Paracellular barrier and channel functions of TJ claudins in organizing biological systems: Advances in the field of barriology revealed in knockout mice

Atsushi Tamura and *Sachiko Tsukita

Laboratory of Biological Science, Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University, Osaka, Japan.

**Corresponding author*

Abstract

Claudin was first identified as a four-transmembrane protein in the tight junctions (TJs) between epithelial cells. The claudin family has 27 members, which are specifically expressed depending on the epithelial cell type. Accumulating evidence has revealed that claudins are responsible for the paracellular barrier that prevents molecules from passing through epithelial cell sheets. In addition, the extracellular domains of some claudins enable them to act as a permselective paracellular channel for specific molecules, including ions and non-ionic solutes. Recent studies using claudin knockout mice revealed that the loss of claudins' specific paracellular barrier and/or channel functions affects particular biological functions and leads to pathological states. In this review, considering recent findings *in vivo*, we describe how, sometimes in concert with canonical transporters and channels, the paracellular barrier and channel functions of claudins sophisticatedly organize biological systems.

1.Introduction:

The role of epithelial barriers caused by tight junctions (TJs) in regulating homeostasis in the compartments of the body: The field of barriology

A multicellular organism enclosed by an epithelial cell sheet is separated into inner and outer parts[1]. The internal part of the body is further divided into many other compartments by sheets of epithelial cells (including endothelial and mesothelial cells), such as body cavities, intestinal lumens, blood vessels, nasal cavities, seminiferous tubules, thyroid gland follicles, and oviducts. Specific small and large compartments exist throughout the body; an organ is essentially a combination of epithelial cell sheet-lined compartments. The mechanism that prevents solutes from leaking between cells in the partition that separates the compartments is called the “paracellular barrier” [2-4]. On the other hand, the epithelial cell body itself serves as a barrier, the “transcellular barrier,” due to its plasma membrane. The homeostasis in each epithelial cell sheet-lined compartment is not static, but is dynamically maintained by the paracellular and transcellular barrier and the selective exchange of molecules across the epithelial cell sheet barrier.

2. Identification of claudins as the basic epithelial TJ proteins

Although the concept of tight junction(TJ)-based paracellular barrier has been developed after 1885 primarily from the morphological and physiological aspects, the contribution of cell-cell adhesive membrane proteins to TJ formation was not established, until the TJ claudins were identified to play this role, in 1998, when it leads to the field of Barriology [5-11].

Although the identity of the unique TJ transmembrane molecules was an intriguing mystery for many years, mainly because the TJ is a very insoluble structure that is not easily accessed by conventional cell biological methods. Thus, the identification of TJ proteins required a novel procedure for

isolating cell-cell junctions[12], as previously described [13]. Since the first TJ essential transmembrane proteins, claudin-1 and -2, were identified as four-transmembrane domain proteins in 1998, a total of 27 claudin family members have been reported in mouse and human [6,7,14]. In addition to the four transmembrane domains, claudins have a long, first extracellular loop that includes a claudin-motif W-GLW-C-C, and a shorter second extracellular loop. The amino acid identity between claudin family members is not high, generally about 30% [15,16]. Thus, claudins are multi-gene family proteins with different properties, that are expressed in different parts of the body, where they perform various functions [9,10,17,18].

Most claudin family members are mainly expressed in epithelial cells, although a few of them are expressed in non-epithelial cells; for example, claudin-11 and -19 are expressed in oligodendrocytes and Schwann cells. Claudin-6 is expressed in pluripotent stem cells, and claudin-8 in undifferentiated spermatogonia [19-22], to have other functions than those as adhesion molecules, for example, as a TJ platform for cell signaling. Such functions have been observed in some cancer cells that originated from epithelial cells [23,24]. The expressions of claudins are regulated by many factors, including epithelial-mesenchymal transition (EMT)-related transcription factors, hormone- and various cytokines, and others [17,25,26]. In general, most claudins are epithelial TJ proteins that are down-regulated by EMT factors.

