In vertebrates, tight junctions (TJs) play a central role in sealing the intercellular space in epithelial and endothelial cellular sheets. In situ membrane proteins must be selectively transported across these cellular sheets. Two distinct pathways are known for this transport: the transcellular and paracellular pathways in which materials move across plasma membranes and TJs, respectively. The molecular machinery involved in transport through the TJs has been suggested to be responsible for the intercellular sealing in epithelial and endothelial cellular sheets.

**Claudins Constitute Paired TJ Strands**

TJ strands have been suggested by some investigators to be predominantly lipid in nature (i.e., inverted cylindrical lipid micelles; Kachar and Reese, 1982; Pinto da Silva and Kachar, 1982; Verkleij, 1984), but many cell biologists have long searched for proteinaceous components constituting TJ strands. Thus, when occludin, a novel ~65-kD integral membrane protein with four transmembrane domains, was identified as the first component of TJ strands (Furuse et al., 1993; Ando-Akatsu et al., 1996), this identification was regarded as the Holy Grail in this field (Gumbiner, 1993), and successive studies emphasized the importance of occludin in the structure and functions of TJs (Furuse et al., 1993; Ando-Akatsu et al., 1996). However, it has gradually become clear that the molecular architecture of TJ strands is more complex than expected (Balda et al., 1996; Hirase et al., 1997; Moroi et al., 1998). Especially, the fact that the occludin-deficient visceral endoderm cells still bear well developed network of TJ strands indicated that, as yet, unidentified membrane proteins or lipid molecules constitute TJ strands (Saitou et al., 1998).

In 1998, two novel integral membrane proteins (~23 kD) with four transmembrane domains were identified from the junction-enriched fraction as components that copartitioned with occludin (Fig. 2; Furuse et al., 1998a). These proteins, named claudin-1 and -2, were structurally related (~30% identical at the amino acid sequence level), but showed no sequence similarity to occludin. The characteristics of these molecules were that they reconstituted TJs in fibroblasts when singly transfected (Furuse et al., 1998b). The reconstituted TJs were morphologically indistinguishable from those in situ, although they were not zonula but puncta occludens; ultrathin and freeze-fracture replica electron microscopy identified the formation of kissing points and paired strands, respectively, between adjacent transfectedants (Furuse et al., 1998b; Kubota et al., 1999).

**Aqueous Pores**

Aqueous pores in the TJ wall were first observed by freeze-fracture electron microscopy of brain endothelial cells (Staehelin, 1973). Each TJ strand laterally and tightly associates with that in the apposing membrane of adjacent cells to form a paired strand, where the intercellular space is obliterated (so-called kissing junctions but puncta occludens; ultrathin and freeze-fracture replica electron microscopy identified the formation of kissing points and paired strands, respectively, between adjacent transfectedants (Furuse et al., 1998b; Kubota et al., 1999).

**Mini-Review**

**Pores in the Wall: Claudins Constitute Tight Junction Strands Containing Aqueous Pores**

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localized at TJs (Martin-Padura et al., 1998). Although the nature of this molecule has remained elusive, preliminary freeze-fracture replica analyses indicated that this molecule itself has no ability to reconstitute TJ strands in fibroblasts (Itoh, M., unpublished data).

The Claudin Family, A Newly Emerging Gene Family

Identification of claudin-1 and -2 indicated the existence of a novel gene family. To date, 18 members of this claudin family have been identified, and the list of claudins is still increasing (Morita et al., 1999a; Simon et al., 1999; Tsukita and Furuse, 1999). Some of these molecules were previously identified as RVP1 (claudin-3; Briehl and Miesfeld, 1991), CPE-R (claudin-4; Katahira et al., 1997), TMVCF (claudin-5; Sirotkin et al., 1997), and OSP (claudin-11; Bronstein et al., 1996), although their physiological functions have remained unclear. All of these claudin family members have not yet been examined in detail, but the data obtained to date by immunolabeling and/or transfection experiments favored the notion that all members of the claudin family are directly involved in the formation of TJ strands in situ.

Northern blotting showed that tissue distribution patterns of each claudin member are distinct (Furuse et al., 1998a; Morita et al., 1999a). For example, claudin-1 and -2 are expressed at high levels in the liver and kidney, whereas claudin-3 mRNA is detected mainly in the lung and liver. Claudin-4, -7, and -8 are primarily expressed in the lung and kidney. These findings together with those of immunofluorescence microscopy suggested that, in various tissues, >2 species of claudins are coexpressed in single cells. On the other hand, it also became clear that some types of cells express their own specific claudin species. One example is claudin-5/TMVCF, which consists of TJ strands specifically in endothelial cells of blood vessels (Morita et al., 1999c). To date, claudin-5/TMVCF has not been found in nonendothelial cells. Another example is claudin-11/OSP, which is primarily expressed in the brain and testis. This claudin species was shown to constitute TJ strands between lamellae of myelin sheaths of oligodendrocytes in the brain and those between adjacent Sertoli cells in the testis (Morita et al., 1999b). Recently, claudin-11/OSP-deficient mice were successfully generated (Gow et al., 1999). In these mice, TJ strands were absent in myelin sheaths of oligodendrocytes and Sertoli cells, conclusively indicating that, in these types of cells, TJ strands are mainly composed of a single specific claudin, claudin-11/OSP. These findings naturally raised questions regarding the physiological relevance of the existence of so many claudin species. This point will be discussed later.

