The Na-K-ATPase α₁β₁ heterodimer as a cell adhesion molecule in epithelia

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The Na-K-ATPase plays a critical role in epithelia by driving transepithelial Na⁺-dependent transport of various solutes and water. For example, in renal tubules the ion gradients generated by the Na-K-ATPase drive Na⁺ reabsorption and the secondary active transport (reabsorption or secretion) of numerous solutes, including other ions, glucose, and amino acids (15). In alveolar epithelium, the transepithelial Na⁺ gradient created by Na-K-ATPase is responsible for water reabsorption from alveolar spaces which is critical for normal gas exchange (46, 68, 79). This driving force of the Na-K-ATPase depends on the tight junctions that maintain polar distribution of basolateral and apical membrane transporters and restrict paracellular diffusion of solutes (66). The tight junctions, in turn, depend on the presence of the adherens junctions that initiate cell-cell adhesion, stabilize intercellular contacts, and trigger signaling pathways required for the formation and maintenance of the tight junctions (3, 21, 28) (Fig. 1).

Both tight and adherens junctions are formed by cell adhesion molecules (CAMs), the integral proteins embedded in the adjacent membranes, that undergo the trans (cell-to-cell)-dimerization via the interactions between their extracellular domains (23). This trans-dimerization triggers association of the cytoplasmic domain of CAM to the cytoskeleton via anchoring proteins, which stabilizes cell-cell junctions (23, 44, 66). In addition, trans-dimerization of CAMs can induce or modulate the interaction between their cytoplasmic domains and signaling molecules and hence initiate junction-associated signal transduction (66). In epithelial cells, the major CAMs of tight junctions are occludins and claudins, while CAMs of adherens junctions include a well-characterized E-cadherin and the more recently identified nectins (1, 16, 45, 62, 69). As found for both E-cadherin and nectin-1, not only trans-dimerization of CAMs, but also their cis-dimerization in the same membrane is crucial for intercellular adhesion (22, 48, 84). Particularly, cis-dimerization of nectin-1 is critical for initiation of cell-cell contact (48), while cis-dimerization of E-cadherin is important for stabilization of adherens junctions (22).

The Na-K-ATPase consists of a catalytic α subunit and an N-glycosylated β subunit required for maturation and membrane targeting of the enzyme. Additional regulatory subunits of the Na-K-ATPase belong to a family of seven single-span transmembrane proteins containing an FXYD motif in the transmembrane region (17, 67). Expressed in a tissue-specific manner, FXYD proteins associate with the Na-K-ATPase αβ-heterodimer in a number of cells as subsidiary subunits and may modulate the kinetic properties of the enzyme (17, 18, 43). Of the four known isoforms of the Na-K-ATPase α subunit (α₁, α₂, α₃, and α₄) and three isoforms of the Na-K-ATPase β subunit (β₁, β₂, and β₃) the α₁ and β₁ are the major isoforms in epithelial cells (6, 13). The Na-K-ATPase β₁ subunit has three N-glycosylation sites, all occupied by N-glycans (42). N-glycans are added to the subunit in the endoplasmic reticulum (ER) and then modified by ER- and Golgi-resident glycosidases and glycosyltransferases. Prevention of N-glycosylation of the β₁ subunit has little effect on α₁-β₁ assembly (4, 87), trafficking of the pump to the plasma membrane (35, 80), or Na-K-ATPase activity (4, 35, 70, 71). However, N-glycans
have been found important for initiation, maintenance, and regulation of epithelial junctions (80, 81).

In the present review, we summarize the data showing that the Na-K-ATPase acts as CAM in adherens junctions (Fig. 2) and thus facilitates transepithelial transport not only by pumping ions, but also by maintaining the integrity of the intercellular junctions (Fig. 1).

