Role of Na-K-ATPase in the assembly of tight junctions

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Rajasekaran, Ayyappan K., and Sigrid A. Rajasekaran. Role of Na-K-ATPase in the assembly of tight junctions. Am J Physiol Renal Physiol 285: F388–F396, 2003; 10.1152/ajprenal.00439.2002.—Na-K-ATPase, also known as the sodium pump, is a crucial enzyme that regulates intracellular sodium homeostasis in mammalian cells. In epithelial cells Na-K-ATPase function is also involved in the formation of tight junctions through RhoA GTPase and stress fibers. In this review, a new two-step model for the assembly of tight junctions is proposed: step 1, an E-cadherin-dependent formation of partial tight junction strands and of the circumferential actin ring; and step 2, active actin polymerization-dependent tethering of tight junction strands to form functional tight junctions, an event requiring normal function of Na-K-ATPase in epithelial cells. A new role for stress fibers in the assembly of tight junctions is proposed. Also, implications of Na-K-ATPase function on tight junction assembly in diseases such as cancer, ischemia, hypomagnesemia, and polycystic kidney disease are discussed.

E-cadherin; stress fibers; ischemia

THE VECTORIAL TRANSPORT FUNCTION (directional transport of molecules across an epithelial cell layer) of epithelial cells largely depends on the transepithelial flow of sodium ions (Na⁺). Na⁺ enters the cell down its electrochemical gradient through channels, exchangers, and cotransporters localized to the apical plasma membrane and is pumped out of the cell by Na-K-ATPase localized to the basolateral plasma membrane. Na-K-ATPase catalyzes an ATP-dependent transport of three Na⁺ ions out and two K⁺ ions into the cell per pump cycle, thereby generating the transmembrane Na⁺ gradient across the plasma membrane that is crucial to regulate the vectorial transport function of epithelial cells.

Na-K-ATPase is a heterodimer, consisting of an α- and β-subunit (reviewed in Ref. 56), but recent studies have demonstrated the presence of an additional γ-subunit (25, 67). The α-subunit (~112 kDa) is the catalytic subunit whereas both the β-subunit (50–60 kDa) and the γ-subunit (~7 kDa) have a modulatory role in Na-K-ATPase activity (4, 31, 64, 96, 97). Of the four α-subunit and the three β-subunit isoforms known, α₁ and β₁ are expressed in most epithelial tissues (reviewed in Refs. 56, 66, 69, and 94). In addition to the role of Na-K-ATPase in transepithelial transport, recent studies have demonstrated a role for Na-K-ATPase function in the assembly of junctional complexes in epithelial cells (83). This short review will focus on insights into how Na-K-ATPase, an ion pump, is involved in the assembly of tight junctions.

STRUCTURE AND MOLECULAR COMPOSITION OF TIGHT JUNCTIONS

Tight junctions (zonula occludens) form a continuous belt at the boundary between the apical and lateral plasma membrane domains of neighboring epithelial cells (Fig. 1A) and are structurally characterized by the close apposition of contiguous plasma membranes (23). They selectively regulate the passage of molecules across the paracellular space (Fig. 1B) (gate function) (21) and passively separate molecules into the apical and basolateral plasma membrane domains (fence function) (59). A tight junction is crucial in maintaining the polarized phenotype and the vectorial transport functions of epithelial cells. The current knowledge of tight junctions is consistent with a view that tight junctions are specialized membrane microdomains that might function as a molecular platform involved in cell signaling, vesicle protein docking, actin organization, and cell polarity in epithelial cells (reviewed in Refs. 68, 98, 99, and 109).

