Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1778 (2008) 631–645
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbamem

Review

Structure and function of claudins

Gerd Krause, Lars Winkler, Sebastian L. Mueller, Reiner F. Haseloff, Jörg Piontek, Ingolf E. Blasig *

Leibniz-Institut für Molekulare Pharmakologie, Robert-Rössle-Str. 10, 13125 Berlin, Germany

Received 21 June 2007; received in revised form 18 October 2007; accepted 19 October 2007
Available online 25 October 2007

Abstract

Claudins are tetraspan transmembrane proteins of tight junctions. They determine the barrier properties of this type of cell–cell contact existing between the plasma membranes of two neighbouring cells, such as occurring in endothelia or epithelia. Claudins can completely tighten the paracellular cleft for solutes, and they can form paracellular ion pores. It is assumed that the extracellular loops specify these claudin functions. It is hypothesised that the larger first extracellular loop is critical for determining the paracellular tightness and the selective ion permeability. The shorter second extracellular loop may cause narrowing of the paracellular cleft and have a holding function between the opposing cell membranes. Sequence analysis of claudins has led to differentiation into two groups, designated as classic claudins (1–10, 14, 15, 17, 19) and non-classic claudins (11–13, 16, 18, 20–24), according to their degree of sequence similarity. This is also reflected in the derived sequence-structure function relationships for extracellular loops 1 and 2. The concepts evolved from these findings and first tentative molecular models for homophilic interactions may explain the different functional contribution of the two extracellular loops at tight junctions.

Keywords: Tight junction; Transmembrane protein; Claudin; Extracellular loop; Cell–cell contact; Paracellular pore; Structural model; Epithelial and endothelial barriers

Contents

1. Introduction

Tight junctions (TJ) are the most apical type of cell–cell contact in the lateral membrane between polarised cells, such as those existing in endothelia and epithelia. TJ show a wide variability of tightness in different organs, ranging from almost

* Corresponding author. Tel.: +49 30 94793 244; fax: +49 30 94793 243.
E-mail address: iblasig@fmp-berlin.de (I.E. Blasig).

0005-2736/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2007.10.018

Table 1
Distribution, function and pathology of claudins with particular consideration of paracellular and extracellular aspects

Claudin	Tissue expression	Paracellular function	Pathologies	Other observations
1	– Typical for tight epithelia, e.g. epidermis [41], distal nephron [97], gallbladder [98] – Mouse prostate [99] – Human ovarian epithelium [100] – Inner ear [57] – Brain capillary endothelium [101][a] – Mouse choroid plexus epithelium TJ [102][a]	– Mammalian epidermal barrier [41]	– Hepatitis C virus co-receptor in ECL1 [95] – Overexpression: melanoma cells, activated by PKC leads to MMP-2 overexpression and cell motility [103]; colorectal ca. [104] – Mutations: ichthyosis, sclerosing cholangitis [105] – Downregulation: squamous cell ca. [106], glioblastoma [101], prostate adeno ca. [107]	– KO demonstrate tightening of epidermis for water [41] – MAPK [32], PKC [33], WNK4 [37] phosphorylate C-terminal tail
2	– Typical for leaky epithelia, e.g. intestinal crypts [108], proximal renal tubule [80,97], choroid plexus [102] – Human ovarian surface epithelium [100] – Inner ear [57]	– Increases paracellular cation permeability in MDCK-C7: $Na^+ = K^+ > choline^+ \gg Cl^-$ [9]	– Upregulation in Crohn's disease [94]	– WNK4 phosphorylates C-terminal tail [37]
3	– Mouse prostate [99] – Human gallbladder [98] – Inner ear [57], brain capillary endothelium [81] – Tighter segments of nephron [80] – Liver/intestinal epithelial cells [108]	– Constitutes TJ strands in endothelium [81]	– Overexpression in ca.: ovarian [100], prostate [107], human colorectal [109], breast [110] – Receptor for CPE [111], binds the ECL2 [96] – Loss from BBB *TJ* in glioblastoma and, encephalomyelitis [81]	– PKA [34], WNK4 [37] phosphorylate C-terminal tail
4	– Mouse prostate [99] – Human gallbladder [98]	– Reduces paracellular cation permeability in MDCK-II [53] – Claudin-4 knock down in bladder tumour cells reduces TER [45]	– Overexpression: ovarian adeno ca. [44,100], human colorectal adeno ca. [109]; prostate ca. [107], human breast ca. [110] – Receptor for CPE [111]	– WNK4 phosphorylates C-terminal tail [37]; EphA2 [38] – ECL1: basic→acid aa exchange elevates paracellular Na^+ permeability [66]
5	– Typical for endothelia, e.g. brain capillary endothelial cells [42,75] – Mouse prostate [99] – Human ovarian surface epithelium [100] – Human colon epithelium [46]	– Reduces paracellular cation permeability in MDCK-II [8] – Constitutes TJ strands in endothelial cells [75] – ECL2: narrowing paracellular cleft, holding of opposing cell/claudin [16]	– Downregulation: Crohn's disease [94], DiGeorge/Velo cardio-facial syndrome [112], hyperplastic vessels in glioblastoma [101] – Upregulation: pancreatic ca. [113]	– KO: small molecules cross BBB [42] – PKA phosphorylates C-terminal tail [35] – ECL1: Cys→Ala reduces tightness [8] – ECL2: oligomerisation [15], contributes to paracellular enrichment [16] – PKCα/ζ phosphorylation: disappearence from membrane, ζ reduces TER [36]
6	– Embryonic epithelia [56] – High expression: neonatal proximal tubule, thick ascending limb, distal convoluted tubule, collecting duct (but not adult kidney) [59]	– May play role in maturational changes in paracellular permeability [59]		– Transgenic mice: skin barrier defect (abnormal epidermal differentiation markers) [114]
7	– Mouse prostate [99] – Duodenum, jejunum, ileum, colon [58] – Human palatine tonsillar epithelium [115] – Nephron segments primarily at the basolateral membrane [116]	– Increases paracellular Na^+-, decreases Cl^--permeability in LLC [51] – Paracellular Cl^--barrier, Na^+-pore [51]	– Downregulation: breast- [117], head-, neck ca. [118] – Elevated: stomach ca. [119]	– Replacement of negatively charged aa by positive ones in ECL1 but not ECL2 increase Cl^--permeability [12] – Knock down in LLC decreases paracellular Cl^--permeability [54]
8	– Mouse prostate [99], inner ear [57] – Duodenum, jejunum, ileum, colon [58] – Distal nephron, late segments of thin descending limbs of long-looped nephrons [116]	– Reduces paracellular cation permeability in MDCK-I [49]	– Downregulation: Crohn's disease [94]	

9	– Inner ear [57] – Neonatal kidney [59]	– May play role in maturational changes of paracellular permeability [59]		
10	– Mouse prostate [99] – Inner ear [57] – Most segments of nephron [13]	– Preferentially paracellular permeation of anions (10A) or cations (10B) in MDCK-II and LLC [13]		– 2 splice variants: 10A (kidney only) without negatively charged aa in ECL1, 10B with negative aa in ECL1 [13]
11	– Schwann' cells, Sertoli cells [14,78] – Mouse coroid plexus epithelium TJ [102]	– Saltatory conduction [14] – Endocochlear potential [57] – Reduces paracellular cation permeability in MDCK-II [53]		– KO: nerve conduction reduced, male sterility, hind limb weakness (no TJ in myelin/between Sertoli cells) [14]; deafness [57]
12	– Inner ear [57], brain endothelial cells [42] – Duodenum, jejunum, ileum, colon [58]			– No PDZ-binding motif at C-terminus[b] [7]
13	– Duodenum, jejunum, ileum, colon [58] – Neonatal kidney [59]	– May play role in the maturational changes in paracellular permeability [59]		
14	– Inner ear [57] – Sensory epithelium of organ of Corti [120]	– Reduces paracellular cation permeability in MDCK-II [43]	– Nonsyndromic deafness, cochlear hair cell degeneration (mutation, e.g., human V85D in TM2) [43,120]	– Palmitoylated at conserved Cys proximal to membrane following TMD2 and 4 [40]; KO: deafness [43]
15	– Kidney endothelial cells [80] – Duodenum, jejunum, ileum, colon [58]	– Increases paracellular Na^+ permeability in LLC [52]		– ECL1: acidic→basic aa exchange reverses preference for Na^+ to Cl^- [66]
16	– Thick ascending limb of Henle's loop [70] – Distal nephron of man [70], rat [67]	– Increases paracellular cation permeability ($K^+ > Na^+ > Mg^{++}$) in LLC [10]; Mg^{++}/Ca^{++} resorption [70]	– Familial hypomagnesaemia, hypercalciuria, nephrocalcinosis [70] by mutations: ECL1 (H141N, R149L, L151F/W), ECL2 (A209T, R216T, S235P) [67], C-terminus [121]	– 73 cytosolic/N-terminal aa (other claudins have ~7 aa) [7] – ECL1: excess of acidic aa, replacement reduces Na^+ permeability increase [10]
17	– mRNA in mouse [50], human kidney [7], human/mouse taste receptor cells [122]			
18	– Mouse lung epithelial cells [60] – Inner ear [57] – Stomach, lung [61]		– Downregulation in gastric ca. [123]	– Downstream target gene for T/EBP/NKX2.1 homeodomain transcription factor; 2 splice variants in ECL1 [61] – Extension in ECL2 [7]
19	– Kidney, retina [102], – Myelinated peripheral neurons, Schwann' cells [79]	– Reduces paracellular cation permeability in MDCK-II, increases TER [48] – Selective paracellular cation barrier at TJ [68] – Involved in electrophysiological 'sealing' [79]	– Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis, visual impairments; hypomagnesaemia mutations in TMD1, 2 and ECL1 (Q57E) [123]	– KO: nerve conduction affected [79]
20	– mRNA: human skin [62], rat duodenum [64]		– mRNA: chondrosarcoma, brain/liver ca. [7]	
21	– Human DNA sequence [65]			
22	– mRNA: human trachea [65], rat duodenum [64]		– mRNA: human breast ca., astrocytoma [7]	
23	– mRNA: colon, stomach, placenta [65], human skin [62], rat duodenum [64], mouse taste receptor cells [122]		– Downregulation: intestinal-type gastric ca. [124]	– 111 cytosolic/C-terminal aa (other claudins have ~25 aa) [7]
24	– Human DNA sequence [65]			

aa, amino acid; BBB, blood-brain barrier; ca., carcinoma; CPE, Clostridium perfringens enterotoxin; ECL, extracellular loop; EphA2, receptor Tyr kinase; KO, knock out mice; LLC: LLC-PK1, renal epithelial cells of porcine proximal tubule (claudin-1, (-2), -3, -4, -7 [51]); MAPK, mitogen-activated protein kinase; MDCK, Madine–Darby canine kidney cells (I: claudin-1, -4, -7; II: -1, -2, -4, -7 [125]); PKA, cAMP depending protein kinase A; PKC, protein kinase C; TJ, tight junctions; TER, *trans* endothelial/epithelial electrical resistance; TM, transmembrane domain; WNK4, a Thr/Ser kinase.

[a] The identity of claudin-1 is questionable (anti-claudin-1 antibodies may cross-react with claudin-3 [81] and simulate claudin-1 in the BBB).

[b] Most C-termini of claudins, except claudin-12 [7], reveal binding motif for PDZ domains [23, 24].

complete tightening of the paracellular cleft for solutes, e.g. in the bladder, to forming paracellular pores for specific cations (and anions), e.g. specific segments of renal tubules. Thus, TJ provide one possibility to form tissue barriers and pores, and, hence, to enable vital functions in all epithelial organs and organs containing TJ-bearing endothelia, e.g., stomach, intestine, liver, gallbladder, prostate, testis, ovary, placenta, brain, retina, inner ear or lung (Table 1). In transmission electron microscopy, TJ appear as fusion of the plasma membranes of opposing cells. Freeze-fracture electron microscopy displays intramembranous networks of strands and complementary grooves [1], i.e. grooves are negative images of strands [2]. TJ strands consist of transmembrane proteins, such as occludin [3], tricellulin [4] and different claudins [5]. The claudins are expressed in a tissue specific combination resulting in tissue specific barrier characteristics. Mammals express ~24 claudins [6].

