPIN with NPRAP/β-catenin. A, the pull-down experiment of NPRAP/β-catenin with the GST fusion protein of PAPIN using the urea/detergent extracts of rat brain synaptosomes. The urea/detergent extract of rat crude synaptosomes was incubated with either GST or GST-PAPIN-9 containing the first and second PDZ domains of PAPIN fixed on the glutathione-Sepharose 4B beads, and the proteins bound to the beads were immunoblotted with the anti-β-catenin antibody. Lane 1, the input; lane 2, the precipitate with GST; and lane 3, the precipitate with GST-PAPIN-9. B, subcellular localization of PAPIN in rat brain. Equal aliquots of the subcellular fractions of rat brain (25 μg of protein each) were immunoblotted with either the anti-PAPIN or anti-β-catenin antibody. Upper panel, the immunoblot with the anti-PAPIN antibody; lower panel, the immunoblot with the anti-β-catenin antibody. Lane 1; the homogenate fraction; lane 2, the crude synaptosomal fraction; lane 3, the nuclear pellet fraction; lane 4, the synaptosomal cytosol fraction; lane 5, the crude synaptosomal pellet fraction; lane 6, the lysed synaptosomal membrane fraction; lane 7, the crude synaptic vesicle fraction; lane 8, the synaptic plasma membrane (SPM) fraction; lane 9, the 0.5% (w/v) Triton X-100 soluble fraction of the SPM; lane 10, the 0.5% (w/v) Triton X-100 insoluble fraction of the SPM; lane 11, the 1% (w/v) Triton X-100 soluble fraction of the SPM; and lane 12, the 1% (w/v) Triton X-100 insoluble fraction of the SPM.

screening using NPRAP/β-catenin as a bait. PAPIN interacts with NPRAP/β-catenin and its ubiquitous isoform, p0071, in vitro and in transfected cells. We could not directly show the interaction of endogenous PAPIN and p0071, because the endogenous p0071 was highly resistant to the detergent extraction and even the detergent/urea extraction. However, because PAPIN and p0071 are localized similarly at cell-cell junctions of NRK cells, both proteins are likely to interact with each other in vivo. The interaction of PAPIN with NPRAP/β-catenin or p0071 is mediated by the second PDZ domain of PAPIN and the PDZ-binding motif of NPRAP/β-catenin or p0071. The PDZ domain is a protein-interacting module and was initially reported to bind the C terminus of various proteins (19). However, the PDZ domain of Drosophila INAD protein binds to the PDZ-binding motif of TRP, which is localized inside the sequence (20). The second PDZ domain of PAPIN similarly binds to the PDZ-binding motif of p0071, which is localized inside the sequence.

FIG. 4. Interaction of PAPIN with p0071. A, the comparison of the C-terminal sequences of p0071 and NPRAP/β-catenin. The armadillo repeats are shown as boxes. The amino acids corresponding to the PDZ-binding motif are underlined. The asterisks indicate the terminus codons. B, the schematic description of various constructs of p0071. The armadillo repeats are shown as boxes. DSWV is the PDZ-binding motif. a, p0071–1; b, p0071–2; and c, p0071–3. C, communoprecipitation of PAPIN with p0071 from COS cells. PAPIN was overexpressed with either the full-length or the C-terminal region lacking p0071 in COS cells. The extracts of these cells were incubated with the anti-p0071 antiserum, and the precipitates were immunoblotted with either the anti-PAPIN or the anti-p0071 antibody. Upper panel, the immunoblot with the anti-PAPIN antibody; lower panel, the immunoblot with the anti-p0071 antibody; and c, communoprecipitation with the anti-p0071 antibody. D, the interaction of various Myc-tagged constructs of p0071 with GST-PAPIN-9 containing the first and second PDZ domains of PAPIN. The extracts of COS cells transfected with pCneo Myc p0071–1,–2, or –3 were incubated with either GST or GST-PAPIN-9 fixed on the glutathione-Sepharose 4B beads, and the proteins bound to the beads were immunoblotted with the anti-Myc antibody. Lane 1, the input; lane 2, the precipitate with GST; and lane 3, the precipitate with GST-PAPIN-9. a, pCneo Myc p0071–1; b, pCneo Myc p0071–2; c, pCneo Myc p0071–3.

FIG. 3. Tissue distribution of PAPIN and p0071. A blot with 2 μg of mRNA from each rat tissue was hybridized with a uniformly labeled probe corresponding to either the amino acids 231–811 of PAPIN or 1–1211 of p0071 and exposed overnight. Lane 1, heart; lane 2, brain; lane 3, spleen; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; and lane 8, testis. A, PAPIN; B, p0071.
protein/(±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor-binding protein, and channel-interacting PDZ domain protein, have more than one PDZ domains (17, 21–31). The multiple PDZ domain-containing proteins are considered to bind various ligands via distinct PDZ domains and provide a scaffold for these ligands (reviewed in 32–34). PAPIN may also interact with various molecules besides NPRAP/β-catenin and p0071 via the PDZ domains. NPRAP/β-catenin and p0071 belong to a family of p120ctn containing 10 armadillo repeats. The functions of NPRAP/β-catenin and p0071 are not clear, but both proteins are localized at cell-cell junctions, suggesting that they play roles as components of cell-cell junctions like p120ctn (3, 11–13, 35).

There are now three reports for the interactions between PDZ domain-containing proteins and armadillo repeat-containing proteins. Adenomatous polyposis coli gene product interacts with PSD-95/SAP90 and SAP97/human Discs-large tumor suppressor gene (36). NPRAP/β-catenin interacts with synaptic scaffolding molecule (15), and NPRAP/β-catenin and p0071 bind to PAPIN. Because both the PDZ domain-containing protein family and the armadillo repeat-containing protein family are localized at cell-cell junctions, these interactions may be important in the architecture of cell-cell junctions.

Presenilin 1 is reported to interact with the armadillo repeats of β-catenin, p0071, and NPRAP/β-catenin (37–39). Recent studies have revealed the implication of presenilin 1 in both Notch and Wnt/Wingless pathways (40–44). Presenilin 1 is localized mainly at the endoplasmic reticulum and Golgi complex (45–47), but a recent study has revealed that presenilin-1 is recruited to the cell-cell contact through the complex formation with E-cadherin and β catenin (48). We have not examined whether p0071 or NPRAP/β-catenin interacts simultaneously with PAPIN and presenilin 1. But ligands of PAPIN may form a complex with p0071/NPRAP/β-catenin and presenilin 1 and may play roles in Notch or Wnt/Wingless pathways.
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