In freeze-fracture electron micrographs, tight junctions appear as anastomosing intramembranous particle strands localized at the apicalolateral side of polarized epithelial cells. The contacts between the tight junction strands of adjacent cells are in such close apposition as to effectively eliminate any intercellular space. These areas of intimate contact can be visualized as membrane contact points (kisses) in transmission electron micrographs (Fig. 1B). Each particle of the tight junction strand is composed of transmem-
brane proteins and cytoplasmic plaque proteins connected to the actin cytoskeleton (Fig. 1C). Occludins, claudins, and the junctional adhesion molecule (JAM) are the three transmembrane proteins localized to the tight junctions. Occludin was the first tight junction transmembrane protein identified (28) but appears to be unnecessary for the formation of tight junction strands (86). The JAM, a member of the immunoglobulin superfamily, appears to have roles in cell adhesion and extravasation of immune cells across tight junctions (7). Recently, claudins have been identified as membrane proteins localized to tight junctions and have been shown to be involved in the formation of tight junction strands (26, 30). Until now, 24 members of the claudin family have been described in various epithelial cells (98). Identification of occludin and claudins has tremendously increased our understanding of the structure and barrier function of tight junctions. Both occludin and claudin have four transmembrane domains and are involved in creating the paracellular barrier (2, 6, 18, 27, 63, 101, 105). The intracellular COOH terminus of both occludin (22, 29) and claudin (43) associates with cytoplasmic plaque proteins such as zonula occludens-1 (ZO-1) (92), ZO-2 (46), and ZO-3 (40). ZO-1 and ZO-2 associate with the actin binding proteins α-catenin (44, 45, 80) and spectrin (61) (Fig. 1C) and link the tight junction plaques to the actin cytoskeleton. Additional plaque proteins identified include cingulin (16), symplekin (49), AF-6 (106), and 7H6 (110). Proteins involved in signal transduction (c-src and c-yes), proteins involved in membrane traffic (VAP-33, Rab3b, Rab13, Rab 8, Sec6, and Sec8), and cell polarity-related proteins (Par3 and Par6) are present in the vicinity of tight junctions (reviewed in Refs. 68, 98, and 109). The structure, protein composition, and regulation of paracellular permeability of tight junctions have been recently described in further detail in excellent reviews (12, 58, 68, 75, 91, 98, 99).

**NA-K-ATPase Enzyme Activity Is Required for Tight Junction Formation**

The formation of tight junctions is a critical event in the biogenesis of polarized epithelial cells during early vertebrate development (reviewed in Ref. 24), during tubular and ductal development in epithelial tissues, and during recovery from tissue damage after ischemic or toxic injury. To understand the molecular events that take place during the formation of junction complexes in epithelial cells, Madin-Darby canine kidney (MDCK) cells have been extensively used (reviewed in Refs. 12 and 20). A Ca²⁺-switch assay (32) is a widely utilized method for investigating the mechanisms involved in the formation of tight junctions. In this approach, single-cell suspensions are allowed to attach to the substratum in a normal-Ca²⁺-containing medium for ~30 min. During this time, the cells are still round and do not establish polarity (Fig. 2). The attached cells are then transferred to a low-Ca²⁺ medium (<5 μM Ca²⁺) for ~12 h to allow for the formation of dense monolayers. The cells are then switched to a normal-Ca²⁺ medium, in which tight junctions and cell polarity will be established within 3 h. Thus the Ca²⁺-switch assay allows for the rapid monitoring of molecular events that occur during tight junction development (Fig. 2). Techniques including immunofluorescence localization of tight junction proteins, electron microscopy, and transepithelial electrical resistance (TER), an indicator of the paracellular permeability function

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**Fig. 1.** Schematic diagram of tight junctions in epithelial cells. **A:** localization of tight junction at the apicolateral side of polarized epithelial cells. **B:** organization of tight junction strands, each strand consisting of tight junction particles (beads). **C:** enlarged view of a tight junction particle showing membrane proteins connected to the cytoplasmic plaque proteins and actin cytoskeleton. For simplicity, only zonula occludens (ZO)-1 and ZO-2 are shown. α-Ct, α-catenin.

**Fig. 2.** Illustration of the calcium-switch assay used to study the role of Na-K-ATPase in the assembly of tight junctions.
of tight junctions, are used to monitor tight junction development.