3. Organ-dependent expressions of claudin family members

Organs include many types of epithelial cells, in which claudins exhibit distinct expression patterns. In most epithelia, more than three species of claudins are expressed, which vary in type and relative amount. At one extreme, only a few (1-3) claudin species are very dominantly expressed in a tissue and at the other, nearly all the claudins are expressed and produce an averaged effect. There are also various intermediate cases that lie between these two extreme ones [16,18].

For example, claudin-11 and claudin-18 (stomach type) is very dominantly expressed in the sertoli cells in testis and the stomach, respectively [19,27]. In the liver, claudins-1, 2, and 3 are dominant, whereas many claudin species are expressed in the kidney [28]. There are also location-dependent differences in the distribution of claudins within an organ. Thus, the expressed claudins are different between organs, between tissues, and between epithelial cells. It is likely that the multiplicity of claudin expression patterns is important for their variety of functions in organs.

4. Characterization of claudins as paracellular barrier proteins, and as paracellular channel proteins in some cases

As described above, claudins were first characterized as TJ proteins because of their TJ localization [6,7]. Although other membrane proteins, like occludin, tricellulin, JAM, and CAR have also been identified as TJ proteins, claudins are the most essential, because they form the TJ strands that create the paracellular barrier [8,29-31]. Claudin is the only protein which can form steady strands in overexpressed fibroblasts which originally don't express claudin molecules.

When exogenously expressed, claudins were integrated into pre-existing TJs, the claudins appeared to contribute mostly to the TJs' paracellular barrier function, because morphologically, the gaps between cells were very narrow at the TJs, almost zero, which would inhibit the free permeation of molecules at least of large size [2]. When the ion transport was physiologically estimated across epithelial cell sheets in which claudins were exogenously expressed, many claudin subtypes decreased the ion permeation, as expected for paracellular barrier proteins. On the other hand, the ion permeation was increased by some claudins, indicating that they acted as paracellular ion channels. This role is exemplified by claudins -2, -10b, and -15 for cations and claudins -4, -7, and -10a for anions[18].

Based on the few epithelial physiological studies examining non-ionic

solute permeation, it is speculated that certain claudin species allow non-ionic solute permeation in a size-dependent manner at least for the small molecules. The regulation of water transport by claudins was recently studied [32,33]. However, it is premature to discuss this point, because another possible mechanism, in which solute permeation is determined by the rearrangement or dynamism of TJ strands, has also been proposed. The mechanism and pattern analyses of the proposed mechanisms, as well as that of channel function of claudins, remain to be challenged.

Regarding the TJ channel pore size, accumulated studies have indicated that TJ channels have a radius of about 4 - 6 Å. For example, a radius of 5.2 Å was reported for the TJ channels in the human colon cancer cell line CaCO2 [34]. A radius of 4.3 Å and 4.5 Å was reported in T8 and CaCO2 cells [35,36], respectively, and another study using PEG analysis suggested that the radius is ~4 Å [37]. In addition, channels with a larger radius, of 8 Å and 6.5 Å, were reported in certain parts of the body [37]. Although the channel size has not been systematically studied with respect to specific claudin species, it was recently shown that claudin-2 forms a pore with a 3.25-Å radius [38].

In summary, claudins are the TJ proteins that form the paracellular barrier in epithelial cell sheets. Some claudins also appear to form TJ paracellular channels in cell sheets to allow the selective permeation of ions, including cations and anions, and of solutes in a size-dependent manner. The mechanisms underlying these restricted permeabilities will be revealed by expanded structure-function studies of specific claudins.

5. Structure of TJ claudin

One of the most powerful approaches to studying the paracellular barrier and channel is the high-resolution crystallography of transmembrane proteins [39]. Recently, the crystal structure of claudin-15 was clarified to 2.4-Å resolution, which revealed a characteristic β -sheet fold comprised of two extracellular segments and five β -strands connected to a four-helix

transmembrane motif [40]. The five β -sheets extend on the outer surface of the membrane, forming a palm-like configuration. The structural analysis further showed that W-GLW and CC of the W-GLW-CC in the claudin motif contributes to the stability of the extracellular loop. The hydrophobic portion around M68 in the extracellular helix (ECH) domain of the first extracellular loop fits into a hydrophobic pocket around F146, F147, and L158 of a neighboring claudin molecule, thus forming the cis-interaction of claudins in the same cell.

