Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1778 (2008) 757–769
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbamem

Review

Interactions of tight junctions with membrane channels and transporters

Sigrid A. Rajasekaran [a], Klaus W. Beyenbach [b], Ayyappan K. Rajasekaran [a,c,*]

[a] *The Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA 90095, USA*
[b] *Department of Biomedical Sciences, Cornell University, Ithaca, NY 14853, USA*
[c] *Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA 90095, USA*

Received 20 June 2007; received in revised form 2 November 2007; accepted 9 November 2007
Available online 19 November 2007

Abstract

Tight junctions are unique organelles in epithelial cells. They are localized to the apico-lateral region and essential for the epithelial cell transport functions. The paracellular transport process that occurs via tight junctions is extensively studied and is intricately regulated by various extracellular and intracellular signals. Fine regulation of this transport pathway is crucial for normal epithelial cell functions. Among factors that control tight junction permeability are ions and their transporters. However, this area of research is still in its infancy and much more needs to be learned about how these molecules regulate tight junction structure and functions. In this review we have attempted to compile literature on ion transporters and channels involved in the regulation of tight junctions.

Keywords: Tight junction; Septate junction; Na,K-ATPase; Channel; Transporter

Contents

* Corresponding author. Nemours Center for Childhood Cancer Research, Alfred I. DuPont Hospital for Children, Wilmington, DE 19803, USA. Tel.: +1 302 651 6593; fax: +1 302 651 6539.
E-mail address: araj@medsci.udel.edu (A.K. Rajasekaran).

0005-2736/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2007.11.007

Epithelial cells form highly polarized boundaries between biological compartments to regulate the movements of water, ions, and other solutes between them. Ion and solute transport across an epithelial cell layer can be either via transcellular or paracellular pathways. Crucial for the highly regulated and coordinated paracellular transport are tight junctions, which are continuous networks of interconnected parallel strands at the apical cell poles of adjacent cells. At these sites the membranes of neighboring cells appear to touch resembling kisses according to Diamond [1] (Fig. 1). The two major functions of the tight junction are the gate and the fence function. The fence function serves to block the intramembrane diffusion of proteins and macromolecules between the apical and basolateral membrane domain while the gate function controls the paracellular pathway. In addition to these functions, recent studies indicate that tight junctions also act as scaffolding platforms for cell signaling as well as docking stations for transport vesicles [2,3]. Dysregulation of the barrier function of epithelial as well of endothelial tight junctions occur in a variety of diseases e.g. chronic inflammatory diseases such as inflammatory bowel disease, multiple sclerosis and allergies, microbial infectious diseases, diabetes and its complications, and cancer [4–6].

Tight junctions are dynamic structures with multi-protein complexes consisting of transmembrane proteins and peripheral membrane proteins. Scaffolding proteins link these proteins to the cytoskeleton, a crucial player in tight junction function [6], while others are involved in signal transduction [2,3]. The barrier properties of tight junctions can be altered either acutely or long-term by physiological and pathological factors. These factors include growth factors (EGF, HGF, VEGF, FGF, TGF-β), cytokines (TNF-α, various interleukins), hormones (glucocorticoids, estrogen, dihydrotestosterone, retinoic acid, C-type natriuretic peptide), drugs and nutrients [2,5,7,8]. The pathways how these factors induce changes in tight junction permeability are diverse. Mechanisms include endocytosis of tight junction proteins, insertion of newly synthesized junctional proteins or altered protein–protein interactions. Post-translational modifications such as phosphorylation appear to be key in regulating these mechanisms. For example tyrosine kinases (src, c-yes), threonine/serine kinases (protein kinase C, protein kinase A, WNK4), phosphatases (protein phosphatase 2A), small GTPases (Rho family, Rabs) as to name a few, have all been described to be involved in phosphorylation of tight junction proteins [9,10].

Properties of the epithelial paracellular barrier include electrical resistance and solute permeability, which can be assessed via measurements of the transepithelial electrical resistance (TER), transepithelial ionic diffusion potentials and the diffusion of labeled charged or un-charged tracer molecules of various sizes [11]. TER measurements allow for assessing fast and continuous changes of the paracellular conductance. Appropriate control studies must be undertaken to distinguish between transcellular and paracellular contributions to the transepithelial resistance. Measurements of transepithelial ionic diffusion potentials can assess the ionic permselectivity of the tight junction pathway while measurements of diffusion of tracer molecules have the given advantage of being size selective. However, due to the duration of the latter assay, vesicular transcellular pathways such as endo- and exo-cytosis and transcytosis might be complicating factors, although the size selectivity of the permeability is generally regarded as proof of paracellular transport [12]. Nevertheless, using TER and tracer diffusion studies as complementary methods are a good measure for paracellular permeability.

Recent studies have shown that Na,K-ATPase, a P-type ATPase, is involved in the regulation of tight junction structure and function. In addition, other ion transporters and channels have been identified in having a function to modulate tight junction structure and paracellular permeability. The purpose of this article is to review these new developments and the emerging role of channels and transporters in tight junction function.

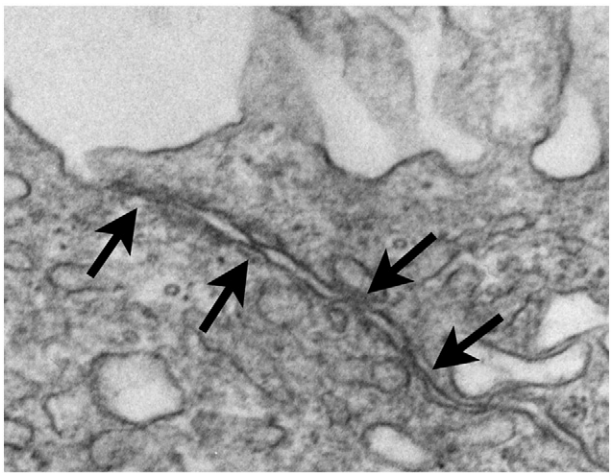

Fig. 1. Electron micrograph of the epithelial tight junction. Arrows indicate the kissing points of the tight junctions of two adjacent cells in primary cultures of human retinal pigment epithelial cells.

1. Na,K-ATPase localizes to and regulates tight junction functions

The Na,K-ATPase catalyzes the ATP-dependent transport of three sodium ions out and two potassium ions into the cell per pump cycle, thereby generating the sodium as well as potassium gradients across the plasma membrane. The function of this enzyme is crucial to maintain intracellular homeostasis. The Na,K-ATPase is a heterodimer consisting of an α- and a β-subunit [13,14] and an optional γ-subunit [15]. The α-subunit (~112 kDa) is the catalytic subunit while both the β-subunit (50–60 kDa) and the γ-subunit (~7 kDa) are modulators of Na,K-ATPase activity. While it is well known that the Na,K-ATPase is critical for the vectorial transport across epithelial cells, recent work indicates an additional role, namely in the formation of tight junctions and in the regulation of tight junction structure and permeability. Among all the ion transporters and channels, the Na,K-ATPase is the most studied as it relates to the regulation of tight junction structure and functions [16].

1.1. Na,K-ATPase and tight junction formation

Studies by our laboratory have shown that the enzymatic function of the Na,K-ATPase is required for epithelial polarization that crucially depends on the formation of the tight junctions [17]. Using MDCK cells in a calcium switch as model system, two independent methods of Na,K-ATPase inhibition (ouabain and potassium depletion) have confirmed that the Na,K-ATPase enzymatic function is necessary for the formation of tight junctions. One of the proteins affected by Na,K-ATPase inhibition was RhoA, a small GTPase required for the formation of stress fibers [18]. Inhibition of Na,K-ATPase resulted in a reduced stress fiber content of the MDCK cells. The effect of Na,K-ATPase inhibition was to a large extent reversed by expressing exogenous wild type RhoA. As inhibition of Na,K-ATPase activity results in a concomitant increase in intracellular sodium, the observed results were mimicked by the sodium ionophore gramicidin [17]. Interestingly, increased glucose uptake via the Na^+-glucose transporter SGLT-1 is accompanied by an increase in intracellular sodium and a decrease in TER (see below). It is tempting to speculate that changes in intracellular sodium concentration during epithelial polarization might affect the assembly of tight junctions.

At this time, the exact molecular mechanisms associated with Na,K-ATPase's role in tight junction formation remain elusive. As a first hypothesis, the Na,K-ATPase might function synergistically with the cell adhesion molecule E-cadherin in assembling the tight junction. E-cadherin is a Ca^{2+}-dependent cell–cell adhesion molecule expressed in epithelial cells shown to be important for tight junction formation [19,20]. In support of this hypothesis are the observations that restoration of E-cadherin expression alone or Na,K-ATPase β-subunit expression alone in Maloney sarcoma virus-transformed (MSV)-MDCK cells was not sufficient to induce an epithelial phenotype [21]. Instead, the exogenous expression of both E-cadherin and Na,K-ATPase β-subunit, together with the restoration of Na,K-ATPase activity and low intracellular sodium levels, were sufficient to induce functional tight junctions and epithelial polarity in this transformed cell line [21]. It is also important to note that Na,K-ATPase β-subunit itself can function as a cell–cell adhesion molecule [21–25]. While further experiments are necessary to investigate how the Na,K-ATPase affects the assembly of tight junctions, we suggest that the Na,K-ATPase is involved in the regulation of RhoA-mediated polymerization of actin. The active polymerization of actin may then provide the necessary force to mobilize tight junction strands at the apico-lateral region in order to establish functional tight junctions [16].