With the use of these approaches, a recent study demonstrated that Na-K-ATPase activity is necessary for the formation of tight junctions and development of polarity in MDCK cells (83). Inhibition of Na-K-ATPase activity by ouabain (a specific inhibitor) or by K+ depletion (inhibition due to the lack of the steep K+ gradient necessary for pump function (10, 77, 78)) in MDCK cells subjected to a Ca2+ switch prevented the formation of tight junctions and the development of epithelial polarity. Tight junctions formed rapidly, and polarity was established after reactivation of Na-K-ATPase pump function by restoring the initial K+ concentration (K+ repletion), indicating that the effects of Na-K-ATPase inhibition were reversible. Inhibition of tight junction formation correlated with increased intracellular Na+ concentration levels ([Na+]i) generated by the inhibition of Na-K-ATPase, and the Na+ ionophore gramicidin, which increases [Na+]i, mimicked the effects of Na-K-ATPase inhibition on tight junction formation and epithelial polarity. Moreover, treatment of cells with ouabain in Na+-free medium did not affect tight junction formation. Because Na-K-ATPase function controls a variety of ions and metabolite transport systems, inhibition of this enzyme might induce multiple biochemical changes in cells, including altered Ca2+ signaling, cell volume, cell pH, and membrane potential. However, this study demonstrated that Na-K-ATPase enzyme activity is involved in the formation of tight junctions in epithelial cells. It is well established that cell-cell and cell-substratum contacts are involved in the establishment of tight junctions and polarity in epithelial cells (reviewed in Ref. 108). Lack of tight junction assembly and polarity in Na-K-ATPase-inhibited cells suggests that the intracellular ionic gradient maintained by Na-K-ATPase is also involved in the assembly of tight junctions and generation of polarity in epithelial cells.

SYNERGISM BETWEEN Na-K-ATPase AND E-CADHERIN IN THE ASSEMBLY OF TIGHT JUNCTIONS

The cell-cell contact mediated by the cell adhesion molecule E-cadherin has been implicated in the formation of tight junctions in MDCK cells. E-cadherin is a single transmembrane protein localized to the basolateral plasma membrane in polarized epithelial cells. The extracellular domain of E-cadherin contains five IgG-like repeats that mediate cell-cell contact between epithelial cells by homophilic interaction in a Ca2+-dependent manner (reviewed in Ref. 95). The cytoplasmic tail of E-cadherin associates with α-, β-, and γ-catenins (76) and p120ctn (89). α-Catenin either directly or through α-actinin links the E-cadherin complex to the actin cytoskeleton, which is crucial for the cell adhesion function of E-cadherin (51, 85).

The function of E-cadherin seems to be necessary for the translocation of tight junction proteins to the plasma membrane and for the formation of tight junction complexes. Inhibition of E-cadherin’s cell-adhesion function by extracellular domain-specific antibodies prevented the assembly of tight junctions (37). In cells maintained in a low-Ca2+ medium, ZO-1 is localized in the cytoplasm. On transfer of the cells to a normal-Ca2+ medium, ZO-1 rapidly translocated to the plasma membrane in control cells, whereas in the presence of anti-E-cadherin extracellular domain antibody this translocation was drastically reduced (37).

In contrast, in Na-K-ATPase-inhibited cells post-Ca2+ switch, ZO-1 was localized to the plasma membrane but failed to show a continuous staining pattern normally seen in MDCK cells (83). This suggests that Na-K-ATPase inhibition does not affect E-cadherin function. Moreover, adherens junctions, the formation of which requires functional E-cadherin (107), were present in ouabain-treated and K+-depleted cells (83). Therefore, plasma membrane localization of tight junction proteins such as ZO-1 and occludin seems to be due to the presence of functional E-cadherin present in Na-K-ATPase-inhibited cells, but the discontinuous ZO-1 staining pattern suggested that Na-K-ATPase function is necessary for formation of the continuous ZO-1 staining pattern in cells with established tight junctions. Consistent with this view, another study showed that in Moloney sarcoma virus-transformed MDCK cells (MSV-MDCK), a cell line with highly reduced E-cadherin and Na-K-ATPase β-subunit levels, ectopic expression of E-cadherin resulted in the localization of ZO-1 to the plasma membrane yet with a discontinuous staining pattern (80, 84). Only ectopic expression of E-cadherin combined with restored Na-K-ATPase function (through ectopic expression of Na-K-ATPase β-subunit) resulted in a continuous ZO-1 staining pattern and the formation of functional tight junctions, substantiating a hypothesis for a synergistic function of E-cadherin and Na-K-ATPase in the formation of tight junctions in epithelial cells. At this point, however, we cannot exclude the possibility that the β-subunit of Na-K-ATPase itself might play a role in the synergistic function of Na-K-ATPase and E-cadherin in establishing tight junctions and polarity in epithelial cells.