In addition, in claudin-15, the 5- β -sheet extracellular domain creates a negatively charged palm-shaped platform, which presumably provides the basis for the paracellular channel selectivity for cations. Thus, the characteristic high-resolution structure of this claudin revealed the basis for the molecular interactions that create the TJ paracellular barrier and channel.

6. TJ claudins as organizers of paracellular and intracellular events

The TJs also play important roles in intracellular events, such as those related to cytoskeletal networks, signaling pathways, and vesicle trafficking. In these processes, claudins are directly associated with scaffold proteins, such as ZO1/2 [8,41]. Hence, TJs play crucial roles in paracellular and intracellular functions, and in their cooperative functions. However, we are focusing on the paracellular role of TJs in organizing biological systems in this review.

7. Paracellular roles of claudins in biological systems as revealed by knockout (KO) mouse studies

The paracellular barrier function of TJ claudins is probably the simplest to interpret experimentally, since their dysfunction leads to the diffusion of some molecules that are normally blocked, although the specificity of the

barrier function for various kinds of molecules differs between claudins. The roles of specific claudins in organizing biological systems that respond to environmental cues, have begun to be revealed by *in vivo* analyses of KO mice. Compared to the paracellular barrier functions of claudins, their paracellular channel functions are much more sophisticated, since in many cases cooperation with other types of claudins and certain channels/transporters is required to elicit the biological function. We note that, except in the case of a single-claudin TJ barrier that disappears upon the claudin's deficiency, the loss of a claudin usually does not disrupt the TJs, because other expressed claudins can form them. However, a property of the epithelium is usually altered to affect a specific biological function, thus suggesting a biological role for the targeted claudin.

8. Specific paracellular barrier functions of claudins in biological systems suggested by knockout (KO) mouse studies

Since claudins are the paracellular barrier proteins, the mechanism for barrier creation should be similar for all claudins; that is, they all closely attach to the two membranes of neighboring epithelial cells at their apical region. On the other hand, accumulating evidence indicates that different specific paracellular barrier functions occur under certain conditions, depending on the types of claudins involved. A paracellular barrier role has been suggested for claudins -1, -3, -4, -5, -11, -18, and -19. KO mouse studies have been performed for claudins -1, -4, -5, -11, and -18.

8.1 Claudin-1 in the skin creates a water barrier

Claudin-1, classified as a paracellular barrier-forming claudin, is expressed in various organs like liver, skin. In the skin, claudin-1 is predominantly expressed, along with other types of claudins, such as claudin-3 and -4. In claudin-1-deficient mice in which the claudin was removed from TJs, water loss from the body was observed by measuring the transepidermal water loss

(TEWL) after birth. These mice die of dehydration within a day after birth [42]. On the other hand, claudin-1 KO mice also show changes in the composition and barrier function of the stratum corneum (SC), suggesting a role for claudin-1 in skin-cell differentiation in addition to blocking water [43]. These findings pointed to the role of claudin-1 of skin epidermal sheets in organizing a waterproof cell sheet system on the entire surface of the body.

8.2. Claudin-4 prevents hydronephrosis in the kidney and may have roles in differentiation in the thymus, intestine, and urinary bladder

Although claudin-4 is generally considered to be a paracellular barrier type of claudin based on studies in cultured cells, there are some cases in which it forms a paracellular cation channel in combination with claudin-8 [44].

The kidney and urinary tract of KO mice of claudin-4 show hyperplasia and thickening of the urothelial layers, leading to lethal hydronephrosis due to urinary tract obstruction [45]. On the other hand, claudin-4 is a specific marker for Aire-expressing thymal epithelial cells and enteroendocrine cells in the intestine [46,47]. It is possible that claudin-4's role in differentiation is indirectly related to its paracellular barrier function.