Role of Ion Transport Activity and Signaling Function of the Na-K-ATPase in Intercellular Adhesion

Transport by the Na-K-ATPase is important for cell-cell adhesion. Inhibition of Na-K-ATPase activity by ouabain in various epithelia prevents tight junction formation (56, 59), triggers disassembly of existing junctions (11, 12, 32), or increases their permeability (57, 58). During blastocyst formation in early mouse embryos, ouabain treatment, K⁺ depletion, or knockdown of the Na-K-ATPase β₁ subunit disrupts the normal formation of tight junctions (41, 82). In Drosophila, the Na-K-ATPase is required for the formation of septate junctions (reviewed in Ref. 30). In most cases, the ouabain-dependent effects on cell adhesion are similar to the effects detected upon incubation of cells at low K⁺ concentration or in the presence of the Na⁺-ionophore gramicidin that increased intracellular concentration of Na⁺ (11, 59), demonstrating that the mainte-
nance of low intracellular Na\(^+\) by the Na-K-ATPase is crucial for intercellular junctions. However, recent studies have shown that low levels of ouabain that do not inhibit ion pumping activity also modulate the degree of sealing of the tight junctions and distribution of the tight junction proteins, claudins (31). Treatment of cells with ouabain and ouabain-like compounds at nontoxic levels is known to activate signaling cascades (reviewed in Ref. 36). Several Na-K-ATPase-mediated signaling pathways, including Src, ERK1/2, MAPK, epidermal growth factor receptor, inositol 1,4,5-triphosphate receptor, phosphatidylinositol 3-kinase, RhoA and PLC\(_\gamma\)-1, are implicated in the regulation of intercellular junctions (reviewed in Refs. 8, 10, 61). Therefore, both the ion-pumping activity and the signaling function of the Na-K-ATPase play a role in regulating intercellular junctions.

The Na-K-ATPase as an Important Component of an Epithelial Junctional Complex

Numerous studies have demonstrated colocalization of the Na-K-ATPase with junctional proteins in epithelial cell monolayers (reviewed in Refs. 8, 61). The Na-K-ATPase remains colocalized with the adherens junction proteins, E-cadherin and \(\beta\)-catenin, even after internalization induced by disruption of intercellular junctions between MDCK cells by Ca\(^{2+}\) depletion (80). Density gradient centrifugation analysis of the epithelial junctional complex shows that the Na-K-ATPase codistributes with the adherens junction proteins, E-cadherin, \(\beta\)-catenin, and \(\alpha\)-catenin, as well as with the tight junction protein, occludin (83). The cytoplasmic domains of both E-cadherin and the Na-K-ATPase \(\alpha_1\) subunit are connected to spectrin/actin cytoskeleton via an anchoring protein, ankyrin (29, 49–51). The high-affinity ankyrin binding site has been identified to be in the second cytoplasmic loop of the Na-K-ATPase \(\alpha_1\) subunit (88) (Fig. 2).

Both E-cadherin and the Na-K-ATPase acquire resistance to nonionic detergents after formation of junctions between epithelial cells (49, 51, 81). Such junction-dependent resistance to nonionic detergents is a common property of all CAMs of both tight and adherens junctions (78). This resistance results from stable association of the cytoplasmic domains of CAMs with the cytoskeleton in response to intercellular trans-interaction of their extracellular domains at the site of cell-cell junctions. MDCK cells transformed with Moloney sarcoma virus (MSV-MDCK), which are not able to form functional tight junctions (5), express low levels of both E-cadherin and the Na-K-ATPase \(\beta_1\) subunit compared with normal MDCK cells (5, 55, 60). Overexpression of the Na-K-ATPase \(\beta_1\) subunit in E-cadherin-transfected MSV-MDCK cells increases resistance of E-cadherin to detergent extraction (60). These results indicate that the presence of the Na-K-ATPase \(\beta_1\) subunit is important for association of E-cadherin with the cytoskeleton. Moreover, detergent resistance of both Na-K-ATPase and E-cadherin in normal MDCK cells is decreased by the removal of N-glycans from the \(\beta_1\) subunit (80, 81). This implies that the intracellular association of the Na-K-ATPase \(\alpha_1\) subunit with the cytoskeleton and E-cadherin is facilitated by N-glycan-mediated interactions of the extracellular domain of the Na-K-ATPase \(\beta_1\) subunit with particular CAMs present at the site of cell-cell contact. No direct interaction between the Na-K-ATPase \(\beta_1\) subunit and E-cadherin or occludin has been detected by coimmunoprecipitation in MDCK cells (74). On the other hand, the presence of interactions between the Na-K-ATPase \(\beta_1\) subunits of neighboring cells in epithelial cell monolayers was first hypothesized (65) and later demonstrated by fluorescence resonance energy transfer (52) and coimmunoprecipitation (52, 74). These results suggest that the Na-K-ATPase \(\alpha\)-\(\beta\) heterodimer itself can act as CAM by undergoing both trans-dimerization via its \(\beta_1\) subunit and association with the cytoskeleton via its \(\alpha_1\) subunit. To prove or disprove this hypothesis, it is essential to establish whether trans-dimerization of \(\beta_1\) subunits is important for either initiation of cell-cell contact, or maintenance of intercellular junctions, or both.

