Characterization of ZO-2 as a MAGUK Family Member Associated with Tight as well as Adherens Junctions with a Binding Affinity to Occludin and α Catennin*

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ZO-2, a member of the MAGUK family, was thought to be specific for tight junctions (TJs) in contrast to ZO-1, another MAGUK family member, which is localized at TJs and adherens junctions (AJs) in epithelial and nonepithelial cells, respectively. Mouse ZO-2 cDNA was isolated, and a specific polyclonal antibody was generated using corresponding synthetic peptides as antigens. Immunofluorescence microscopy with this polyclonal antibody revealed that, similarly to ZO-1, in addition to TJs in epithelial cells, ZO-2 was also concentrated at AJs in nonepithelial cells such as fibroblasts and cardiac muscle cells lacking TJs. When NH₂-terminal dlg-like and COOH-terminal non-dlg-like domains of ZO-2 (N-ZO-2 and C-ZO-2, respectively) were separately introduced into cultured cells, N-ZO-2 was colocalized with endogenous ZO-1/ZO-2, i.e. at TJs in epithelial cells and at AJs in non-epithelial cells, whereas C-ZO-2 was distributed along actin filaments. Consistently, occludin as well as α catennin directly bound to N-ZO-2 as well as the NH₂-terminal dlg-like portion of ZO-1 (N-ZO-1) in vitro. Furthermore, immunoprecipitation experiments revealed that the second PDZ domain of ZO-2 was directly associated with N-ZO-1. These findings indicated that ZO-2 forms a complex with ZO-1/occludin or ZO-1/α catennin to establish TJ or AJ domains, respectively.

Generation and maintenance of specialized membrane domains are required for cells to exert their physiological functions, and the underlying molecular mechanisms of these processes are attracting increasing interest from cell biologists. As components of the machinery responsible for membrane specialization, a new gene family was identified which is now called the MAGUK family (membrane-associated guanylate kinase homologues) (for reviews, see Refs. 1–3). MAGUKs are roughly divided into two functional portions: the NH₂-terminal half, which shows similarity to Drosophila lethal (1) discs large-1 (dlg) consisting of three PDZ, one SH3, and one GUK domain; and the COOH-terminal half with no sequence similarity to dlg (20–22). Consistent with the subcellular distribution of ZO-1 in epithelial and nonepithelial cells, its NH₂-terminal dlg-like half bound directly to the cytoplasmic domain of occludin (23, 24) as well as α catennin (24) that associates with the cytoplasmic domain of cadherin via β catennin (25–32). However, how ZO-1 is excluded out from AJs in epithelial cells where α catennin is highly concentrated remains unclear. In addition, the COOH-terminal non-dlg-like half of ZO-1 was shown to be directly associated with actin filaments in vitro as well as in vivo (24, 44). As compared with ZO-1, our knowledge of ZO-2 is still limited. ZO-2 with a molecular mass of 220 kDa was first identified as a peripheral membrane protein with a molecule of about 160 kDa and was concentrated at TJs in epithelial cells (17, 18). However, in nonepithelial cells lacking TJs, such as cardiac muscle cells and fibroblasts, it was precisely colocalized with cadherins (19, 20). ZO-1 molecule is roughly divided into two functional portions: the NH₂-terminal half, which shows similarity to Drosophila lethal (1) discs large-1 (dlg) consisting of three PDZ, one SH3, and one GUK domain; and the COOH-terminal half with no sequence similarity to dlg (20–22). Consistent with the subcellular distribution of ZO-1 in epithelial and nonepithelial cells, its NH₂-terminal dlg-like half bound directly to the cytoplasmic domain of occludin (23, 24) as well as α catennin (24) that associates with the cytoplasmic domain of cadherin via β catennin (25–32). However, how ZO-1 is excluded out from AJs in epithelial cells where α catennin is highly concentrated remains unclear. In addition, the COOH-terminal non-dlg-like half of ZO-1 was shown to be directly associated with actin filaments in vitro as well as in vivo (24, 44). As compared with ZO-1, our knowledge of ZO-2 is still limited. ZO-2 with a molecular mass of 160 kDa was first identified as a ZO-1-binding protein by immunoprecipitation with anti-ZO-1 mAb (33). Cloning and sequencing of dog ZO-2 cDNA revealed that it also contains a dlg-like domain containing three PDZ, one SH3, and one GUK domain, and that the second PDZ domain of ZO-2 was directly associated with N-ZO-1. These findings indicated that ZO-2 forms a complex with ZO-1/occludin or ZO-1/α catennin to establish TJ or AJ domains, respectively.