8.3. Claudin-5 in the brain forms the blood-brain barrier

Claudin-5, which shows paracellular barrier activity when transferred into cultured cells, is expressed in endothelial cells, particularly in the brain [48,49]. Claudin-5 KO mice exhibit a lethal phenotype within 10 hours after birth, in which relatively small molecules (<800 Da) but not larger ones, selectively leak into the brain through the dysregulated TJs of endothelial cells ([50] Nitta et al. 2003). This result clearly showed that claudin-5 plays a critical role in the blood-brain barrier (BBB). Such changes in the BBB provide a strategy for drug delivery through the BBB for the diseases like brain tumor or encephalitis. Furthermore, another report showed that the

targeted suppression of claudin-5 decreased post-traumatic brain edema, an important finding that could lead to new therapies for the injured brain. Manipulation of the BBB is an important issue in drug delivery, for which claudin-5 is a promising target, due to its dominant role as a constitutive BBB paracellular barrier protein with size selectivity.

8. 4. Claudin-11 is important for saltatory conduction in the central nervous system and for spermatogenesis in the testis

Claudin-11, which was originally identified as oligodendrocyte-specific protein (OSP), is expressed in the TJs of oligodendrocytes in the central nervous system (CNS), and of sertoli cells in the testis, and of basal cells in the inner ear. The deletion of claudin-11 in mice causes slowed nerve conduction with hindlimb weakness, due to the loss of TJs in oligodendrocytes, which affects their physical shielding function [19]. In addition, these KO mice show defects in spermatogenesis due to the TJ barrier loss in sertoli cells [19]. Thus, although clear mechanisms remain unknown, claudin-11 KO mice show both slowed nerve conductance and male sterility due to TJ paracellular barrier loss.

8. 5. Claudin-11 and claudin-14 in the inner ear prevent deafness

In the inner ear, the endolymph potential (EP) is affected by a deficiency of claudin-11 in basal cells that results in TJ loss [51,52]. Consequently, a defect in the EP was caused without K⁺ concentration changes in the cochlear duct, resulting in deafness. In KO mice lacking claudin-14, which is expressed in the outer hair cells (OHCs), a high K⁺ concentration around the basolateral side of the epithelial OHCs causes prolonged OHC depolarization that leads to cell death. Both Claudin-11 KO and claudin-14 KO cause deafness, but the deafness results from deficiencies in different parts of the inner ear. Thus, for hearing, the TJs of several kinds of cochlear duct-surrounding epithelial cells are required to maintain the EP in the inner ear.

8.6. Claudin-18 controls inflammation in the stomach and lung

Claudin-18 has two major alternative splicing forms, which are dominantly expressed in the stomach (claudin-18 stomach type: claudin-18-2), and in the lung (claudin-18 lung type: claudin-18-1)[53].

The stomach of claudin-18 KO mice shows the paracellular leakage of H⁺ ions, which causes prolonged acute inflammation as gastritis [27] whereas the molecular mechanism in detail was not clarified. In the lungs of the claudin-18 KO mice, solute permeability and alveolar fluid clearance are increased [54]. Thus, the claudin-18-based paracellular barrier plays a role in controlling inflammation in both the stomach and lungs.

8.7. Claudin-19 in peripheral nerves is involved in saltatory nerve conduction

Claudin-19 is expressed in the kidney and lung. It is also expressed in the TJs of peripheral nerves, and in TJ-like structures in Schwann cells that are detected by electron microscopy.

The general morphology of claudin-19-deficient Schwann cells appears normal, at both the internodal regions and the nodes of Ranvier, except that the TJs are completely missing from the outer and inner mesaxons. This tissue composition is thought to be important for the saltatory conduction in neural transmission, by blocking the leakage of ions between the neural axon and Schwann cells. In KO mice of claudin-19, this saltatory conductance is delayed, suggesting that claudin-19 acts in the non-epithelial Schwann cells, to electrically seal the compartment between the neural axon and Schwann cells, which is critical for saltatory conduction in the peripheral nervous system[20].