2. Topology and posttranslational modification of claudins

Fig. 1 summarises structural and possible interaction features and properties of the claudin protein family. Claudins share the tetraspan transmembrane topology with occludin, tricellulin and connexins that form gap junctions, without considerable sequence homology. Very great sequence homology is found between claudins 1–10, 14, 15, 17 and 19. Combined with functional findings, we therefore define this group as classic claudins, as concluded from the phylogenetic tree in Fig. 2. All other claudins are named non-classic claudins.

Intracellularly, mammalian claudins exhibit ~7 N-terminal amino acids, ~12 loop amino acids, and 25–55 C-terminal amino acids. Exceptions are claudin-12 (loop 25, human), claudin-16 (N-terminal 73, human) and claudin-23 (C-terminal 111, human) [7]. The extracellular loop (ECL) 1 consists of ~50 amino acids with two conserved cysteines involved in the barrier function [8]. Negative [9–11] and positive [12,13] charges in ECL1 contribute to pore formation. The ECL2 usually has ~25 amino acids, but fewer in claudin-11 [14] and more in claudin-18 [57]. The ECL2 has been less investigated. It may associate with itself [15] and possess a holding function, narrowing the paracellular cleft [16].

Transfection of TJ-free fibroblasts has demonstrated that, in contrast to most of the other TJ proteins, claudins reconstitute membranous strands similar to those found in epithelial cells [17]. Occludin has also some potential to form strands. However, the occludin strands are shorter and less complex than those of claudins [18]. This indicates that claudins are major constituents of TJ and that they can potentially polymerise. However, only *in vitro* data are available and only oligomers have been identified so far, i.e. dimers up to hexamers for claudin-5 [15,19] and for claudin-4 [20]. In non-denaturing gel electrophoresis, hexamers seem to be the preferred oligomer for claudin-4 and -5 [19,20] whereas claudin-1 and -3 exhibit smaller oligomers [19].

The scaffolding proteins zonula occludens (ZO)-1 and -2 are essential for the spatial organisation of claudin-based membranous strands in epithelial cells [21], but not for basic strand formation in claudin-transfected unpolar cells [22]. At the C-terminal positions -3, -2, -1, 0, most claudins reveal a PDZ-binding motif, such as –K/R/H–X–Y–V. For instance, ZO-1-PDZ1 preferentially associates with the C-terminal patterns –K/R(T/S)–T/S–Y/W–V(L/I) [23]. Accordingly, PDZ1 of ZO-1, ZO-2 and ZO-3 binds to claudins 1–8 [24], the multi

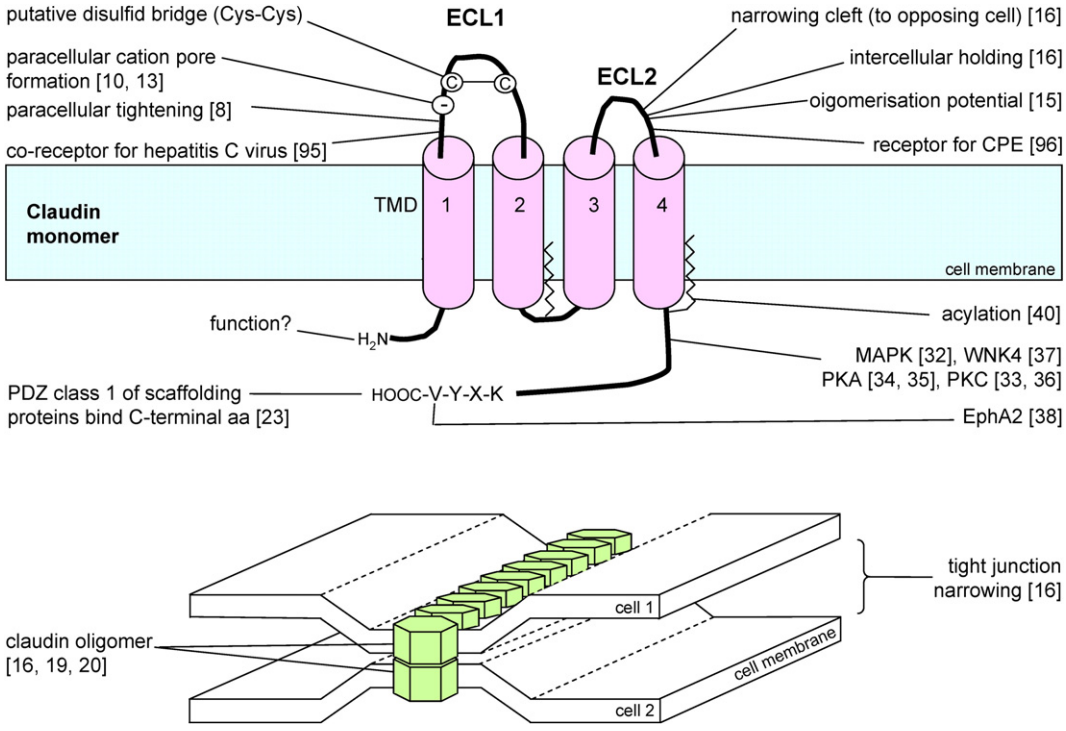


Fig. 1. Schematic view of tight junction strands (lower part) and their major components, the claudins (top). The upper part shows possible properties on different parts of the claudin molecule, as listed in Table 1. ECL, extracellular loop; θ, negatively charged residue; for further explanations see Table 1.

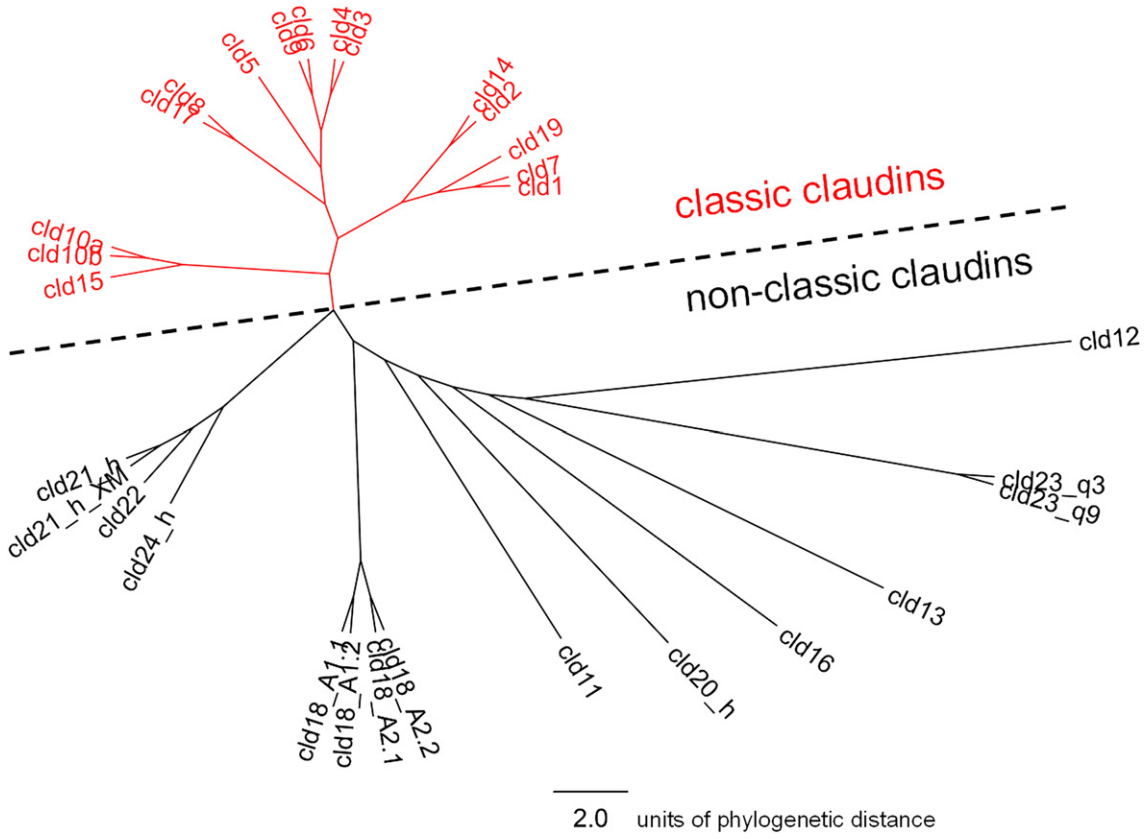


Fig. 2. Phylogenetic tree for the full-length sequences of claudins. Classic claudins 1–10, 14, 15, 17, and 19 with high common sequence similarity are clustered in red, non-classic claudins 11–13, 16, 18, and 20–24 are shown in black. For known mammalian, claudins in the mouse-sequences were taken as representative. Claudin-20, -21, and -24 are only known in human (h). For each sequence, the full Swissprot accession number is stated in Fig. 4. Claudin-21 and -24 were translated (tr) from the sequences located within human genomic contig NT_022792.13 and contig NT_033899.3, respectively [65]. The database entry XM_210581 is also called claudin-21). This is likely a different splice variant, because most of the first extracellular loop and later sequences are identical to claudin-21_tr. Sequences were aligned by the GCG program package (GCG Wisconsin package, Accelrys Inc., San Diego, CA). The phylogenetical distances (indicated by line length and branching) were calculated with the program Jalview [39] and depicted with the program figtree (http://evolve.zoo.ox.ac.uk/software.html?id=figtree).

PDZ domain protein 1 (MUPP1) binds to claudin-1 [25] and claudin-8 [26], and the Pals1-associated TJ protein PATJ binds to claudin-1 [27]. Among the classic claudins, the –Y–V motif in the C-terminal positions -1 and 0 exhibits 100% conservation, in contrast to a greater variety in non-classic claudins (H/S/Y/D/E/R–V/L). This is consistent with the fact that C-terminal peptides of claudin-2, -8, -10, -14, -17, and -20 (all containing the C-terminus -Y-V), have binding values for the PDZ1 of ZO-1 between 2 and 61 μM [23]. Although claudin-16 (non-classic) contains C-terminal –R–V, it also associates with ZO-1 [28], since it has been observed that the residues at position -3 (T) can compensate for the binding ability [23,29].

Claudins and their tightness and pore functions are highly dynamic, as indicated by the short half life of, e.g., claudin-4 with about 4 h [30]. In comparison, the turnover of many other transmembrane proteins is much longer [31]. Manifold phosphorylations (Table 1, last column) also point to short-term regulation of claudins. Phosphorylation can be catalyzed by mitogen-activated protein kinase (Thr203, rat) [32] and protein kinase C [33] in claudin-1, or by protein kinase A in claudin-3 (Thr192, human) [34] and claudin-5 (Thr207, rat) [35]. Claudin-5 phosphorylation by protein kinases Cα and ζ results in its disappearance from the cell membrane; activation of protein kinase Cα increased paraendothelial permeability [36]. Claudin-1 to -4 are phosphorylated via threonine/serine kinase WNK4 [37]. The receptor tyrosine kinase EphA2 phosphorylates the PDZ-binding C-terminus of claudin-4 (Tyr208, human), resulting in its dislocation from cell–cell contacts, due to reduced ZO-1 association [38]. Phosphorylation of claudin-3 may decrease the TJ strength in ovarian cancer cells [34] and of claudin-5 in endothelial cells [35].

S-acylation has been reported in claudin-14. In epithelial cells and fibroblasts, palmitoylation has been identified at conserved cysteines proximal to the membrane, following the transmembrane domains 2 and 4. The palmitoylation is required for efficient localisation/trafficking into TJ, but not for stability or strand assembly [40].

3. Functional properties of claudins

Table 1 gives an overview of the organ and tissue distribution, functions and pathologies of individual claudins. A general property of claudins in the TJ is the paracellular sealing function, which is tissue-, size- and charge-selective. Knock out mice have proven that claudin-1, -5, -11, and -14 tighten skin [41], blood-brain barrier [42], myelin sheaths and Sertoli cell

layers [14], and epithelium in the inner ear [43], respectively. Claudin-3 could be relevant for tightening as a mutation mimicking the phosphorylated state decreases the TJ strength in an ovarian cancer cell line [34]. Claudin-4 overexpression in ovarian cancer epithelium is positively correlated with the paracellular resistance, which is decreased by *Clostridium perfringens* enterotoxin, a ligand of this claudin [44]. In a bladder tumour cell line, inhibition of methyl transferases increases claudin-4 membrane expression and the resistance, which can be prevented by claudin-4 siRNA [45], also supporting a sealing function. Claudin-5 transfected in Caco-2 cells contributed to the barrier function [46].