1.2. Na,K-ATPase in established epithelial monolayers

The Na,K-ATPase evidently also plays a role in regulating tight junction permeability in established epithelial monolayers with functional tight junctions. Inhibition of Na,K-ATPase activity resulted in increased permeability to both ions and non-ionic molecules in polarized primary cultures of human retinal pigment epithelial cells [26] and in the well-differentiated pancreatic ductal carcinoma cell line HPAF-II [27]. The associated changes in tight junction structure were evident only at the ultrastructural level with reduced numbers of kissing points and condensation of tight junction strands. While no change in the localization of tight junction proteins was observed at the light microscopy level, biochemical studies revealed the hyperphosphorylation of the tight junction protein occludin due to the inhibition of protein phosphatase 2A (PP2A) [27]. PP2A is a serine/threonine protein phosphatase known to be localized to tight junctions, to associate with and dephosphorylate occludin, and to regulate tight junction function in MDCK cells [28]. In contrast, in HPAF-II cells PP2A was not associated with occludin but rather co-immunoprecipitated with both, Na,K-ATPase α- and β-subunits [27], that was also observed in human A549 cells expressing wild type rat Na,K-ATPase α-subunit [29]. Furthermore, immunoelectron microscopy revealed the Na,K-ATPase β-subunit at the apical junctional complex in addition to its described basolateral localization [27]. While additional experiments are necessary, we suggest that PP2A, occludin and Na,K-ATPase form a microdomain at the apical junctional complex that – via altering the phosphorylation status of occludin – regulates tight junction permeability depending on the cytoplasmic ionic milieu of cells.

1.3. Na,K-ATPase and tight junctions in development

Studies in the laboratory of Watson have established a critical role for Na,K-ATPase in the regulation of tight junctions during mouse development [30]. Inhibition of Na,K-ATPase by either ouabain or potassium depletion prevented the formation of functional tight junctions and increased tight junction permeability which was accompanied by a discontinuous staining pattern of ZO-1 and occludin [31]. Specific knockdown of Na,K-ATPase β_1-subunit in mouse one-cell zygotes by an RNAi approach inhibited blastocyst formation, also accompanied by altered localization of the tight junction proteins ZO-1 and occludin [32].

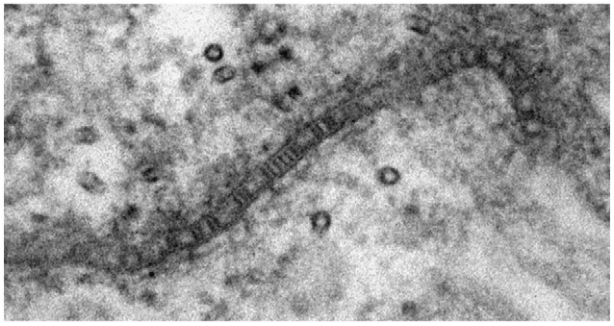

Fig. 2. The septate junction in Malpighian (renal) tubules of the yellow fever mosquito *Aedes aegypti*.

In zebra fish the α1β1 subunit of Na,K-ATPase is encoded by the heart and mind (had) locus which is required for heart morphogenesis and brain ventricle formation during embryonic development [33–35]. A recent study showed that the Had/Na, K-ATPase ion transport activity is necessary for the maintenance of apical ZO-1 junction belts during heart morphogenesis [36].

Together these studies document the essential role of the Na, K-ATPase in the development and function of tight junctions. Part of this essential role appears to involve the intracellular ion homeostasis as necessary condition for maintaining epithelial integrity.

2. Septate junctions

Septate junctions in invertebrates are the functional equivalent of tight junctions in vertebrates [37]. Both are part of the paracellular transport pathway in epithelial tissues. While tight junctions appear as sites where the plasma membranes of two cells touch, septate junctions maintain a cleft of about 15 nm between the two cells. The cleft may be smooth (continuous) or bridged (pleated) with variably spaced groups of septa [38] as shown in Fig. 2. One hypothesis views pleated regions as a barrier to the transepithelial movement of solute [39]. If this is so, then the septa must be rather dynamic, opening and closing to meet the salt and water challenges of the animal [40]. Another hypothesis poses the idea that septa bridge the cytoplasm of adjacent cells, like gap junctions [41].

In physiological studies of transepithelial transport, the laboratory of Beyenbach has come across a highly dynamic paracellular pathway in Malpighian tubules of the yellow fever mosquito (Beyenbach, 2003 #104). Under control conditions the isolated tubule secretes an approximately isosmotic fluid consisting largely of NaCl, KCl and water (Fig. 3a). The secretion takes place against lumen-positive transepithelial voltages of 59 mV across an epithelium with a transepithelial resistance of 58 Ωcm^2 [42]. The cations Na^+ and K^+ are secreted into the tubule lumen against their electrochemical potentials via energy-dependent transepithelial transport pathways through principal cells of the tubule (Fig. 3a). Significantly, transcellular active transport of Na^+ and K^+ is powered by the V-type H^+ATPase and not the classical Na^+/K^+ATPase (Fig. 3a).

The literature on the development of tight and septate junctions is substantial [2,16,43–52]. Upon differentiation of the epithelium some transcriptional mechanisms are probably used for the normal turnover of tight junction proteins or the remodeling of the tight junction as, for example, secretory crypt cells in the intestine become absorptive tip cells in the course of a few days [53]. In contrast, the hormone-induced, switch-like on/off behavior of the Cl^- conductance observed in septate junctions of Malpighian tubules must reflect post-translational modifications (Fig. 3). The conformational change of a septate junction protein that brings about the sudden increase in the paracellular Cl^- conductance may be particularly well developed in the female mosquito challenged by the enormous salt and water load of the blood meal. The smaller the animal, the greater the threat of desiccation. The threat of desiccation is opposed by a moderately tight renal epithelium (the Malpighian tubule) that serves the conservation of body fluids. Here, septate junctions have primarily a barrier function, and secondarily a permeation function for the homeostatic turnover of the extracellular fluid compartment (Fig. 3a). However, after the

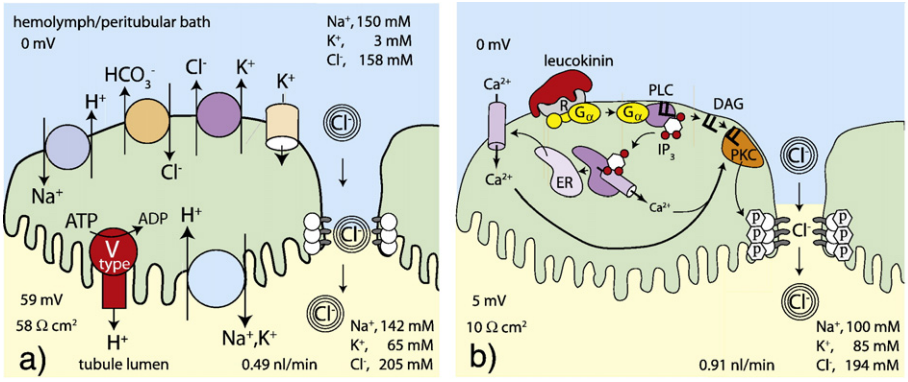

Fig. 3. Effects of the diuretic peptide leucokinin on paracellular Cl^- secretion in Malpighian tubules of the yellow fever mosquito. (a) Cl^- passes through an aqueous pathway with low conductance under control conditions; (b) Cl^- passes through an ionic pathway with high conductance after the tubule is stimulated with the diuretic peptide leucokinin. The signaling pathway of leucokinin leads to the hypothetical phosphorylation of septate junctional proteins. R, G-protein coupled receptor; Gα, subunit of G-protein; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; IP_3, inositol triphosphate; ER, calcium-depleted endoplasmic reticulum; P, phosphate. Data from [40,42,141–143].

mosquito has ingested a volume of blood ten times her own body weight, a leaky epithelium with a high paracellular Cl^- conductance is useful in the elimination of the unwanted salt and water fraction of the blood meal (Fig. 3b). Indeed, the high rates of isosmotic NaCl and KCl secretion by Malpighian tubules stimulated with the diuretic peptide leucokinin, reflect the wide functional dynamic range of the septate junction.

The gene for the Na/K ATPase is present in the yellow fever mosquito, however the enzyme activity (ATP hydrolysis) is not detected in Malpighian tubules [54]. What is more, the diuretic hormone leucokinin continues to increase the Cl^- conductance of the paracellular pathway during metabolic inhibition with cyanide, when the cytoplasmic ATP has dropped from 0.9 mM to 0.08 mM in Malpighian tubules [55]. Apparently, enzyme activity of the Na/K ATPase and normal ATP concentrations are not required for mediating the sudden, switch-like increase in paracellular Cl^- conductance upon stimulation by leucokinin (Fig. 3b). If the Na/K ATPase is expressed in Malpighian tubules of the yellow fever mosquito, then it may have ATP- and transport-independent functions, as also suggested by recent studies in Drosophila septate junctions [56,57].

In Drosophila, both α-(ATPα) and β-(NRV2) subunits of the sodium pump are concentrated at the septate junctions. Mutations in either one of these genes are associated with a structural loss of septate junctions accompanied by the disruption of the paracellular barrier of the embryonic salivary gland. The two subunits of the Na/K ATPase are part of a protein complex consisting of Coracle, Neurexin, Gliotactin, and Neuroglian localized to the septate junctions [58]. Besides the salivary gland, Na,K-ATPase was also found to be essential for septate junction formation and function in epithelia from Drosophila trachea and epidermis and in the control of the tube-size of the trachea [59]. In a later study, the formation of septate junctions and the diameter of the tubes was found to be independent of the pump function of the Na,K-ATPase [57].

