Multi-PDZ Domain Protein 1 (MUPP1) Is Concentrated at Tight Junctions through Its Possible Interaction with Claudin-1 and Junctional Adhesion Molecule*

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Claudins, most of which end in valine at their COOH termini, constitute tight junction (TJ) strands, suggesting that TJ strands strongly attract PDZ-containing proteins. Indeed, ZO-1, -2, and -3, each of which contains three PDZ domains, were shown to directly bind to claudins. Using the yeast two-hybrid system, we identified ZO-1 and MUPP1 (multi-PDZ domain protein 1) as binding partners for the COOH terminus of claudin-1. MUPP1 has been identified as a protein that contains 13 PDZ domains, but it has not been well characterized. In vitro binding assays with recombinant MUPP1 confirmed the interaction between MUPP1 and claudin-1 and identified PDZ10 as the responsible domain for this interaction. A polyclonal antibody specific for MUPP1 was then generated. Immunofluorescence confocal microscopy as well as immunoelectron microscopy with this antibody revealed that in polarized epithelial cells MUPP1 was exclusively concentrated at TJs. Furthermore, in vitro binding and transfection experiments showed that junctional adhesion molecule, another TJ adhesion molecule, also bound to the PDZ9 domain of MUPP1. These findings suggested that MUPP1 is concentrated at TJs in epithelial cells through its binding to claudin and junctional adhesion molecule and that it may function as a multivalent scaffold protein that recruits various proteins to TJs.

Tight junctions (TJs)§ constitute the epithelial and endothelial junctional complex together with adherens junctions and desmosomes and are located at the most apical part of the complex (1). TJs have dual barrier and fence roles. They create the primary barrier to the diffusion of solutes through the paracellular pathway and maintain cell polarity as a boundary between the apical and basolateral plasma membrane domains (1–5). On ultrathin section electron microscopy, TJs appear as a series of discrete sites of apparent fusion, involving the outer leaflet of the plasma membranes of adjacent cells (1). On freeze-fracture electron microscopy, TJs appear as a set of continuous, anastomosing intramembranous particle strands (TJ strands) (6, 7).

The molecular architecture of TJs has been unraveled rapidly in recent years. Two distinct types of integral membrane proteins, occludin and claudins, have been identified as constituents of TJ strands (8–10). Both occludin and claudins bear four transmembrane domains but do not show any sequence similarity with each other. Claudins and occludin are thought to constitute the backbone of TJ strands and to modulate some functions of TJs, respectively (5, 10–15). Claudins comprise a multigene family consisting of more than 20 members (9, 10, 16–19). It was recently shown that heterogeneous claudin species (and also occludin) are co-polymerized to form individual TJ strands as heteropolymers and that between adjacent cells claudin molecules adhere with each other in both homotypic and heterotypic manners except in some combinations (20, 21). In addition to claudins and occludin, another type of integral membrane protein, JAM (junctional adhesion molecule) belonging to the immunoglobulin superfamily, was also reported to be concentrated at TJs (22), but this molecule did not appear to constitute TJ strands per se but to laterally associate with strands (23).

Interestingly most claudin species (and also JAM) end in valine at their COOH termini (5). This suggests that these COOH termini bind directly to PDZ domains (24, 25). As the cytoplasmic surface of individual TJ strands is expected to appear as a toothbrush consisting of numerous densely packed short COOH-terminal cytoplasmic tails of claudins, TJ strands including laterally associated JAM molecules may strongly attract and recruit many PDZ domain-containing proteins (5, 23). Indeed, three structurally related PDZ domain-containing proteins, ZO-1, ZO-2, and ZO-3, have been shown to be concentrated at the cytoplasmic surface of TJs (26–30). Cloning and sequencing of cDNAs encoding these molecules showed that all have three PDZ domains (PDZ1 to 3), one Src homology 3 domain, and one guanylate kinase-like domain in this order from their NH₂ termini (29–32). Recently the PDZ1 domains of ZO-1/2/3 were shown to bind directly to the COOH termini of claudins (33). In addition, several PDZ domain-containing proteins such as MAGI (membrane-associated guanylate kinase inverted)-1/2/3 (34–37) and mammalian homologues of Caenorhabditis elegans PAR (partitioning)-3/6 (38–42) were also reported to be concentrated at TJs. Recently PDZ domains of PAR-3 were shown to be associated with JAM (and not with claudins) (23, 43), but it remains unknown how the other PDZ domain-containing proteins are recruited to TJs.