After claudin-8 transfection in MDCK-II cells, the paracellular barrier was intensified to alkali metal and divalent cations, to protons, ammonium and bicarbonate [47]. Transfected claudin-19 increased transepithelial resistance and decreased cation permeability [48]. Taken together, a tightness function can be demonstrated for claudin-1, -5, -11, and -14. For claudin-3, -4, -8, and -19, a tightening potential is indicated. The distinct tightness properties of a given tissue and a given claudin, however, seem to be largely dependent on the combination of the claudins that are expressed and on the manner in which they copolymerise [6,49,88].

Paracellular cation pores are formed by claudin-2 for monovalent cations [9], by claudin-7 for Na^+ [51], by claudin-10B preferentially for cations [13], by claudin-15 for Na^+ [52], and by claudin-16 for mono- and divalent cations [10]. These claudins, except claudin-10, have been shown to increase paracellular cation permeability in TJ-containing MDCK or LLC-PK1 cells. In contrast, sealing claudins, such as claudin-4 [53], -5 [8], -8 [49], -11 [53], -14 [43], and -19 [48], selectively decrease the paracellular cation permeability through TJ. Claudins 2, 7, 10, 15, and 16 can therefore be identified as pore forming claudins. In MDCK-II, the activation energy for paracellular proton permeation was similar to that in bulk aqueous solution. This suggests a Grotthuss mechanism implying that the pores are sufficiently wide to accommodate water molecules in a freely mobile state [47].

The evidence for paracellular anion pores is less clear than that for cation pores. Claudin-10A may preferentially constitute pores for anions [13]. The knock down of claudin-7 in LLC-PK1 cells, renal epithelial cells of porcine proximal tubule expressing claudin-1, (-2), -3, -4, and -7 [51], depressed Cl^- permeation [54]. In contrast, others report that the overexpression of the same claudin decreases the Cl^- permeability [12,51]. Nevertheless, these findings are only possible if a paracellular Cl^- pore exists. If negatively charged amino acids in the ECL1 of claudin-7 are replaced by positive ones, the Cl^- permeability increases [12]. This is a further hint that certain claudins might generate or support Cl^- pores.

Apart from the paracellular transfer of ions over the TJ barrier, the flux of uncharged molecules may take place at TJ. Those molecules seem to take another route, through TJ-strand-brakes, opened during the highly dynamic rearrangement of TJ [55].

No function has been identified so far for claudins 6, 9, 12, 13, 17, 18, and 20–24. Claudin-6 [56], -9 [57], and -13 [58] are expressed in different types of cells and may play a role in the maturation of the epidermis or the paracellular permeability [59]. For claudin-12 [42,57,58] and -18 [57,60,61], the protein has been detected in epithelia and endothelia of gastrointestine, inner ear, or brain endothelial cells. For claudin-17 [7,50], -20 [7,62,63], -22 [7,63,64], and -23 [62,64,65], only the mRNA has been identified, e.g., in kidney, colon, stomach or placenta of different species, such as human, mouse or opossum. The existence of claudin-21 and -24 has been solely derived from the analysis of the human genome [62].

4. Extracellular loops of claudins and their cell–cell contact function

To understand the function of claudins in TJ one has to consider their extracellular loops. TJ are most probably constituted via interactions of the ECLs which are exposed to the opposing membrane within the cell–cell contact. This is supported by mutations in the ECL1 [8,10,66–68] and ECL2 [16,67], both affecting cell–cell contact functions. Comparison of the different claudin properties listed in Table 1 shows that the ECLs mainly determine paracellular solute tightness and paracellular ion permeability.

4.1. ECL1, sealing and pore forming function

Several studies on paracellular tightness have demonstrated that claudins with tightening properties, such as claudin-4 [53], -5 [8], -8 [49], -11 [53], -14 [43], and -19 [48], reduce paracellular cation permeability when transfected into TJ-containing cells, such as MDCK-II. On the other hand, pore forming claudin-2 [9], -15 [52], or -16 [10] may facilitate paracellular cation permeability when transfected in pore-containing epithelia. In addition, replacement of basic by acidic residues in claudin-4 ECL1 increases cation permeability. Exchange of acidic to basic residues in claudin-15 ECL1 reversed paracellular charge selectivity from a preference for cations to anions [66]. The claudin-16 ECL1 shows an excess of negatively charged amino acids. Substitution of most of them by non-charged ones removed their Na^+ facilitating permeability while all substitutions of positively charged amino acids did not [10]. This underlines the significance of negative residues in ECL1 for the cation pore formation.

Mutations in the ECL1 (H71D, L75P, R79L, L81F/W) or ECL2 (A139T, R146T, S165P) of claudin-16 [67,69] result in familial hypomagnesaemia, hypercalciuria and nephrocalcinosis [70]. A mutation in claudin-19 ECL1 (Q57E) causes hypomagnesaemia [68]. These mutations lead to a better molecular understanding of the malfunctions. For claudin-7, replacement of negatively charged amino acids against positively charged ones in the ECL1 but not in the ECL2, increased the Cl^- permeability without changing the targeting to cell junctions [12]. This supports the assumption that the ECL1 and not the ECL2, determines whether a pore is formed.

4.2. ECL2 function

The ECL2 seems to be of general relevance, at least in the group of classic claudins. For the classic claudin-5, a holding

and narrowing function has been proposed for this ECL [16]. However, for a proper TJ function, cooperation between both loops seems to be necessary. It was shown that a specific motif in ECL1 and ECL2 and their proper arrangement to each other are involved in claudin-3/-4 interaction [71].

Other claudin segments may also contribute to the TJ function, as indicated after phosphorylation of the intracellular C-terminal tail [34,35] (Section 2). Similarly, intramembranous parts might play a role, as suggested by mutations in transmembrane domains of claudin-14 [72], -16 [69] and -19 [68]. However, the mutations exhibit intracellular accumulation which is most probably due to misfolding and does not prove their involvement in barrier function.

5. Claudin–claudin interactions and the formation of tight junction strands

Claudins may self-associate in two orthogonal orientations, as shown for claudin-5 [15,16] as is known for the adherens junction proteins cadherins [73]. We therefore propose adopting the nomenclature of cadherin–cadherin interaction to that of claudins. Possible interactions for claudins via their ECLs are outlined in Fig. 3, lower part. Thus, claudin-5 molecules can interact along the plasma membrane of the same cell (*cis*-interaction), as demonstrated by fluorescence resonance energy transfer [15,16]. In addition, the ECLs may associate between the plasma membranes of opposing cells (*trans*-interaction). As far as has been analysed, the *cis*-interaction of claudin-5 cannot be affected by amino acid exchange in the ECL2. This indicates that other claudin parts are primarily involved. In contrast, the *trans*-interaction, exclusively occurring in the TJ, can be influenced by amino acid substitutions in the binding core of this ECL2 [16]. This supports a strong contribution of the ECL2 to the *trans*-interaction of claudin-5 (holding and narrowing function).

Moreover, homo- and heterophilic associations have been observed [74]. Molecules of one claudin family member may bind to each other (homophilic interaction) or to molecules of other family members (heterophilic interaction). However, little is known about how claudins interact at the molecular level. Table 2, upper part, summarises homophilic *trans*-interactions (also termed homotypic interactions [71]) for claudins 1, 2 [17,74], 3 [19,74], 5 [15,19,75], 6 [56,76,77], 9 [77], 11 [78], 14 [40,72,77] and 19 [79]. These claudins are able to form TJ when transfected into TJ-free cells (schematic view see Fig. 3A).

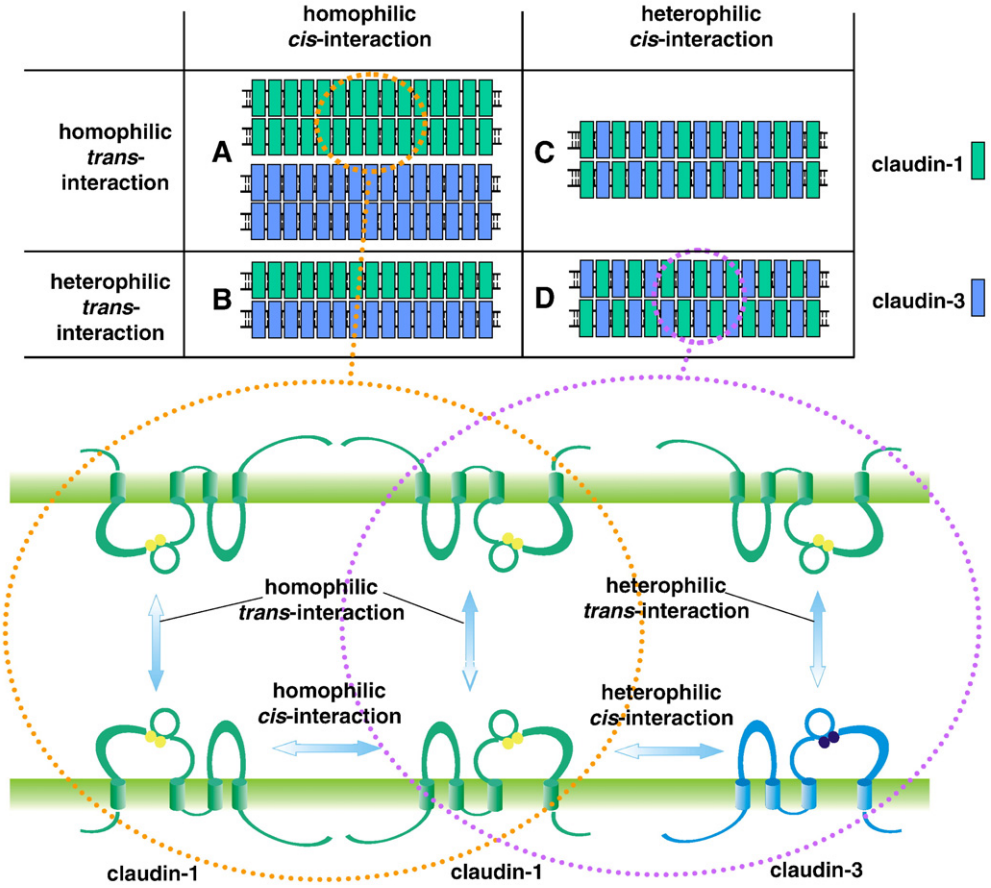


Fig. 3. Tabulated interaction possibilities between claudins as, for example, reported for claudin-1 and -3 [74]. Lower part: homophilic and heterophilic *cis*- and *trans*-interactions based on a nomenclature described for cadherins [73], a group of adherens junction proteins. Upper part: schematic tight junction strands consisting of (A) homophilic *cis*- and *trans*-interactions, (B) heterophilic *trans*-interaction and homophilic *cis*-interaction (C) homophilic *trans*-interaction and heterophilic *cis*-interaction as well as (D) heterophilic *cis*- and *trans*-interactions (modified according to [74]).

