Unexpected Roles of the Na-K-ATPase and Other Ion Transporters in Cell Junctions and Tubulogenesis

Recent work shows that transport-independent as well as transport-dependent functions of ion transporters, and in particular the Na-K-ATPase, are required for formation and maintenance of several intercellular junctions. Furthermore, these junctional and nonjunctional functions of ion transporters contribute to development of epithelial tubes. Here, we consider what has been learned about the roles of ion pumps in formation of junctions and epithelial tubes in mammals, zebrafish, Drosophila, and C. elegans. We propose that asymmetric association of the Na-K-ATPase with cell junctions early in metazoan evolution enabled vertebral transcellular ion transport and control of intraorganismal environment. Ion transport-independent functions of the Na-K-ATPase with cell junctions early in metazoan evolution enabled vectorial transcellular ion transport and control of intraorganismal environment. Ion transport-independent functions of the Na-K-ATPase arose as junctional complexes evolved.

It is well established that ion pumps move ions across cellular membranes and generate electrochemical gradients that are utilized for a wide variety of processes, including signaling, cell volume regulation, and electrolyte transport. Similarly, ion channels are generally described as facilitating passage of specific ions across the membranes to achieve physiological requirements. However, in recent years, it has become clear that many pumps and channels serve additional functions outside of their traditional roles in ion transport. Although the ability of ion transporters to move ions is critical in junction formation and tubulogenesis, there is compelling evidence that some pumps have ion transport-independent roles in cell adhesion and tube morphogenesis.

The ion pump that features most prominently in studies of junctions and tubulogenesis is the Na-K-ATPase. This intensively studied transporter is a heterodimer of α- and β-subunits that transports three Na+ out of and two K+ into the cell for each ATP hydrolyzed. It plays a central role in generating the electrochemical gradient across the plasma membrane that drives transport of water and a diverse array of solutes (reviewed in Refs. 26, 48, 63). In addition, with a total of 11 transmembrane domains and ~1,400 amino acids, the Na-K-ATPase has substantial intracellular and extracellular domains that interact with an ever expanding number of proteins and signaling molecules to mediate ion transport-independent functions (9, 24, 42, 43, 67). The genomes of most metazoans encode multiple α- and β-subunit isoforms with distinct transport characteristics, subcellular targeting information, and protein-protein interactions. For example, in humans, there are four α-subunit and at least three β-subunit genes (10). In Drosophila, there are also three β-subunit genes (56, 71) but only one α-subunit locus that generates at least 12 distinct isoforms (53, 56). Thus Na-K-ATPase isoforms within a given cell can have significantly different functions.

The Na-K-ATPase and Other Transporters in Cell Junction Formation

In the following sections, we discuss the role of the Na-K-ATPase and other ion transporters in cell adhesion. Although there are many types of junctional complexes, the majority of reports on the Na-K-ATPase and ion transporters in cell junctions involve epithelial barrier junctions: tight junctions (TJs) in vertebrates and世家 instances (SHs) in invertebrates such as insects. Both junctions prevent free diffusion of solutes across epithelia via the spaces between cells and have additional roles such as organizing polarity complexes (reviewed in Refs. 26, 73, 74, 81; see FIGURE 1). TJs localize apical of the adherens junctions (FIGURE 1, LEFT), and although their organization requires many proteins, barrier function is provided by the transmembrane claudins that oligomerize into strands. Multiple ion channels can regulate TJ permeability; however, since this topic has recently been thoroughly reviewed (66), here we focus on barrier junction formation. Even though SHs also require claudin family proteins for barrier function, they are not the simple homologs of TJs. In contrast to TJs, SHs are basal to adherens junctions and contain basolateral polarity proteins (FIGURE 1, RIGHT). These junctions also have considerable ultrastructural differences (73, 74), and, as described below, there appear to be significant differences in the roles of ion transporters in formation of TJs and SHs.