In this study, we used the yeast two-hybrid system to screen for PDZ domain-containing molecules that interact with the COOH terminus of claudin-1 and identified MUPP1 (multi-PDZ domain protein 1), which contains 13 PDZ domains in...
tandem (44, 45). This molecule was concentrated at TJs in polarized epithelial cells. Interestingly, MUPP1 bound not only to claudin-1 but also to JAM at its PDZ10 and 9, respectively. These findings provide new insight into the molecular architecture of TJs.

EXPERIMENTAL PROCEDURES

Antibodies and Cells—A GST fusion protein with part of MUPP1 (aa 1570–1699) containing PDZ10 was produced in *Escherichia coli*, purified, and used as an antigen to raise polyclonal antibodies (pAbs) in rabbits. One of these pAbs (B4) was used after affinity purification with the GST fusion protein. Mouse anti-ZO-1 mAb (TS-754) and rabbit anti-JAM pAb (C4) were generated and characterized previously (23, 31). Rat anti-E-cadherin mAb (ECCD2) was a generous gift from Dr. M. Takeichi (Kyoto University, Kyoto, Japan). Rabbit anti-ZO-1 pAb, mouse anti-Myc tag mAb, and rabbit anti-maltose-binding protein (MBP) pAb were purchased from Zymed Laboratories Inc. (San Francisco, CA), MBL (Nagoya, Japan), and New England BioLabs Inc. (Beverly, MA), respectively. MDCK cells, F9 cells, EpH4 cells, L cells, and BJ cells (23) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

*Yeast Two-hybrid Screening*—To construct the bait vector, the cDNA fragment encoding the COOH-terminal region (aa 188–211) of mouse claudin-1 was amplified by PCR and subcloned into pBTM116 containing the LexA DNA-binding domain and a tryptophan marker (pBTM116-Cld1). This vector was introduced into yeast cells (strain L40) followed by selection on medium lacking tryptophan. Then, the yeast cells harboring bait plasmids were transformed with a mouse embryo cDNA library that had been cloned into pVP16 containing the VP16 transactivating domain and a leucine marker. This library was a generous gift from Dr. J. Behrens (Max-Delbruck Center for Molecular Biology, Berlin). About 2 × 10⁶ yeast transformants were plated on synthetic complete medium lacking histidine, leucine, and tryptophan, and ~500 colonies were picked up after 48–96 h of incubation. After the assay for β-galactosidase activity on filters, seven positive clones were obtained. Prey plasmids were recovered from these positive clones (Prey #1–#7), and the sequences of their inserts were determined. To confirm their interactions, the prey plasmids or a control plasmid (pVP16) were retransformed into L40 with pBTM116-Cld1 or pBTM116. Their interactions were quantified in liquid culture by monitoring the conversion of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol and D-galactose due to the β-galactosidase enzyme encoded by the reporter plasmid.

*Cloning of Mouse MUPP1 cDNA and Transfection*—To isolate a full-length cDNA encoding mouse MUPP1, a mouse F9 a ZAP cDNA library was screened by plaque hybridization using the digoxigenin-labeled NotI fragment of prey #4 as a probe. This screening yielded several positive clones, and the longest clone contained a complete open reading frame (ORF) of MUPP1 (pSK-MUPP1). To construct a vector for Myc-tagged MUPP1 transfection, the cDNA fragment encoding the entire open reading frame of mouse MUPP1 was

![Fig. 1. Yeast-two hybrid screening for claudin-1-binding proteins in a mouse embryo cDNA library.](http://www.jbc.org/)

