Molecular Dissection of Tight Junctions

Shoichiro Tsukita, Mikio Furuse, and Masahiko Itoh
Department of Cell Biology, Faculty of Medicine, Kyoto University, Sakyoku, Kyoto 606, Japan

ABSTRACT. In epithelial and endothelial cells, the tight junction (TJ) seals cells to create a primary barrier to the diffusion of solutes across the cell sheet, and it also works as a boundary between the apical and basolateral membrane domains to create their polarization. An integral membrane protein working at TJ is postulated to exist, but it has remained elusive for quite some time. Most recently, using mAbs, we identified an integral membrane protein named occludin that was exclusively localized at TJ both in epithelial and endothelial cells. Here we overview our recent studies on the structure and function of occludin.

Tight junction (TJ), an element of epithelial and endothelial junctional complexes, is directly involved in the establishment of compositionally distinct fluid compartments in the body by sealing cells to create the primary barrier to the diffusion of solutes through the paracellular pathway (10, 11, 21). TJ also functions as a boundary between the apical and basolateral plasma membrane domains, which differ in proteins, lipid composition, and physiological functions, to create and maintain epithelial and endothelial cell polarity (20). Therefore, TJ is a very important structure for epithelia and endothelial cells to exert their physiological functions.

Some unique proteins reportedly constitute TJ (1, 3). The first protein identified as a TJ constituent was ZO-1 with a molecular mass of 220 kD (2, 26). This protein is a peripheral membrane protein that is localized in the immediate vicinity of the plasma membrane of TJ in epithelial and endothelial cells (25, 26), whereas it is colocalized with cadherins in cells lacking TJ, such as fibroblasts and cardiac muscle cells (13, 14, 15, 32). A ZO-1 binding protein, another peripheral protein called ZO-2 with a molecular mass of 160 kD has been identified (12). Unlike ZO-1, the distribution of this protein is restricted to TJ (16). Both ZO-1 and ZO-2 reportedly show sequence similarity to the product of lethal [1] discs large-1 (dlg), one of the tumor suppressor molecules in Drosophila (15, 16, 28). In addition to ZO-1 and ZO-2, two other TJ-specific peripheral membrane proteins have been identified so far: cingulin and the 7H6 antigen (4, 35). They are distributed more distantly from the membrane than ZO-1 (25, 35).

In thin section electron microscopy, TJ appears as a series of discrete sites of apparent fusion, involving the outer leaflet of the plasma membrane of adjacent cells (5). In freeze-fracture electron microscopy of glutaraldehyde-fixed samples, this junction appears as a set of continuous, anastomosing intramembrane strands or fibrils in the P-face (the outwardly facing cytoplasmic leaflet) with complementary grooves in the E-face (the inwardly facing extracytoplasmic leaflets) (23). On unfixed samples, however, the intramembrane strands are reportedly seen as a linear series of individual intramembranous particles (22). There has been considerable debate about the chemical nature of these strands, and it remains controversial whether the particles in the strands are predominantly lipidic in nature, i.e., cylindrical lipid micelles, or represent units of integral membrane proteins linearly aggregated (17, 19, 33). However, given the detergent stability of TJ strands visualized by negative staining (24) and freeze fracture (27), it is unlikely that these elements are composed solely of lipids. Therefore, it was widely accepted that the identification of the integral membrane protein localizing at TJ is an important breakthrough, because it opens the investigation of TJ to molecular approaches.

Identification of Occludin, a Novel Integral Membrane Protein Localizing at Tight Junctions

We developed an isolation procedure for adherens junctions (AJ) from the rat liver (29), and using this isolated AJ fraction, we identified some novel plaque proteins such as tenuin, radixin, α-catenin, and 220 kD protein (6, 14, 18, 30, 31, 32). Recent cDNA cloning revealed that this 220 kD protein is identical to ZO-1, which was originally thought to be exclusively localized just beneath the plasma membrane of TJ (2, 15, 26). This indicated that the 220 kD/ZO-1 protein is involved in both AJ and TJ, suggesting an intimate relationship between them. Furthermore, in most endothelial and in some epithelial cells such as those of liver, TJ is spatial-
ly intermingled with AJ. We thus speculated that the putative integral membrane protein associated with TJ is present in our isolated AJ fractions from liver cells. In other words, our isolated AJ fraction should offer a good system with which to search for the TJ membrane proteins. We have so far raised many monoclonal antibodies in mice using the membrane fraction prepared from isolated rat AJ as an antigen, but we failed to identify the integral membrane protein localizing at TJ (14).