REGULATION OF TIGHT JUNCTION FORMATION BY Na-K-ATPase THROUGH MAPK

The MAPK pathway has been implicated in tight junction assembly in various cell lines. Chen et al. (15) have shown that downregulation of MAPK signaling in ras-transformed MDCK cells restored epithelial morphology and tight junctions. In pig thyrocytes, activation of MAPK by EGF and transforming growth factor (TGF)-β1 resulted in the loss of tight junctions, and the inhibition of MAPK activation by MEK inhibitor prevented this tight junction loss (35). In salivary gland epithelial cells, activation of MAPK by constitutive expression of Raf resulted in the reduction of occludin levels and disruption of tight junctions (55). In endothelial cells, H2O2-mediated activation of ERK1/ERK2 leads to the disruption of endothelial tight junctions (50). In these cell lines, low MAPK activity seems to be
associated with formation of tight junctions and epithelial polarization, whereas increased MAPK activity leads to the disruption of tight junctions. Interestingly, inhibition of Na-K-ATPase activity by ouabain activated MAPK in cardiac myocytes and other cell types (38) as well as in polarized epithelial cells (Rajasekaran SA, Espineda C, and Rajasekaran AK and Landon I and Rajasekaran AK, unpublished observations). It is tempting to speculate that increased MAPK activity induced by Na-K-ATPase inhibition has an inhibitory role on the assembly of functional tight junctions. Signaling pathways induced by the inhibition of Na-K-ATPase function and their impact on tight junction assembly will be an important area to pursue in the future.

ROLE OF STRESS FIBERS IN THE ASSEMBLY OF TIGHT JUNCTIONS

Several earlier studies showed that tight junction structure and permeability are regulated by the perijunctional actomyosin ring (reviewed in Refs. 3, 58, and 99). This ring is located at the apical pole of polarized epithelial cells and can be visualized by light microscopy as a circumferential actin ring that colocalizes with tight junction and adherens junction proteins. Earlier studies showed that disruption of tight junction structure and permeability correlated with disruption of the circumferential actin ring (3, 58, 99). However, in Na-K-ATPase-inhibited MDCK cells that did not form tight junctions, no apparent change in the circumferential actin ring was detected at the light microscopic level (83). Although less prominent, it has also been shown that stress fibers projecting from the perijunctional actin ring interface at the cytoplasmic surface of tight junction membrane contact points (41, 57).

A role for RhoA GTPase (a small GTP-binding protein) involved in the formation of stress fibers (39, 47, 100) has been demonstrated to be involved in the regulation of tight junction structure function (42, 48, 74). Interestingly, the impediment of tight junction assembly in Na-K-ATPase-inhibited MDCK cells was accompanied by a drastic reduction in stress fibers and correlated with diminished RhoA activity (83). Exogenous overexpression of wild-type RhoA GTPase bypassed the inhibitory effect of Na-K-ATPase on tight junction formation, indicating that RhoA GTPase is an essential component downstream of Na-K-ATPase function linking Na-K-ATPase to the formation of functional tight junctions (83). A similar effect on stress fibers, RhoA GTPase, and tight junction assembly was found in cells treated with gramicidin, a Na⁺ ionophore that, like inhibition of Na-K-ATPase, increases [Na⁺]. This indicated that intracellular Na⁺ homeostasis regulated by Na-K-ATPase function is involved in the formation of stress fibers and regulation of RhoA GTPase in MDCK cells. In polarized monolayers of retinal pigment epithelial cells, inhibition of Na-K-ATPase resulted in an increase in tight junction permeability. This increased permeability correlated with decreased tight junction membrane contact points and reduced actin stress fibers without an apparent change in the circumferential actin ring (82). Therefore, it seems likely that both the perijunctional actin ring and stress fibers are involved in the assembly and function of tight junctions. The perijunctional actin ring might regulate paracellular permeability and provide stability to tight junctions, whereas stress fibers might be involved in more dynamic functions related to the assembly and function of tight junctions. These dynamic functions during tight junction assembly may include proper molecular alignment of tight junction proteins at the tight junction region and tethering of tight junction strands to establish functional tight junctions (see model below). In cells with established tight junctions, the stress fibers might have a role in maintaining the tight junction membrane contact points through their association with tight junction membrane and cytoplasmic proteins. Reduced stress fibers associated with minimal change in the circumferential actin ring in Na-K-ATPase-inhibited cells highlighted for the first time that stress fibers are also involved in the regulation of tight junction assembly and function.