Table 2
Tight junction-like strand formation of tight junction free-cells transfected with exogenous claudins — Indications for *trans*-interactions between claudin's extracellular loops presented from the cell surface of opposing cells

Claudin	*trans*-interaction	Cell type	Condition	Freeze-fracture electron microscopy of tight junctions			References
				Strand morphology	Association	Comment	
1	Homophilic	L	Transfected	Continuous strands	P-face		[17,82]
				Continuous grooves	E-face	Complementary	
1	Homophilic	NIH/3T3	Transfected	Linear strands with connections			[19]
2	Homophilic	L	Transfected	Discontinuous strands, particles in grooves	E-face		[17,74]
				Ridges with particles	P-face		
3	Homophilic	L	Transfected	Continuous strands	P-face		[108]
				Continuous grooves	E-face	Complementary	
3	Homophilic	NIH/3T3	Transfected	Non-linear strands, few connections			[19]
5	Homophilic	L	Transfected	Discontinuous strands, particles in grooves	E-face		[75]
				Particle-free ridges	P-face		
5	Homophilic	NIH/3T3	Transfected	Discontinuous strands			[19]
5	Homophilic	HEK	Transfected	Discontinuous strands, branched	E-face	*cis*-interaction simultaneously shown	[16]
6	Homophilic	L	Transfected	Discontinuous strands, particles in grooves	E-face		[76]
				Ridges with particles	P-face		
6	Homophilic	HEK	Transfected				[56]
6	Homophilic	COS-7	Transfected	Meandering, anastomosing strands			[77]
9	Homophilic	COS-7	Transfected	Meandering, anastomosing strands			[77]
11	Homophilic	L	Transfected	Discontinuous strands (few branched, often parallel, varying particle distance)	P-face		[78]
14	Homophilic	L	Transfected	Continuous, short strands			[72]
				Continuous, short grooves		Complementary	
14	Homophilic	COS-7	Transfected	Parallel strands	E-face		[77]
19	Homophilic	L	Transfected	Continuous strands			[79]
1/2		L	Cotransfected	Fragmented continuous strands	P-face	Heterophilic *cis*-interaction assumed	[74]
				Scattered particles in grooves	E-face		
	No heterophilic	L	Coculture [a]	No strands			[74]
1/3	+	L	Cotransfected	Continuous strands	P-face		[74]
				Continuous grooves	E-face	Complementary	
	Heterophilic	L	Coculture [a]	Continuous strands	P-face		[74]
				Continuous grooves	E-face	Complementary	
1/3	+	NIH/3T3	Cotransfected	Continuous strands (as polarised epithelia)	P-face	Heterophilic *cis*- and/or *trans*-interaction	[19]
2/3	+	L	Cotransfected	Fragmented continuous strands	P-face	Heterophilic *cis*-interaction	[74]
				Scattered particles in grooves	E-face		
	Heterophilic	L	Coculture [a]	Continuous claudin-3 strands	P-face		[74]
				Discontinuous claudin-2 strands	E-face		
3/4		HeLa	Cotransfected			Heterophilic *cis*-interaction	[71]
3/5	+	NIH/3T3	Cotransfected	Continuous strands (as polarised epithelia)	P-face	Heterophilic *cis*- and/or *trans*-interaction	[19]
	Heterophilic	HeLa	Coculture [a]				[71]
4/1, 4/3, 4/5	No heterophilic	HeLa	Coculture [a]				[71]

L, mouse L-fibroblasts; NIH/3T3, mouse fibroblasts; HEK, human embryonic kidney cell line 293; COS-7, African green monkey kidney cells (fibroblast-like); HeLa, human cervix epithelial carcinoma cells; P- or E-face association, tight junction strands found in the *p*rotoplasmic or *e*xoplasmic face of the freeze-fracture replica.
[a] Coculture of monotransfected cells.

Heterophilic *trans*-interaction (or heterotypic interaction [71]) between different claudins (Fig. 3B) has been observed for claudins 1↔3, 2↔3 [74] and 3↔5 [19,71]. The heterophilic interactions imply that compatible structural features of the ECLs are conserved, at least, throughout these claudins. The heterophilic interactions are sensitive to small changes in the ECLs [71]. However, how claudins interact at the molecular and structural level to seal the paracellular cleft and to form pores, is an unsolved question. On the other hand, it has not been possible to demonstrate a heterophilic *trans*-interaction for claudins 1↔2 [74], 1↔4, 3↔4, and 4↔5 [71] (Table 2, lower part). This demonstrates that only certain claudins are able to interact with each other.

Homophilic *cis*-interaction (or homomeric interaction [71]) is described for claudin 5↔5 using a FRET assay [15,16]. Heterophilic *cis*-interactions (or heteromeric interactions [71]) are found for claudins 2↔3 [74], 3↔4 [71] and assumed for 1↔2 [74], respectively. Most epithelia and endothelia express a mixture of different claudins which is specific for the function of a tissue or parts thereof (e.g., variation along the nephron [80]). Consequently, heteropolymers are most likely and these can be formed in different ways (possibilities see Fig. 3B–D).

The analysis of TJ strands is possible by transition and freeze-fracture electron microscopy [1]. The latter shows claudin strands in different layers of the TJ membrane [81,75]. However, molecular considerations and discrimination between different claudins are difficult in TJ-expressing cells or tissues. To overcome this difficulty, TJ-free fibroblasts [17,74–76,78,79,82], COS-7 [77], NIH/3T3 [19], and HEK-293 [16,56] were introduced to analyse homophilic and heterophilic *trans*-interactions in TJ after the transfection of claudins. In addition, *trans*-interaction is investigated as claudin enrichment in cell–cell contacts (TJ) [16]. *cis*-interactions can be studied in living cells by fluorescence resonance energy transfer after exogenous expression of claudins C-terminally fused with fluorescent proteins [15,16]. Non-denaturing gel electrophoresis may give hints of the size of claudin oligomers [19,20] and coimmuno-precipitation provides hints of heterophilic interactions [19]. Analysis of recombinant claudin segments with size-exclusion chromatography, light scattering or mass spectrometry [15], as well as the effect of peptides [83], may further characterise claudin interactions.

6. Alignment of the extracellular loops of claudins

The alignment and phylogenetic tree analysis of whole-length sequences of claudins led to differentiation of two groups; the classic claudins have very high common sequence similarity, but non-classic claudins possess low similarity (Fig. 2). This is even sustained and affirmed for the extracellular loop sequences of ECL1 and ECL2, by inspecting their alignment separately (Fig. 4). Classic claudins exhibit a much stronger consensus sequence than non-classic claudins.

As shown in Fig. 4, left alignment, the ECL1 of the complete claudin protein family consists of 50–56 amino acids, wherein the non-classic group exhibits a much higher sequence variety than the classic group, which has a higher number of consensus amino acids. Two cysteines (e.g., positions 54 and 64, human claudin-1) are conserved in all claudins and are reported to be essential for tightness function [8]. Non-conserved negatively charged residues in the ECL1 of claudin-2 [9], -15 [11], and -16 [10] are thought to be responsible for cation pore formation at TJ. In general, it seems that in the ECL1 an excess of either

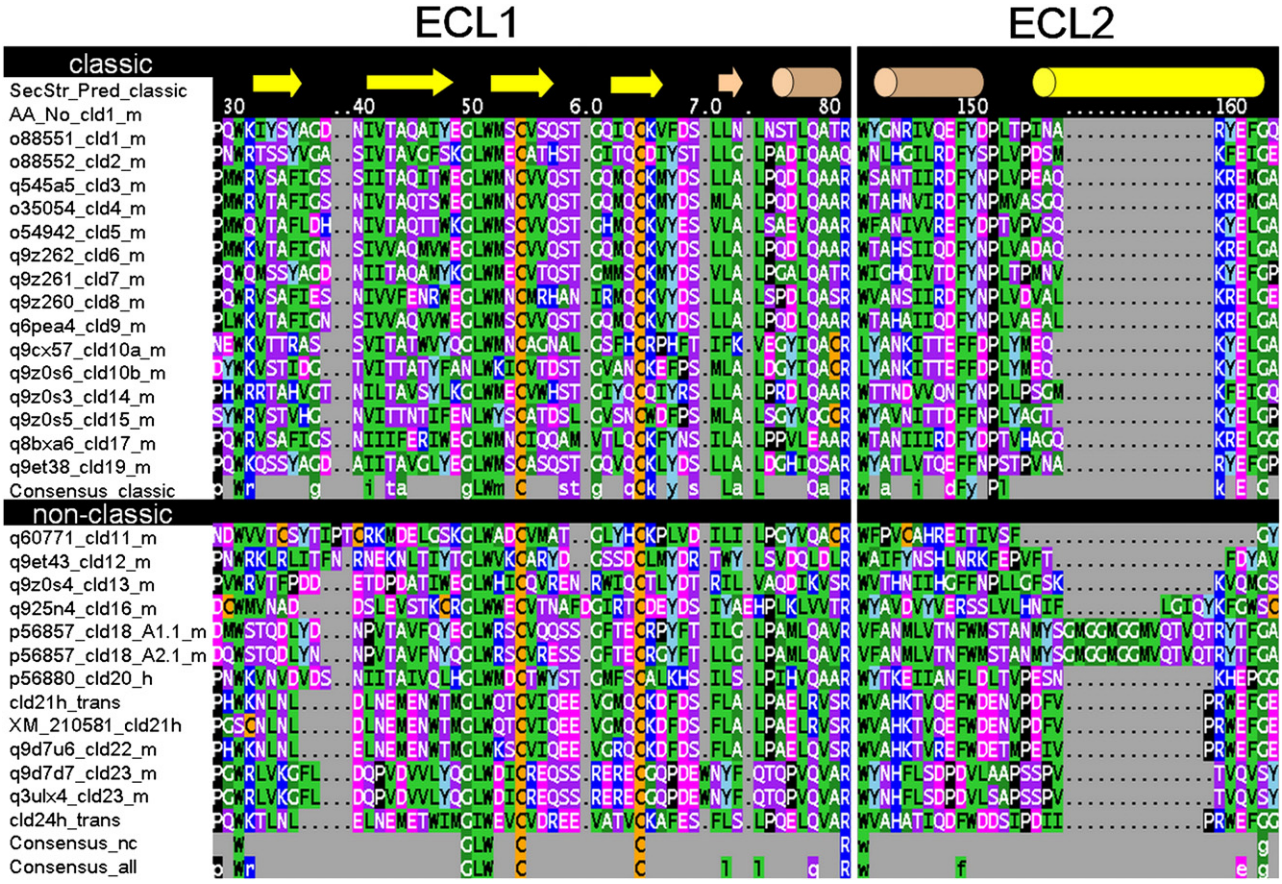


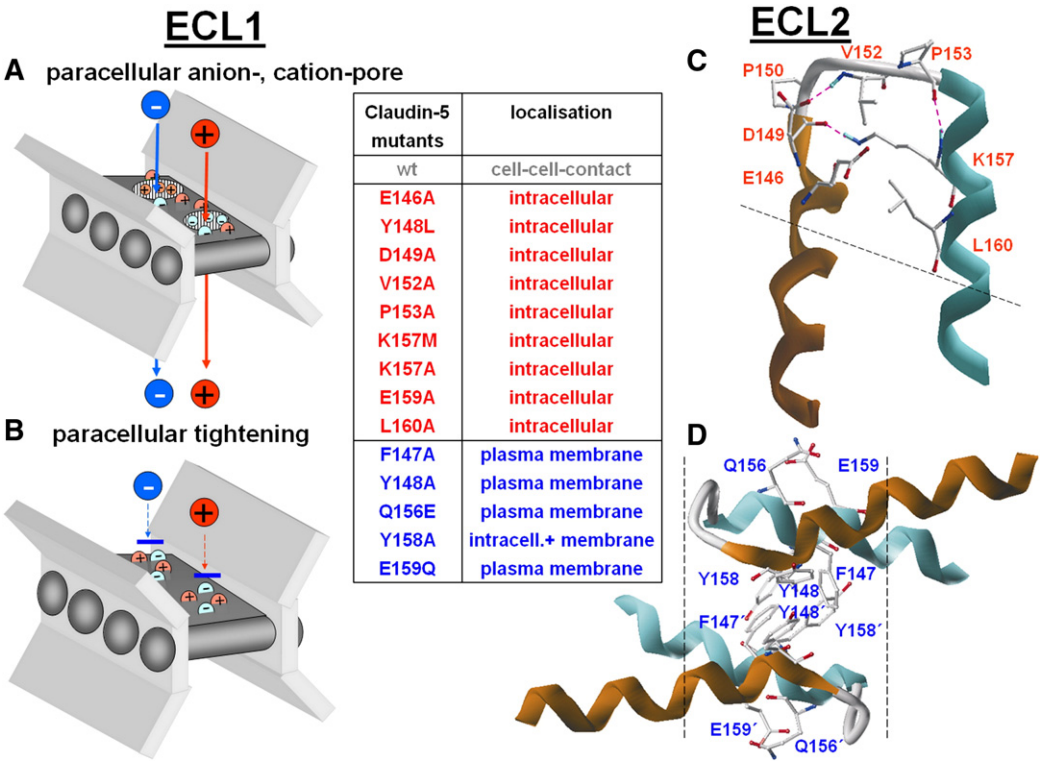
Fig. 4. Alignment of sequences of the extracellular loops (ECL) 1 and 2 from claudins with the GCG program package (GCG Wisconsin package, Accelrys Inc., San Diego, CA). The claudins are grouped by sequence similarity in classic and non-classic ones (see Fig. 2). For each mammalian claudin, the mouse-sequences (m) were taken as representative. Claudin-20, -21, and -24 are only know in human (h). Numbers of amino acids are taken from cld1_m. For each sequence the Swissprot accession number is stated. From claudin-10 and -18, two isoforms with differences in ECL1 are known. The two claudin-23 sequences possess an amino acid exchange in the ECL2. The consensus sequences for the classic and non-classic as well as for all claudins are given in capital letters (>90% sequence identity) and small letters (>70% identity) sequence identity, respectively. Green: hydrophobic, blue: basic, magenta: acidic, violet: hydrophilic residues, yellow: cysteines, cyan: tyrosines, black: prolines. Classic claudins showed in ECL1 predictions of β-strands and in ECL2 a prediction of two α-helices. Secondary structure element predictions were applied by multiple methods using program jpred [90]. Arrows, β-strands; tubes, α-helices; yellow, strong prediction; beige, weak prediction.

negatively or positively charged residues supports formation of pores with preference for the passage of cations or anions, respectively. This can be exemplified by the two splice variants of claudin-10, which are moderately charge selective. Claudin-10A has an excess of positive charges in this ECL and that of claudin-10B an excess of negative charges. Thus, claudin-10A is reported to convert the selectivity more to Cl^- than to Na^+, and claudin-10B more to Na^+ than to Cl^- [13].