Simple epithelial cells contain three specialized membrane domains at the most apical part of lateral membranes for intercellular adhesion, tight junctions (TJs), adhesive junctions (AJs) and desmosomes (8). In these domains, occludin/claudin (9–11), cadherin (12–14), and desmoglein/desmocollin (15, 16) were identified as major integral membrane proteins (adhesion molecules), respectively, but our knowledge regarding how these integral membrane proteins are sorted into three distinct junctional membrane domains is still fragmentary. To date, three MAGUKs have been shown to be associated with these intercellular junctions, and these molecules are now called ZO-1, ZO-2, and ZO-3.

ZO-1 was first identified as a peripheral membrane protein with a molecular mass of 220 kDa and was concentrated at TJs in epithelial cells (17, 18). However, in nonepithelial cells lacking TJs, such as cardiac muscle cells and fibroblasts, it was precisely colocalized with cadherins (19, 20). ZO-1 molecule is roughly divided into two functional portions: the NH₂-terminal half, which shows similarity to Drosophila lethal (1) discs large-1 (dlg) consisting of three PDZ, one SH3, and one GUK domain; and the COOH-terminal half with no sequence similarity to dlg (20–22). Consistent with the subcellular distribution of ZO-1 in epithelial and nonepithelial cells, its NH₂-terminal dlg-like half bound directly to the cytoplasmic domain of occludin (23, 24) as well as α catennin (24) that associates with the cytoplasmic domain of cadherin via β catennin (25–32). However, how ZO-1 is excluded out from AJs in epithelial cells where α catennin is highly concentrated remains unclear. In addition, the COOH-terminal non-dlg-like half of ZO-1 was shown to be directly associated with actin filaments in vitro as well as in vivo (24, 44). As compared with ZO-1, our knowledge of ZO-2 is still limited. ZO-2 with a molecular mass of 160 kDa was first identified as a ZO-1-binding protein by immunoprecipitation with anti-ZO-1 mAb (33). Cloning and sequencing of dog ZO-2 cDNA revealed that it also contains a dlg-like domain containing three PDZ, one SH3, and one GUK domain, and that the second PDZ domain of ZO-2 was directly associated with N-ZO-1. These findings indicated that ZO-2 forms a complex with ZO-1/occludin or ZO-1/α catennin to establish TJ or AJ domains, respectively.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) AF113065.

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1 The abbreviations used are: TJ, tight junction; AJ, adherens junction; N-ZO-2, NH₂-terminal dlg-like domain of ZO-2; C-ZO-2, COOH-terminal non-dlg-like domain of ZO-2; F-ZO-2, full-length ZO-2; mAb, monoclonal antibody; pAb, polyclonal antibody; RT-PCR, reverse transcriptase polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; GST, glutathione S-transferase; HA, hemagglutinin; MDCK, Madin-Darby canine kidney.
Characterization of ZO-2 in Comparison with ZO-1

At the initial phase of junction formation of epithelial cells, ZO-1 was precisely colocalized with cadherins in prordinal spot-like AJs, where TJs were not yet assembled, and then at the later stage, ZO-1 was transferred from AJs to TJs (38). Although these findings suggest the direct involvement of ZO-1 in the establishment of two distinct membrane domains, AJs and TJs, in epithelial cells, lack of information concerning ZO-2 has hampered more direct assessment of the junction sorting mechanism at the molecular level. In this study, we isolated a mouse ZO-2 cDNA and characterized its product in detail.

**EXPERIMENTAL PROCEDURES**

**Cloning of Mouse ZO-2 cDNA**—Based on the human cDNA clone X104, which was later recognized as human ZO-2 cDNA (39), a partial human ZO-2 cDNA fragment (2897–3241) was obtained by RT-PCR using mRNA from human T84 cells. This fragment was used as a probe to screen a mouse lung cDNA library. Eleven positive clones were isolated, one of which, clone 10, contained the entire open reading frame of mouse ZO-2.