How Na-K-ATPase function is involved in the regulation of tight junction assembly through RhoA GTPase activity and stress fiber formation in epithelial cells is not currently known. Rho function is modulated by a set of regulatory proteins. Rho is activated through GDP-GTP exchange, which is promoted by guanine nucleotide exchange factors and is inactivated through GTPase-activating proteins (reviewed in Refs. 39 and 100). Stabilization of the inactive GDP-bound form of Rho is mediated by Rho guanine nucleotide dissociation inhibitors (39, 100). It is possible that inhibition of Na-K-ATPase function may either inhibit the function of guanine nucleotide exchange factors, resulting in the accumulation of the inactive form of Rho (Rho-GDP), or promote the functions of GTPase-activating proteins or Rho guanine nucleotide dissociation inhibitors, affecting the activation of Rho. Na-K-ATPase-mediated signaling mechanisms involved in the regulation of RhoA GTPase and actin assembly will be an important area of future research that will provide more insights into how ion homeostasis might regulate the role of stress fibers in the assembly and function of tight junctions.

TWO-STEP MODEL FOR THE ASSEMBLY OF TIGHT JUNCTIONS

From these recent observations on the Na-K-ATPase function in tight junction assembly, we propose a two-step model for the formation of tight junctions in epithelial cells (Fig. 3). Step 1 is dependent on the cell-cell adhesion function of E-cadherin. On E-cadherin-mediated cell-cell interactions, tight junction proteins are translocated from the cytoplasm to the plasma membrane and circumferential actin ring and the adherens junctions are established (Fig. 3, A and C). The translocation of ZO-1 might be due to its association with catenins that, in turn, are associated with E-cadherin-containing transport vesicles (80). Inhibition of E-cad-
herin-mediated cell-cell adhesion function by extracellular domain-specific anti-E-cadherin antibodies inhibited the translocation of ZO-1 to the plasma membrane as well as prevented the formation of the perijunctional actin ring (37). Also, during this initial step of tight junction assembly, ZO-1 association with the actin cytoskeleton as well as with occludin and claudin might take place to form the discontinuous tight junction strands, as seen in Na-K-ATPase-inhibited cells. Formation of discontinuous tight junction strands after the Ca$^{2+}$ switch has been described earlier (32).

**Step 2** of tight junction assembly is dependent on active actin polymerization probably regulated by RhoA GTPase. Active polymerization of actin filaments associated with ZO-1, ZO-2, ZO-3, or occludin (104) might provide the propulsive force to mobilize discontinuous tight junction strands in the plane of the membrane and facilitate tethering of tight junction strands at the apicolateral region to establish functional tight junctions (Fig. 3, B and D). Thus this model predicts that both stress fibers and circumferential actin are involved in the assembly and function of tight junctions. Because inhibition of Na-K-ATPase reduced RhoA GTPase activity and prevented tight junction assembly, we suggest that normal intracellular ionic homeostasis maintained by NA-K-ATPase is necessary for step 2 of the tight junction assembly. Future experiments are necessary to identify molecular components and signaling pathways involved in these two steps of tight junction assembly described in this model.