The ECL2 of classic claudins (Fig. 4, right alignment) consists of ~25 amino acids. In non-classic claudins, the ECL2 varies in length from 17 (claudin-11) [14] to 39 (claudin-18) [7] and, to a larger extent, in the amino acid sequences. A defined sequence length of ECL2 for all claudins is difficult to state, since the predictions of the borderlines for transmembrane (TM) regions vary for different claudins, even among the classic ones. This can be exemplified for claudin-5, where more hydrophobic residues at the N-terminal side of the ECL2 lead to extension of the predicted TM3 region and a decreased ECL2 length. This prediction is shown in the annotation to the claudins in the Swissprot database and by other TM prediction methods, such as SPLIT4 [84], HMMTOP2 [85], and TMHMM2 [86]. The different TM length may indicate either different TM tilt within the membrane or different elongation of potential transmembrane helices into the extracellular side for some claudins. The occurring consensus "dFy.Pl" (Fig. 4, right upper part) in the middle of the ECL2 amino acid sequence is part of a predicted helix with a following turn structure at the classic group [16].

7. Interaction models of claudin extracellular loops

Due to the lack of X-ray or NMR structural data for interacting claudins, no detailed information down to atom level is available. Only global schemes have been proposed [87,88]. These point to general participation of charged residues aligned in the ion pore, as demonstrated by mutations [12,13,66,89]. To gain insight in potential structures of the ECLs of claudins, we performed secondary structure predictions using the program jpred [90]. They indicate mainly β-



Claudin-5 mutants	localisation
wt	cell-cell-contact
E146A	intracellular
Y148L	intracellular
D149A	intracellular
V152A	intracellular
P153A	intracellular
K157M	intracellular
K157A	intracellular
E159A	intracellular
L160A	intracellular
F147A	plasma membrane
Y148A	plasma membrane
Q156E	plasma membrane
Y158A	intracell.+ membrane
E159Q	plasma membrane

Fig. 5. Suggested molecular interactions between extracellular domains of homophilic claudins in tight junctions, explaining pore formation and tightening by the first extracellular loop (ECL) 1 as well as narrowing and holding function of the ECL2. Schemes for ECL1: (A) The charge depending pore formation likely by charge repulsion that allows paracellular anion or cation permeation is supported by favoured spatial placement of an overplus of either positively or negatively charged residues at the ECL, lining the respective pore. (B) The tightness between claudins is likely produced by a differently distribution and facing of charged amino acids (likely by charge attraction), preventing the paracellular ion permeation. Blue bar, blocked ion permeability; + in red circle, cation; − in blue circle, anion; dark grey, general representation of claudin polymer consisting of oligomers (columns) assumed. Comparative molecular model for ECL2 adapted from [16]: (C) A sequence portion with a helix–turn–helix structure of the protein 2BDV identified at the Protein Data Bank (http://www.rcsb.org/pdb/) is highly homologous to the ECL2 sequence of claudin-5. Two α-helical structures (orange, cyan) linked by a turn are likely extracellular extensions of transmembrane helices TMH3 and 4. This monomer structure is highly consistent to all mutants disturbing the fold (intracellular phenotype, red in the inserted table) since the side chains of wild type residues are stabilising the turn conformation by H-bonds or hydrophobic interaction. (D) Based on the monomer, a tentative anti-parallel dimer model is suggested for homophilic *trans*-interaction of two ECL2 that is partly consistent with mutant phenotypes that are localised at the membrane and disturbed the *trans*-interaction (blue in inserted table, disjunction phenotype). Aromatic amino acids are likely forming an aromatic core, which hold onto each other between opposing cells [16]. Dashed lines indicate the border between TMH and ECL. Amino acid positions, mouse claudin-5.

strands in ECL1 and a helix–turn–helix motif for the ECL2 of classic claudins (Fig. 4).

For the ECL1, the two following schemes can be summarised: (i) The pore forming property supporting the paracellular ion permeation is favoured, probably because of charge repulsion by spatial placement of equally charged residues in the ECL1, since pore formation depends on the spatial location, distribution and facing of equally charged residues (Fig. 5A). (ii) The tightness property is supported by a different distribution and facing of charged amino acids, preventing pore formation and paracellular cation permeation via the ECL1, probably due to charge attraction (Fig. 5B).

In the case of ECL2, more detailed information is available [16]. Based on mutagenesis and morphological data of claudin-5, molecular modelling led to a homology model of a monomer, which explains folding/transport defects caused by the mutants (Fig. 5C). The experimental data suggest that the ECL2 is involved in TJ strand formation via *trans*- but not *cis*-interaction. Based on the monomer model, a tentative anti-parallel homodimer model has been proposed (Fig. 5D). This first dimer model is consistent with the finding that three aromatic amino acids (F147, Y148, Y158) are involved in the *trans*-interaction. The results still do not explain how two further residues (Q156, E159) are also involved in the *trans*-interaction. Possibly, the Q and E interact with other parts of the molecule (e.g. ECL1). Since nearly all the residues which were of relevance for the interaction are conserved in the classic claudin group, the proposed mechanism might be of general importance for classic claudins.

8. Schemes for strand formation

As shown in Table 2, two types of strand morphology have been described after transfection of claudins in TJ-free cells. Continuous strands are observed for claudin-1 [17,19,74], -3 [74], -14 [40,72] and -19 [79]. This means that these claudins form continuous strands associated with the protoplasmic face (P-face) of the membrane when visualised by freeze-fracture electron microscopy [17]. On the other hand, rows of particles of up to about 10 nm in size [11,19,20], separated by spaces of mainly similar length, are obtained with claudin-2 [11,74], -5 [16,19,75], -6 [76] and -11 [78] (discontinuous strands). The particles in discontinuous strands are associated with the exoplasmic face (E-face) of the membrane after freeze-fracturing [75]. For claudin-2 [17,74], -6 [76] and -11 [78], intramembranous particles were seen on the P-face also.

This diversity might be due to different properties in *cis*-oligomerisation. *In vitro* evidence suggests that claudins possess a general potential for oligomerisation. In semi-native

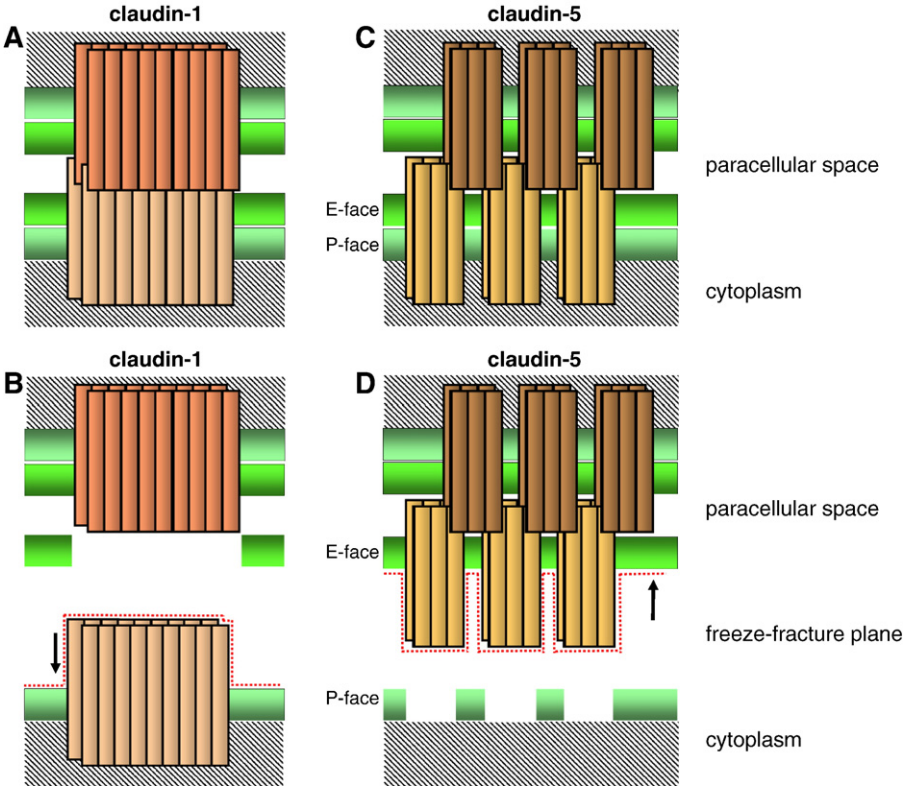


Fig. 6. Scheme for the formation of tight junction strands by a single claudin and their appearance after freeze-fracture electron microscopy. (A) *trans*-interaction of claudin-1 triggers the formation of polymeric strands and continuous *cis*-interaction supports this polymerisation. (B) During freeze-fracturing, the claudin-1 strands cannot slide out of the protoplasmic(P)-face due to the continuity and are observed on the P-face of the membrane as continuous strands. (C) Due to *cis*-interaction, claudin-5 monomers form oligomers, e.g. hexamers in one membrane. *trans*-interaction triggers the formation of polymeric discontinuous strands. (D) During freeze-fracturing, the claudin-5 oligomer slides out of the P-face and is seen on the exoplasmic(E)-face of the membrane as a line of particles. Black arrows indicate the direction of observation in the freeze-fracture replica; red dotted line, freeze-fracture plane. Rectangle, claudin monomer. For explanation of *cis*- and *trans*-interactions see Fig. 3.

electrophoresis with perfluorooctanoate gels, claudin-5 is detected as a larger oligomer than claudin-1 [19]. Claudin-5 preferentially forms hexamers [19], but does not polymerise in one cell [16]. Only *trans*-interaction between oligomers at TJ triggers the formation of polymeric strands between the cells [16]. As polarised cells express different claudins, one has to assume that heterophilic claudin–claudin interactions also take place. Strand formation is, at least, caused by interactions of ECLs. For claudin-5, this is documented by amino acid replacement studies in the ECL2, which led to abolishment of strands or marked alterations in strand morphology [16].

The 10 nm size of the discontinuous particles [17,19,20] matches the size of connexin hexamers determined by electron microscopy, size-exclusion chromatography or density gradient centrifugation [91–93]. Since claudin molecules are roughly the same size as those of connexins and also consist of 4 transmembrane helices, it is conceivable that they may also form hexamers *in vivo* even though the configuration would be different. This is supported by biochemical data indicating that claudins such as claudin-4 and -5 can occur as oligomers of different sizes, but preferentially as hexamers [19,20].

The oligomerisation data mentioned above result in schemes for strand formation, which were developed for classic claudins (Fig. 6). Claudin-1 forms continuous polymers within the TJ membrane [17,19,82], triggered by *trans*-interaction and probably supported by continuous *cis*-interaction (Fig. 6A). Maybe due to the continuity, the polymers are not able to slide out of the P-face and are therefore detected as continuous strands on the P-face in freeze-fracture replica (Fig. 6B). On the contrary, claudin-5 generates oligomers (e.g. hexamers) but not polymers in the TJ membrane. These particles (Fig. 6C) are able to slide out of the P-face and, therefore, appear on the E-face in freeze-fracture replica [75,16,19] (Fig. 6D). This could be due to strong *trans*-interaction and the lack of continuity in the membrane. Claudin specific anchorage to the cytoskeleton cannot explain the P-/E-face association, since it is also observed when the anchorage is blocked [16]. In contrast to claudin-transfected TJ-free cells, different claudins probably copolymerise *in vivo*, resulting in continuous strands, as found for epithelial [94] and endothelial cells [81]. Hence, the discontinuity of claudin-5 strands in TJ-free cells [16,19,75] could be due to the fact that other claudins are missing and supports the concept of heteropolymerisation *in vivo*.