9. Specific paracellular channel functions of claudins in biological systems suggested by knockout (KO) mouse studies

A paracellular channel role has been suggested for claudins -2, -7, -10, -15, -16, and in limited cases for claudin-4. KO mouse studies supporting this role have been performed for claudins -2, -10, -15, and -16.

9.1. Claudin-2 in the kidney acts as a paracellular Na⁺ channel for Na⁺-reabsorption in proximal convolute tubules

Claudin-2 was the first channel-forming claudin to be described [29]. Claudin-2 increases the conductance of TJs when it is exogenously expressed in cultured MDCK epithelial cells; specifically, it increases the paracellular permeability for inorganic and organic cations[55]. It was recently suggested that claudin-2 also increases the permeability for water [32]. Mice in which claudin-2 is systemically lost by gene knockout are fertile, grow normally, and have a normal appearance [33,56]. However, their kidneys show a Na⁺-reabsorption defect due to loss of the claudin-2-based Na⁺ channel function in renal proximal tubules.

9.2. Claudin-2 in the liver plays a role in the hepatobiliary system

Claudin-2 is specifically expressed in the perivenous regions of the hepatic lobule in the liver. In contrast, claudin-1 and -3 are expressed in both the perivenous and periportal zones.

In claudin-2 KO mice, the concentrations of bile acids, phospholipids, and cholesterol are significantly increased in the bile, although the ion concentrations are not greatly changed [28]. The bile flow rate in the claudin-2 KO mice is about half that of wild-type mice, suggesting that the paracellular ion and water permeation are decreased as a result of claudin-2 paracellular channel loss. In this respect, the ion and water permeabilities were shown to decrease in these mice, in experiments using isolated intrahepatic bile ducts and primary cultured hepatocytes. Under a lithogenic diet, the claudin-2-deficient mice develop gallstones.

These observations revealed the existence of a claudin-2-based hepatobiliary system, which is particularly important for the composition and flow of bile in the bile canaliculi. Although these changes of bile leads to gallstones, it is more remarkable that the paracellular channel role of claudin-2 provides the motive force for the flow from the blind terminal of the bile canicular counter-current system, through the paracellular flow of cations and water from the sinusoidal vessels in the hepatobiliary system.

9.3. Claudin-2 and claudin-15 in the intestine play a role in coupling nutrient absorption to nutrient transporters

Claudin expression is spatiotemporally regulated in the intestine [57-59]. Claudin-3 and -7 are expressed in epithelial cells in both crypts and villi throughout the intestine, and claudin-4 is expressed in villi tip epithelial cells and enteroendocrine cells. Notably, the pattern of claudin-2 expression in the intestine changes with age. In infant mice before weaning, claudin-2 is expressed in the crypts and villi, while in adult mice it is expressed only in the crypts. The expression of claudin-15 shows the complementary pattern: in infants only in crypts, and in adults in crypts and villi. Since claudin-2 and -15 are the TJ claudin channels in the intestine, their deficiencies were systematically examined in single and double knockout (DKO) mice [56,59,60]. The Na⁺-permeability across the intestinal epithelia was decreased compared to that of wild-type mice, slightly and greatly in the claudin-2 and claudin-15 single KO mice, respectively in adult stage [56]. The effect was severe in the DKO case [60]. Hence, the Na⁺ concentration in the intestinal lumen decreased according to the reduction in Na⁺ permeability, resulting in lethality in the DKO mice. This is because in the DKO mice, the nutrition absorption was almost completely blocked due to the inactivation of nutrient transporters, which depend on Na⁺ concentration gradient from intestinal lumen to cytoplasm of intestinal epithelial cells. The single-KO mice of claudin-15 and -2 showed moderate and mild tendencies toward these effects, respectively.