**NA-K-ATPase, Tight Juncions, and Disease**

**Cancer**

In carcinoma (cancer derived from epithelial cells), the polarized epithelial phenotype is lost (9). Events that lead to the loss of tight junctions and epithelial polarity might eventually lead to proliferation and metastasis of carcinoma cells. Interestingly, in patient tumor samples of renal clear cell carcinoma, an invasive and aggressive form of renal cancer, expression of Na-K-ATPase β-subunit and Na-K-ATPase activity are highly reduced (81). A morphometric analysis of tight junctions in these tumor tissues revealed the loss of tight junctions early during the development of cancer (Kim G, Thomas G, Rajasekaran SA, Rosen E, Shintaku P, Lassman C, Said J, and Rajasekaran AK, unpublished observations). It is tempting to speculate that reduced Na-K-ATPase activity or its subunit expression might play a role in the loss of tight junctions and possibly in the transition from the polarized epithelial phenotype to a more mesenchymal phenotype (epithelial-mesenchymal transition) often found in cancer (79). In addition, reduced Na-K-ATPase activity might activate MAPK signaling, which is known to induce cell proliferation in a wide variety of cells (13). In fact, partial inhibition of Na-K-ATPase by ouabain in endothelial cells (5) and epithelial cells (Rajasekaran SA, Espinosa C, and Rajasekaran AK, unpublished observations) activated MAPK and increased

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**Fig. 3. Two-step model for the assembly of tight junctions in epithelial cells.**

**A**: diagrammatic representation of tight junction assembly mediated by E-cadherin function. Note the partial tight junction strands (red), the circumferential actin ring (grey), and adherens junction (blue) **B**: fully formed tight junction strands at the apicolateral side of epithelial cells (red). Note stress fibers (black) localized at tight junction membrane contact points. **C**: immunofluorescence of ZO-1 after 3 h of Ca$^{2+}$ switch in ouabain-treated cells. Note the gaps in the ZO-1 staining pattern (arrows). **D**: immunofluorescence of ZO-1 after 3 h of calcium switch in control cells. Bar, 10 μm.
Ischemia

Ischemia is a condition caused by the deficiency of oxygenation of a body part caused by an obstruction in or the constriction of a blood vessel. Ischemic events in the kidney in vivo (53, 70, 71) and in vitro (11, 59) are associated with the loss of tight junction integrity. A role of Na-K-ATPase in re-establishing tight junctions and epithelial polarity might have important clinical implications during recovery from ischemic injury. During renal ischemia, the ATP content of the affected epithelial cells is rapidly depleted, resulting in the inhibition of Na-K-ATPase function (17, 60, 87). The consequences of renal ischemia in vivo can be mimicked in cultured epithelial cells by depleting ATP by glycolytic (2-deoxy-D-glucose) or oxidative phosphorylation (antimycin A) inhibitors (20, 33, 59). Recent studies revealed striking similarities between inhibition of tight junction formation after Na-K-ATPase inhibition and after ATP depletion. First, expression of constitutively active RhoA GTPase in ATP-depleted MDCK cells prevented tight junction disassembly induced by ATP depletion (34). Second, the levels of stress fibers and of active RhoA GTPase were drastically reduced in ATP-depleted cells, followed by disruption of tight junctions and cell polarity. Third, fewer changes were observed at the light microscopic level in the organization of the circumferential actin ring (Dr. S. J. Atkinson, Indiana School of Medicine, Indianapolis, IN, personal communication). It is possible that these effects on tight junction integrity observed on ATP depletion are due, at least in part, to the inhibition of Na-K-ATPase. In fact, decreased surface levels of Na-K-ATPase have been reported in ischemia-induced acute renal failure (54) and during postischemic renal injury (52). In addition, during ischemia Na-K-ATPase relocates to the apical plasma membrane due to its detachment from the cytoskeleton (72), and heat shock protein-70 is involved in the restoration of the cytoskeletal linkage of the Na-K-ATPase (8). Because Na-K-ATPase is known to associate with the actin cytoskeleton (73), it is possible that alterations in the actin cytoskeleton might affect the localization of this protein. We recently observed a change in the polarity of Na-K-ATPase in Na-K-ATPase-inhibited cells (82). In retinal pigment epithelial cells, Na-K-ATPase is predominantly localized to the apical plasma membrane. Inhibition of Na-K-ATPase resulted in an increased basolateral localization of Na-K-ATPase. A change in polarity of the Na-K-ATPase correlated with dramatic changes in the amount of actin stress fibers, suggesting that Na-K-ATPase function somehow regulates its localization through its association with the actin cytoskeleton. The inhibition of Na-K-ATPase during ischemia might be one of the mechanisms involved in the loss of tight junction integrity and polarity in ischemic cells. An impediment in the reestablishment of Na-K-ATPase function during recovery from ischemic or toxic injury may inhibit the process of reestablishing tight junctions and thus may delay the healing process or may even lead to hyperplasia of the affected tissue. Defining the role of Na-K-ATPase on tight junction disassembly during ischemia and tight junction reassembly during ischemic recovery should provide insights into therapeutic modalities for the treatment of ischemia.