**Antibodies**—Anti-ZO-2 pAbs, pAb59 and pAb62, were raised in rabbits using synthetic peptides corresponding to the mouse ZO-2 sequences encoding amino acids 407–419 and 1093–1108, as antigens, respectively. The pAbs were affinity purified with the GST-ZO-2 fusion protein that was produced in *Escherichia coli*, separated by SDS-PAGE, and transferred onto nitrocellulose membranes. Mouse anti-ZO-1 mAb (TS–754) and rat anti-catenin mAb (a18) were generated and characterized previously (20, 40). Rat anti-E-cadherin mAb (ECCD-2) and rat anti-P-cadherin mAb (PCD-1) were generously provided by Dr. M. Takeichi (Kyoto University, Kyoto, Japan). Mouse anti-c-myc tag mAb and rabbit anti-c-myc tag pAb were purchased from MBL (Nagoya, Japan). Mouse anti-HA-tag mAb was purchased from Boehringer Mannheim (Indianapolis, IN).

**Constructs and Transfection**—For expression of NH2-terminally HA-tagged proteins in mammalian cells, an oligonucleotide encoding an HA epitope was subcloned into the eukaryotic expression vector pME18S containing GST fusion proteins was produced in *Escherichia coli*, separated by SDS-PAGE, and transferred onto nitrocellulose membranes. Mouse anti-ZO-1 mAb (TS–754) and rat anti-catenin mAb (a18) were generated and characterized previously (20, 40). Rat anti-E-cadherin mAb (ECCD-2) and rat anti-P-cadherin mAb (PCD-1) were generously provided by Dr. M. Takeichi (Kyoto University, Kyoto, Japan). Mouse anti-c-myc tag mAb and rabbit anti-c-myc tag pAb were purchased from MBL (Nagoya, Japan). Mouse anti-HA-tag mAb was purchased from Boehringer Mannheim (Indianapolis, IN).

**RESULTS**

**Isolation of Mouse ZO-2 cDNA and Generation of Anti-ZO-2 pAb**—When we began this study, only a partial dog ZO-2 cDNA had been isolated (34). Similarity searches in data bases identified a human cDNA (X104) which showed marked similarity to dog ZO-2 cDNA (39). Based on this sequence, we isolated a part of human ZO-2 cDNA by PCR using the first strand cDNA generated from total RNA of cultured human epithelial cells (T84). Using this cDNA fragment as a probe, we screened a λZAP cDNA library of mouse lung, and obtained a full-length cDNA encoding mouse ZO-2 (data are available from GenBank/EBI/DDBJ under accession number AF113005). Its open reading frame encoded a protein of 1,167 amino acids with a calculated molecular mass of 132 kDa. During the course of this study, however, a full-length cDNA encoding dog ZO-2 was reported (35). Mouse ZO-2 was 85% identical to dog ZO-2 at the amino acid sequence level, and consisted of three PDZ, one SH3, and one GUK domain from the NH2-terminal end (Fig. 1).

Based on the deduced amino acid sequence of mouse ZO-2, we synthesized two polypeptides corresponding to the middle and COOH-terminal end portions of ZO-2 that showed no sequence similarity to ZO-1 and raised pAbs (pAb59 and pAb62, respectively) in rabbits using them as antigens. Because both pAbs showed the same properties on immunoblotting as well as immunofluorescence microscopy, we will report here only the data obtained with pAb59. As shown in Fig. 2a, the affinity-purified pAb59 specifically recognized recombinant ZO-2 but not recombinant ZO-1 produced in Sf9 cells by baculovirus infection.

**Subcellular Distribution of ZO-2 in Comparison with ZO-1 in Cultured Cells and Tissues**—Using the affinity-purified anti-ZO-2 pAb, we first examined the expression levels of ZO-2 in various cultured cells by immunoblotting and compared them with those of ZO-1 (Fig. 2b). As previously reported, ZO-1 was detected in all the cell types examined including epithelial, fibroblastic, and myeloma cells. ZO-2 was also detected in cultured epithelial cells as well as in fibroblasts such as 3Y1, NIH 3T3, and Swiss 3T3 cells, but not in L fibroblasts or P3 myeloma cells.

We next examined the subcellular localization of ZO-2 in cultured MDCK cells and 3Y1 cells using affinity-purified anti-ZO-2 pAb. In MDCK cells, as expected from previous studies, ZO-2 was precisely colocalized with ZO-1 in a linear pattern at cell-cell borders, and computer-generated cross-sectional views confirmed that ZO-2 was colocalized with occludin at TJs and concentrated more apically than E-cadherin (data not shown).