Barrier Junction Ion Transporters

The Na-K-ATPase

In vitro experiments have shown that the Na-K-ATPase, and can trigger requirement for further demonstration in cell junctions and tubulogenesis. Moreover, Rajotte and colleagues have shown that inhibition of the Na-K-ATPase by the Na-K-ATPase inhibitors does not prevent TJs in epithelial cell lines (23). However, recent work shows that transport-independent as well as transport-dependent functions of the Na-K-ATPase in cell junctions are critical in the formation and maintenance of the Na-K-ATPase (9, 24, 42, 43, 67). The genomes of most metazoans encode multiple Na-K-ATPase genes (10), including signaling, cell volume regulation, and extracellular domains that interact with many proteins and signaling molecules to mediate ion transport-independent functions (9, 24, 42, 43, 67). The genomes of most metazoans encode multiple α- and β-subunit isoforms with distinct transport characteristics, subcellular targeting information, and protein-protein interactions. For example, in humans, there are four α-subunit and at least three β-subunit genes (10). In Drosophila, there are also three β-subunit genes (56, 71) but only one α-subunit locus that generates at least 12 distinct isoforms (53, 56). Thus Na-K-ATPase isoforms within a given cell can have significantly different functions.
We begin by discussing ion transport-dependent and -independent roles of transporters in barrier junction formation and then discuss the roles of ion transporters in cell adhesion. We then propose a model that could explain why the Na-K-ATPase is associated with junctions and how ion transport-independent functions of the Na-K-ATPase could have evolved. We finish by considering ion transporters in tubulogenesis and in polycystic kidney disease (PKD).

Barrier junction formation: ion transport-dependent roles

The Na-K-ATPase plays essential roles in TJ formation. In vitro experiments using MDCK cells have demonstrated that inhibiting Na-K-ATPase pump activity with the hormone ouabain prevents TJ formation (62) and can trigger disassembly of existing TJs (20). The requirement for the pump activity in TJ formation was further demonstrated by use of a low K+ media that blocks pump function and disrupts TJ formation (62). Moreover, Rajasekearan et al. showed that TJ formation defects resulted from high intracellular Na+ as inhibition of Na-K-ATPase in a low Na+ culture medium did not disrupt TJ formation, whereas addition of Na+ ionophore gramicidin caused TJ defects (62). The mechanism by which high Na+ disrupts TJ formation is unclear, but it does not appear to result from a local effect on TJs since in most epithelial cells the ATPase primarily localizes basal to the TJs (reviewed in Ref. 16). Furthermore, high intracellular Na+ also caused loss of stress fibers, desmosomes, and misalignment of apical polarity markers. Although high Na+ would therefore appear to cause fairly extensive perturbations of cell physiology, these perturbations may result from effects on only one or few central regulators, since overexpressing RhoA blocks the effects of inhibiting the pump and allows the cells to maintain normal TJs, stress fibers, desmosomes, and epithelial polarity (62). Thus, in some epithelia, ion transport activity by the Na-K-ATPase may regulate TJ formation through RhoA.

In other vertebrate tissue types, the Na-K-ATPase may have a more direct role in TJ formation. For example, in the retinal pigment epithelial cells and the pancreatic ductal carcinoma cell line HPaC-II, inhibition of Na-K-ATPase activity by ouabain or K+ depletion did not prevent TJ formation but did increase TJ permeability and the strand content (58, 61). However, in contrast to the conventional basolateral localization of the Na-K-ATPase, in HPaC-II and retinal cells, the Na-K-ATPase was also found at TJs and in HPaC-II cells could be co-immunoprecipitated with PP2, a protein phosphatase that localizes to the TJ. These experiments raise an interesting possibility that the negative ion transport-dependent role of the Na-K-ATPase could have additional transport-independent roles that contribute to TJ formation in specific cell types, including regulating PP2's dephosphorylation of TJ proteins such as occludin (58).