The proteins forming and associated with septate and tight junctions are remarkably conserved considering that insects and vertebrates last shared a common ancestor at least 600 million years ago [60]. Table 1 lists some of the junctional proteins that are thought to define the permselectivity of the paracellular transport pathway. These can broadly be grouped into a) transmembrane proteins that may define the barrier/permselectivity/conductance properties of the paracellular pathway, and b) cytoplasmic proteins that structurally and functionally link these integral membrane proteins to the cytoskeleton, to signal pathways to and from the junction, and to the nucleus.

Table 1
Proteins of the invertebrate septate junction and the vertebrate tight junction, adopted from [132]

Septate junctional protein		Tight junctional protein	Reference
Sinuous (Sinu)	transmembrane	claudin	[64]
Megatrachea (Mega)	transmembrane	claudin	[63]
Coracle (Cor)	transmembrane	Band 4.1 s	[133,134]
Na/K ATPase, (ATPα)	transmembrane	Na/K ATPase α subunit	[17,27,59]
Na/K ATPase, β subunit (Nrv2)	transmembrane	Na/K ATPase, β subunit	[21,27,57]
Disc large (Dlg)	cytoplasmic	Dlg, ZO-1	[135,136]
Scribble (Scrib)	cytoplasmic	Scribble	[137]
Lethal giant larvae (Lgl)	cytoplasmic	Lgl	[138]
Neurexin	cytoplasmic	Caspr/Paranodin	[139,140]

The most significant advance in understanding how ions pass through tight junction was made when the laboratory of Lifton discovered by positional cloning mutations in paracellin-1 (claudin-16), that render the tight junction of the human thick ascending limb of the Loop of Henle permeable to Mg^{2+} [61]. This manifests itself in the profound Mg^{2+} wasting in the autosomal recessive disease of familial renal hypomagnesamia. Mutational analysis of the extracellular domain of claudin-4 and-15 supported a model in which claudins act as charge-selective channels in the paracellular space [62]. As to gating such an extracellular ion pore, the conformational change of claudin consequent to phosphorylation and dephosphorylation can be imagined to change the charge/size selectivity filter to yield ion-permeable and -impermeable states. Alternatively, a conformational change could turn an aqueous route with low conductance for Cl^- under control conditions to an ionic route with high Cl^- conductance in the presence of the diuretic hormone leucokinin (Fig. 3). Significantly, two claudin-like proteins, sinuous and megatrachea, have been localized at the septate junction in insect epithelia suggesting that septate and tight junctions may have a common molecular basis [63,64].

3. A conserved role for the Na,K-ATPase in tight junctions

The recent flurry of papers on the role of Na,K-ATPase in tight and septate junction structure and function demonstrates that Na,K-ATPase has a conserved role in regulating junctions in vertebrates and insects. The next step will be to decipher how the signals from this ion transporter are transmitted in order to regulate the structure and function of junctions. We can envision that the Na,K-ATPase enzyme activity and the associated intracellular ion homeostasis might target the phosphorylation status of key tight junction protein/s leading to alteration in tight junction permeability. In addition, Na,K-ATPase subunits by themselves, either α or β or both together, independent of the enzyme's ion transport function might regulate tight junctions by direct or indirect association with tight junction proteins. However, in the light of the recent studies, it is quite possible that both enzyme activity and subunit interactions are involved in the regulation of tight junction function.

Experimental evidence suggests that the Na,K-ATPase α-subunit associates with signaling molecules such as IPR1/3 [65], the p85 subunit of phosphatidylinositol-3-kinase [66,67], PP2A [27,29], Src [68] and PLC-γ1 [65]. The Na,K-ATPase β-subunit subunit binds to annexin II [66] and PP2A [27]. By forming a multiprotein complex with these signaling molecules and the actin cytoskeleton, directly via the α-subunit/ankyrin/spectrin [69,70] and indirectly via the β-subunit/annexin II [66] complex, the Na,K-ATPase could either facilitate the formation of or function itself as a scaffolding platform for cell signaling in the vicinity of tight junctions (Fig. 4). Indeed, PP2A [28], annexin II [71] and the Na,K-ATPase β-subunit [27] all have been found

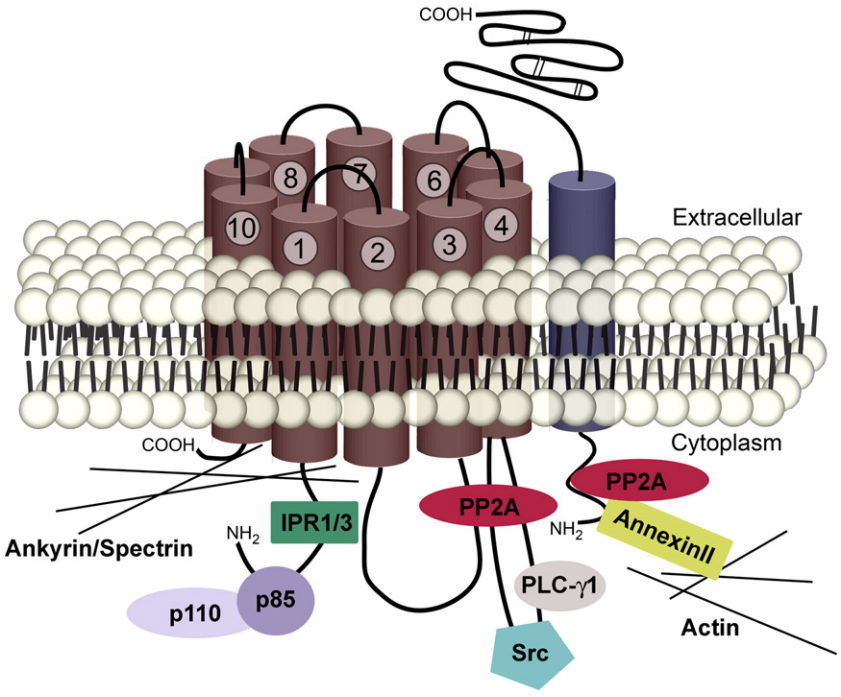

Fig. 4. Model of Na,K-ATPase as a scaffolding signaling platform.

localized to tight junctions. This signaling scaffolding complex might sense changes in the intracellular ionic milieu, probably changes in Na^+ concentrations to modulate tight junction structure and function in response. Whether the Na,K-ATPase in this complex is enzymatically active or whether it serves as an independent pool in the role of a signaling molecule rather than an ion transporter, remains to be determined. Such a "non-pumping" pool has recently been identified in LLC-PK1 cells, a porcine kidney proximal tubule cell line that forms tight junctions [72]. The α-subunit of the Na,K-ATPase has at least three binding sites for phosphates, that could serve as a local phosphate donor/acceptor. Physically associated with other junctional proteins, the α-subunit could donate phosphate to or receive phosphate from junctional proteins locally, in order to regulate tight junction permeability, independently of the cytoplasmic phosphate and ATP concentrations.

4. Other channels and transporters associated with and/or regulating tight junctions and paracellular permeability

Next to the Na,K-ATPase several channels and ion transporters have been implicated in the regulation of TJ permeability. Some of these are described below.

4.1. Na^+-glucose cotransport and other Na^+-nutrient transporters

In both small intestine and renal tubules, the absorption of nutrients and water are critically dependent on the intact epithelium. While a substantial amount of absorption occurs via active epithelial transport, it has been suggested that paracellular glucose transport could modulate transcellular nutrient absorption [73,74]. The paracellular glucose transport is probably the best-described coupling between an apical transporter and paracellular permeability.

4.1.1. SGLT-1

SGLT-1, found in intestinal enterocytes and in the late proximal tubule of the kidney, is a Na^+-glucose co-transporter belonging to the SLC5 gene family, a sodium/glucose cotransporter family with 220 or more members in animals and bacteria [75]. Glucose and Na^+ enter the cells together via the apical SGLT using the potential energy inherent in the Na^+ gradient across the cell membrane. On the transcellular route, glucose exits the cell across the basolateral membrane via the glucose transporter GLUT2 or by exocytosis, and Na^+ is pumped out of the cell via the basolateral Na,K-ATPase. However, apical glucose uptake through SGLT-1 also induced a drop in TER [76,77] and increased the flux of small molecules through small paracellular pores. The Na^+-glucose cotransport-dependent regulation of paracellular permeability has not only been described in vivo in rats and in cultures of Caco-2 cells [78] but also in healthy human subjects [79]. In mucosal sheets of mammalian small intestinal epithelium, reduced TER in cells with increased glucose uptake was accompanied by the localized disruption of the tight junction strands and the condensation of the microfilaments within the perijunctional actomyosin ring [76,80]. In addition, immunoelectron microscopy revealed a spatial dissociation between ZO-1 and the morphologically identified tight junctions [81].

The changes in paracellular permeability and tight junction morphology in the presence of active Na^+-glucose cotransport

were associated with increased phosphorylation of the perijunctional myosin II regulatory light chain (MLC) [78,82]. Activation of the regulatory MLC acts as a biochemical marker of actomyosin contraction as phosphorylated myosin associated with actin induces filament sliding and contraction [83]. While MLC phosphorylation upon expression of constitutively active MLC kinase resulted in the redistribution of ZO-1 and occludin and in increased tight junction permeability quantitatively similar to those following Na^+-glucose cotransport [84], the connection between Na^+-glucose cotransport and MLC phosphorylation remains to be determined [12].

4.1.2. Na^+/H^+ exchanger NHE3

Transepithelial transport of glucose through SGLT-1 results in net Na^+ and glucose absorption, but is also accompanied by Cl^-, HCO_3^- and water uptake into the epithelial cell. The resulting mild cytoplasmic alkalinization and cell swelling upon activation of the SGLT-1 Na^+-glucose cotransport results in activation of the apical NHE_3 Na^+/H^+ exchanger as a regulatory response. Indeed, the inhibition of Na^+/H^+ exchange by amiloride in Caco-2 monolayers resulted in an increase of TER and decreased MLC phosphorylation [85]. However, in these studies NHE_3 did not influence basal TER in the absence of active SGLT-1, suggesting that NHE_3-mediated Na^+/H^+ exchange and Na^+-glucose transport pathways may interact in regulating paracellular permeability.