Fig. 1. Yeast-two hybrid screening for claudin-1-binding proteins in a mouse embryo cDNA library. A, the COOH-terminal 24-amino acid sequence of mouse claudin-1 was used as bait, and seven positive clones were obtained. Three of these clones contained plasmids encoding the PDZ1 domain-containing region (aa 11–142) of ZO-1 (prey #1, #3, and #5), and two contained plasmids encoding the PDZ10-containing regions of MUPP1 (prey #2 and #4, aa 1600–1698 and aa 1570–1699, respectively). MUPP1 was initially identified as a protein containing 13 PDZ domains that interacts with the serotonin 5-HTc receptor (44). B, β-galactosidase assay to confirm the specific interaction of the COOH-terminal region of claudin-1 (Cld-1) with prey #4 (MUPP1; see A) and prey #1 (ZO-1; see A). pBTM116 and pVP16, control plasmids (see “Experimental Procedures”).

![Fig. 2. In vitro binding assay between MUPP1 and claudin-1.](http://www.jbc.org/)

Fig. 2. In vitro binding assay between MUPP1 and claudin-1. The GST protein (GST), the GST fusion protein with the cytoplasmic domain of claudin-1 (GST-Cld1), or the GST fusion protein with the cytoplasmic domain of claudin-1 lacking its COOH-terminal YV (GST-Cld1ΔYV), which was bound to glutathione-Sepharose beads, was incubated with the lysate of *E. coli* expressing the MBP fusion protein with PDZ1–5, PDZ-D6–9, PDZ10–13, PDZ10, PDZ11, PDZ12, or PDZ13 of MUPP1. After washing, the proteins associated with GST fusion proteins or GST were eluted from the beads with a buffer containing glutathione. The eluates were separated by SDS-PAGE followed by immunoblotting with anti-MBP pAb. MBP-PDZ10–13 showed binding affinity to GST-Cld1 (A), and among these four PDZ domains, only MBP-PDZ10 bound to GST-Cld1 (B). Binding was abolished when the COOH-terminal YV was deleted.
bovine serum albumin, samples were incubated with primary antibodies for 1 h in a moist chamber. They were then washed three times with PBS followed by incubation for 30 min with secondary antibodies. Samples were washed with PBS for three times, embedded in 95% glycerol–PBS containing 0.1% para-phenylenediamine and 1% n-propyl gallate, and then examined with a Zeiss Axioscope II photomicroscope (Carl Zeiss) or MRC1024 confocal fluorescence microscope (Bio-Rad) equipped with a Zeiss Axioscope II photomicroscope.

**Immunoelectron Microscopy**—Immunoelectron microscopy using ultrathin cryosections was performed essentially according to the method developed by Tokuyasu (48). Small pieces of small intestine were dissected out from adult mice and fixed in 1.2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 20 min at 4°C. After being infused with 2 M sucrose containing 20% polyvinylpyrrolidone at 4°C overnight, samples were rapidly frozen in liquid nitrogen and then cut into ultrathin cryosections at ~90°C using a glass knife with an FC-S low temperature sectioning system (Reichert-Jung, Vienna, Austria). Cryosections ~0.5 μm thick were processed for immunofluorescence microscopy as described above. Cryosections ~70 nm thick were collected on carbon-coated Formvar-filmed grids and then put on a 2% gelatin bed in PBS for 30 min at 37°C. Grids were washed six times with PBS containing 10 mM glycine (PBS-G) and incubated with PBS-G containing 1% Block-Ace (Dainippon Pharmaceutical Co., Ltd.) for 10 min. Samples were again washed with PBS-G four times and then incubated with rabbit anti-MUPP1 pAb for 1 h. After washing with PBS containing 0.1% bovine serum albumin seven times, sections were incubated with goat anti-rabbit IgG coupled to 10-nm gold colloid (British BioCell International Ltd.) for 1 h. Sections were washed with PBS containing 0.1% bovine serum albumin seven times and with PBS six times. Sections were then fixed with 1% glutaraldehyde in PBS for 10 min and then washed with distilled water six times followed by staining with 2% methylcellulose containing 0.5% uranyl acetate for 10 min. Samples were air-dried and examined with a 1200EX electron microscope (JEOL) at an accelerating voltage of 100 kV.