Interestingly, ZO-2 was also precisely colocalized with ZO-1 in...
a punctate or serrated pattern at cell-cell borders of 3Y1 fibroblasts lacking TJs (Fig. 3, a and b). This characteristic distribution of ZO-2 was identical to P-cadherin (Fig. 3, c and d). This finding prompted us to re-examine the expression and subcellular distribution of ZO-2 in cardiac muscle cells. Frozen sections were doubly stained with anti-ZO-1 mAb and affinity-purified anti-ZO-2 pAb, and intense signals of ZO-2 as well as ZO-1 were detected from intercalated discs (Fig. 3, e and f). Both ZO-1 and ZO-2 were co-concentrated at intercalated discs (arrows), whereas ZO-2 signal was very weak from ZO-1-positive blood vessels (arrowheads). Bars, 10 μm.

Characterization of Dlg-like and Non-dlg-like Domains of ZO-2 in Vivo and in Vitro—

Previously, we divided ZO-1 into the NH2-terminal dlg-like (N-ZO-1) and COOH-terminal non-dlg-like (C-ZO-1) portions and characterized them both in vivo and in vitro (24). In this study, the full-length ZO-2 (F-ZO-2) was also divided into the NH2-terminal dlg-like (N-ZO-2) and COOH-terminal non-dlg-like (C-ZO-2) domains (see Fig. 1). First, F-ZO-2, N-ZO-2, and C-ZO-2 were tagged with HA peptide at their NH2 ends and introduced into cultured MDCK cells as well as 3Y1 cells (Fig. 4). Under transient expression conditions, immunofluorescence microscopy with anti-HA mAb revealed that in both MDCK and 3Y1 cells, F-ZO-2 as well as N-ZO-2 were recruited to the cell-cell borders where endogenous ZO-2 was concentrated, i.e. TJs in MDCK cells and P-cadherin-based spot AJs in 3Y1 cells (Fig. 4, a–d).
Characterization of ZO-2 in Comparison with ZO-1

In marked contrast, C-ZO-2 appeared to be colocalized with actin filaments. In 3Y1 fibroblasts, C-ZO-2 was clearly distributed along stress fibers (Fig. 4f), although in MDCK cells it was distributed diffusely with some concentration at cell-cell borders (along circumferential actin bundles) and plasma membranes (along microvilli) (Fig. 4e).

We have previously reported that N-ZO-1 directly binds to the cytoplasmic domain of occludin as well as σ-catenin in vitro (24). The binding of N-ZO-2 to occludin and/or σ-catenin was compared with that of N-ZO-1 (Fig. 5). N-ZO-1 and N-ZO-2 were produced in Sf9 cells by baculovirus infection, and the cell lysate of Sf9 cells containing almost the same amounts of N-ZO-1 or N-ZO-2 was incubated with a GST fusion protein with the cytoplasmic domain of occludin (Fig. 5a). CBB staining of the eluates from the GST/occludin fusion protein beads revealed that the cytoplasmic domain of occludin directly bound to N-ZO-2 as well as N-ZO-1. Next, we examined the binding of N-ZO-2 to σ-catenin, but in this case the electrophoretic mobilities of recombinant N-ZO-2 (and also that of N-ZO-1) were almost the same as that of GST-σ-catenin fusion protein, making it difficult to estimate the amount of bound N-ZO-2 (and of bound N-ZO-1) by CBB staining (Fig. 5b). Thus, the total proteins of Sf9 cells containing almost the same amounts of N-ZO-1 or N-ZO-2 were biotinylated, then the in vitro binding assay with GST-σ-catenin fusion protein was performed. The amounts of bound N-ZO-1 and N-ZO-2 were estimated by detection with alkaline phosphatase-avidin. As shown in Fig. 5b, GST-σ-catenin fusion proteins bound directly to N-ZO-2 as well as N-ZO-1.