Trans-Dimerization of the Na-K-ATPase Is Important for Both Initiation and Maintenance of Intercellular Junctions

Overexpression of the Na-K-ATPase \(\beta_1\) subunit and E-cadherin, but not of E-cadherin alone, facilitates formation of tight junctions in MSV-MDCK cells (60). In normal MDCK cell monolayers, transient induction of the \(\beta_1\) subunit biosynthesis increases the rate of developing high transepithelial resistance (9). These results are consistent with possible contribution of the interactions between the Na-K-ATPase \(\beta_1\) subunits to the formation and/or stabilization of epithelial junctions. To determine whether this is the case, the effects of modulating these \(\beta_1\)-\(\beta_1\) interactions on stability of adherens junctions and paracellular permeability of tight junctions have been studied in MDCK cell monolayers (74). When stably expressed in these cells, the exogenous Na-K-ATPase \(\beta_1\) subunits, either dog or rat, or their unglycosylated mutants, replace approximately half of the endogenous \(\beta_1\) subunits in the \(\alpha_1\)-\(\beta_1\) complexes without a change in the total amount or ion-pumping activity of the Na-K-ATPase (74, 76, 81). This occurs because of competition of the endogenous and exogenous \(\beta_1\) subunits for binding to the endogenous \(\alpha_1\) subunits in the ER. The ER quality control system allows export only of assembled \(\alpha\)-\(\beta\) complexes to the Golgi, thereby maintaining a constant ratio of \(\alpha\) and \(\beta\) subunits in the plasma membrane, whereas the number of \(\alpha_1\) subunits in the ER determines the amount of the \(\alpha\)-\(\beta\) complexes (76). The presence in the plasma membrane of both endogenous \(\beta_1\) subunit and yellow fluorescent protein (YFP)-tagged exogenous \(\beta_1\) subunit enables assessment of \(\beta_1\)-\(\beta_1\) interactions by detecting the amount of the endogenous \(\beta_1\) subunits that co-immunoprecipitate with YFP-\(\beta_1\) (74).

Such assessment does not discriminate between cis- and trans- \(\beta_1\)-\(\beta_1\) interactions, since both endogenous and exogenous \(\beta_1\) subunits are expressed in the same cell. Disruption of intercellular junctions by Ca\(^{2+}\) depletion decreases the amount of \(\beta_1\)-\(\beta_1\) complexes by 50% (74), showing that at least half of them are formed between adjacent cells. In agreement with these data, increasing the quantity of non-transfected MDCK cells in mixed monolayers of these cells with MDCK cells expressing both the exogenous and endogenous dog \(\beta_1\) subunits proportionally increases the amount of the endogenous \(\beta_1\) subunits that co-immunoprecipitates with the exogenous \(\beta_1\) subunits (74). The results show that a significant portion of \(\beta_1\)-\(\beta_1\) complexes formed is intercellular. If the exogenous and endogenous \(\beta_1\) subunits interacted only within the same membrane, addition of nontransfected cells would not change the...
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Fig. 3. The integrity of intercellular junctions depends on the Na-K-ATPase β1 subunit dimerization. A: the interactions between yellow fluorescent protein (YFP)-linked β1 or β2 subunits expressed in MDCK cells with the endogenous β1 subunits were assessed by communoprecipitation (co-IP) using anti-YFP antibody followed by a Western blot analysis of precipitated YFP-linked subunits and coprecipitated endogenous subunits. Densitometric quantification for each cell line was performed by dividing the signal from the anti-YFP antibody followed by a Western blot analysis of precipitated YFP-linked subunits and coprecipitated endogenous subunits. Error bars, ±SD (n = 3). The results presented in the figure were originally published in Ref. 74 (Journal of Biological Chemistry, ©The American Society for Biochemistry and Molecular Biology).