Hypomagnesemia

In dominant renal hypomagnesemia, a dominant negative mutation in the Na-K-ATPase \(\gamma\)-subunit (FXYD2) has been linked to the loss of Mg\(^{2+}\) associated with this disease (65). A conserved glycine-41 within the putative transmembrane domain is mutated to arginine in this disease. The \(\gamma\)-subunit mutant protein localized intracellularly and did not codistribute with \(\alpha\) and \(\beta\)-subunits at the plasma membrane. Whether the mutant-expressing cells have reduced Na-K-ATPase activity that contributes to increased tight junction permeability to Mg\(^{2+}\) remains to be tested. Interestingly, claudin-16 mutations have been demonstrated as the basis of recessive hypomagnesemia in humans (90). Whether a functional link exists between claudin-16 and the Na-K-ATPase \(\gamma\)-subunit remains to be seen.

Polycystic Kidney Disease

Polycystic kidney disease is characterized by the appearance of fluid-filled cysts within the parenchyma of the kidney, eventually leading to kidney failure. Autosomal dominant polycystic kidney disease (ADPKD) is the major hereditary type and is characterized by the loss of renal function in the fifth or sixth decade of life. Mutations in PKD1 and PKD2 genes encoding polycystin-1 and polycystin-2 have been linked to the onset of this disease (reviewed in Ref. 93). One of the characteristic features of this disease is the loss of polarity of several proteins (reviewed in Ref. 102). For example, Na-K-ATPase is mislocalized to the apical plasma membrane yet is fully functional (102, 103). A recent study demonstrated that the integrity of tight junctions is not altered in ADPKD (14). Whether the integrity of the tight junctions is due to the presence of functional Na-K-ATPase remains to be seen.

MDCK cells grown on collagen gels form microcysts and have been utilized as a model for studying mechanisms involved in fluid secretion in ADPKD (62). MDCK microcysts are filled with fluid, and the cells in the monolayer lining are polarized, with the apical surfaces facing the lumen. Inhibition of Na-K-ATPase by ouabain decreased fluid secretion and caused stratification of cells within the cysts (36). Electron micrographs of the ouabain-treated cysts showed increased intercellular spaces between the cells. It is not clear whether these cells have tight junctions or whether the tight junction permeability is altered in these cells.
Future research using this model should provide more insights into the role of Na-K-ATPase on tight junction structure and function in polycystic kidney disease.

CONCLUSIONS

Recent studies on the implications of Na-K-ATPase in tight junction formation strongly suggest that Na-K-ATPase may not only regulate vectorial transport function but also, directly or indirectly, the cell structure of epithelial cells. In addition to the new role in epithelial cell structure, several new functions of Na-K-ATPase have been reported in the last three years (reviewed in Ref. 88), including a signaling role in NF-kB activation (1) and MAPK activation (38), a role in the induction of cell proliferation through EGF receptor transactivation (5, 38), and a role in cell sub-stratum attachment (19). These recent findings indicate that in addition to its ionic pump function, Na-K-ATPase might have multiple functions in epithelial cells. The multifunctional nature of Na-K-ATPase might be due to its enzymatic activity in controlling intracellular Na⁺ homeostasis. In addition, the α-, β-, and γ-subunits themselves may have individual roles in cell regulation independent of their role in pump function. Understanding the multiple functions of Na-K-ATPase is crucial to obtaining more insight into the role of Na-K-ATPase in normal and disease states.

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DISCLOSURES

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