To obtain powerful antigens, we then isolated the so-called AJ fraction from the liver of the chick, which is evolutionally distant from mouse and rat. To escape confusion, we refer to this fraction not as ‘AJ fraction’ but as ‘junctional fraction’. Using the membrane preparation from the junctional fraction as an antigen, we raised a monoclonal antibody in rats. We found three monoclonal antibodies which recognized distinct epitopes of the same integral membrane protein with an apparent molecular mass of 65 kD by immunoblotting. Immunostaining revealed that this membrane protein was exclusively localized at TJ both at the light and electron microscopic level (Fig. 1). Furthermore, using these monoclonal antibodies, we cloned the cDNA encoding this antigen. Sequence analysis revealed no homology between this membrane protein and other proteins so far identified. An interesting feature of its predicted sequence was that like connexin, this membrane protein contains four major hydrophobic, potentially membrane-embedded domains (Fig. 2). Therefore, we concluded that the integral membrane protein localizing at TJ is now identified, and named it ‘occludin’ from the Latin word ‘occlude’ (8).

**Molecular Architecture of Tight Junctions**

The following structural characteristics of occludin molecules were clarified by cDNA cloning and sequencing (Fig. 2). (a) In the NH₂-terminal half, occludin contains four transmembrane domains that segment the molecule into five domains (domains A-E). (b) A COOH-terminal half (domain E) consisting of approximately 250 amino acid residues resides in cytoplasm. (c) Charged amino acids mostly locate at domain E. (d)
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Fig. 2. Membrane folding model for occludin. Occludin molecule is divided into five domains (domains A–E).

The content of tyrosine and glycine residues is very high in the extracellular domains (domains B and D).

Since occludin has been identified and its cDNA has been obtained, the following issues on the structure of TJ require resolution: how the newly synthesized occludin molecules are delivered and localized at TJ and how occludin interacts with TJ-specific peripheral proteins such as ZO-1 and ZO-2. To answer these questions, we first performed transfection experiments, and showed that chick occludin introduced into human and bovine epithelial cells was correctly delivered to and localized at TJ, and that domain E of occludin was necessary for the localization of the newly synthesized occludin at TJ.

Secondly, through in vitro binding using glutathione-S-transferase (GST)-domain E fusion protein, we revealed that occludin directly binds to ZO-1, and that domain E is necessary for the occludin-ZO-1 association. Furthermore, we narrowed down the sequences necessary for TJ localization and ZO-1 association, and found that both sequences fell within the same region (the COOH-terminal 150 a.a.) in domain E. This coincidence suggests that the association with underlying cytoskeletons through ZO-1 is required for occludin to be localized at TJ.

Taking it into consideration that ZO-1 is specifically bound to tetrameric forms of spectrin, we were led to the hypothetical model for the molecular architecture of TJ as shown in Fig. 3 (9).

Occludin is a Good Candidate for an Adhesion Molecule Working at Tight Junctions

Compared with adhesion molecules working at other intercellular junctions such as adherens junctions and desmosomes, those at TJ should be structurally and functionally unique. They must tightly obliterate the in-

Fig. 3. Possible molecular architecture of tight junctions.
tercellular space for the barrier function in epithelial and endothelial cell sheets (5). They must form a continuous strand within the membrane to work as a fence against membranous lipids and proteins (23). To evaluate whether occludin is a good candidate for an adhesion molecule working at TJ, chicken occludin was overexpressed in insect cells by recombinant baculovirus infection. Most of the overexpressed occludin molecules did not appear on the cell surface, and instead, they were concentrated in peculiar multilamellar structures in the cytoplasm to form TJ-like structures, that is, to fuse the opposing membranes (Fig. 4). Immuno-replicas showed that short TJ-like intramembranous particle strands occur in the membranes of multilamellar structures, and that these strands contain chicken occludin molecules. These data support the notion that occludin is an adhesion molecule at TJ and that it plays a key role in the formation of TJ (7).

Perspective

TJ is an very important cell adhesion apparatus not only from cell biological perspective but also from medical perspective. For example, the precise characterization of the regulation mechanism of the permeability of endothelial and epithelial cells is an area of current active investigation. Unanswered questions include how TJ is involved in the blood-brain barrier system, how the permeability of endothelial cells is elevated during an inflammatory reaction, and how the permeability of intestinal epithelial cells is controlled during absorption. So far, the lack of information about the integral membrane protein at TJ has made it impossible to answer these questions in molecular terms. However, now that we have identified occludin and obtained its cDNA, we should be able to start dissecting the structure and functions of TJ at the molecular level. Unfortunately, none of our anti-chicken occludin mAbs recognizes human or mouse TJ, and chicken is not advantageous for us to modulate TJ functions at the cellular level as well as at the organ level. Human and mouse homologues of occludin should be identified. This is now being studied in our laboratory using chick occludin cDNA.

REFERENCES


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