The results of the DKO mouse study revealed that there exists a sophisticated biological system for controlling the paracellular flow of Na^+ from the serosal to the mucosal sides of the intestinal wall, through paracellular TJ channels of claudin-2 and -15 across epithelial cell sheets. This system is coupled with the activity of Na^+ -dependent nutrient transporters, to allow nutrient absorption from the intestinal lumen across the intestinal epithelial cells, pointing to that the cooperation of paracellular and transcellular transport systems is involved in the organization of the biological systems in the body, a novel framework of biological system.

9.4. Claudin-7 in kidney for urinary Na^+ , Cl^- , and K^+ wasting and in intestine for control of inflammation

Claudin-7 is expressed in various organs like intestine, lung and kidney. While claudin-7 overexpression induce Cl^- permeability decrease and Na^+ permeability increase in LLC-PK1 cells, claudin-7 knockdown induce Na^+ permeability in MDCK cell and decrease Cl^- permeability in LLC-PK cells [31,61]. The some inconsistency between these results might be suggested by various expression level of claudin-17, anion channel type claudin.

In claudin-7 KO mice, urinary Na^+ , K^+ and Cl^- waste, dehydration was observed due to the decrease in the paracellular Cl^- permeability. This mouse in intestine shows severe intestinal inflammation and lethal phenotype within pnd 12. In this mouse, integrin-2 expression and normally formed claudin-7/integrin-2/claudin-1 complex was disrupted, which induce up-regulation of matrix metalloproteinases (MMPs) inducing intestinal epithelial tissue damage and inflammation [62,63].

9.5. Claudin-10 loss in the kidneys causes hypermagnesemia and nephrocarcinosis

Although claudin-10 has several alternative splicing forms, its major expression patterns are classified into two splicing forms, claudin-10a and

-10b [64]. Whereas claudin-10b is expressed in various organs, claudin-10a is expressed mainly in the kidney, especially in the thick ascending limb (TAL) of Henle's loop. Although claudin-10a and -10b use the same exons except for the first coding exon, which contributes to the first transmembrane region and first half of the extracellular regions, they show different paracellular permselectivities: claudin-10b acts as a cation-selective channel, and claudin-10a as an anion-selective one.

In claudin-10 KO mice in which both of the splicing forms are deleted specifically in the TAL, hypermagnesemia and nephrocarcinosis are observed due to a decrease in the paracellular Na⁺ permeability and an increase in the paracellular Mg²⁺ and Ca²⁺ permeability [65].

9.6. Claudin-16 in the kidney promotes Mg²⁺ and Ca²⁺ reabsorption by generating a lumen-positive transepithelial potential in the TAL

Claudin-16 is also called paracellin. The analysis of human paracellin, which provided the first description of an *in vivo* function of claudins, was followed by analyses in gene-targeted mice. In patients with mutations in paracellin genes, Mg²⁺ reabsorption in the TAL is disturbed, leading to hypomagnesemia.

In claudin-16 knockdown mice [66], the Mg²⁺ and Ca²⁺ reabsorption is decreased due to the reduced lumen-positive transepithelial potential and decreased paracellular NaCl dilution potential. Although claudin-16 was suggested to be a Mg²⁺ and Ca²⁺ channel in early studies, its Na⁺ channel activity was clearly revealed in this mouse study. An interaction between claudin-16 and claudin-19 in the TAL is probably critical for the high TJ cation selectivity, which generates enough lumen-positive transepithelial potential for Mg²⁺ and Ca²⁺ reabsorption.

In contrast, in claudin-16 KO mice, the paracellular Na⁺ permeability was not changed in the KO mice, suggesting that claudin-16 alone performs the divalent cation channel role in the TAL [67]. In any case, there appear to be at least two molecular systems for claudin-16's role.