4.2. K^+-ATP channels

K^+-ATP channels are ATP-sensitive K^+ channels that are widely distributed throughout mammalian tissues and play important roles in coupling cell metabolic status to electrical activity [86]. K^+-ATP channels are protein complexes composed of the Kir6.0 subunit (a member of the inwardly rectifying K^+ channel subfamily Kir6.0) and the sulfonylurea receptor (SUR) subunit (a member of the ATP-binding cassette (ABC) superfamily). Kir6/SUR complexes are known to be involved in the regulation of the gap junction permeability [87]. Recent studies suggested an additional role in the regulation of epithelial tight junction permeability [88]. Kir6.1/SUR2A complexes are restricted to tight junctions in human gastric mucosa and co-immuno-precipitate and colocalize with occludin in the liver [88]. In rat small intestine tolbutamide, a blocker of the SUR component of the Kir6 ion channel permeability, provoked an increase in tight junction permeability, whereas the activation of SUR with diazoxide resulted in decreased paracellular permeability. Whether the paracellular permeability changes mediated by the K^+-ATP channel are associated with changes in cellular energy status remains to be determined.

4.3. Chloride channels

Anion channels allow the diffusion of negatively charged ions along their electrochemical gradient. They are often called chloride channels since chloride is the most abundant anion in organisms. Nevertheless these channels may conduct other ions, sometimes even better than chloride [89]. The plasma membrane chloride channels function in volume regulation and ionic homeostasis and transepithelial transport. Chloride channels might play an additional role in paracellular permeability, since some of their family members have been localized to tight junctions (see below).

4.3.1. ClC-2 chloride channel

ClC-2 is a member of the ClC chloride channel family and its activity is regulated by a variety of factors that include hyperpolarization, hypotonicity-induced cell swelling and low extracellular pH [89]. ClC-2 has a wide tissue distribution, with highest expression in the brain, kidney and intestine. While the ClC-2 channel is localized to the apical membrane of airway epithelial cells [90] and to the basolateral membrane in mouse colon [91], most notable is its predominant localization to tight junctions in intestinal epithelial cells [91] and Caco-2 cells [92]. Interestingly, Moeser et al. [93] reported recently that activation of ClC-2 using lubiprostone stimulated rapid recovery of TER and significantly reduced paracellular permeability in ischemia-injured porcine intestinal mucosa. In addition, during recovery, in ischemic tissues treated with lubiprostone occludin was localized exclusively to the tight junction whereas it was diffuse in untreated tissue. However, how the unique localization of ClC-2 functions to regulate the permeability barrier of the tight junction remains to be determined [94].

4.3.2. CLIC chloride intracellular channels

Chloride intracellular channels are structurally distinct from the members of the CLIC chloride channel family and are unique in being able to assume both soluble and membrane forms [95]. While recently CLIC4 has been shown to form poorly selective, redox-regulated ion channels [96], the functions of the CLIC family members remain poorly understood. In an interesting study, Berryman and Goldenring [97] showed that in addition to its localization to the centrosome and midbody, CLIC4 co-localizes with the tight junction protein ZO-1 in the apical region of polarized epithelial cells, and was not detected on the lateral plasma membrane. Another member of the CLIC family, CLIC5 has been shown to be in a protein complex containing F-actin, ezrin, α-actinin and other proteins associated with the cortical actin cytoskeleton [98,99]. Whether CLIC channels regulate tight junctions through regulating the cortical actin cytoskeleton, an essential component of functional tight junctions, is not known. Given CLIC's unique features as channel proteins that are both soluble and membrane bound and their possible function during mitosis and cytokinesis [97], these channels might also be involved in the complex and poorly understood regulation of tight junctions and cell polarity during cell division.

4.3.3. Cystic fibrosis transmembrane conductance regulator (CFTR)

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel localized to the apical membrane and is expressed most prominently in cells of the intestine, airways, secretory glands, bile ducts and epididymis. While the correct apical localization of CFTR depends on its

interaction with apical PDZ proteins including EBP50/ezrin [100,101], microscopic studies on HT-29 monolayers revealed proximity but no co-localization of CFTR with ZO-1 [102]. While the localizations of ClC-2 and CLIC-4 to tight junctions are well documented, to our knowledge, a role of CFTR in tight junction function is not known.

4.4. Non-selective cation channels of the transient receptor potential (TRP) superfamily

The superfamily of transient receptor potential (TRP) channels includes more than 20 related cation channels that may be grouped into six mammalian subfamilies (TRPA, TRPC, TRPM, TRPML, TRPP and TRPV) based on their amino acid sequence homology. TRP channels are calcium-permeable cation channels with polymodal activation properties and mediate the transmembrane flux of cations down their electrochemical gradients, thereby raising intracellular Ca^{2+} and Na^{+} concentrations and depolarizing the cell. Most TRP channels seem to be responsive to multiple distinct stimuli and they might function as cellular sensors and signal integrators (for a comprehensive review on TRP channels see [103]).

4.4.1. TRPV4 and TRPV1

TRPV4 is a constitutively active Ca^{2+}-permeable cation channel that can be activated by phorbol esters and endocannabinoid anandamide in addition to moderate heating and changes in extracellular osmolarity. TRPV4 can be found in many different epithelial cells that include airway epithelia, plexus choroideus, endothelial cells, keratinocytes of the skin, sweat gland, cochlear hair cells and in kidney, where it localizes to the basolateral side of water-impermeant nephron segments. Recently, it has been shown that activation of TRPV4 by phorbol ester in the mouse mammary gland cell line HC11, resulted in a TER decrease [104]. Although the initial TER decrease was due to changes in transcellular conductance, it was accompanied by a slower increase in paracellular permeability for small molecules that could be blocked by ruthenium red, a TRPV channel inhibitor. TRPV4 did not colocalize with occludin at the apical pole but was found at the lateral plasma membrane codistributing with E-cadherin. Nevertheless, activation of TRPV4 resulted in a rearrangement of the tight junction structure with break in the tight junction strands and decreases in the expression of claudins, most strikingly claudin-4.

While there is no evidence for a functional role of TRPV4 as a regulator of paracellular permeability *in vivo*, it has been recently reported that activation of another member of the TRPV subfamily, the capsaicin receptor TRPV1, results in the disruption of the blood–brain barrier in rats [105]. It is possible that activation of TRPV1 alters tight junction permeability of cerebrovascular cells like TRPV4.

4.4.2. TRPC4 and TRPC1

TRPC4, which is highly homologous to TRPC5, functions as nonselective cation channel that is activated by the Gq/11 family of GPCRs and receptor tyrosine kinases. PLC enzymatic activity and intracellular Ca^{2+} are required for channel activation. TRPC4 is widely distributed, but is highly expressed in the central nervous system. In cultured human fetal astrocytes, TRPC4 is expressed on the plasma membrane with a distinctive focal staining pattern similar to ZO-1 [106]. TRPC4 colocalized with ZO-1 in immunofluorescence and immunogold labeled cells. Further co-immunoprecipitation and GST pull-down experiments confirmed the association of TRPC4 with ZO-1 through the TRL motif of TRPC4 and the first PDZ domain of ZO-1. These molecular interactions have been suggested as part of a signaling complex at junctional sites of astrocytes involved in Ca^{2+} homeostasis. In isolated and cultured lung endothelial cells from mice, thrombin exposure results in a decrease of TER. However, cells isolated from TRPC4 knockout mice lacked this response [107]. In addition, lung vascular permeability in response to thrombin did not increase in TRPC4 knockout mice to the degree observed in control mice. The intracellular Ca^{2+}-influx through TRPC4 and Ca^{2+}-dependent actin reorganization might be involved in regulating tight junction permeability in vivo [107]. Whether this link between thrombin, a potent proinflammatory mediator, TRPC4 and the impairment of tight junction function in chronic inflammatory disease has biological significance remains to be determined.

Although it was the first mammalian TRP channel identified, the function of TRPC1 is still enigmatic. When expressed in heteromeric complexes with TRPC3, 4, or 5, TRPC1 functions as part of a Gq/11 receptor-operated cation channel. TNF-α-induced and cDNA expression studies point to a role of TRPC1 as being a key determinant of endothelial permeability response [108]. In human artery endothelial cells (HPAEC), TNF-α exposure increases TRPC1 expression without significantly altering expression of other TRPC isoforms. Thrombin challenge in TNF-α treated HPAEC cells decreased TER to a greater extend compared to control cells. Similarly, in TRPC1 transfected HMEC cells, thrombin produced a higher decrease in TER than in control cells and the decrease persisted for a prolonged time. Therefore, not only the activity but also expression levels of TRPC channels might be important in the regulation of paracellular permeability. Thus, tight junction regulation by TRP channels is an emerging idea and should add an exciting dimension to the field.