Claudins Are Functionally Involved in the TJ Barrier

Now that the paired TJ strands can be reconstituted from a single gene product, claudin, the relationship between the TJ strand formation and the TJ barrier function could be experimentally evaluated. However, as noted above, since the reconstituted TJ strands did not surround individual fibroblast transfectants continuously, the barrier function of these TJ strands could not be measured using these cellular sheets. Gene knockout is a promising approach to analyze the functions of TJ strands, as recently shown in claudin-11/OSP-deficient mice (Gow et al., 1999), but it is technically difficult to directly examine the barrier functions of TJ strands in mice in detail.

Recently, to solve these technical difficulties, the bacterial peptide toxin, Clostridium perfringens enterotoxin (CPE), was used (Sonoda et al., 1999). As described above, claudin-4 was initially identified as a CPE receptor (CPE-R), and it was reported that the COOH-terminal half of this peptide (C-CPE) specifically bound to claudin-4/CPE-R (Katahira et al., 1997). When this C-CPE was applied to cultured epithelial cells (i.e., MDCK cells that express mainly claudin-1 and -4), claudin-4 was specifically
removed from TJs with a concomitant decrease in transepithelial electrical resistance as well as in the number of TJ strands. These findings provided concrete evidence of the direct involvement of claudins in the barrier functions of TJs. We have now concluded that claudins constitute the wall between adjacent epithelial/endothelial cells, which is responsible for sealing in the paracellular pathway. However, as noted above in situ, this wall contains pores, leading to the question of the chemical natures of these pores.

**Aqueous Pores within TJ Strands**

A nalysis of hereditary hypomagnesemia yielded insight that may allow us to answer this question. The renal resorption of $\text{Mg}^{2+}$ is known to occur predominantly through the paracellular pathway in the thick ascending limb of the Henle but, in these patients, this paracellular flux is blocked, resulting in severe hypomagnesemia. Positional cloning has identified a member of the claudin family (claudin-16/paracellin-1) as a gene responsible for this disease (Simon et al., 1999), and this claudin species was shown to be exclusively expressed in the thick ascending limb of the Henle. This finding led to a very intriguing conclusion: claudin-11/paracellin-1 is directly involved in a selective paracellular conductance for $\text{Mg}^{2+}$ ions of claudin-based TJ strands. It is possible that only claudin-16/paracellin-1, but not other claudins, can form pores through its homotypic interaction on the claudin-based wall. However, as the permeability properties of individual paired TJ strands appear to be fairly variable in different epithelia (Diamond, 1977; Gumbiner, 1993), it is more likely that most claudin species can constitute not only the wall, but also the pores in the wall. Recent detailed analyses of the manner of interaction of heterogeneous claudin species within and between TJ strands provided important information to clarify this point (Furuse et al., 1999). (1) When two of claudin-1, -2, and -3 were coexpressed in L fibroblasts, claudins were copolymerized into individual TJ strands (heteropolymers) in any combination (Fig. 3a). (2) When two of the L transfectants singly expressing claudin-1, -2, or -3 were cocultured, claudin-3-based strands (heteropolymers) laterally associated with claudin-1– or claudin-2–based strands (homopolymers) in a heterotypic manner, but such heterotypic paired strands were not formed between claudin-1– and claudin-2–based strands (Fig. 3b). (3) When L transfectants singly expressing claudin-1 were cocultured with those coexpressing claudin-1 and -2, claudin-1 homopolymers laterally associated with claudin-1/2 heteropolymers to form paired strands (Furuse, M., and S. Tsukita unpublished data; Fig. 3c). These observations suggested a possible explanation as to how claudins constitute the wall and the pore simultaneously. In Fig. 4, two types of paired strands are supposed, claudin-1/3-based and claudin-1/2-based paired strands, in which individual strands, i.e., heteropolymers consisting of claudin-1 and -3 or claudin-1 and -2, respectively, are laterally associated. In the former type of paired strands, claudin-1 and -3 could adhere both in homotypic and heterotypic manners (Fig. 3b). However, in the latter type, claudin-1 could not adhere with claudin-2 in a heterotypic manner, resulting in the formation of pores within paired strands. This is purely speculative at this stage, but as in situ TJ strands are composed of various combinations of at least 18 claudin species, it is tempting to hypothesize that the tightness of paired TJ strands is determined by the number/type of species of claudins and their mixing ratio in strands.

**Perspective**

Individual TJ strands (paired TJ strands) differ in permeability depending on cell type. For example, as a model system, the tightness of MDCK cells has been compared between type I and II clones; MDCK I cells have a fairly tighter TJ barrier than MDCK II cells, but no significant difference was detected in the number of TJ strands between these two clones (Stevenson et al., 1988). In this mini-review, we proposed a model that may explain the variation of the tightness of individual paired TJ strands based on the combination of claudin species. This model may be oversimplified. In most of the TJ strands in situ, occludin is incorporated in the claudin-based strands (Fujimoto, 1995; Furuse et al., 1998b). It is not clear how claudins and occludin are arranged to form individual TJ strands. Furthermore, the information is not available re-
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