Fig. 4. The 198–207 amino acid region of the dog β1 subunit is important for β1-β1 interaction. A: computational alignment of amino acid sequences of the Na-K-ATPase dog β1 subunit (NP_001003283.1) and rat β1 subunit (NP_037245.2). The amino acid residues that are identical, similar, and different are shown in black, green, and purple font, respectively. The most variable region between dog and rat sequence (red box) overlaps with the epitope of the cell-adhesion-blocking anti-β1 subunit antibody (green arrow). The species-specific residues exposed at the surface of the extracellular domain (stars) were identified by mapping on a high-resolution structure of the Na-K-ATPase (2ZXE) (64). N-glycans are shown in blue. B: positions of the amino acid residues mutated in the 198–207(8) amino acid regions of YFP-dog β1 and YFP-rat β1 are shown at the left. The wild-type (wt) and mutated fusion proteins were stably expressed in MDCK cells. Communoprecipitation of the endogenous β1 subunits with expressed YFP-linked wild-type and mutated subunits followed by a Western blot analysis was used to assess β1-β1 interactions. The results of densitometric quantification shown at the right indicate that the rat-like mutations in the 198–207 region of YFP-dog β1 decrease its binding to the endogenous β1 subunit, while the dog-like mutations in the 198–208 region of YFP-rat β1 improve its interaction with the endogenous β1 subunit. Error bars, ±SD (n = 3). *Significant difference from the dog wt, P < 0.01. Student’s t-test. C: multiple alignment of various isoforms of the β subunits of the P1-type ATPases shows that the amino acid sequence downstream of the second N-glycosylation site that has been found important for trans-dimerization of the Na-K-ATPase β subunits (74) is absent in other β subunit isoforms of the Na-K-ATPase and homologous H-K-ATPase. CD, cytoplasmic domain; TMD, transmembrane domain. [From Tokhtaeva et al. (75).]
A
Cytoplasmic domain  Transmembrane domain  Extracellular domain

Dog β₁
Rat β₁

Dog β₁
Rat β₁

Extracellular domain

198-207(8)

T202

B
Exogenous YFP-linked β₁ subunits

Dog wt: YFP-dog β₁

Rat wt: YFP-rat β₁

Dog/rat (+T): T insertion between V201 and M202 in YFP-dog β₁

Dog/rat (198-208): rat 198-208 sequence in YFP-dog β₁

Rat/dog (-T): T202 removal from YFP-rat β₁

Rat/dog (198-207): dog 198-207 sequence in YFP-rat β₁

C

CD  TMD  Extracellular domain

Dog Na,K-β₁
Rat Na,K-β₁

Human Na,K-β₂
Human H,K-β

Human Na,K-β₃

Dog Na,K-β₁  Dog Na,K-β₂  Human Na,K-β₁  Human Na,K-β₂  Human Na,K-β₃

178 180 181 173

IKLNRLGFKPKNESLAEAYPV-MKYSYVLPVQC
IKLNRLGFKPKNESLEYPPTMLKYNPNVLPVQC
IKMNRFVNYAGA--NGS-----------MNVIC
IKMNRFVNYAGA--NGS-----------APRVDC
VKMNRNGLKPEG-----------VPRIDC
The impairment of $\beta_1$-$\beta_1$ binding by removing N-glycans or by altering the amino acid sequence in one of the interacting subunits decreases detergent resistance of the Na-K-ATPase itself and also of the adherens junctional proteins, E-cadherin and its cytoplasmic partner $\beta$-catenin (Fig. 3B) (74, 81), indicating that stability of the adherens junctional complexes does depend on $\beta_1$-$\beta_1$ dimerization. It is known that stable adherens junctions are required for normal functioning of the tight junctions (3, 21, 28). Accordingly, the impairment of $\beta_1$-$\beta_1$ binding also increases the paracellular permeability (Fig. 3C) (74, 81). Conversely, improvement of $\beta_1$-$\beta_1$ binding by reduction of N-glycan branching with specific inhibitors decreases both extractability of junctional proteins and paracellular permeability (Fig. 5) (74, 81). Therefore, trans-dimerization of $\beta_1$ subunits is important for integrity of intercellular junctions.