4.5. Human ATP binding cassette (ABC) transporters

ABC (ATP binding cassette) proteins form one of the largest protein families, defined by the presence of the ABC unit with two short conserved peptide motifs involved in ATP binding (Walker A and Walker B) and a third conserved sequence, the 'ABC signature'. Most ABC proteins are membrane transporters being responsible for the translocation of various substrates, such as lipids, bile salts, toxic compounds, and peptides, across membranes. Most ABC proteins are active pumps, however, some family members are channels, such as the chloride channel CFTR and sensors, such as the sulphonylurea receptors (SUR1 and SUR2) that function as intracellular ATP sensors regulating the permeability of potassium channels (for review see [109–111]). Although there is a wealth of knowledge on transcellular transport functions of ABC transporters, studies on

a potential role in the regulation of tight junction function and paracellular permeability are still in their infancy. Only recently, the ABC transporter Mrp3, a member of the ABCC subfamily of multidrug resistance-associated proteins (MRPs), was described to be co-localized with ZO-1 in mouse choroid plexus, intestine and kidney [112]. Mrp3 is expressed in the pancreas, adrenal gland, placenta, gut, liver, kidney and prostate. It has been proposed to play a role in protecting the liver from accumulation of bile salts, which is reflected in its substrate specificity of conjugated bile salts and monovalent bile salts such as cholate, taurocholate, and glycocholate. The significance of Mrp3 colocalization to ZO-1 is unknown. Further, it remains to be determined whether ABC transporters are as important for paracellular transport as they are for transcellular transport. It is possible that regulation of the paracellular transport requires a higher degree of transport of ions, which could explain that the best described function of an ABC transporter in this regard is the subfamily of SUR proteins that associate with the K^+-ATP channel Kir6.0 subunit (see above). In addition, Mrp3 is to our knowledge the only other ABC transporter described to be localized at junctional complexes.

4.6. Aquaporins

Water transport across epithelial barriers can occur via transcellular and paracellular routes. While water can be transported by varying degrees across the membrane by co-transporters such as the Na^+-glucose transporter [113], aquaporins are capable of high rates of water transport independently of solute transport. The permeability characteristics and distribution of aquaporins in tissues reflects their role in the regulation of water homeostasis (for review see [114]). The twelve aquaporins known to date can be divided into two groups, one whose members are only permeated by water and second, aquaglyceroporins, that are permeated by water and other small solutes, in particular glycerol. Aquaporin-5 (AQP5), a member of the first group of aquaporins, is localized to the apical plasma membrane in salivary glands, lacrimal glands, and airway epithelium, plays an important role in fluid secretion. Recently, Kawedia et al. [115] reported that the decrease in transcellular water transport by deletion of AQP5 affects paracellular permeability in parotid glands of AQP5 knockout mice. While there were only small differences in tight junction strands between control and AQP5−/− mice, a significant decrease in claudin-7 and occludin expression was observed in the knockout mice. While the molecular pathways remain elusive, these studies indicated that AQP5 could function to link paracellular and transcellular pathways. These findings also support the hypothesis that aquaporins can regulate paracellular water transport as proposed by the Hill laboratory [116,117] as well as the Fischbarg's laboratory [118].

4.7. Connexins

Gap junctions, composed of the channel-forming integral membrane proteins termed connexins, mediate cell-to-cell communication coordinating numerous physiological processes of multicellular systems. Connexins, of which about 20 have been identified, form intercellular protein channels between adjacent cells to allow the direct exchange of ions and small molecules important for the rapid transmission of action potentials in heart and neuronal tissues, the diffusion of metabolites and nutrients, and the diffusion of second messengers. Besides the connexin–connexin interactions to form gap junctions, diverse other proteins have been found to associate with connexins (for review see [119–122]). One of these proteins is the tight junction protein ZO-1. Interaction and/or colocalization of connexins with ZO-1 have been described for connexins Cx30, Cx31.9, Cx32, Cx36, Cx43, Cx45, Cx46, Cx47, and Cx50 (reviewed in [122]). Cx32 has also been found to associate with ZO-2 [123] and Cx45 with ZO-3 [124].

It is generally believed that ZO-1 could function in organizing gap junctions and/or recruiting signaling molecules that regulate gap junction intercellular communication. The fact, however, that tight junction proteins other than ZO-1 interact with connexins points to a possible role of connexins in tight junction function. Cx32 was found not only to colocalize and associate with ZO-1 and ZO-2 but also with occludin and claudin-1 [123,125]. Cx26 interacted with the coiled-coil domain of occludin [126], and Cx40 and Cx43 were shown to associate with occludin and claudin-5 in addition to ZO-1 [127]. In freeze fracture replica, gap junction plaques were closely associated with a well-developed network of tight junction strands in hepatocytes [123].

Connexins are not only structurally but also functionally associated with tight junction proteins. Expression of Cx32 in the immortalized mouse hepatocyte cell line CHST8 induced the expression of the tight junction proteins such as occludin, claudin-1 and claudin-2 [125,128] and of MAGI-1, a member of the membrane associated guanylate kinase (MAGUK) localized to tight junctions [129]. Further, Cx32 expression in mouse hepatocytes increased the number of tight junction strands [125], accompanied by reduced paracellular permeability to inulin and mannitol [128,129] and polarized distribution of BODIPY-spingomyelin [128]. Treatment with the gap junctional intercellular communication inhibitor 18β-glycyrrhetinic acid (18β-GA), on the other hand, resulted in decreases of occludin and claudin-1 at the cell-cell border [128]. Similar, expression of Cx26 in Caco-2 cells resulted in reduced mannitol permeability, and increased TER and claudin-4 expression [130]. Inhibition of the gap junctional intercellular communication by 18β-GA abolished the effects of Cx26 expression on paracellular permeability, suggesting that the function of gap junctions also affects tight junction function. Interestingly, a subsequent study from Sawada's group [131] showed that overexpression of Cx26 in Calu-3 cells prevented the downregulation of the barrier and fence functions of tight junctions induced by the Na,K-ATPase inhibitor ouabain. Whether Na,K-ATPase functions through connexins in the regulation of tight junction function remains to be determined.

5. Conclusions

Tight junctions are crucial for epithelial cell functions. Depending on the extracellular and intracellular environment,

epithelial cells have adopted intriguing mechanisms to rapidly change the structure and permeability of tight junctions. Although growth factors, cytokines, hormones, drugs and nutrients are described to have specific roles in the regulation of tight junction permeability, the studies described in this review strongly indicate that ions and ion transporters are also involved in the regulation of tight junction structure and functions. These transporters might function by being physically present at the junctions and modulating a local ionic milieu or changes in the ionic content of the cells might transmit signals to eventually alter tight junction structure and functions. As indicated in this review our understanding of the roles of most of the transporters in the regulation of tight junction structure and function are still in infancy and detailed future research in this area should enable us to understand the intricate mechanisms by which ions regulate tight junctions.

Acknowledgements

This work was supported by DK56216 to A.K.R. and NSF IOB-0542797 to K.W.B. We thank Dr. Sonali Barwe for useful suggestions and critical reading of the manuscript.