10. Conclusion and Perspectives

The TJ paracellular barrier proteins are claudins, of which there are 27 family members. Some claudin family members form paracellular channels for select molecules, such as ions and non-ionic solutes. The paracellular barrier and channel functions of claudins are critical microenvironmental regulators in the body, and their roles should be systematically analyzed as “barriology” [7,9]. The claudins determine the functional parameters of epithelial cell sheets, which not only enclose the large and small compartments of the body but also define the compartments’ specific homeostasis. Thus, the claudins in epithelial cells help to organize biological systems, many of which have been revealed by KO mouse studies.

The functional analyses of claudins combined with their structural biological analyses will lead to new therapeutic strategies for various diseases as well as preventative approaches for maintaining and promoting good health. In this respect, the control of inflammation by claudin 7 or 18 is particularly important for preventing various cancer states. The regulation of claudin-5 should lead to methods for controlling the BBB to ease drug delivery to the brain. The regulation of nutrition absorption by claudins 2 and 15 revealed a new basic physiological system, which will be applicable to maintaining good health, including appropriate body weight. The regulation of bile flow and the associated hepatobiliary system by claudin-2 and the kidney ion reabsorption system by claudin-16 also have important implications for health. Considering that functional analyses using gene KO strategies are beginning to reveal new physiological functional schemes, further studies will reveal higher-ordered biological systems and new methods for manipulating biological systems in highly sophisticated ways to lead to a novel scheme for preventive medicine and for medical therapy.

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Figure Legends

Fig. 1.

Epithelial tight junctions (TJs), which are composed of TJ claudin.

- A. Location of TJs between epithelial cells.
- B. Schematic drawing of a TJ membrane. The plasma membranes of two neighboring cells are closely attached to each other by adhesion molecules (claudins) at the TJs (arrows).
- C. Thin section electron micrograph of TJs. The close adherence of two plasma membranes at TJs (arrows) is clearly seen. Bar, 50 nm.
- D. Freeze-fracture electron micrograph of a TJ. The freeze-fractured plane of the plasma membrane of a small intestinal epithelial cell is shown. The TJ transmembrane proteins (claudins) form TJ strands. Bar, 50 nm.
- E. Crystal structure of claudin-15. The crystal structure of claudin-15 was determined at 2.4-Å resolution. It revealed a characteristic β -sheet fold consisting of five β -sheets connected to four α -helical transmembrane motifs. Please see ref.[40]. C: Cytoplasmic space, E: Extracellular space.
- F. Schematic drawing of the molecular structures of claudin-15.

Fig. 2. Variations in the expression patterns of 27 members of the claudin family, according to organ type. The vertical axes indicate the ratio of the claudin mRNA level to that of GAPDH.

Fig. 3. Functions of claudin-based paracellular barriers, revealed by analyses of KO mice.

- A. In the skin, claudin-1 is expressed in the second of three granular cell layers. In claudin-1 KO mice, water inside the body leaks through the skin, and the mice die of dehydration after birth.
- B. In claudin-5 KO mice, the blood-brain-barrier, which consists of claudin-5-expressing capillary vessels, is disrupted to allow size-selective solute permeation. The mice die within 10 hours after birth.
- C. In stomach-type claudin-18 KO mice, the secreted protons from parietal cells infiltrate the sub-mucosal space, and induce pseudo-pyloric gastritis.

Fig. 4. Functions of claudin-based paracellular channels, revealed by analyses of KO mice.

- A. In the proximal tubules of the kidney in claudin-2 KO mice, Na^+ reabsorption is disturbed and urinary Na^+ is wasted, leading to hypernatruria.
- B. In claudin-2/15 double KO mice, the paracellular Na^+ channel function in the small intestine is severely decreased. As a result, the supply of Na^+ from the sub-mucosal space to the intestinal lumen is inhibited, and $[\text{Na}^+]$ in the intestinal lumen is decreased. The function of Na^+ -dependent nutritional transporters of glucose, amino acids, and bile acids that play roles in lipid absorption, is decreased, leading to lethal malabsorption.

Figure 1 Tamura & Tsukita

Figure 2 Tamura & Tsukita

Figure 3 Tamura & Tsukita

Figure 4 Tamura & Tsukita