In vivo data from multiple organisms also provide evidence for ion transport-dependent roles of the Na-K-ATPase in TJ formation. In early mouse embryos, during blastocyst formation, ouabain treatment or K+ depletion disrupted the normal formation of TJs and increased the paracellular permeability (76). Similarly, knockdown of Na-K-ATPase β1 subunit in the one-cell mouse embryo mislocalized the TJ proteins ZO-1 and occludin (48). In zebrafish, Na-K-ATPase activity along with the TJ component nagie oko (nok) is required to maintain apical ZO-1 junction belts in myocardial cells (19). Taken together, these results strongly support ion transport-dependent roles of the Na-K-ATPase in TJ formation. Although there are many reports of ion transporters that modulate TJ permeability (60), so far the only ion transporter other than the Na-K-ATPase to be required for TJ formation or integrity is the TRPV4 Ca2+ channel.
Like the Na-K-ATPase, TRPV4 localizes to lateral membranes in mammalian epithelial cells. Activation of TRPV4 causes TJ strand breaks and reduces claudin levels (37, 64).

Na-K-ATPase-mediated ion transport required for formation of barrier junctions in invertebrates? The role of the Na-K-ATPase in C. elegans barrier junctions has not been reported, but, as discussed below, in Drosophila, the Na-K-ATPase is required for formation of SJs (29, 33, 55, 56). However, in contrast to TJ formation, ion transport does not appear to be required because a maternal deletion of Na-K-ATPase subunit mutants could be rescued with a catalytically inactive isoform (55). Thus, with the caveat that residual ion transport by a small maternal contribution of Na-K-ATPase could not be ruled out in Drosophila experiments, it is not obvious that there is a conserved role for Na-K-ATPase-mediated ion transport in barrier junction formation.

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Barrier junction formation: ion transport-independent roles

The clearest example of an ion transporter having a transport-independent role in barrier junctions is the involvement of the Na-K-ATPase in formation of Drosophila barrier junctions, the SJs. In contrast to most vertebrate epithelia, Na-K-ATPase isoforms localize specifically to SJs (5 [29, 56] (FIGURE 1, RIGHT)). In embryos homozygous for either ATPalpha α-subunit or Nrv2 β-subunit mutations, SJs fail to form and SJ proteins are mislocalized along the lateral membrane (29, 33, 54). The Na-K-ATPase appears to be a structural component of SJMs since it colocalizes and can be coIPed with other SJ proteins, such as Coracle, Neurexin, and Neurouglian (28). Consistent with a transport-independent role, of the three Drosophila β-subunits, only Nrv2 can provide junctional function (55). By contrast, experiments with pumps from species ranging from torpedo rays to humans have suggested most α/β combinations form functional ion pumps (reviewed in Ref. 40). Moreover, genetic rescue experiments demonstrated that the cytoplasmic NH2-terminus of the "long" α-subunit isoform is required for barrier junction formation (55), although this domain appears dispensable for Na-K-ATPase pump activity in the mammalian Na-K-ATPase (68). Furthermore, a mutant ATPalpha isoform that lacks catalytic activity can nonetheless provide full junctional activity (55). These results, in combination with chimera experiments demonstrating that the extracellular domain of Nrv2 is specifically required for SJ formation, strongly suggest that in Drosophila the Na-K-ATPase functions as a scaffold independent of canonical Na-K-ATPase ion transport functions.

Given the apparent localization of the mammalian Na-K-ATPase to TJ1 in HPAF-II cells (58), is it possible that the Na-K-ATPase also has a structural role in TJ formation? Direct evidence in mammalian cells is lacking, but, remarkably, expression of the rat epithelial α1 subunit in the Drosophila ATPalpha mutant background rescued SJ formation and function, which strongly suggests conservation of ion transport-independent functional junctions of the α-subunit (55). Consistent with this, Cibrian-Ulharte et al. found that, during zebrafish heart formation, mutation of several NH2-terminal serine residues in the α1B1 subunit in the zebrafish mutant background disrupted TJ formation (19). The effect of these α-subunit mutations on ion transport was not determined; however, previous studies have shown this region of the rat α1 to be dispensable for alpha-subunit catalytic function (68). Together, the above results suggest that there may be both pump-dependent and pump-independent roles for the Na-K-ATPase in vertebrate junction formation.

Are there pump-independent roles for the Na-K-ATPase in C. elegans? This has not been carefully studied, but RNAi knockdown of one of the three β-subunits causes a body morphology defect (39) reminiscent of those caused by mutations in components of the apical junctional complex such as E-cadherin and β-catenin (21). However, detailed investigations of the phenotypes remain to be performed.