References

[1] J.M. Diamond, Tight and leaky junctions of epithelia: a perspective on kisses in the dark, Fed. Proc. 33 (1974) 2220–2224.
[2] E.E. Schneeberger, R.D. Lynch, The tight junction: a multifunctional complex, Am. J. Physiol., Cell Physiol. 286 (2004) C1213–C1228.
[3] K. Shin, V.C. Fogg, B. Margolis, Tight junctions and cell polarity, Annu. Rev. Cell Dev. Biol. 22 (2006) 207–235.
[4] M. Cereijido, R.G. Contreras, D. Flores-Benitez, C. Flores-Maldonado, I. Larre, A. Ruiz, L. Shoshani, New diseases derived or associated with the tight junction, Arch. Med. Res. 38 (2007) 465–478.
[5] N.S. Harhaj, D.A. Antonetti, Regulation of tight junctions and loss of barrier function in pathophysiology, Int. J. Biochem. Cell Biol. 36 (2004) 1206–1237.
[6] J.R. Turner, Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application, Am. J. Pathol. 169 (2006) 1901–1909.
[7] M.G. Laukoetter, M. Bruewer, A. Nusrat, Regulation of the intestinal epithelial barrier by the apical junctional complex, Curr. Opin. Gastroenterol. 22 (2006) 85–89.
[8] A. Nusrat, J.R. Turner, J.L. Madara, Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells, Am. J. Physiol.; Gasterointest. Liver Physiol. 279 (2000) G851–G857.
[9] G.J. Feldman, J.M. Mullin, M.P. Ryan, Occludin: structure, function and regulation, Adv. Drug Deliv. Rev. 57 (2005) 883–917.
[10] C.M. Van Itallie, J.M. Anderson, Claudins and epithelial paracellular transport, Annu. Rev. Physiol. 68 (2006) 403–429.
[11] L.G. Johnson, Applications of imaging techniques to studies of epithelial tight junctions, Adv. Drug Deliv. Rev. 57 (2005) 111–121.
[12] A. Kapus, K. Szaszi, Coupling between apical and paracellular transport processes, Biochem. Cell. Biol. 84 (2006) 870–880.
[13] G.E. Shull, L.K. Lane, J.B. Lingrel, Amino-acid sequence of the beta-subunit of the (Na++K+)ATPase deduced from a cDNA, Nature 321 (1986) 429–431.
[14] G.E. Shull, A. Schwartz, J.B. Lingrel, Amino-acid sequence of the catalytic subunit of the (Na++K+)ATPase deduced from a complementary DNA, Nature 316 (1985) 691–695.
[15] P. Beguin, X. Wang, D. Firsov, A. Puoti, D. Claeys, J.D. Horisberger, K. Geering, The gamma subunit is a specific component of the Na,K-ATPase and modulates its transport function, EMBO J. 16 (1997) 4250–4260.
[16] A.K. Rajasekaran, S.A. Rajasekaran, Role of Na-K-ATPase in the assembly of tight junctions, Am. J. Physiol., Renal Physiol. 285 (2003) F388–F396.
[17] S.A. Rajasekaran, L.G. Palmer, S.Y. Moon, A. Peralta Soler, G.L. Apodaca, J.F. Harper, Y. Zheng, A.K. Rajasekaran, Na,K-ATPase activity is required for formation of tight junctions, desmosomes, and induction of polarity in epithelial cells, Mol. Biol. Cell 12 (2001) 3717–3732.
[18] A.J. Ridley, A. Hall, The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors, Cell 70 (1992) 389–399.
[19] B. Gumbiner, B. Stevenson, A. Grimaldi, The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex, J. Cell Biol. 107 (1988) 1575–1587.
[20] M. Takeichi, Cadherins: a molecular family important in selective cell–cell adhesion, Ann. Rev. Biochem. 59 (1990) 237–252.
[21] S.A. Rajasekaran, L.G. Palmer, K. Quan, J.F. Harper, W.J. Ball Jr., N.H. Bander, A. Peralta Soler, A.K. Rajasekaran, Na,K-ATPase beta-subunit is required for epithelial polarization, suppression of invasion, and cell motility, Mol. Biol. Cell 12 (2001) 279–295.
[22] S.P. Barwe, S. Kim, S.A. Rajasekaran, J.U. Bowie, A.K. Rajasekaran, Janus model of the Na,K-ATPase beta-subunit transmembrane domain: distinct faces mediate alpha/beta assembly and beta-beta homo-oligomerization, J. Mol. Biol. 365 (2007) 706–714.
[23] N. Kitamura, M. Ikekita, T. Sato, Y. Akimoto, Y. Hatanaka, H. Kawakami, M. Inomata, K. Furukawa, Mouse Na+/K+-ATPase beta1-subunit has a K+-dependent cell adhesion activity for beta-GlcNAc-terminating glycans, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 2796–2801.
[24] L. Shoshani, R.G. Contreras, M.L. Roldan, J. Moreno, A. Lazaro, M.S. Balda, K. Matter, M. Cereijido, The polarized expression of Na+,K+-ATPase in epithelia depends on the association between beta-subunits located in neighboring cells, Mol. Biol. Cell 16 (2005) 1071–1081.
[25] O. Vagin, E. Tokhtaeva, G. Sachs, The role of the beta1 subunit of the Na,K-ATPase and its glycosylation in cell–cell adhesion, J. Biol. Chem. 281 (2006) 39573–39587.
[26] S.A. Rajasekaran, J. Hu, J. Gopal, R. Gallemore, S. Ryazantsev, D. Bok, A.K. Rajasekaran, Na,K-ATPase inhibition alters tight junction structure and permeability in human retinal pigment epithelial cells, Am. J. Physiol., Cell Physiol. 284 (2003) C1497–C1507.
[27] S.A. Rajasekaran, S.P. Barwe, J. Gopal, S. Ryazantsev, E.E. Schneeberger, A.K. Rajasekaran, Na-K-ATPase regulates tight junction permeability through occludin phosphorylation in pancreatic epithelial cells, Am. J. Physiol.: Gasterointest. Liver Physiol. 292 (2007) G124–G133.
[28] V. Nunbhakdi-Craig, T. Machleidt, E. Ogris, D. Bellotto, C.L. White III, E. Sontag, Protein phosphatase 2A associates with and regulates atypical PKC and the epithelial tight junction complex, J. Cell Biol. 158 (2002) 967–978.
[29] E. Lecuona, L.A. Dada, H. Sun, M.L. Butti, G. Zhou, T.-L. Chew, J.I. Sznajder, Na,K-ATPase {alpha}1-subunit dephosphorylation by protein phosphatase 2A is necessary for its recruitment to the plasma membrane, FASEB J. 20 (2006) 2618–2620.
[30] G.M. Kidder, A.J. Watson, Roles of Na,K-ATPase in early development and trophectoderm differentiation, Semin. Nephrol. 25 (2005) 352–355.
[31] M.I. Violette, P. Madan, A.J. Watson, Na+/K+-ATPase regulates tight junction formation and function during mouse preimplantation development, Dev. Biol. 289 (2006) 406–419.
[32] P. Madan, K. Rose, A.J. Watson, Na/K-ATPase beta1 subunit expression is required for blastocyst formation and normal assembly of trophectoderm tight junction-associated proteins, J. Biol. Chem. 282 (2007) 12127–12134.
[33] L.A. Lowery, H. Sive, Initial formation of zebrafish brain ventricles occurs independently of circulation and requires the nagie oko and snakehead/atp1a1a.1 gene products, Development 132 (2005) 2057–2067.
[34] X. Shu, K. Cheng, N. Patel, F. Chen, E. Joseph, H.J. Tsai, J.N. Chen, Na, K-ATPase is essential for embryonic heart development in the zebrafish, Development 130 (2003) 6165–6173.
[35] S. Yuan, E.M. Joseph, The small heart mutation reveals novel roles of Na+/K+-ATPase in maintaining ventricular cardiomyocyte morphology and viability in zebrafish, Circ. Res. 95 (2004) 595–603.
[36] E. Cibrian-Uhalte, A. Langenbacher, X. Shu, J.N. Chen, S. Abdelilah-Seyfried, Involvement of zebrafish Na+,K+ATPase in myocardial cell junction maintenance, J. Cell Biol. 176 (2007) 223–230.

[37] M. Furuse, S. Tsukita, Claudins in occluding junctions of humans and flies, Trends Cell Biol. 16 (2006) 181–188.
[38] T.J. Bradley, A.M. Stuart, P. Satir, The ultrastructure of the larval malpighian tubules of a saline–water mosquito, Tissue Cell 14 (1982) 759–773.
[39] P. Satir, N.B. Gilula, The fine structure of membranes and intercellular communication in insects, Annu. Rev. Entomol. 18 (1973) 143–166.
[40] K.W. Beyenbach, Regulation of tight junction permeability with switch-like speed, Curr. Opin. Nephrol. Hypertens. 12 (2003) 543–550.
[41] N.B. Gilula, D. Branton, P. Satir, The septate junction: a structural basis for intercellular coupling, Proc. Natl. Acad. Sci. U. S. A. 67 (1970) 213–220.
[42] T.L. Pannabecker, T.K. Hayes, K.W. Beyenbach, Regulation of epithelial shunt conductance by the peptide leucokinin, J. Membr. Biol. 132 (1993) 63–76.
[43] S.T. Ballard, J.H. Hunter, A.E. Taylor, Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium, Annu. Rev. Nutr. 15 (1995) 35–55.
[44] J. Berkes, V.K. Viswanathan, S.D. Savkovic, G. Hecht, Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation, Gut 52 (2003) 439–451.
[45] K. Matter, M.S. Balda, Signalling to and from tight junctions, Nat. Rev., Mol. Cell Biol. 4 (2003) 225–236.
[46] D.A. Nguyen, M.C. Neville, Tight junction regulation in the mammary gland, J. Mammary Gland Biol. Neoplasia 3 (1998) 233–246.
[47] N. Sawada, M. Murata, K. Kikuchi, M. Osanai, H. Tobioka, T. Kojima, H. Chiba, Tight junctions and human diseases, Med. Electron Microsc. 36 (2003) 147–156.
[48] J. Stein, G. Kottra, Intestinal intercellular tight junctions. I. Structure and molecular mechanisms of regulation, Z. Gastroenterol. 35 (1997) 205–220.
[49] B.R. Stevenson, J.M. Anderson, S. Bullivant, The epithelial tight junction: structure, function and preliminary biochemical characterization, Mol. Cell. Biochem. 83 (1988) 129–145.
[50] U. Tepass, G. Tanentzapf, R. Ward, R. Fehon, Epithelial cell polarity and cell junctions in Drosophila, Annu. Rev. Genet. 35 (2001) 747–784.
[51] S. Tsukita, M. Furuse, M. Itoh, Multifunctional strands in tight junctions, Nat. Rev., Mol. Cell Biol. 2 (2001) 285–293.
[52] J.R. Turner, 'Putting the squeeze' on the tight junction: understanding cytoskeletal regulation, Semin. Cell Dev. Biol. 11 (2000) 301–308.
[53] 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.
[54] X.H. Weng, M. Huss, H. Wieczorek, K.W. Beyenbach, The V-type H(+)-ATPase in Malpighian tubules of *Aedes aegypti*: localization and activity, J. Exp. Biol. 206 (2003) 2211–2219.
[55] D.S. Wu, K.W. Beyenbach, The dependence of electrical transport pathways in Malpighian tubules on ATP, J. Exp. Biol. 206 (2003) 233–243.
[56] J.H. Kaplan, A moving new role for the sodium pump in epithelial cells and carcinomas, Sci. STKE 2005 (2005) pe31.
[57] S.M. Paul, M.J. Palladino, G.J. Beitel, A pump-independent function of the Na,K-ATPase is required for epithelial junction function and tracheal tube-size control, Development 134 (2007) 147–155.
[58] J.L. Genova, R.G. Fehon, Neuroglian, Gliotactin, and the Na+/K+ATPase are essential for septate junction function in Drosophila, J. Cell Biol. 161 (2003) 979–989.
[59] S.M. Paul, M. Ternet, P.M. Salvaterra, G.J. Beitel, The Na+/K+ATPase is required for septate junction function and epithelial tube-size control in the Drosophila tracheal system, Development 130 (2003) 4963–4974.
[60] M.J. Benton, F.J. Ayala, Dating the tree of life, Science 300 (2003) 1698–1700.
[61] 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 Mg2+ resorption, Science 285 (1999) 103–106.
[62] 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.
[63] M. Behr, D. Riedel, R. Schuh, The claudin-like megatrachea is essential in septate junctions for the epithelial barrier function in Drosophila, Dev. Cell 5 (2003) 611–620.
[64] V.M. Wu, J. Schulte, A. Hirschi, U. Tepass, G.J. Beitel, Sinuous is a Drosophila claudin required for septate junction organization and epithelial tube size control, J. Cell Biol. 164 (2004) 313–323.
[65] Z. Yuan, T. Cai, J. Tian, A.V. Ivanov, D.R. Giovannucci, Z. Xie, Na/K-ATPase tethers phospholipase C and IP3 receptor into a calcium-regulatory complex, Mol. Biol. Cell 16 (2005) 4034–4045.
[66] S.P. Barwe, G. Anilkumar, S.Y. Moon, Y. Zheng, J.P. Whitelegge, S.A. Rajasekaran, A.K. Rajasekaran, Novel role for Na,K-ATPase in phosphatidylinositol 3-kinase signaling and suppression of cell motility, Mol. Biol. Cell 16 (2005) 1082–1094.
[67] C.H. Pedemonte, R. Efendiev, A.M. Bertorello, Inhibition of Na,K-ATPase by dopamine in proximal tubule epithelial cells, Semin. Nephrol. 25 (2005) 322–327.
[68] M. Haas, H. Wang, J. Tian, Z. Xie, Src-mediated inter-receptor cross-talk between the Na+/K+-ATPase and the epidermal growth factor receptor relays the signal from ouabain to mitogen-activated protein kinases, J. Biol. Chem. 277 (2002) 18694–18702.
[69] P. Devarajan, D.A. Scaramuzzino, J.S. Morrow, Ankyrin binds to two distinct cytoplasmic domains of Na,K-ATPase alpha subunit, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 2965–2969.
[70] W.J. Nelson, P.J. Veshnock, Ankyrin binding to (Na++K+)ATPase and implications for the organization of membrane domains in polarized cells, Nature 328 (1987) 533–536.
[71] D.B. Lee, N. Jamgotchian, S.G. Allen, F.W. Kan, I.L. Hale, Annexin A2 heterotetramer: role in tight junction assembly, Am. J. Physiol., Renal Physiol. 287 (2004) F481–F491.
[72] M. Liang, J. Tian, L. Liu, S. Pierre, J. Liu, J. Shapiro, Z.J. Xie, Identification of a pool of non-pumping Na/K-ATPase, J. Biol. Chem. 282 (2007) 10585–10593.
[73] J.R. Pappenheimer, On the coupling of membrane digestion with intestinal absorption of sugars and amino acids, Am. J. Physiol. 265 (1993) G409–G417.
[74] J.R. Turner, Show me the pathway! Regulation of paracellular permeability by Na(+)-glucose cotransport, Adv. Drug Deliv. Rev. 41 (2000) 265–281.
[75] E.M. Wright, E. Turk, The sodium/glucose cotransport family SLC5, Pflugers Arch. 447 (2004) 510–518.
[76] J.L. Madara, J.R. Pappenheimer, Structural basis for physiological regulation of paracellular pathways in intestinal epithelia, J. Membr. Biol. 100 (1987) 149–164.
[77] J.R. Pappenheimer, Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters, J. Membr. Biol. 100 (1987) 137–148.
[78] J.R. Turner, B.K. Rill, S.L. Carlson, D. Carnes, R. Kerner, R.J. Mrsny, J.L. Madara, Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation, Am. J. Physiol. 273 (1997) C1378–C1385.
[79] J.R. Turner, D.E. Cohen, R.J. Mrsny, J.L. Madara, Noninvasive in vivo analysis of human small intestinal paracellular absorption: regulation by Na+-glucose cotransport, Dig. Dis. Sci. 45 (2000) 2122–2126.
[80] K. Atisook, S. Carlson, J.L. Madara, Effects of phlorizin and sodium on glucose-elicited alterations of cell junctions in intestinal epithelia, Am. J. Physiol. 258 (1990) C77–C85.
[81] K. Atisook, J.L. Madara, An oligopeptide permeates intestinal tight junctions at glucose-elicited dilatations. Implications for oligopeptide absorption, Gastroenterology 100 (1991) 719–724.
[82] J.J. Berglund, M. Riegler, Y. Zolotarevsky, E. Wenzl, J.R. Turner, Regulation of human jejunal transmucosal resistance and MLC phosphorylation by Na(+)-glucose cotransport, Am. J. Physiol.: Gasterointest. Liver Physiol. 281 (2001) G1487–G1493.
[83] A.P. Somlyo, A.V. Somlyo, Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase, Physiol. Rev. 83 (2003) 1325–1358.
[84] L. Shen, E.D. Black, E.D. Witkowski, W.I. Lencer, V. Guerriero, E.E. Schneeberger, J.R. Turner, Myosin light chain phosphorylation regulates