Interaction of ZO-2 with ZO-1—Because ZO-2 was first identified in the ZO-1 immunoprecipitate (33), ZO-1 is thought to be directly associated with ZO-2. Recently, the ZO-2 binding domain on ZO-1 was narrowed down to its PDZ2 domain (44). We attempted to identify the ZO-1 binding domain on ZO-2. First, we examined the in vitro binding of GST fusion proteins with N-ZO-1 or C-ZO-1 with recombinant N-ZO-2 or C-ZO-2 produced in Sf9 cells, but we detected no significant binding. These findings suggested that modifications on ZO-1 or ZO-2 molecules within cells are required for the ZO-1/ZO-2 interaction. Therefore, we introduced HA-tagged F-ZO-2 cDNA into EL cells (L cells transfected with E-cadherin) expressing myc-tagged N-ZO-1 (NZ-EL cells) or myc-tagged C-ZO-1 (CZ-EL cells), and F-ZO-2 in the total cell lysate was immunoprecipitated with alkaline phosphatase-avidin. As shown in Fig. 5c, N-ZO-1 and N-ZO-2 bound to the GST/occludin fusion protein with the cytoplasmic domain of occludin (arrow). N-ZO-2 bound to occludin and/or σ-catenin, but in this case the electrophoretic mobilities of recombinant N-ZO-2 (and also that of N-ZO-1) were almost the same as that of GST-σ-catenin fusion protein, making it difficult to estimate the amount of bound N-ZO-2 (and of bound N-ZO-1) by CBB staining (Fig. 5d). Thus, the total proteins of Sf9 cells containing almost the same amounts of N-ZO-1 or N-ZO-2 was incubated with a GST fusion protein with the cytoplasmic domain of occludin (Fig. 5a). CBB staining of the eluates from the GST/occludin fusion protein beads revealed that the cytoplasmic domain of occludin directly bound to N-ZO-2 as well as N-ZO-1. Next, we examined the binding of N-ZO-2 to σ-catenin, but in this case the electrophoretic mobilities of recombinant N-ZO-2 (and also that of N-ZO-1) were almost the same as that of GST-σ-catenin fusion protein, making it difficult to estimate the amount of bound N-ZO-2 (and of bound N-ZO-1) by CBB staining (Fig. 5b). Thus, the total proteins of Sf9 cells containing almost the same amounts of N-ZO-1 or N-ZO-2 were biotinylated, then the in vitro binding assay with GST-σ-catenin fusion protein was performed. The amounts of bound N-ZO-1 and N-ZO-2 were estimated by detection with alkaline phosphatase-avidin. As shown in Fig. 5b, GST-σ-catenin fusion proteins bound directly to N-ZO-2 as well as N-ZO-1.
Characterization of ZO-2 in Comparison with ZO-1

ZO-1 is expressed not only in epithelial/endothelial cells but also in nonepithelial/endothelial cells such as cardiac muscle cells, fibroblasts, and astrocytes (19, 20, 42) and that, in these nonepithelial/endothelial cells, ZO-1 is precisely colocalized with cadherins (19, 20). In contrast to the ubiquitous expression and the peculiar subcellular distribution of ZO-1, ZO-2, another MAGUK family member, was reported to be specific for TJs, and to be absent from AJs in cardiac muscle cells (34). However, in the present study using anti-ZO-2 pAb, we found that ZO-2 was precisely co-concentrated with ZO-1 at intercalated discs (AJs). At present, the reason for this discrepancy remains unclear, but the following observations favored the notion that ZO-2 is very similar to ZO-1 in terms of AJ-association in nonepithelial/endothelial cells. First, a human cDNA called X104, which was later recognized to be human ZO-2 cDNA, was ubiquitously detected in various tissues and was abundant in heart (39). Second, our anti-ZO-2 pAb also detected concentration of ZO-2 at the P-cadherin-based spot-like AJs in cultured fibroblasts, which had not been examined in the previous report (34). Third, when HA-tagged F-ZO-2 and N-ZO-2 were introduced into cultured fibroblasts, it was correctly targeted to the P-cadherin-based spot-like AJs.