**In Vitro Binding Assay**—The DNA fragments encoding the cytoplasmic domain of claudin-1, the cytoplasmic domain of JAM, and the cytoplasmic domain of claudin-1 lacking its COOH-terminal YV were amplified using specific primers and subcloned into pGEX vector as described previously (16, 29). For production of MBP fusion proteins with individual PDZ domains of MUPP1, the following cDNAs were amplified by PCR and subcloned into pMAL-CRI (New England Biolabs Inc.): PDZ1 (aa 124–235), PDZ2 (aa 244–347), PDZ3 (aa 363–472), PDZ4 (aa 531–637), PDZ5 (aa 678–779), PDZ6 (aa 992–1027), PDZ7 (aa 1124–1241), PDZ8 (aa 1323–1430), PDZ9 (aa 1456–1551), PDZ10 (aa 1600–1700), PDZ11 (aa 1865–1801), PDZ12 (aa 1932–1942), PDZ13 (aa 1957–2055), PDZ15 (aa 134–179), PDZ26–9 (aa 992–1515), and PDZ10–13 (aa 1610–2055). These recombinant proteins were expressed in E. coli (DH5α). GST fusion proteins expressed in E. coli were purified using glutathione-Sepharose 4B beads (Amersham Biosciences, Inc.). After washing with PBS, the beads were incubated with lysate of E. coli expressing MBP fusion proteins followed by washing with PBS containing 1% Triton X-100. Bound proteins were then eluted with 300 μl of 50 mM Tris-HCl buffer (pH 8.0) containing 200 mM glutathione. Each eluate was processed for SDS-PAGE followed by immunoblotting with anti-MBP pAb.

**Gel Electrophoresis and Immunoblotting**—One-dimensional SDS-PAGE (10–12.5% gel) was performed based on the method of Laemmli (49), and proteins were electrophoretically transferred from gels onto nitrocellulose membranes. The membranes were soaked in 5% skimmed milk and incubated with the primary antibodies. After washing with PBS, the membranes were incubated with biotinylated secondary antibodies for rabbit, rat, or mouse IgG (Amersham Biosciences, Inc.). They were then washed with PBS followed by incubation with streptavidin-conjugated alkaline phosphatase (Amersham Biosciences, Inc.). The enzyme reaction was visualized using nitroblue tetrazolium and bromochloroindolyl phosphate.

**RESULTS**

**Identification of MUPP1 as a Claudin-1-binding Protein by Yeast Two-hybrid Screening**—We generated a LexA-binding domain fusion protein containing the COOH-terminal 24 amino acids of mouse claudin-1. This fusion protein was expressed in yeast and did not activate the His or β-galactosidase reporter genes when expressed alone or in combination with control constructs. Approximately 2 × 10^7 clones from a mouse embryo cDNA library were screened with the claudin-1-LexA

**amplified by PCR using pSK-MUPP1 as a template and subcloned into pSK-7myc (46). The Myc-tagged MUPP1 fragment was isolated from pSK-7myc and subcloned into the mammalian expression vector pME18S or pCAGGSneodelEcoRI (47) to produce pME18S-MUPP1–7myc or pCAGGSneodelEcoRI-MUPP1–7myc, respectively.

L cells and JL cells were co-transfected with 3 μg of pME18S-MUPP1–7myc and 0.3 μg of pSV2hes. Eph4 cells were transfected with 3 μg of pCAGGSneodelEcoRI-MUPP1–7myc. After 43 h in culture, cells were washed three times with PBS and then examined with a Zeiss Axioscope II photomicroscope.
construct. Screening under selective conditions (His*, Leu*, and Trp*) yielded seven positive clones. To determine the identity of each of the seven clones, sequencing was carried out. As shown in Fig. 1A, three of the clones were found to contain plasmids encoding the PDZ1 domain of ZO-1. Considering that the COOH terminus of claudins interacts directly with the PDZ1 domain of ZO-1 (33), the isolation of a ZO-1-PDZ1-encoding plasmid confirmed that the library screening had worked. Two other plasmids were found to encode the PDZ10-containing region of MUPP1 (Fig. 1A). This molecule was initially identified as a binding protein for the 5-HT1c receptor (44), and sequencing of its cDNA showed that unusually it contains 13 PDZ domains. As shown in Fig. 1B, the β-galactosidase assay confirmed the specific interaction of the COOH-terminal region of claudin-1 with MUPP1 as well as ZO-1.