barrier function by remodeling tight junction structure, J. Cell Sci. 119 (2006) 2095–2106.
[85] J.R. Turner, E.D. Black, J. Ward, C.M. Tse, F.A. Uchwat, H.A. Alli, M. Donowitz, J.L. Madara, J.M. Angle, Transepithelial resistance can be regulated by the intestinal brush-border Na(+)/H(+) exchanger NHE3, Am. J. Physiol., Cell Physiol. 279 (2000) C1918–C1924.
[86] N. Inagaki, S. Seino, ATP-sensitive potassium channels: structures, functions, and pathophysiology, Jpn. J. Physiol. 48 (1998) 397–412.
[87] A. Velasco, A. Tabernero, B. Granda, J.M. Medina, ATP-sensitive potassium channel regulates astrocytic gap junction permeability by a Ca2+-independent mechanism, Jpn. J. Physiol. 74 (2000) 1249–1256.
[88] T. Jons, D. Wittschieber, A. Beyer, C. Meier, A. Brune, A. Thomzig, G. Ahnert-Hilger, R.W. Veh, K+-ATP-channel-related protein complexes: potential transducers in the regulation of epithelial tight junction permeability, J. Cell Sci. 119 (2006) 3087–3097.
[89] T.J. Jentsch, V. Stein, F. Weinreich, A.A. Zdebik, Molecular structure and physiological function of chloride channels, Physiol. Rev. 82 (2002) 503–568.
[90] C.B. Murray, M.M. Morales, T.R. Flotte, S.A. McGrath-Morrow, W.B. Guggino, P.L. Zeitlin, CIC-2: a developmentally dependent chloride channel expressed in the fetal lung and downregulated after birth, Am. J. Respir. Cell Mol. Biol. 12 (1995) 597–604.
[91] K. Gyomorey, H. Yeger, C. Ackerley, E. Garami, C.E. Bear, Expression of the chloride channel ClC-2 in the murine small intestine epithelium, Am. J. Physiol., Cell Physiol. 279 (2000) C1787–C1794.
[92] R. Mohammad-Panah, K. Gyomorey, J. Rommens, M. Choudhury, C. Li, Y. Wang, C.E. Bear, ClC-2 contributes to native chloride secretion by a human intestinal cell line, Caco-2, J. Biol. Chem. 276 (2001) 8306–8313.
[93] A.J. Moeser, P.K. Nighot, K.J. Engelke, R. Ueno, A.T. Blikslager, Recovery of mucosal barrier function in ischemic porcine ileum and colon is stimulated by a novel agonist of the ClC-2 chloride channel, lubiprostone, Am. J. Physiol.: Gasterointest. Liver Physiol. 292 (2007) G647–G656.
[94] A.T. Blikslager, A.J. Moeser, J.L. Gookin, S.L. Jones, J. Odle, Restoration of barrier function in injured intestinal mucosa, Physiol. Rev. 87 (2007) 545–564.
[95] R.H. Ashley, Challenging accepted ion channel biology: p64 and the CLIC family of putative intracellular anion channel proteins (Review), Mol. Membr. Biol. 20 (2003) 1–11.
[96] H. Singh, R.H. Ashley, CLIC4 (p64H1) and its putative transmembrane domain form poorly selective, redox-regulated ion channels, Mol. Membr. Biol. 24 (2007) 41–52.
[97] M.A. Berryman, J.R. Goldenring, CLIC4 is enriched at cell–cell junctions and colocalizes with AKAP350 at the centrosome and midbody of cultured mammalian cells, Cell Motil. Cytoskelet. 56 (2003) 159–172.
[98] M. Berryman, A. Bretscher, Identification of a novel member of the chloride intracellular channel gene family (CLIC5) that associates with the actin cytoskeleton of placental microvilli, Mol. Biol. Cell 11 (2000) 1509–1521.
[99] M. Berryman, J. Bruno, J. Price, J.C. Edwards, CLIC-5A functions as a chloride channel in vitro and associates with the cortical actin cytoskeleton in vitro and in vivo, J. Biol. Chem. 279 (2004) 34794–34801.
[100] B.D. Moyer, J. Denton, K.H. Karlson, D. Reynolds, S. Wang, J.E. Mickle, M. Milewski, G.R. Cutting, W.B. Guggino, M. Li, B.A. Stanton, A PDZ-interacting domain in CFTR is an apical membrane polarization signal, J. Clin. Invest. 104 (1999) 1353–1361.
[101] D.B. Short, K.W. Trotter, D. Reczek, S.M. Kreda, A. Bretscher, R.C. Boucher, M.J. Stutts, S.L. Milgram, An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton, J. Biol. Chem. 273 (1998) 19797–19801.
[102] J.R. Broughman, L. Sun, S. Umar, J.H. Sellin, A.P. Morris, Chronic PKC-beta2 activation in HT-29 Cl.19a colonocytes prevents cAMP-mediated ion secretion by inhibiting apical membrane CFTR targeting, Am. J. Physiol.: Gasterointest. Liver Physiol. 291 (2006) G331–G344.
[103] I.S. Ramsey, M. Delling, D.E. Clapham, An introduction to TRP channels, Annu. Rev. Physiol. 68 (2006) 619–647.
[104] B. Reiter, R. Kraft, D. Gunzel, S. Zeissig, J.D. Schulzke, M. Fromm, C. Harteneck, TRPV4-mediated regulation of epithelial permeability, FASEB J. 20 (2006) 1802–1812.
[105] D.E. Hu, A.S. Easton, P.A. Fraser, TRPV1 activation results in disruption of the blood–brain barrier in the rat, Br. J. Pharmacol. 146 (2005) 576–584.
[106] X. Song, Y. Zhao, L. Narcisse, H. Duffy, Y. Kress, S. Lee, C.F. Brosnan, Canonical transient receptor potential channel 4 (TRPC4) co-localizes with the scaffolding protein ZO-1 in human fetal astrocytes in culture, Glia 49 (2005) 418–429.
[107] C. Tiruppathi, M. Freichel, S.M. Vogel, B.C. Paria, D. Mehta, V. Flockerzi, A.B. Malik, Impairment of store-operated Ca2+ entry in TRPC4(−/−) mice interferes with increase in lung microvascular permeability, Circ. Res. 91 (2002) 70–76.
[108] B.C. Paria, S.M. Vogel, G.U. Ahmmed, S. Alamgir, J. Shroff, A.B. Malik, C. Tiruppathi, Tumor necrosis factor-alpha-induced TRPC1 expression amplifies store-operated Ca2+ influx and endothelial permeability, Am. J. Physiol. Lung Cell. Mol. Physiol. 287 (2004) L1303–L1313.
[109] P. Borst, R.O. Elferink, Mammalian ABC transporters in health and disease, Ann. Rev. Biochem. 71 (2002) 537–592.
[110] R.G. Deeley, C. Westlake, S.P. Cole, Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins, Physiol. Rev. 86 (2006) 849–899.
[111] I. Klein, B. Sarkadi, A. Varadi, An inventory of the human ABC proteins, Biochim. Biophys. Acta 1461 (1999) 237–262.
[112] A. Soontornmalai, M.L. Vlaming, J.M. Fritschy, Differential, strain-specific cellular and subcellular distribution of multidrug transporters in murine choroid plexus and blood–brain barrier, Neuroscience 138 (2006) 159–169.
[113] D.D. Loo, E.M. Wright, T. Zeuthen, Water pumps, J. Physiol. 542 (2002) 53–60.
[114] L.S. King, D. Kozono, P. Agre, From structure to disease: the evolving tale of aquaporin biology, Nat. Rev., Mol. Cell Biol. 5 (2004) 687–698.
[115] J.D. Kawedia, M.L. Nieman, G.P. Boivin, J.E. Melvin, K. Kikuchi, A.R. Hand, J.N. Lorenz, A.G. Menon, Interaction between transcellular and paracellular water transport pathways through Aquaporin 5 and the tight junction complex, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3621–3626.
[116] A.E. Hill, B. Shachar-Hill, A new approach to epithelial isotonic fluid transport: an osmosensor feedback model, J. Membr. Biol. 210 (2006) 77–90.
[117] M. Murakami, K. Murdiastuti, K. Hosoi, A.E. Hill, AQP and the control of fluid transport in a salivary gland, J. Membr. Biol. 210 (2006) 91–103.
[118] J. Fischbarg, F.P. Diecke, P. Iserovich, A. Rubashkin, The Role of the Tight Junction in Paracellular Fluid Transport across Corneal Endothelium. Electro-osmosis as a Driving Force, J. Membr. Biol. 210 (2006) 117–130.
[119] M. Chanson, B.A. Kotsias, C. Peracchia, M. O'Grady, S, Interactions of connexins with other membrane channels and transporters, Prog. Biophys. Mol. Biol. 94 (2007) 233–244.
[120] B.N. Giepmans, Gap junctions and connexin-interacting proteins, Cardiovasc. Res. 62 (2004) 233–245.
[121] J.C. Herve, N. Bourmeyster, D. Sarrouilhe, Diversity in protein–protein interactions of connexins: emerging roles, Biochim. Biophys. Acta 1662 (2004) 22–41.
[122] J.C. Herve, N. Bourmeyster, D. Sarrouilhe, H.S. Duffy, Gap junctional complexes: From partners to functions, Prog. Biophys. Mol. Biol. 94 (2007) 29–65.
[123] T. Kojima, Y. Kokai, H. Chiba, M. Yamamoto, Y. Mochizuki, N. Sawada, Cx32 but not Cx26 is associated with tight junctions in primary cultures of rat hepatocytes, Exp. Cell Res. 263 (2001) 193–201.
[124] P.J. Kausalya, M. Reichert, W. Hunziker, Connexin45 directly binds to ZO-1 and localizes to the tight junction region in epithelial MDCK cells, FEBS Lett. 505 (2001) 92–96.
[125] T. Kojima, N. Sawada, H. Chiba, Y. Kokai, M. Yamamoto, M. Urban, G.H. Lee, E.L. Hertzberg, Y. Mochizuki, D.C. Spray, Induction of tight junctions in human connexin 32 (hCx32)-transfected mouse hepatocytes: connexin 32 interacts with occludin, Biochem. Biophys. Res. Commun. 266 (1999) 222–229.
[126] A. Nusrat, J.A. Chen, C.S. Foley, T.W. Liang, J. Tom, M. Cromwell, C. Quan, R.J. Mrsny, The coiled-coil domain of occludin can act to organize structural and functional elements of the epithelial tight junction, J. Biol. Chem. 275 (2000) 29816–29822.