This trans-dimerization of $\beta_1$ subunits is also important for initiation of cell-cell contact. Overexpression of the $\beta_1$ subunit increases adhesiveness of nonpolarized Chinese hamster ovary cells as detected by a cell aggregation assay (65). Furthermore, cell junction formation between surface-attached MDCK cells is inhibited by an antibody against the extracellular domain of the $\beta_1$ subunit (80). The mutagenic removal of N-glycans from the $\beta_1$ subunit reduces the rate of cell-cell contact formation between dispersed MDCK cells (80). Also, aggregation of rat lung epithelial cells is inhibited by the rat-like mutant of the extracellular domain of the dog $\beta_1$ subunit added to the cell culture media (75).

The detection of $\beta_1$-$\beta_1$ complexes in MDCK cells separated from each other by Ca$^{2+}$ depletion (74) indicates the presence of not only trans-, but also cis-$\beta_1$-$\beta_1$ interactions within the same membrane. Mutating Gly48 in the transmembrane domain of the Na-K-ATPase $\beta_1$ subunit inhibits oligomerization of the transmembrane domains $\beta_1$ subunit fused to the maltose binding protein within Escherichia coli membranes (2). The same mutation in the full-length Na-K-ATPase $\beta_1$ subunit inhibits cell aggregation of $\beta_1$-overexpressing MSV-MDCK cells (2), suggesting that the Na-K-ATPase $\beta_1$ subunits undergo cis-oligomerization within the same plasma membrane (Fig. 2) and that this interaction facilitates cell aggregation.

Therefore, the Na-K-ATPase acts as CAM in adherens junctions, i.e., it facilitates both initiation and stabilization of cell-cell junctions via intracellular trans-dimerization followed by intracellular association with the cytoskeleton. Remarkably, the two main functions of CAM, trans-dimerization and anchorage to the cytoskeleton, are segregated between two subunits of the Na-K-ATPase, the $\beta_1$ and $\alpha_1$, respectively (Fig. 2). It is possible that cis-dimerization of the Na-K-ATPase molecules within the same membrane via transmembrane domains of the $\beta_1$ subunits also contributes to the formation and/or stabilization of the junctional complex.

The Putative $\beta_1$-$\beta_1$ Trans-Dimerization Domain

The interaction between two dog subunits is more effective than the interaction between rat and dog subunits (Fig. 3A), suggesting the importance of species-specific amino acid regions in $\beta_1$-$\beta_1$ interaction. Mapping the residues that are different in dog and rat subunits on the high-resolution structure of the Na-K-ATPase (2ZXE) (64) identified the highly variable between dog and rat region that is exposed on the surface of the extracellular domain of the $\beta_1$ subunit facing a neighboring cell (Fig. 2). It contains four residues that are different in the dog and rat subunits and one residue (Thr) that is present in rat, but not in dog subunits (Fig. 4A). This region overlaps with the epitope for the antibody (Fig. 4A, green arrow) that reacts with dog, but not rat, $\beta_1$ subunits and inhibits formation of cell-cell contacts between dispersed MDCK cells (74, 80).

To determine whether this region is important for $\beta_1$-$\beta_1$ interaction, rat-like and dog-like mutations in dog and rat $\beta_1$ subunits, respectively, have been introduced into these species-specific sequences. Similar to the wild-type exogenous $\beta_1$ subunits, stably expressed mutants replace about half of the endogenous $\beta_1$ subunits with no change in the total amount of the pumps in the membrane (75). Insertion of a rat-specific Thr202 into the exogenous dog $\beta_1$ subunit, either alone or together with four rat-like amino acid substitutions in the 198–207 sequence, impairs its interaction with the endogenous dog $\beta_1$ subunit down to the level observed between rat $\beta_1$ and dog $\beta_1$ subunits (Fig. 4B). Conversely, the removal of a Thr-unique Thr202 from the rat $\beta_1$ subunits improves its binding to the dog $\beta_1$ subunit. Four dog-like replacements in the 198–208 region performed in addition to the Thr removal further improved binding of the rat exogenous $\beta_1$ subunit to the endogenous dog $\beta_1$ subunit up to the level observed between the exogenous and endogenous dog $\beta_1$ subunits (Fig. 4B) (75). These results show the importance of the 198–207 amino acid sequence for binding between dog $\beta_1$ subunits (Fig. 2).