Non-barrier cell junctions and adhesion: roles of the Na-K-ATPase and other transporters

Although the Na-K-ATPase has not been shown to have a direct role in forming mammalian epithelial barrier junctions, considerable evidence indicates that the β1-subunit acts as a cell adhesion molecule on basolateral surfaces of epithelial cells. The Na-K-ATPase has long been observed at the lateral contacts of MDCK cells, but only recently has its adhesive role been demonstrated. Shoshani et al. showed that, in co-cultures of MDCK cells and Chinese Hamster Ovary (CHO) cells, the Na-K-ATPase only localizes to the membrane interface between two cells when both cells express the β1-subunit (69). Furthermore, they used aggregation assays to show that the β1-subunit could increase the adhesivity of nonpolarized CHO cells. Importantly, Vagin et al. showed that cell junction formation between MDCK cells was inhibited by an antibody against the extracellular domain of the β1-subunit (75). This adhesive interaction depends on N-linked glycosylation of β1-subunit but not on Na-K-ATPase functioning as an anti-β1 antibody.

Outside of junctions, the Na-K-ATPase also mediates homophilic and heterophilic interactions (Table 1). For example, the adhesion of the Na-K-ATPase can promote migration (74). In C. elegans, the α3-subunit locates to the posterior and ventral midline and in embryos heterozygous for a deletion in this adhesion component, the head is not properly arrested (30). Conversely, activation of the Na-K-ATPase causes a body morphology defect (39) reminiscent of those caused by mutations in components of the apical junctional complex such as E-cadherin and β-catenin (21). However, detailed investigations of the phenotypes remain to be performed.

Other transporters also modulate adhesion. For example, these are the Na-K-ATPase isoforms of the nonvertebrate Na+-dependent homophilic adhesion molecules agrin (34). AGRIN is secreted, activates the Na-K-ATPase activity, and inhibits adhesion through a direct interaction with α3-subunit. The Na-K-ATPase also modulates cell adhesion through interaction with agrin in C. elegans. In wild type, the Na-K-ATPase is colocalized with agrin. In Nrv2 null mutants, these proteins are not colocalized, suggesting that the Na-K-ATPase is required for interactions of agrin with the membrane. As the Na-K-ATPase also modulates cell adhesion through interaction with agrin in C. elegans, it is possible that the Na-K-ATPase is required for adhesion through the same mechanism in mammalian epithelial cells.
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In summary, nonadhesive activity appears to be
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septa or a paracellular diffusion barrier in cardiac tissue. Whether this adhesion depends on Na-K-ATPase-mediated ion transport remains to be determined, but the molecular similarity between cardiac and Sj adhesion complexes suggests that cardiac adhesion is ion transport independent.

**The Na-K-ATPase and epithelial cell junctions: Together from the beginning?**

The Na-K-ATPase is associated with adhesion and/or barrier junctions in a wide diversity of species. This raises the question of whether these associations result from convergent evolution, with the Na-K-ATPase being a useful component that was added to different junctions at different times, or from an ancient association of the Na-K-ATPase with primordial cell junctions that has persisted in present-day junctions. To unify many otherwise disparate observations and to outline the following working model that provides a functional explanation for why the Na-K-ATPase would be associated with cell junctions from the earliest times of metazoan evolution. This model also suggests a logical path for evolution of ion transport-independent functions of the Na-K-ATPase (FIGURE 2). Before the transition from simple unicellular eukaryotes (FIGURE 2A) to primitive metazoans consisting of adhering cells (FIGURE 2B), primitive metazoans developed the Na-K-ATPase (2, 65), numerous ion channels, and the cadherin family of transmembrane proteins (2). Primitive metazoans used cadherin-based adhesion to form multicellular animals (FIGURE 2B). In the hypothetical ancestral metazoan, we propose that cadherin localized to simple junctional structures, possibly organized by lateral oligomerization of cadherins (13, 83, 84) or by intracellular complexes. However, since apical/basal polarity mechanisms had not yet evolved, the Na-K-ATPase was distributed uniformly on the cell surface (FIGURE 2B). An alternative possibility is that the homophilic adhesion properties of the present-day mammalian β1-containing Na-K-ATPase (69) actually arose early in evolution and that adhesion by both the Na-K-ATPase and cadherin may have helped unicellular metazoans aggregate into primitive multicellular metazoans. Thus, in a primitive metazoan, even though the Na-K-ATPase would not have a polarized apical/basal localization, it could have localized laterally to the points of cell-cell contact or been part of a cadherin-based junctional complex.