In Vitro Binding of MUPP1 with Claudin-1—The results obtained from yeast two-hybrid screening suggested that PDZ10 of MUPP1 binds to the COOH terminus of claudin-1. Then we performed in vitro binding assays to examine whether claudin-1 binds to MUPP1 and if so, which PDZ domain(s) of MUPP1 is responsible for this binding. First, MUPP1 was divided into three pieces from PDZ1 to 5 (PDZ1–5), from PDZ6 to 9 (PDZ6–9), and from PDZ10 to 13 (PDZ10–13). We then produced MBP fusion protein with these three segments of MUPP1 in E. coli, and their crude lysates containing recombinant MBP-PDZ1–5, MBP-PDZ6–9, or MBP-PDZ10–13 were mixed with beads conjugated with the GST fusion protein with the cytoplasmic domain of claudin-1 (GST-Cld-1). Bound proteins were then eluted from beads, and each eluate was subjected to SDS-PAGE followed by immunoblotting with anti-MBP pAb. As shown in Fig. 2A, PDZ10–13, but not MBP-PDZ1–5 or MBP-PDZ6–9, was specifically associated with GST-Cld-1. This binding was abolished when the COOH-terminal YV was deleted from GST-Cld-1. Then to narrow down the domain responsible for the interaction between PDZ10–13 and GST-Cld-1, MBP fusion proteins with PDZ10, 11, 12, and 13 were produced separately in E. coli. As shown in Fig. 2B, only PDZ10 bound to GST-Cld-1, and again this interaction required the COOH-terminal YV of GST-Cld-1. These findings indicated that among 13 PDZ domains of MUPP1 PDZ10 showed strong affinity to the COOH terminus of claudin-1.

Generation and Evaluation of Anti-MUPP1 pAb—To examine whether MUPP1 is localized at TJs, we raised a pAb against the GST fusion protein with a part of mouse MUPP1 (aa 1570–1699) containing PDZ10 (GST-PDZ10/MUPP1). As shown by immunoblotting, the affinity-purified pAb specifically recognized GST-PDZ10/MUPP1 in E. coli lysates (but not recombinant full-length ZO-1) as well as a single band around 250 kDa in lysates of cultured epithelial cells (Fig. 3A). Judging from the calculated molecular mass of mouse MUPP1 (~250 kDa), we concluded that this pAb specifically recognized the band of MUPP1. As shown in Fig. 3A, the molecular mass of MUPP1 appeared to be slightly different between mouse cells and dog MDCK cells, but it was not clear whether this difference was attributable to species-related differences or to alternative splicing as reported previously (50).

Next we evaluated this pAb as a tool for immunofluorescence microscopy. For this purpose, we cloned full-length cDNA encoding MUPP1 from the mouse F9 cell cDNA library, constructed an expression vector for Myc-tagged MUPP1, and then introduced it into cultured mouse epithelial Eph4 cells. Stable transfectants were mixed and co-cultured with parental non-transfected cells followed by double staining with anti-Myc mAb and anti-MUPP1 pAb. Anti-MUPP1 pAb detected the cell-cell borders of Myc-positive transfectants more intensely than those of nontransfected cells (Fig. 3B, c and d). These findings indicated that this anti-MUPP1 pAb specifically recognized exogenous as well as endogenous MUPP1 in Eph4 cells.