The same region is involved in the interaction between two rat $\beta_1$ subunits, since the rat-like mutations in the 198–207 sequence of a secreted protein containing the extracellular domain of the dog $\beta_1$ subunit increase its binding to the YFP-linked rat $\beta_1$ subunit in vitro (75). In addition, the same mutations resulted in less efficient aggregation between rat epithelial cells followed by addition of the extracellular domain of the $\beta_1$ subunit to the cell suspension (75). The data indicate that the extracellular domain of the dog $\beta_1$ subunit with rat-like mutations in the 198–207 sequence competitively inhibits intercellular binding between rat $\beta_1$ subunits and hence reduces cell-cell adhesion, while the wild-type extracellular domain of the dog $\beta_1$ subunit binds poorly to the rat $\beta_1$ subunit and does not prevent cell aggregation. Therefore, the interaction between two rat $\beta_1$ subunits is also mediated by amino acid residues within the rat 198–208 sequence that determines species specificity (Fig. 2).

The absence of a Thr residue between residues 201 and 202 is critical for binding between two dog $\beta_1$ subunits (75). On the other hand, the two rat $\beta_1$ subunits effectively interact with one another (52), and their 198–208 regions, which contain the Thr residues, are important for this interaction (75). These results indicate that the amino acid residues critical for $\beta_1$-$\beta_1$ binding are located both upstream and downstream of the Thr insertion position. The insertion or removal of the Thr residues in one of the two interacting subunits likely misaligns these binding residues. Remarkably, the 198–207/8 region is present only in the $\beta_1$ subunit, which is the major $\beta$ subunit isofrom in epithelial cells, but not in the other $\beta$ subunit isoforms of the Na-K-ATPase and the homologous H-K-ATPase (Fig. 4C). It is possible that, during evolution, this region was conserved in the $\beta_1$ subunits to enable an additional role for the Na-K-ATPase in the formation and maintenance of epithelial juncture.
Fig. 5. The integrity of intercellular junctions can be regulated by remodeling of N-glycans linked to the Na-K-ATPase β₁ subunit. A: a scheme showing how the expression of the Golgi glycosyltransferases controls branching of N-glycans during formation of the mature cell monolayers from dispersed MDCK cells. The stop-branching enzyme, N-acetylglucosamine-glycosyltransferase (GnT)-III, is upregulated, while the branching promoting enzymes, GnT-V and GnT-IVC, are downregulated in confluent cells compared with dispersed cells. As a result, the degree of branching of N-glycans, including those linked to the Na-K-ATPase β₁ subunit, is less in confluent cells than in dispersed cells. Accordingly, the β₁ subunit present in the basolateral membranes of confluent cells has a higher electrophoretic mobility compared with that in dispersed cells. B–D: decreasing the degree of N-glycan branching by exposure of cells to the inhibitor of N-glycan processing, swainsonine, improves β₁-β₁ interactions (B), increases the resistance of E-cadherin to Triton X-100 extraction (C), and decreases the paracellular permeability of MDCK cell monolayers for BCECF (D). Conversely, increasing the degree of N-glycan branching by silencing of a stop-branching enzyme, GnT-III, increases the paracellular permeability (D). siRNA, small interfering RNA. The figure summarizes results originally published in Refs. 74 and 81 (Journal of Biological Chemistry, ©The American Society for Biochemistry and Molecular Biology).
Modulation of Intercellular Adhesion by Glossoytransferase-Dependent Remodeling of N-Glycans of the Na-K-ATPase β1 Subunit

N-linked glycosylation is a very common modification of the transmembrane and secreted proteins. This modification is initiated cotranslationally in the ER lumen by covalent addition of a 14-residue N-glycan to the asparagine of the N-glycosylation site in the polypeptide chain and is completed posttranslationally in the Golgi. The majority of known membrane proteins contain N-glycosylation sites, suggesting that N-glycans have important biological functions. The significance of N-glycans, though, has been underestimated, limiting their roles to modulating stability, solubility, and susceptibility of proteins to aggregation. However, recent studies have shown that N-glycans have critical roles in protein folding, ER quality control, protein trafficking, intracellular signaling, cell adhesion, cell migration, and carcinogenesis (7, 24, 25, 53, 54).