We propose that after multicellularization the next breakthrough in metazoan evolution was establishment of barrier junctions that allowed “simple” metazoans to control solute environments inside their bodies. For example, in FIGURE 2 we show differential Na+ concentrations to represent a possible shift of a simple metazoan from a salt-water to a fresh-water habitat, but using barrier junctions to maintain its ancestral high-salt internal environment. Note, however, that, although necessary, barrier junctions are not sufficient for controlling an organism’s internal environment. An organism must also be able to vectorially move solutes across a simple epithelium. Thus effective control over ion transport requires some form of asymmetrical Ion transporters. We propose that the Na-K-ATPase is the company that evolved the Na-K-ATPase. In vertebrates, Na-K-ATPase localized primarily to the Na-K-ATPase and cadherin (FIGURE 2B). However, in Drosophila, the Na-K-ATPase would be more evenly distributed (D, 29, 56). Evolution of robust apical/basal polarity mechanisms allowed the Na-K-ATPase to lose strict junctional localization in some species such as vertebrates and instead localize throughout basal or, less commonly, apical membranes (D, vertebrates). However, in Drosophila epithelium, the Na-K-ATPase remains predominantly junctional (29, 56) (D, Drosophila), and there is evidence that some Na-K-ATPase localizes to TJs in particular vertebrate epithelia (58). See text for additional details.

**FIGURE 2.** A model for the evolution of junctional functions of the Na-K-ATPase. During the evolution of metazoans (A–D), the Na-K-ATPase may have associated with cell junctions during the transition from “primitive” to “simple” metazoans (B and C). Primitive metazoans lacked apical/basal polarity and barrier junctions and could not control their intraorganellar fluid composition. In contrast, barrier junctions and asymmetric localization of the Na-K-ATPase, possibly by asymmetric association with junctional complexes, allowed “simple” metazoans to control their internal environment (C). Once associated with junctions, the Na-K-ATPase would be positioned to evolve ion transport-independent functions, such as homophilic adhesion (69) and junctional scaffolding (29, 56). Evolution of robust apical/basal polarity mechanisms allowed the Na-K-ATPase to lose strict junctional localization in some species such as vertebrates and instead localize throughout basal or, less commonly, apical membranes (D, vertebrates). However, in Drosophila epithelial cells, the Na-K-ATPase remains predominantly junctional (29, 56) (D, Drosophila), and there is evidence that some Na-K-ATPase localizes to TJs in particular vertebrate epithelia (58). See text for additional details.

**Roles of Ion Transporters in Tubulogenesis**

Epithelial tube classes are complex multicellular structures that function in fluid and ion transport. To form these structures, epithelial cells require extensive invagination and fusion of membrane domains to create a new luminal space. This luminal space may function as a fluid-filled lumen, a vessel lumen, or a fluid-filled space in muscles. The roles of ion transporters in epithelial tubulogenesis have been reviewed elsewhere (53, 56). Here, we will focus on epithelial tubulogenesis in vertebrates, focusing each class of epithelium on ion transporters and tubulogene-
We propose that a straightforward way to establish vectorial ion transport would have been to asymmetrically localize the Na-K-ATPase to one side of a junctional complex and quite possibly to the barrier junction itself since it would define the apical/basal boundary. We envision such an early junctional complex to be a conglomerate of the present-day adherens and barrier junctions, perhaps similar to the apical junctional complex in present-day C. elegans (47).