Subcellular Localization of MUPP1 in Polarized Epithelial Cells—We then examined the subcellular distribution of endogenous MUPP1 in fully polarized MDCK cells by confocal microscopy. As shown in Fig. 4, when cells were double stained with anti-MUPP1 pAb and anti-ZO-1 mAb, the MUPP1 signal precisely coincided with the ZO-1 signal at the most apical region of lateral membranes both in on-face and vertical sectional images. In contrast, E-cadherin was distributed along

![Image](http://www.jbc.org/)

**Fig. 4. Concentration of MUPP1 at TJs in MDCK cells.** Confluent cultures of MDCK cells were double stained with anti-MUPP1 pAb (a) or anti-MUPP1 pAb (b) or anti-ZO-1 mAb (c) or anti-E-cadherin mAb (d) and observed under a confocal microscope. Vertical sectional images were generated and are shown in each lower panel. ap, the level of apical membranes. As shown in merged images (composite), MUPP1 was precisely colocalized with ZO-1 at TJs but not with E-cadherin that is distributed at adherens junctions and lateral membranes (arrowheads). Bar, 10 μm.
was concentrated more apically than E-cadherin (arrows, precisely colocalized with ZO-1 at TJs (arrows)). MUPP1 was localized at TJs (Fig. 5), cryosections ~0.5 μm thick were double stained with anti-MUPP1 pAb (a) and anti-ZO-1 mAb (b) or anti-MUPP1 pAb and anti-E-cadherin mAb (c). Broken lines and arrowheads in a and d indicate the levels of basement membranes and microvillar tips of epithelial cells, respectively. As shown in merged images (composite), MUPP1 was precisely colocalized with ZO-1 at TJs (arrows in c), whereas MUPP1 was concentrated more apically than E-cadherin (arrows in f). Bar, 5 μm. g and h, cryosections ~70 nm in thickness were labeled with anti-MUPP1 pAb followed by colloidal gold-conjugated secondary antibodies and examined by electron microscopy. MUPP1 appeared to be exclusively localized at TJs. In both images, the MUPP1 signals appear to be at the apical-most end of the TJ regions, but in other images they were detected more diffusely along TJs. Mv, microvilli; AJ, adherens junction; DS, desmosome. Bars, 200 nm.

lateral membranes with some concentration at the junctional region, and vertical sectional images showed that MUPP1 was concentrated more apically. Although the degree of concentration of MUPP1 varied depending on epithelial cell type (data not shown), these findings suggested that MUPP1 is concentrated at TJs.

As shown in Fig. 4, d–f, however, the MUPP1/E-cadherin co-staining showed a significant overlap by confocal microscopy. Therefore, to clarify this point, we next examined the subcellular localization of MUPP1 in mouse intestinal epithelial cells. When cryosections ~0.5 μm thick were double stained with anti-MUPP1 pAb/anti-ZO-1 mAb or anti-MUPP1 pAb/anti-E-cadherin mAb, MUPP1 was concentrated at the ZO-1-positive dots that were located more apically than E-cadherin (Fig. 5, a–f). Then cryosections ~70 nm thick were labeled with anti-MUPP1 pAb followed by colloidal gold-conjugated secondary antibodies and examined by electron microscopy. As shown in Fig. 5, g and h, MUPP1 was exclusively localized at TJs.

Interaction of MUPP1 with JAM—In addition to Claudins, in TJs another adhesion molecule called JAM was also reported to interact with PDZ domain-containing proteins such as ZO-1 and PAR-3 (23, 43, 51). Therefore, to examine the interaction of MUPP1 with JAM, we performed in vitro binding experiments using GST fusion proteins with the cytoplasmic domain of JAM (GST-JAM) and recombinant PDZ1–13 of MUPP1. As shown in Fig. 6A, among the 13 PDZ domains only PDZ9 of MUPP1 appeared to bind to JAM specifically.

Next to confirm this PDZ-mediated interaction of MUPP1 with JAM within cells, we transfected Myc-tagged MUPP1 into L cells exogenously expressing JAM (JL cells) as well as parental L cells, and stable transfectants were obtained followed by double staining with anti-JAM pAb and anti-Myc mAb. As shown in Fig. 6B, a and b in JL cells expressing myc-MUPP1, JAM was concentrated at cell-cell borders, which recruited myc-MUPP1 to the cell-cell adhesion sites. Considering that in parental L cells expressing myc-MUPP1 no concentration of MUPP1 at cell-cell borders was detected (Fig. 6B, c and d), we concluded that JAM binds to PDZ9 of MUPP1 (Fig. 7).