[127] K. Nagasawa, H. Chiba, H. Fujita, T. Kojima, T. Saito, T. Endo, N. Sawada, Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells, J. Cell. Physiol. 208 (2006) 123–132.
[128] T. Kojima, D.C. Spray, Y. Kokai, H. Chiba, Y. Mochizuki, N. Sawada, Cx32 formation and/or Cx32-mediated intercellular communication induces expression and function of tight junctions in hepatocytic cell line, Exp. Cell Res. 276 (2002) 40–51.
[129] M. Murata, T. Kojima, T. Yamamoto, M. Go, K. Takano, H. Chiba, T. Tokino, N. Sawada, Tight junction protein MAGI-1 is up-regulated by transfection with connexin 32 in an immortalized mouse hepatic cell line: cDNA microarray analysis, Cell Tissue Res. 319 (2005) 341–347.
[130] H. Morita, T. Katsuno, A. Hoshimoto, N. Hirano, Y. Saito, Y. Suzuki, Connexin 26-mediated gap junctional intercellular communication suppresses paracellular permeability of human intestinal epithelial cell monolayers, Exp. Cell Res. 298 (2004) 1–8.
[131] M. Go, T. Kojima, K. Takano, M. Murata, J. Koizumi, M. Kurose, R. Kamekura, M. Osanai, H. Chiba, D.C. Spray, T. Himi, N. Sawada, Connexin 26 expression prevents down-regulation of barrier and fence functions of tight junctions by Na+/K+-ATPase inhibitor ouabain in human airway epithelial cell line Calu-3, Exp. Cell Res. 312 (2006) 3847–3856.
[132] V.M. Wu, G.J. Beitel, A junctional problem of apical proportions: epithelial tube-size control by septate junctions in the Drosophila tracheal system, Curr. Opin. Cell Biol. 16 (2004) 493–499.
[133] R.G. Fehon, I.A. Dawson, S. Artavanis-Tsakonas, A Drosophila homologue of membrane-skeleton protein 4.1 is associated with septate junctions and is encoded by the coracle gene, Development 120 (1994) 545–557.
[134] R.S. Lamb, R.E. Ward, L. Schweizer, R.G. Fehon, Drosophila coracle, a member of the protein 4.1 superfamily, has essential structural functions in the septate junctions and developmental functions in embryonic and adult epithelial cells, Mol. Biol. Cell 9 (1998) 3505–3519.
[135] E. Willott, M.S. Balda, A.S. Fanning, B. Jameson, C. Van Itallie, J.M. Anderson, The tight junction protein ZO-1 is homologous to the Drosophila discs-large tumor suppressor protein of septate junctions, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 7834–7838.
[136] D.F. Woods, P.J. Bryant, The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions, Cell 66 (1991) 451–464.
[137] D. Bilder, N. Perrimon, Localization of apical epithelial determinants by the basolateral PDZ protein Scribble, Nature 403 (2000) 676–680.
[138] D. Strand, I. Raska, B.M. Mechler, The Drosophila lethal(2)giant larvae tumor suppressor protein is a component of the cytoskeleton, J. Cell Biol. 127 (1994) 1345–1360.
[139] S. Baumgartner, J.T. Littleton, K. Broadie, M.A. Bhat, R. Harbecke, J.A. Lengyel, R. Chiquet-Ehrismann, A. Prokop, H.J. Bellen, A Drosophila neurexin is required for septate junction and blood–nerve barrier formation and function, Cell 87 (1996) 1059–1068.
[140] S.D. Carlson, J.L. Juang, S.L. Hilgers, M.B. Garment, Blood barriers of the insect, Annu. Rev. Entomol. 45 (2000) 151–174.
[141] M. Yu, K.W. Beyenbach, Leucokinin and the modulation of the shunt pathway in Malpighian tubules, J. Insect Physiol. 47 (2001) 263–276.
[142] M.J. Yu, K.W. Beyenbach, Effects of leucokinin-VIII on Aedes Malpighian tubule segments lacking stellate cells, J. Exp. Biol. 207 (2004) 519–526.
[143] M.J. Yu, K.W. Beyenbach, Leucokinin activates Ca(2+)-dependent signal pathway in principal cells of *Aedes aegypti* Malpighian tubules, Am. J. Physiol., Renal Physiol. 283 (2002) F499–F508.