Pathological conditions may influence strand morphology. Discontinuous strands can be found in colon biopsies of Crohn's disease, due to changes in expression and distribution of claudins 2, 5 and 8 [94]. Brain degeneration may lead to a loss of claudin-3, but not of claudin-5, at TJ in cerebral endothelial cells [81], also changing the claudin composition of the TJ. In conclusion, a defined claudin composition and stoichiometry seems to be essential for the formation of continuous strands.

Acknowledgements

We would like to thank Hartwig Wolburg/Tübingen for the valuable discussion on the schematic models of tight junction strand formation and Rodney Yeats/Berlin for preparing the manuscript.

References

[1] L.A. Staehelin, Structure and function of intercellular junctions, Int. Rev. Cytol. 39 (1974) 191–283.
[2] B.E. Hull, L.A. Straehelin, Functional significance of the variations in the geometrical organization of tight junction networks, J. Cell Biol. (1976) 688–704.
[3] M. Furuse, T. Hirase, M. Itoh, M. Nagafuchi, S. Yonemura, S. Tsukita, Occludin: a novel integral membrane protein localizing at tight junctions, J. Cell Biol. 123 (1993) 1777–1788.
[4] J. Ikenouchi, M. Furuse, K. Furuse, H. Sasaki, S. Tsukita, S. Tsukita, Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells, J. Cell Biol. 171 (2005) 939–945.
[5] K. Morita, M. Furuse, K. Fujimoto, S. Tsukita, Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 511–516.
[6] M. Furuse, S. Tsukita, Claudins in occluding junctions of humans and flies, Trends Cell Biol. 16 (2006) 181–188.
[7] K.J. Hewitt, R. Agarwal, P.J. Morin, The claudin gene family: expression in normal and neoplastic tissues, BMC Cancer 6 (186) (2006) 1–8.
[8] H. Wen, D.D. Watry, M.C.G. Marcondes, H.S. Fox, Selective decrease in paracellular conductance of tight junctions: role of the first extracellular domain of claudin-5, Mol. Biol. Cell 24 (2004) 8408–8417.
[9] S. Amasheh, N. Meiri, A.H. Gitter, T. Schoneberg, J. Mankertz, J.D. Schulzke, M. Fromm, Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells, J. Cell Sci. 115 (2002) 4969–4976.
[10] J. Hou, D.L. Paul, D.A. Goodenough, Paracellin-1 and the modulation of ion selectivity of tight junctions, J. Cell Sci. 118 (2005) 5109–5118.
[11] O.R. Colegio, C.M. Van Itallie, C. Rahner, J.M. Anderson, Claudin extracellular domains determine paracellular charge selectivity and resistance but not tight junction fibril architecture, Am. J. Physiol., Cell Physiol. 284 (2003) C1346–C1354.
[12] M.D. Alexandre, B.G. Jeansonne, R.H. Renegar, R. Tatum, Y.H. Chen, The first extracellular domain of claudin-7 affects paracellular Cl^- permeability, Biochem. Biophys. Res. Commun. 357 (2007) 87–91.
[13] C.M. Van Itallie, S. Rogan, A. Yu, L.S. Vidal, J. Holmes, J.M. Anderson, Two splice variants of claudin-10 in the kidney create paracellular pores with different ion selectivities, Am. J. Physiol., Renal Physiol. 291 (2006) F1288–F1299.
[14] A. Gow, C.M. Southwood, J.S. Li, M. Pariali, G.P. Riordan, S.E. Brodie, J. Danias, J.M. Bronstein, B. Kachar, R.A. Lazzarini, CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice, Cell 99 (1999) 649–659.
[15] I.E. Blasig, L. Winkler, B. Lassowski, S.L. Mueller, N. Zuleger, E. Krause, G. Krause, K. Gast, M. Kolbe, J. Piontek, On the self-association potential of transmembrane tight junction proteins, Cell. Mol. Life Sci. 63 (2006) 505–514.
[16] J. Piontek, L. Winkler, H. Wolburg, S.L.Mueller, N. Zuleger, C.Piehl, B. Wiesner, G. Krause, I.E. Blasig. Formation of tight junction: determinants of homophilic interaction between classic claudins, FASEB J. 22 (2008).
[17] M. Furuse, H. Sasaki, K. Fujimoto, S. Tsukita, A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts, J. Cell Biol. 143 (1998) 391–401.
[18] M. Furuse, K. Fujimoto, N. Sato, T. Hirase, S. Tsukita, S. Tsukita, Overexpression of occludin, a tight junction-associated integral membrane protein, induces the formation of intracellular multilamellar bodies bearing tight junction-like structures, J. Cell Sci. 109 (1996) 429–435.
[19] C.B. Coyne, T.M. Gambling, R.C. Boucher, J.L. Carson, L.G. Johnson, Role of claudin interactions in airway tight junctional permeability, Am. J. Physiol., Lung Cell Mol. Physiol. 285 (2003) L1166–L1178.
[20] L.L. Mitic, V.M. Unger, J.M. Anderson, Expression, solubilization, and biochemical characterization of the tight junction transmembrane protein claudin-4, Protein Sci. 12 (2003) 218–227.

[21] K. Umeda, J. Ikenouchi, S. Katahira-Tayama, K. Furuse, H. Sasaki, M. Nakayama, T. Matsui, S. Tsukita, M. Furuse, S. Tsukita, ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation, Cell 126 (2006) 741–754.
[22] H. Sasaki, C. Matsui, K. Furuse, Y. Mimori-Kiyosue, M. Furuse, S. Tsukita, Dynamic behavior of paired claudin strands within apposing plasma membranes, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 3971–3976.
[23] Y.N. Zhang, S. Yeh, B.A. Appleton, H.A. Held, P.J. Kausalya, D.C.Y. Phua, W.L. Wong, L.A. Lasky, C. Wiesmann, W. Hunziker, S.S. Sidhu, Convergent and divergent ligand specificity among PDZ domains of the LAP and zonula occludens (ZO) families, J. Biol. Chem. 281 (2006) 22299–22311.
[24] M. Itoh, M. Furuse, K. Morita, K. Kubota, M. Saitou, Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2 and ZO-3, with the COOH termini of claudins, J. Cell Biol. 147 (1999) 1351–6136.
[25] Y. Hamazaki, M. Itoh, H. Sasaki, M. Furuse, S. Tsukita, Multi-PDZ domain protein 1 (MUPP1) is concentrated at tight junctions through its possible interaction with claudin-1 and junctional adhesion molecule, J. Biol. Chem. 277 (2002) 455–461.
[26] B. Jeansonne, Q. Lu, D.A. Goodenough, Y.H. Chen, Claudin-8 interacts with multi-PDZ domain protein 1 (MUPP1) and reduces paracellular conductance in epithelial cells, Cell. Mol. Biol. (Noisy-le-grand) 49 (2003) 13–21.
[27] M.H. Roh, C.J. Liu, S. Laurinec, B. Margolis, The carboxyl terminus of zona occludens-3 binds and recruits a mammalian homologue of discs lost to tight junctions, J. Biol. Chem. 277 (2002) 27501–62709.
[28] A. Ikari, N. Hirai, M. Shiroma, H. Harada, H. Sakai, H. Hayashi, Y. Suzuki, M. Degawa, K. Takagi, Association of paracellin-1 with ZO-1 augments the reabsorption of divalent cations in renal epithelial cells, J. Biol. Chem. 279 (2004) 54826–54832.
[29] U. Wiedemann, P. Boisguerin, R. Leben, D. Leitner, G. Krause, K. Mölling, R. Volkmer-Engert, H. Oschkinat, Quantification of PDZ domain specificity, prediction of ligand affinity and rational design of super-binding peptides, J. Mol. Biol. 343 (2004) 703–718.
[30] C.M. Van Itallie, O.R. Colegio, J.M. Anderson, The cytoplasmic tails of claudins can influence tight junction barrier properties through effects on protein stability, J. Membr. Biol. 199 (2004) 29–38.
[31] R.F. Fallon, D.A. Goodenough, 5-hour half-life of mouse-liver gap-junction protein, J. Cell Biol. 90 (1981) 521–526.
[32] M. Fujibe, H. Chiba, T. Kojima, T. Soma, T. Wada, T. Yamashita, N. Sawada, Thr203 of claudin-1, a putative phosphorylation site for MAP kinase, is required to promote the barrier function of tight junctions, Exp. Cell. Res. 295 (2004) 36–47.
[33] V. Nunbhakdi-Craig, T. Machleidt, E. Ogris, D. Bellotto, C.L. White, E. Sontag, Protein phosphatase 2A associates with and regulates atypical PKC and the epithelial tight junction complex, J. Cell Biol. 158 (2002) 967–978.
[34] T. D'Souza, R. Agarwal, P.J. Morin, Phosphorylation of claudin-3 at threonine 192 by cAMP-dependent protein kinase regulates tight junction barrier function in ovarian cancer cells, J. Biol. Chem. 280 (2005) 26233–26240.
[35] T. Soma, H. Chiba, Y. Kato-Mori, T. Wada, T. Yamashita, T. Kojima, N. Sawada, Thr(207) of claudin-5 is involved in size-selective loosening of the endothelial barrier by cyclic AMP, Exp. Cell Res. 300 (2004) 202–212.
[36] S.M. Stamatovic, O.B. Dimitrijevic, R.F. Kepp, A.V. Andjelkovic, Protein kinase Cα-RhoA cross talk in CCL2-induced alterations in brain endothelial permeability, J. Biol. Chem. 281 (2006) 8379–8388.
[37] K. Yamauchi, T. Rai, K. Kobayashi, E. Sohara, T. Suzuki, T. Itoh, S. Suda, A. Hayama, S. Sasaki, S. Uchida, Disease-causing mutant WNK4 increases paracellular chloride permeability and phosphorylates claudins, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4690–4694.
[38] M. Tanaka, R. Kamata, R. Sakai, EphA2 phosphorylates the cytoplasmic tail of claudin-4 and mediates paracellular permeability, J. Biol. Chem. 280 (2005) 42375–42782.
[39] M. Clamp, J. Cuff, S.M. Searle, G.J. Barton, The Jalview Java alignment editor, Bioinformatics 20 (2004) 426–427.
[40] C.M. Van Itallie, T.M. Gambling, J.L. Carson, J.M. Anderson, Palmitoylation of claudins is required for efficient tight-junction localization, J. Cell Sci. 118 (2005) 1427–1436.
[41] M. Furuse, M. Hata, K. Furuse, Y. Yoshida, A. Haratake, Y. Sugitani, T. Noda, A. Kubo, S. Tsukita, Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice, J. Cell Biol. 156 (2002) 1099–1111.
[42] T. Nitta, M. Hata, S. Gotoh, Y. Seo, H. Sasaki, N. Hashimoto, M. Furuse, S. Tsukita, Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice, J. Cell Biol. 161 (2003) 653–660.
[43] T. Ben-Yosef, I.A. Belyantseva, T.L. Saunders, E.D. Hughes, K. Kawamoto, C.M. Van Itallie, L.A. Beyer, K. Halsey, D.J. Gardner, E.R. Wilcox, J. Rasmussen, J.M. Anderson, D.F. Dolan, A. Forge, Y. Raphael, S.A. Camper, T.B. Friedman, Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration, Hum. Mol. Genet. 12 (2003) 2049–2061.
[44] B. Litkouhi, J. Kwong, C.-M. Lo, J.G. Smedley, B.A. McClane, M. Aponte, Z. Gao, J.L. Sarno, J. Hinners, W.R. Welch, R.S. Berkowitz, S.C. Mok, E.I.O. Garner, Claudin-4 overexpression in epithelial ovarian cancer is associated with hypomethylation and is a potential target for modulation of tight junction barrier function using a C-terminal fragment of *Clostridium perfringens* enterotoxin, Neoplasia 9 (2007) 304–314.
[45] S. Boireau, M. Buchert, M.S. Samuel, J. Pannequin, J.L. Ryan, A. Choquet, H. Chapuis, X. Rebillard, C. Avances, M. Ernst, D. Joubert, N. Mottet, F. Hollande, DNA-methylation-dependent alterations of claudin-4 expression in human bladder carcinoma, Carcinogenesis 28 (2007) 246–258.
[46] S. Amasheh, T. Schmidt, M. Mahn, P. Florian, J. Mankertz, S. Tavalali, A. Gitter, J.D. Schulzke, Contribution of claudin-5 to barrier properties in tight junctions of epithelial cells, Cell Tissue Res. 321 (2005) 89–96.
[47] S. Angelow, K.J. Kim, A.S.L. Yu, Claudin-8 modulates paracellular permeability to acidic and basic ions in MDCK II cells, J. Physiol.-London 571 (2006) 15–26.
[48] S. Angelow, R. El-Husseini, S.A. Kanzawa, A.S. Yu, Renal localization and function of the tight junction protein, claudin-19, Am. J. Physiol., Renal Physiol. 293 (2007) F166–F177.
[49] A.S. Yu, A.H. Enck, W.I. Lencer, E.E. Schneeberger, Claudin-8 expression in Madin–Darby canine kidney cells augments the paracellular barrier to cation permeation, J. Biol. Chem. 278 (2003) 17350–17359.
[50] A. Reymond, V. Marigo, M.B. Yaylaoglu, A. Leoni, C. Ucla, N. Scamuffa, C. Caccioppoli, E.T. Dermitzakis, R. Lyle, S. Banfi, G. Eichele, S.E. Antonarakis, A. Ballabio, Human chromosome 21 gene expression atlas in the mouse, Nature 420 (2002) 582–586.
[51] M.D. Alexandre, Q. Lu, Y.H. Chen, Overexpression of claudin-7 decreases the paracellular Cl^- conductance and increases the paracellular Na^+ conductance in LLC-PK1 cells, J. Cell Sci. 118 (2005) 2683–2693.
[52] C.M. Van Itallie, A.S. Fanning, J.M. Anderson, Reversal of charge selectivity in cation or anion-selective epithelial lines by expression of different claudins, Am. J. Physiol., Renal Physiol. 285 (2003) F1078–F1084.
[53] C.M. Van Itallie, C. Rahner, J.M. Anderson, Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability, J. Clin. Invest. 107 (2001) 1319–1327.
[54] J Hou, A.S. Gomes, D.L. Paul, D.A. Goodenough, Study of claudin function by RNA interference, J. Biol. Chem. 281 (2006) 36117–36123.
[55] P. Guo, A.M. Weinstein, S. Weinbaum, A dual-pathway ultrastructural model for the tight junction of rat proximal tubule epithelium, Am. J. Physiol. Renal Physiol. 285 (2003) F241–F257.
[56] K. Turksen, T.C. Troy, Claudin-6: a novel tight junction molecule is developmentally regulated in mouse embryonic epithelium, Dev. Dyn. 222 (2001) 292–300.
[57] S.I. Kitajiri, M. Furuse, K. Morita, Y. Saishin-Kiuchi, H. Kido, J. Ito, S. Tsukita, Expression patterns of claudins, tight junction adhesion molecules, in the inner ear, Hear. Res. 187 (2004) 25–34.
[58] H. Fujita, H. Chiba, H. Yokozaki, N. Sakai, K. Sugimoto, T. Wada, T. Kojima, T. Yamashita, N. Sawada, Differential expression and subcellular localization of claudin-7, -8, -12, -13, and -15 along the mouse intestine, J. Histochem. Cytochem. 54 (2006) 933–944.