For both dog and rat β1 subunits expressed in MDCK cells, the amount of coprecipitated endogenous β1 subunits is significantly greater with normally glycosylated exogenous subunits than with their unglycosylated mutants. Since N-glycans are not required for proper folding of the β1 subunits (73), these results indicate that N-glycans are involved in β1-β1 interaction. This interaction is not mediated by lectins that bind either sialic acid residue or galactose residue on N-glycan termini, because β1-β1 binding is not impaired by removal of sialic acid or galactose residues or by cell incubation with lactose that is known to inhibit galactose-lectin binding (73). Furthermore, the involvement of other lectins that bind to mannose or N-acetylgalactosamine residues is doubtful, since these residues are not exposed on the N-glycan termini of the β1 subunits (77). In addition, the importance of the 198–207/8 amino acid domain for trans-dimerization is an argument against lectin involvement, since the presence of a lectin molecule between bulky hydrophilic N-glycans would not allow direct amino acid-mediated interaction between two β1 subunits of adjacent cells. Therefore, N-glycans are presumably required for stabilizing the amino acid-mediated interactions between extracellular domains of the two β1 subunits, suggesting that β1-β1 interactions can be modified by alterations in the β subunit N-glycosylation status.

Recent studies have demonstrated that both β1-β1 dimerization and intercellular adhesion indeed can be modulated by alterations in the structure of N-glycans. The highly diverse structures of N-glycans are generated during their processing in the Golgi due to the activity of various glycosyltransferases. The heterogeneity of N-glycans arises from variations in the number of branches (from 1 to 5) and the length and/or composition of individual branches (Fig. 5A). The action of N-acetylgalactosamine-glycosyltransferases (GaNTs) determines the number of branches in an N-glycan, while the activity of other glycosyltransferases, such as sialyltransferase and β-1,4-galactosyltransferase, is responsible for elongation of individual branches. The degree of N-glycan branching can be reduced pharmacologically, by exposing cells to the inhibitor of N-glycan processing, swainsonine. This treatment increases coprecipitation of the endogenous β1 subunit with the exogenous β1 subunit, indicating the improvement of β1-β1 interactions (Fig. 5B). In parallel, swainsonine treatment increases resistance of E-cadherin to extraction by digitonin (74) or by Triton X-100 (Fig. 5C) (81) and decreases the paracellular permeability (Fig. 5D) (81). Conversely, promoting N-glycan branching by silencing GaNT-III, the enzyme that stops branching of N-glycans, increases the paracellular permeability of MDCK cell monolayers (Fig. 5D) (81). Cell exposure to swainsonine affects glycosylation of not only the Na-K-ATPase β1 subunit, but also many other cellular glycoproteins, including E-cadherin. Alterations in N-glycosylation of E-cadherin also influence cell-cell adhesion (37, 89). Importantly, the effects of swainsonine on detergent resistance of E-cadherin and paracellular permeability of MDCK cell monolayers are significantly diminished in a cell line expressing the unglycosylated Na-K-ATPase β1 subunit (81), indicating that the decreased branching of the β1 subunit N-glycans contributes to the tightening and stabilization of cell-cell junctions. Therefore, the fewer branches are in N-glycans of the β1 subunit, the tighter are intercellular junctions.

During formation of mature MDCK cell monolayers from dispersed cells, along with a gradual decrease in the paracellular permeability of cell monolayers, N-glycans linked to E-cadherin gradually decrease in size (81). Similarly, the size of N-glycans linked to E-cadherin is decreased in confluent cells compared with dispersed cells (37, 81). Consistent with these observations, the relative molecular weight of N-glycans linked to the N-cadherin decreases as nonconfluent retinal pigmented epithelial cells become confluent (86). Also, the N-glycans linked to E-cadherin, Na-K-ATPase β1 subunit, β1-integrin, Na+/Ca2+ exchanger, and polycystin-2 are smaller in relatively tight renal epithelia compared with the leaky epithelium of small intestine (81), suggesting the inverse correlation between the size of N-glycans and tightness of epithelia.