Our previous study suggested that ZO-1 functions as a cross-linker between occludin and actin filaments in epithelial/endothelial cells or between α catenin and actin filaments in nonepithelial/endothelial cells (24). The present study revealed that the cytoplasmic domain of occludin and α catenin also bound to N-ZO-2 in vitro. Exogenously expressed C-ZO-2 was distributed along stress fibers in cultured fibroblasts similarly to exogenously expressed C-ZO-1. From these observations, we concluded that ZO-2 is very similar to ZO-1 also as a cross-linker. At present, it remains unknown why two similar cross-linkers, ZO-1 and ZO-2, exist in intercellular junctions such as AJs and TJs. Furthermore, the recent knockout study of occludin revealed that ZO-1 is still exclusively concentrated at occludin-deficient TJs (43). Preliminary experiments revealed that ZO-2 also remained at occludin-deficient TJs (data not shown). These findings indicate that ZO-1 as well as ZO-2 are recruited to normal TJs through direct or indirect interactions not only with occludin but also with other TJ-specific membrane proteins such as the recently identified claudins (10). The possible interaction of ZO-1 and ZO-2 would make the relationship of these two similar cross-linker proteins more complex. Recent immunoprecipitation analyses indicated that the PDZ2 domain of ZO-1 was responsible for ZO-1/ZO-2 interaction although it was not clear whether this interaction was direct or indirect (44). L cells and their transfectants gave a good model in which to examine the ZO-1/ZO-2 interaction because they lack endogenous expression of ZO-2. In the present study, immunoprecipitation experiments using metabolically labeled cells demonstrated that N-ZO-1 binds to the PDZ2 domain of ZO-2. Of course, although the possibility cannot be completely excluded that a third protein mediates this binding, these findings favored the notion that ZO-1 and ZO-2 directly form a heterodimer (or oligomer) through PDZ2/PDZ2 interaction. This type of PDZ/PDZ interaction has been reported be-
between neuronal nitric oxide synthase and PSD-95 (or PSD-93) (45).

In this study, two intercellular-associated MAGUK family members, ZO-1 and ZO-2, were compared in detail. The elucidation of the molecular mechanism behind the peculiar behavior of ZO-1 and ZO-2, i.e., their respective localization at TJs and AJs in epithelial/endothelial and nonepithelial/endothelial cells, is necessary to better understand the molecular mechanism behind the polarization of epithelial/endothelial cells. Because information concerning the molecular components of intercellular junctions is rapidly accumulating, further analyses of ZO-1 and ZO-2 including knocking-out their genes will lead to a better understanding of the physiological functions of MAGUK family members in general.