Once the Na-K-ATPase was associated with the primitive junctional complex, it would be well positioned to evolve adhesive functions as in the mammalian β1-subunit (69), junctional scaffold functions as in the Drosophila Na-K-ATPase (41). Furthermore, regulation of Na-K-ATPase activity to control intracellular Na+ levels either locally or throughout the cell could have been used to regulate barrier junction formation, such as TJs in mammalian MDCK cells (62). Consistent with conserved junctional roles for the Na-K-ATPase, Paul et al. showed that the rat α1-subunit can substitute for the Drosophila ATPα subunit during embryonic development (55), suggesting that the mammalian Na-K-ATPase retains junctional functions even though the ultrastructure of Drosophila and human epithelial junctions have diverged considerably, and there is not yet any evidence that the Na-K-ATPase functions as a scaffold in mammalian junctional complexes.

Roles of Ion Transporters in Tubulogenesis

Epithelial tubes are critical for the existence of complex multicellular organisms because they transport fluids and gases, and provide the interface for and regulate exchange between compartments. The extensive involvement of cell junctions and ion transporters in these functions provides multiple opportunities for ion transporters to have both transport-dependent and transport-independent roles in tubulogenesis. Mechanisms of tubulogenesis have been detailed in multiple reviews (14, 36), so here we will focus on examples and aspects of tubulogenesis involving ion transporters.

Tubulogenesis is the process of forming and shaping cellular tubes. Several distinct classes of tubes exist, and there are multiple mechanisms for generating each class of tubes. A major distinction between tube classes is whether the lumen of the tube is extracellular and the tube wall is comprised of one or more cells joined by junctions (e.g., FIGURE 3A, A–C) or whether the lumen is intracellular with the tube wall being contiguous cytoplasm (e.g., FIGURE 3D). As discussed below, ion transporters play important roles in morphogenesis of both multicellular and intracellular tubes. Among multicellular tubes, a major distinction between tubes is how the lumen forms. Lumens can be formed by invagination of a preexisting epithelium such as during morphogenesis of the Drosophila trachea (airway), by formation of a single lumen within a cluster of cells such as in morphogenesis of the zebrafish brain ventricles, or by formation of multiple lumens in a cluster of cells that resolve to a single lumen such as in the zebrafish gut and in mammalian MDCK cell culture. To date, ion transporters do not have known roles in initial lumen formation, however, as discussed below, they are essential in multiple aspects of morphogenesis once tubes have formed.

Tubulogenesis: ion transport-dependent roles

One of the more surprising roles of ion transporters in tubulogenesis is to help create a single lumen out of multiple lumens. In zebrafish gut development and in MDCK cells cultured in a three-dimensional matrix, lumen morphogenesis initiates with formation of multiple small lumens that resolve into a single larger one (FIGURE 3A). In zebrafish lacking the Tcf2 transcription factor, expression of the Na-K-ATPase was severely reduced in gut cells. Although multiple small lumens formed in these mutants, they did not resolve into a single lumen (4). This phenotype was not due to effects on apical/basal polarity or gross organization of TJs but was due to a loss of function of the Na-K-ATPase pump activity, which is important for regulating tube size in multiple tissue types. For example, Lowery and Sive showed that the zebrafish snakehead mutation, which is predicted to compromise atp1a1a.1 pump activity, prevented apically localized Na-K-ATPase from “inflating” the lumen at the center of the developing brain ventricles (FIGURE 3B) (46). In this case, mechanical inflation of the ventricles using a syringe demonstrated that fluid transport rather than ion...
homeostasis was the underlying defect in the mutant (45). Fluid transport also appears to play a critical role in tube-size control in the zebrafish gut and MDCK cell cysts since ouabain treatment reduces lumen diameter, whereas Forskolin stimulation of chloride channels increases diameter (4). Consistent with these observations, Forskolin-regulated chloride channels contribute to lumen growth in in vitro models of PKD (50). Importantly, Forskolin treatment of zebrafish tfg mutants or combined treatment of MDCK cells with ouabain and Forskolin did not increase lumen diameter, indicating that Na-K-ATPase pump activity is required for the chloride channels to mediate fluid accumulation. Interestingly, these results show that the Na-K-ATPase can drive lumen inflation by multiple ionic circuits since the Na-K-ATPase is basolateral in the gut and in MDCK cells but apical in the zebrafish brain ventricle epithelium.