DISCUSSION

TJ strands are regarded as heteropolymers of claudins (5, 21). Thus, numerous short COOH-terminal cytoplasmic domains of claudins with valine at their ends are expected to originate from the cytoplasmic surface of TJ strands, which led us to speculate that this surface strongly attracts various PDZ domain-containing proteins. Therefore, we searched for PDZ domain-containing proteins that specifically bind to claudin-1 by yeast two-hybrid screening. As a result, in addition to ZO-1 that was previously reported to bind directly to claudin-1, a very unusual PDZ domain-containing molecule, MUPP1, was picked up as a claudin-1-binding protein; this molecule contained 13 PDZ domains. The claudin-1/MUPP1 interaction was confirmed by the in vitro binding assay. MUPP1 was initially identified as a protein that interacts with the COOH terminus of the serotonin 5-hydroxytryptamine type 2C receptor (44, 45, 52). This protein was also reported to be associated with c-Kit (50), the membrane-spanning proteoglycan NG2 (53), and adenovirus E4-ORF1 and high-risk papillomavirus type 18 E6 oncoproteins (54) but has not been well characterized, especially in terms of its subcellular localization. In this study, we generated a pAb specific for MUPP1, and by immunofluorescence as well as immunoelectron microscopy we concluded that in simple epithelial cells MUPP1 is concentrated at TJs, favoring the in vivo interaction between MUPP1 and claudins.

JAM is another integral membrane protein that is concentrated at TJs in epithelial cells (22). Recently the spatial relationship between JAM and claudin-based TJ strands have been examined in detail, leading to the following molecular architectural model for TJs (23): the cytoplasmic tails of claudins, which originated from the surface of TJ strands, directly bind to the PDZ1 domain of ZO-1 (and also ZO-2/ZO-3) (33). On the other hand, the COOH terminus of JAM showed affinity to the PDZ3 domain of ZO-1 (and probably also ZO-2/ZO-3) (23, 51). Through these interactions, JAM might be recruited and tethered to TJ strands through ZO-1/ZO-2/ZO-3. Furthermore, JAM molecules laterally aggregate to form oligomers (55), which would allow the recruitment of additional JAM molecules around TJ strands. As these JAM molecules might be free of ZO-1/ZO-2/ZO-3, they could recruit other PDZ domain-containing proteins such as PAR-3 and then also its binding proteins such as atypical protein kinase C, PAR-6, and Cdc42 to TJs (39–42). This study showed that MUPP1 is similar to ZO-1 in several points. Both MUPP1 and ZO-1 are concentrated at...
TJs but not at cadherin-based adherens junctions in polarized epithelial cells. Furthermore, both bind to claudins and JAM at nearly located PDZ domains within individual molecules (see Fig. 7). Thus, in a manner similar to ZO-1, MUPP1 might also function as a cross-linker between claudin-based TJ strands and JAM oligomers in TJs.

However, the most characteristic feature of MUPP1, i.e. the most characteristic difference of MUPP1 from ZO-1, is the occurrence of 13 PDZ domains in tandem within single MUPP1 molecules. This would indicate that many integral membrane proteins other than claudins and JAM can be tethered to the claudin-based TJ strands through MUPP1 molecules. Indeed, as mentioned above, a serotonin receptor (44, 45, 52), c-Kit (50), and the membrane-spanning proteoglycan NG2 (53) were reported to bind to MUPP1, although it remained unclear whether these membrane proteins are concentrated at TJs in epithelial cells. In addition to membrane proteins, MUPP1 would also recruit various cytoplasmic proteins to TJs. For example, as mentioned above, MUPP1 was reported to bind to viral oncoprotein products (54). Therefore, it is tempting to speculate that MUPP1 is involved in the formation of macromolecular complexes just beneath the plasma membranes at TJs, which might play an important role in the regulation of the growth and/or differentiation of epithelial cells. It is premature to further discuss the physiological functions of MUPP1 at junctions. One way to clarify the physiological functions of MUPP1 would be to search for the binding partners of the individual PDZ domains of MUPP1.

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