[59] G. Abuazza, A. Becker, S.S. Williams, S. Chakravarty, H.T. Truong, F. Lin, M. Baum, Claudins 6, 9, and 13 are developmentally expressed renal tight junction proteins, Am. J. Physiol. Renal. Physiol. 291 (2006) F1132–F1141.

[60] Y. Gon, M.R. Wood, W.B. Kiosses, E. Jo, M.G. Sanna, J. Chun, H. Rosen, S1P3 receptor-induced reorganization of epithelial tight junctions compromises lung barrier integrity and is potentiated by TNF, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9270–9275.

[61] T. Niimi, K. Nagashima, J.M. Ward, P. Minoo, D.B. Zimonjic, N.C. Popescu, S. Kimura, Claudin-18, a novel downstream target gene for the T/EBP/NKX2.1 homeodomain transcription factor, encodes lung- and stomach-specific isoforms through alternative splicing, Mol. Cell. Biol. 21 (2001) 7380–7390.

[62] The MGC Project Team, The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC), Genome Res. 14 (2004) 2121–2127.

[63] T. Ota, Y. Suzuki, T. Nishikawa, T. Otsuki, T. Sugiyama, R. Irie, A. Wakamatsu, K. Hayashi, H. Sato, K. Nagai, K. Kimura, H. Makita, M. Sekine, M. Obayashi, T. Nishi, T. Shibahara, T. Tanaka, S. Ishii, J. Yamamoto, K. Saito, Y. Kawai, Y. Isono, Y. Nakamura, K. Nagahari, K. Murakami, T. Yasuda, T. Iwayanagi, M. Wagatsuma, A. Shiratori, H. Sudo, T. Hosoiri, Y. Kaku, H. Kodaira, H. Kondo, M. Sugawara, M. Takahashi, K. Kanda, T. Yokoi, T. Furuya, E. Kikkawa, Y. Omura, K. Abe, K. Kamihara, N. Katsuta, K. Sato, M. Tanikawa, M. Yamazaki, K. Ninomiya, T. Ishibashi, H. Yamashita, K. Murakawa, K. Fujimori, H. Tanai, M. Kimata, M. Watanabe, S. Hiraoka, Y. Chiba, S. Ishida, Y. Ono, S. Takiguchi, S. Watanabe, M. Yosida, T. Hotuta, J. Kusano, K. Kanehori, A. Takahashi-Fujii, H. Hara, T.-O. Tanase, Y. Nomura, S. Togiya, F. Komai, R. Hara, K. Takeuchi, M. Arita, N. Imose, K. Musashino, H. Yuuki, A. Oshima, N. Sasaki, S. Aotsuka, Y. Yoshikawa, H. Matsunawa, T. Ichihara, N. Shiohata, S. Sano, S. Moriya, H. Momiyama, N. Satoh, S. Takami, Y. Terashima, O. Suzuki, S. Nakagawa, A. Senoh, H. Mizoguchi, Y. Goto, F. Shimizu, H. Wakebe, H. Hishigaki, T. Watanabe, A. Sugiyama, M. Takemoto, B. Kawakami, M. Yamazaki, K. Watanabe, A. Kumagai, S. Itakura, Y. Fukuzumi, Y. Fujimori, M. Komiyama, H. Tashiro, A. Tanigami, T. Fujiwara, T. Ono, K. Yamada, Y. Fujii, K. Ozaki, M. Hirao, Y. Ohmori, A. Kawabata, T. Hikiji, N. Kobatake, H. Inagaki, Y. Ikema, S. Okamoto, R. Okitani, T. Kawakami, S. Noguchi, T. Itoh, K. Shigeta, T. Senba, K. Matsumura, Y. Nakajima, T. Mizuno, M. Morinaga, M. Sasaki, T. Togashi, M. Oyama, H. Hata, M. Watanabe, T. Komatsu, J. Mizushima-Sugano, T. Satoh, Y. Shirai, Y. Takahashi, K. Nakagawa, K. Okumura, T. Nagase, N. Nomura, H. Kikuchi, Y. Masuho, R. Yamashita, K. Nakai, T. Yada, Y. Nakamura, O. Ohara, T. Isogai, S. Sugano, Complete sequencing and characterization of 21,243 full-length human cDNAs, Nat. Genet. 36 (2004) 40–45.

[64] N. Charoenphandhu, K. Wongdee, K. Tudpor, J. Pandaranandaka, N. Krishnamra, Life Sci. 80 (2007) 1729–1737.

[65] M. Katoh, M. Katoh, CLDN23 gene, frequently down-regulated in intestinal-type gastric cancer, is a novel member of CLAUDIN gene family, Int. J. Mol. Med. 11 (2003) 683–689.

[66] O.R. Colegio, C.M. Van Itallie, H.J. McCrea, C. Rahner, J.M. Anderson, Claudins create charge-selective channels in the paracellular pathway between epithelial cells, Am. J. Physiol., Cell Physiol. 283 (2002) C142–C147.

[67] S. Weber, L. Schneider, M. Peters, J. Misselwitz, G. Ronnefarth, M. Boswald, K.E. Bonzel, T. Seeman, T. Sulakova, E. Kuwertz-Broking, A. Gregoric, J.B. Palcoux, V. Tasic, F. Manz, K. Scharer, H.W. Seyberth, M. Konrad, Novel paracellin-1 mutations in 25 families with familial hypomagnesemia with hypercalciuria and nephrocalcinosis, J. Am. Soc. Nephrol. 12 (2001) 1872–1881.

[68] M. Konrad, A. Schaller, D. Seelow, A.V. Pandey, S. Waldegger, A. Lesslauer, H. Vitzthum, Y. Suzuki, J.M. Luk, C. Becker, K.P. Schlingmann, M. Schmid, J. Rodriguez-Soriano, G. Ariceta, F. Cano, R. Enriquez, H. Juppner, S.A. Bakkaloglu, M.A. Hediger, S. Gallati, S.C. Neuhauss, P. Nurnberg, S. Weber, Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement, Am. J. Hum. Genet. 79 (2006) 949–957.

[69] P.J. Kausalya, S. Amasheh, D. Gunzel, H. Wurps, D. Muller, M. Fromm, W. Hunziker, Disease-associated mutations affect intracellular traffic and paracellular Mg^{2+} transport function of claudin-16, J. Clin. Invest. 116 (2006) 878–891.

[70] D.B. Simon, Y. Lu, K.A. Choate, H. Velazquez, E. Al-Sabban, M. Praga, G. Casari, A. Bettinelli, G. Colussi, J. Rodriguez-Soriano, D. McCredie, D. Milford, S. Sanjad, R.P. Lifton, Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption, Science 285 (1999) 103–106.

[71] B.L. Daugherty, C. Ward, T. Smith, J.D. Ritzenthaler, M. Koval, Regulation of heterotypic claudin compatibility, J. Biol. Chem. 282 (2007) 30005–30013.

[72] M. Wattenhofer, A. Reymond, V. Falciola, A. Charollais, D. Caille, C. Borel, R. Lyle, X. Estivill, M.B. Petersen, P. Meda, S.E. Antonarakis, Different mechanisms preclude mutant CLDN14 proteins from forming tight junctions in vitro, Human Mutat. 25 (2005) 543–544.

[73] T. Ahrens, O. Pertz, D. Haussinger, C. Fauser, T. Schulthess, J. Engel, Analysis of heterophilic and homophilic interactions of cadherins using the c-Jun/c-Fos dimerization domains, J. Biol. Chem. 277 (2002) 19455–19460.

[74] M. Furuse, H. Sasaki, S. Tsukita, Manner of interaction of heterogeneous claudin species within and between tight junction strands, J. Cell Biol. 147 (1999) 891–903.

[75] K. Morita, H. Sasaki, M. Furuse, S. Tsukita, Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells, J. Cell Biol. 147 (1999) 185–194.

[76] K. Morita, M. Furuse, Y. Yoshida, M. Itoh, H. Sasaki, S. Tsukita, Y. Miyachi, Molecular architecture of tight junctions of periderm differs from that of the maculae occludentes of epidermis, J. Invest. Dermatol. 118 (2002) 1073–1079.

[77] F.D. Nunes, L.N. Lopez, H.W. Lin, C. Davies, R.B. Azevedo, A. Gow, B. Kachar, Distinct subdomain organization and molecular composition of a tight junction with adherens junction features, J. Cell Sci. 119 (2006) 4819–4827.

[78] K. Morita, H. Sasaki, K. Fujimoto, M. Furuse, S. Tsukita, Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis, J. Cell Biol. 145 (1999) 579–588.

[79] T. Miyamoto, K. Morita, D. Takemoto, K. Takeuchi, Y. Kitano, T. Miyakawa, K. Nakayama, Y. Okamura, H. Sasaki, Y. Miyachi, M. Furuse, S. Tsukita, Tight junctions in Schwann cells of peripheral myelinated axons: a lesson from claudin-19-deficient mice, J. Cell Biol. 169 (2005) 527–538.