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REFERENCES

1. Woods, D. A., and Bryant, P. J. (1993) Mech. Dev. 44, 85–89
2. Kim, S. K. (1995) Curr. Opin. Cell Biol. 7, 641–649
3. Anderson, J. M. (1995) Biochem. Soc. Trans. 23, 470–475
4. Kim, E., Niethammer, M., Rothschild, A., Jan, Y. N., and Sheng, M. (1995) Nature 378, 85–88
5. Kornau, H. C., Schenker, L. T., Kennedy, M. B., and Seeburg, P. H. (1989) Science 249, 1737–1740
6. Niethammer, M., Kim, E., and Sheng, M. (1996) J. Neurosci. 16, 2157–2163
7. Irie, M., Hata, Y., Takeuchi, M., Ichtchenko, K., Toyoda, A., Hirao, K., Takai, Y., Rosaihl, T. W., and Sudhof, T. C. (1997) Science 277, 1511–1515
8. Farquhar, M. G., and Palade, G. E. (1963) J. Cell Biol. 17, 375–409
9. Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S., Teuktit, S., and Tsukita, S. (1993) J. Cell Biol. 123, 1773–1778
10. Furuse, M., Fujita, K., Hiragi, T., Fujimoto, K., and Tsukita, S. (1996) J. Cell Biol. 141, 1539–1550
11. Ando-Akatsu, Y., Saitou, M., Hirase, T., Kishi, M., Sakakibara, A., Itoh, M., Yonemura, S., Furuse, M., and Tsukita, S. (1996) J. Cell Biol. 133, 43–47
12. Boiler, K., Vestweber, D., and Kemler, R. (1985) J. Cell Biol. 106, 327–332
13. Takeichi, M. (1991) Science 251, 1451–1455
14. Kemler, R. (1992) Semin. Cell Biol. 3, 149–155
15. Holton, J. L., Kenny, T. P., Logan, P. K., Collins, J. E., Keen, J. N., Sharma, R., and Garrod, D. R. (1996) J. Cell Sci. 97, 239–246
16. Koch, P. J., Walsh, M. J., Schmelz, M., Goldschmidt, M. D., Zimbembaum, R., and Franke, W. W. (1990) Eur. J. Cell Biol. 53, 1–12
17. Stevenson, B. R., Siliciano, J. D., Moseker, M. S., and Goodenough, D. A. (1988) J. Cell Biol. 103, 755–766
18. Anderson, J. M., Stevenson, B. R., Jesaitis, L. A., Goodenough, D. A., and Moseker, M. S. (1998) J. Cell Biol. 106, 1114–1149
19. Itoh, M., Yonemura, S., Nagafuchi, A., Tsukita, S., and Tsukita, S. (1991) J. Cell Biol. 115, 1449–1462
20. Itoh, M., Nagafuchi, A., Yonemura, S., Kitani-Yasuda, T., Tsukita, S., and Tsukita, S. (1993) J. Cell Biol. 121, 491–502
21. Tsukita, Sh., Itoh, M., Nagafuchi, A., Yonemura, S., and Tsukita, S. (1995) J. Cell Biol. 123, 1049–1053
22. Willett, E., Balda, M. S., Fanning, A. S., Jameson, B., Van Itallie, C., and Anderson, J. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7834–7831
23. Furuse, M., Itoh, M., Hirase, T., Nagafuchi, A., Yonemura, S., Tsukita, S., and Tsukita, S. (1994) J. Cell Biol. 127, 1617–1626
24. Itoh, M., Nagafuchi, A., Moroi, S., and Tsukita, S. (1997) J. Cell Biol. 138, 181–192
25. Ozawa, M., Baribault, H., and Kemler, R. (1989) EMBO J. 8, 1711–1717
26. Nagafuchi, A., and Takeichi, M. (1988) EMBO J. 7, 3679–3684
27. Ozawa, M., and Kemler, R. (1992) J. Cell Biol. 116, 989–996
28. Auerle, H., Butz, S., Stappart, J., Weissig, H., Kemler, R., and Hoschuetzky, H. (1994) J. Cell Sci. 107, 3655–3663
29. Hinck, L., Nathke, I. S., Papkoff, J., and Nelson, W. J. (1994) J. Cell Biol. 123, 1327–1340
30. Nathke, I. S., Hinck, L., Sweidlow, J. R., Papkoff, J., and Nelson, W. J. (1994) J. Cell Biol. 123, 1341–1352
31. Oyama, T., Kanai, Y., Ochiiai, A, Akimoto, S., Oda, T., Yanagihara, K., Nagafuchi, A., Tsukita, S., Shibamoto, S., Ito, F. et al. (1994) Cancer Res. 54, 6282–6287
32. Jou, T. S., Stewart, D. B., Stappart, J., Nelson, W. J., and Marrs, J. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5067–5071
33. Gumbiner, B., Lowenkopf, T., and Apatrissa, D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3460–3464
34. Jesaitis, L. A., and Goodenough, D. A. (1994) J. Cell Biol. 124, 949–961
35. Beatch, M., Jesaitis, L. A., Gallin, W. J., Goodenough, D. A., and Stevenson, B. R. (1996) J. Biol. Chem. 271, 25723–25726
36. Balda, M. S., Gonzalez-Mariscal, L., Matter, K., Cereijido, M., and Anderson, J. M. (1993) J. Cell Biol. 123, 293–302
37. Haskins, J., Gu, L, Witzchen, E. S., Hubbard, J., and Stevenson, B. R. (1998) J. Cell Biol. 141, 199–208
38. Yonemura, S., Itoh, M., Nagafuchi, A., and Tsukita, S. (1995) J. Cell Sci. 106, 127–142
39. Duclos, F., Rodius, F., Rogemann, K. W. M., and Keenig, M. (1994) Hum. Mol. Genet. 3, 909–914
40. Nagafuchi, A., Takeichi, M., and Tsukita, S. (1991) Cell 65, 849–857
41. Laemmli, U. K. (1970) Nature 277, 680–685
42. Howarth, A. G., Singer, K. L., and Stevenson, B. R. (1994) J. Membr. Biol. 137, 261–270
43. Saitou, M., Fujimoto, K., Doi, Y., Itoh, M., Fujimoto, T., Furuse, M., Takano, H., Noda, T., and Tsukita, S. (1998) J. Cell Biol. 141, 397–408
44. Fanning, A. S., Jameson, B., Jesaitis, L. A., and Anderson, J. M. (1998) J. Biol. Chem. 273, 29745–29753
45. Brennan, J. E., Chao, D. S., Gee, S. H., McGee, A. W., Craven, S. E., Santillano, D. R., Wu, Z., Huang, F., Xia, H., Peters, M. F., Froehner, S. C., and Bredt, D. S. (1996) Cell 84, 757–767