Both the expression level and the activity of GaNT-III, the enzyme that stops branching of N-glycans, are increased in epithelial GE11 cells cultured under dense conditions compared with dispersed cells (26). Similarly, expression of GaNT-III gradually increases during development of mature cell monolayers from dispersed MDCK cells (Fig. 5A) (81). In parallel, two of the enzymes that promote N-glycan branching, GaNT-V and GaNT-IVC, are downregulated (Fig. 5A) (81). In contrast, the expression of the two key enzymes involved in elongation of individual branches, sialyltransferase and β-1,4-galactosyltransferase, does not change (81).

Upregulation of a stop-branching enzyme and downregulation of branching-promoting enzymes would decrease the number of branches in N-glycans, including those attached to the Na-K-ATPase β1 subunit. The reduced degree of N-glycan branching can explain the increase in electrophoretic mobility of the Na-K-ATPase β1 subunit observed during transformation of dispersed cells to confluent cell monolayers (Fig. 5A) (81). Decrease in branching of the β1 subunit N-glycans, in

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Concluding Remarks and Future Directions

The ion-pumping activity of the Na-K-ATPase and its role in signal transduction are important for formation and maintenance of intercellular junctions in epithelial cell monolayers. In addition, the Na-K-ATPase is a structural component of the epithelial junctional complex. The Na-K-ATPase acts as CAM in adherens junctions by undergoing intercellular trans-dimerization via its β1 subunit and by linking to the cytoplasmic domain of E-cadherin via its α1 subunit and ankyrin/spectrin cytoskeleton. Intercellular trans-interactions between the β1 subunits are required for integrity of epithelial junctions. The species-specific, extracellular 198–207(8) amino acid sequence of the β1 subunit is important for β1-β1 trans-dimerization, while N-glycans stabilize the amino acid-mediated interactions between β1 subunits. Intergcellular adhesion can be regulated by N-glycan-mediated modulation of β1-β1 interactions. This modulation occurs because of cell-adhesion-dependent regulation of the level of the specific glycosyltransferases that control the degree of N-glycan branching of the Na-K-ATPase β1 subunit. The precise signaling pathways involved

in the relationship between cell-cell adhesion, cell motility, and the activities of specific glycosyltransferases remain to be elucidated.

An additional layer of regulation of β1-β1 bridges and intercellular adhesion can arise from the interaction of the Na-K-ATPase with FXYD proteins. Overexpression of FXD5 in cancer cell lines impairs cell-cell adhesiveness and promotes experimental cancer metastasis in animals following injection of the transfectants (27, 47). Both an E-cadherin-dependent mechanism and an E-cadherin-independent mechanism have been proposed to explain these effects of FXD5 (47). Interestingly, overexpression of FXD5 in renal epithelial cells decreases transepithelial electrical resistance, increases paracellular permeability to macromolecules, and modifies the glycosylation status of the Na-K-ATPase β1 subunit without any effect on expression of E-cadherin (38–40). These data suggest that FXD5 may weaken the β1-β1 bridges by inhibiting amino acid-mediated intercellular β1-β1 interactions and/or by preventing normal glycosylation of the β1 subunit and, hence, impair intercellular adhesion. Similarly, the presence of FXD3 delays the processing to a fully glycosylated β1 subunit in Xenopus oocytes (14), which implies a potential role of FXD3 in modulating β1-β1 interactions. However, the nature of the involvement of FXD proteins in regulation of β1-β1 interactions and cell adhesion in epithelia remains to be explored.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

O.V. conception and design of research; O.V. and L.A.D. analyzed the data; O.V., L.A.D., and G.S. interpreted the results of the experiments; O.V. prepared the figures; O.V. drafted the manuscript; O.V., L.A.D., E.T., and G.S. edited and revised the manuscript; O.V. approved the final version of the manuscript; E.T. performed the experiments.

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Na-K-ATPase AS A CELL ADHESION MOLECULE