[80] Y. Kiuchi-Saishin, S. Gotoh, M. Furuse, A. Takasuga, Y. Tano, S. Tsukita, Differential expression patterns of claudins, tight junction membrane proteins, in mouse nephron segments, J. Am. Soc. Nephrol. 13 (2002) 875–886.

[81] H. Wolburg, K. Wolburg-Buchholz, J. Kraus, G. Rascher-Eggstein, S. Liebner, S. Hamm, F. Duffner, E.H. Grote, W. Risau, B. Engelhardt, Localization of claudin-3 in tight junctions of the blood-brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme, Acta Neuropathol. (Berl.) 105 (2003) 586–592.

[82] N. Sonoda, M. Furuse, H. Sasaki, S. Yonemura, J. Katahira, Y. Horiguchi, S. Tsukita, *Clostridium perfringens* enterotoxin fragment removes specific claudins from tight junction strands: evidence for direct involvement of claudins in tight junction barrier, J. Cell Biol. 147 (1999) 195–204.

[83] A. Nusrat, G.T. Brown, J. Tom, A. Drake, T.T.T. Bui, C. Quan, R.J. Mrsny, Multiple protein interactions involving proposed extracellular loop domains of the tight junction protein occludin, Mol. Biol. Cell 16 (2005) 1725–1734.

[84] D. Juretic, L. Zoranic, D. Zucic, SPLIT4, J. Chem. Inf. Comput. Sci. 42 (2002) 620–632.

[85] G.E. Tusnády, I. Simon, HMMTOP2, Bioinformatics 17 (2001) 849–850.

[86] A.L.B. Krogh, G. von Heijne, E.L.L. Sonnhammer, TMHMM2, J. Mol. Biol. 305 (2001) 567–580.

[87] C.M. Van Itallie, J.M. Anderson, The molecular physiology of tight junction pores, Physiology 19 (2004) 331–338.

[88] A.S.L. Yu, Claudins and epithelial paracellular transport: the end of the beginning, Curr. Opin. Nephrol. Hypertens. 12 (2003) 503–509.

[89] C.M. Van Itallie, J.M. Anderson, The role of claudins in determining paracellular charge selectivity, Proc. Am. Thorac. Soc. 1 (2004) 38–41.
[90] J.A. Cuff, M.E. Clamp, A.S. Siddiqui, M. Finlay, G.J. Barton, Jpred: a consensus secondary structure prediction server, Bioinformatics 14 (1998) 892–893.
[91] Y. Schmitz, H. Wolburg, Gap junction morphology of retinal horizontal cells is sensitive to pH alterations in vitro, Cell Tissue Res. 263 (1991) 303–310.
[92] G. Perkins, D. Goodenough, G. Sosinsky, Three-dimensional structure of the gap junction connexon, Biophys. J. 72 (1997) 533–544.
[93] J.C. Saez, V.M. Berthoud, M.C. Branes, A.D. Martinez, E.C. Beyer, Plasma membrane channels formed by connexins: their regulation and functions, Physiol. Rev. 83 (2003) 1359–1400.
[94] S. Zeissig, N. Bürgel, D. Günzel, J. Richter, J. Mankertz, U. Wahnschaffe, A.J. Kroesen, M. Zeitz, M. Fromm, J.D. Schulzke, Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease, Gut 56 (2007) 61–72.
[95] M.J. Evans, T. von Hahn, D.M. Tscherne, A.J. Syder, M. Panis, B. Wolk, T. Hatziioannou, J.A. McKeating, P.D. Bieniasz, C.M. Rice, Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry, Nature 446 (2007) 801–805.
[96] K. Fujita, J. Katahira, Y. Horiguchi, N. Sonoda, M. Furuse, S. Tsukita, *Clostridium perfringens* enterotoxin binds to the second extracellular loop of claudin-3, a tight junction integral membrane protein, FEBS Lett. 476 (2000) 258–261.
[97] J.L. Reyes, M. Lamas, D. Martin, M. del Carmen Namorado, S. Islas, J. Luna, M. Tauc, L. Gonzalez-Mariscal, The renal segmental distribution of claudins changes with development, Kidney Int. 62 (2002) 476–487.
[98] J.J. Laurila, T. Karttunen, V. Koivukangas, P.A. Laurila, H. Syrjala, J. Saarnio, Y. Soini, T.I. Ala-Kokko, Tight junction proteins in gallbladder epithelium: different expression in acute acalculous and calculous cholecystitis, J. Histochem. Cytochem. 55 (2007) 567–573.
[99] N. Sakai, H. Chiba, H. Fujita, Y. Akashi, M. Osanai, T. Kojima, N. Sawada, Expression patterns of claudin family of tight-junction proteins in the mouse prostate, Histochem. Cell Biol. 27 (2007) 457–462.
[100] Y. Zhu, M. Brannstrom, P.O. Janson, K. Sundfeldt, Differences in expression patterns of the tight junction proteins, claudin 1, 3, 4 and 5, in human ovarian surface epithelium as compared to epithelia in inclusion cysts and epithelial ovarian tumours, Int. J. Cancer 118 (2006) 1884–1891.
[101] S. Liebner, A. Fischmann, G. Rascher, F. Duffner, E.H. Grote, H. Kalbacher, H. Wolburg, Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme, Acta Neuropathol. (Berl.) 100 (2000) 323–231.
[102] H. Wolburg, K. Wolburg-Buchholz, S. Liebner, B. Engelhardt, Claudin-1, claudin-2 and claudin-11 are present in tight junctions of choroid plexus epithelium of the mouse, Neurosci. Lett. 307 (2001) 77–80.
[103] P.D. Leotlela, M.S. Wade, P.H. Duray, M.J. Rhode, H.F. Brown, D.T. Rosenthal, S.K. Dissanayake, R. Earley, F.E. Indig, B.J. Nickoloff, D.D. Taub, O.P. Kallioniemi, P. Meltzer, P.J. Morin, A.T. Weeraratna, Claudin-1 overexpression in melanoma is regulated by PKC and contributes to melanoma cell motility, Oncogene 26 (2007) 3846–3856.
[104] N. Miwa, M. Furuse, S. Tsukita, N. Niikawa, Y. Nakamura, Y. Furukawa, Involvement of claudin-1 in the beta-catenin/Tcf signaling pathway and its frequent upregulation in human colorectal cancers, Oncol. Res. 12 (2001) 469–476.
[105] S. Hadj-Rabia, L. Baala, P. Vabres, D. Hamel-Teillac, E. Jacquemin, M. Fabre, S. Lyonnet, Y. De Prost, A. Munnich, M. Hadchouel, A. Smahi, Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease, Gastroenterology 127 (2004) 1386–1390.
[106] K. Morita, S. Tsukita, Y. Miyachi, Tight junction-associated proteins (occludin, ZO-1, claudin-1, claudin-4) in squamous cell carcinoma and Bowen's disease, Br. J. Dermatol. 151 (2004) 328–334.
[107] G.M. Sheehan, B.V. Kallakury, C.E. Sheehan, H.A. Fisher, R.P. Kaufman Jr., J.S. Ross, Loss of claudins-1 and -7 and expression of claudins-3 and -4 correlate with prognostic variables in prostatic adenocarcinomas, Hum. Pathol. 38 (2007) 564–569.
[108] C. Rahner, L.L. Mitic, J.M. Anderson, Heterogeneity in expression and subcellular localization of claudins 2, 3, 4, and 5 in the rat liver, pancreas, and gut, Gastroenterology 120 (2001) 411–422.
[109] S.S. de Oliveira, I.M. de Oliveira, W. De Souza, J.A. Morgado-Diaz, Claudins upregulation in human colorectal cancer, FEBS Lett. 579 (2005) 6179–6185.
[110] S.L. Kominsky, M. Vali, D. Korz, T.G. Gabig, S.A. Weitzman, P. Argani, S. Sukumar, *Clostridium perfringens* enterotoxin elicits rapid and specific cytolysis of breast carcinoma cells mediated through tight junction proteins claudin 3 and 4, Am. J. Pathol. 164 (2004) 1627–1633.
[111] J. Katahira, H. Sugiyama, N. Inoue, Y. Horiguchi, M. Matsuda, N. Sugimoto, *Clostridium perfringens* enterotoxin utilizes two structurally related membrane proteins as functional receptors in vivo, J. Biol. Chem. 272 (1997) 26652–26658.
[112] H. Sirotkin, B. Morrow, B. Saint-Jore, A. Puech, R. DasGupta, S.R. Patanjali, A. Skoultchi, S.M. Weissman, R. Kucherlapati, Identification, characterization, and precise mapping of a human gene encoding a novel membrane-spanning protein from the 22q11 region deleted in velo-cardio-facial syndrome, Genomics 42 (1997) 245–251.
[113] L.S. Nichols, R. Ashfaq, C.A. Iacobuzio-Donahue, Claudin 4 protein expression in primary and metastatic pancreatic cancer: support for use as a therapeutic target, Am. J. Clin. Pathol. 121 (2004) 226–230.
[114] K. Turksen, T.C. Troy, Permeability barrier dysfunction in transgenic mice overexpressing claudin 6, Development 129 (2002) 1775–1784.
[115] M. Go, T. Kojima, K. Takano, M. Murata, S. Ichimiya, H. Tsubota, T. Himi, N. Sawada, Expression and function of tight junctions in the crypt epithelium of human palatine tonsils, J. Histochem. Cytochem. 52 (2004) 1627–1638.
[116] W.Y. Li, C.L. Huey, A.S. Yu, Expression of claudin-7 and -8 along the mouse nephron, Am. J. Physiol. Renal Physiol. 286 (2004) F1063–F1071.
[117] S.L. Kominsky, P. Argani, D. Korz, E. Evron, V. Raman, E. Garrett, A. Rein, G. Sauter, O.P. Kallioniemi, S. Sukumar, Loss of the tight junction protein claudin-7 correlates with histological grade in both ductal carcinoma in situ and invasive ductal carcinoma of the breast, Oncogene 22 (2003) 2021–2033.
[118] A.E. Al Moustafa, M.A. Alaoui-Jamali, G. Batist, M. Hernandez-Perez, C. Serruya, L. Alpert, M.J. Black, R. Sladek, W.D. Foulkes, Identification of genes associated with head and neck carcinogenesis by cDNA microarray comparison between matched primary normal epithelial and squamous carcinoma cells, Oncogene 21 (2002) 2634–2640.
[119] A.H. Johnson, H.F. Frierson, A. Zaika, S.M. Powell, J. Roche, S. Crowe, C.A. Moskaluk, W. El-Rifai, Expression of tight-junction protein claudin-7 is an early event in gastric tumorigenesis, Am. J. Pathol. 167 (2005) 577–584.
[120] E.R. Wilcox, Q.L. Burton, S. Naz, S. Riazuddin, T.N. Smith, B. Ploplis, I. Belyantseva, T. Ben-Yosef, N.A. Liburd, R.J. Morell, B. Kachar, D.K. Wu, A.J. Griffith, S. Riazuddin, T.B. Friedman, Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29, Cell 104 (2001) 165–172.
[121] D. Mueller, P.J. Kausalya, F. Claverie-Martin, I.C. Meij, P. Eggert, V. Garcia-Nieto, W. Hunziker, A novel claudin 16 mutation associated with childhood hypercalciuria abolishes binding to ZO-1 and results in lysosomal mistargeting, Am. J. Hum. Genet. 73 (2003) 1293–1301.
[122] S. Michlig, S. Damak, J. Le Coutre, Claudin-based permeability barriers in taste buds, J. Comp. Neurol. 502 (2007) 1003–1011.
[123] Y. Sanada, N. Oue, Y. Mitani, K. Yoshida, H. Nakayama, W. Yasui, Down-regulation of the claudin-18 gene, identified through serial analysis of gene expression data analysis, in gastric cancer with an intestinal phenotype, J. Pathol. 208 (2006) 633–642.
[124] S. Hasegawa, Y. Furukawa, M. Li, S. Satoh, T. Kato, T. Watanabe, T. Katagiri, T. Tsunoda, Y. Yamaoka, Y. Nakamura, Genome-wide analysis of gene expression in intestinal-type gastric cancers using a complementary DNA microarray representing 23,040 genes, Cancer Res. 62 (2002) 7012–7017.
[125] M. Furuse, K. Furuse, H. Sasaki, S. Tsukita, Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin–Darby canine kidney I cells, J. Cell Biol. 153 (2001) 263–272.