In addition to driving fluid transport, Na-K-ATPase ion transport activity is required for other processes in tubulogenesis. As discussed above, in the developing zebrafish heart, blocking Na-K-ATPase pump activity in the background of a null mutation disrupts apical junctions, which disrupts tube morphogenesis and causes a small heart phenotype (19, 70). Ion transport by the Na-K-ATPase is also required for morphogenesis of zebrafish semicircular canals, but details of the role of the Na-K-ATPase are unclear (11). Similarly, in mammalian HK2 cells, ion transport by the K+ channel KCNAl, the Ca2+ channel TRPV6, and the Na+/H+ exchanger NHE1 is required for hepatocyte growth factor (HGF)-induced tubulogenesis. These channels appear to be required downstream of HGF-signaling, but their functions in tube morphogenesis have not yet been delineated (60). Does ion transport by the Na-K-ATPase and other transporters have specific roles in invertebrate tube morphogenesis? In Drosophila, although the ENaC family of sodium channels is required to prevent the tracheal system from filling with liquid (44), there have been no reports of transport-dependent roles for ion channels or pumps in tube formation. In C. elegans, as described at the end of the next section, the EXC-4 chloride channel is required for morphogenesis of the canal cell, but it is unclear whether this is a transport-dependent or -independent role.

Tubulogenesis: ion transport-independent roles

To date, there are no reports of transport-independent roles for ion transporters during initiation of tube formation. However, during development of the Drosophila tracheal system, a transport-independent function of the Na-K-ATPase is required for assembly of a transient apical extracellular matrix (aECM) that controls tracheal tube length (72). Loss of specific Na-K-ATPase subunits disrupts this aECM and causes excessive elongation of tracheal tubes (29, 56, 77). The transport independence of this Na-K-ATPase function was demonstrated by Paul et al. (56) who showed that expression of a catalytically inactive α-subunit could rescue the tracheal defects. The role of the Na-K-ATPase in aECM organization is intimately involved in membrane fusion e.g., preventing Ca2+-induced fusion of the extracellular domain (ex-domain) of aECM in the developing tracheal system from filling with liquid (72). A transport-independent role for the Na-K-ATPase in regulating tracheal tube length may have a role in regulating the size of the tracheal system to meet the needs of the embryo.

The Na-K-ATPase and matrix modification

One of the most critical matrix-modifying proteins is the extracellular matrix (ECM) protein veriiform (Verm) into the tracheal system from filling with liquid (44). Without Verm, tracheal tube length is drastically reduced, aECM becomes disorganized, and tracheal tubes become overly elongated (reviewed in Ref. 81). Whether the Na-K-ATPase has a direct role in the Verm secretion process or whether it is just required for SJ assembly remains to be determined.

Does morphogenesis of epithelial tubes in vertebrates or C. elegans depend on ion transport-independent functions of ion transporters? As discussed in the previous section on barrier junctions, the NH2-terminal (NOX) domain of the zebrafish Na-K-ATPase α1B1 subunit may have a role in regulating the H+/H2O exchange activity of the Na-K-ATPase (50). Importantly, the NH2-terminal (NOX) domain of the zebrafish Na-K-ATPase is required for the interaction of the Na-K-ATPase with other proteins involved in tracheal tube formation (54). The NH2-terminal (NOX) domain of the Na-K-ATPase may have a role in regulating the H+/H2O exchange activity of the Na-K-ATPase (50). Importantly, the NH2-terminal (NOX) domain of the zebrafish Na-K-ATPase is required for the interaction of the Na-K-ATPase with other proteins involved in tracheal tube formation (54). The NH2-terminal (NOX) domain of the Na-K-ATPase may have a role in regulating the H+/H2O exchange activity of the Na-K-ATPase (50). Importantly, the NH2-terminal (NOX) domain of the zebrafish Na-K-ATPase is required for the interaction of the Na-K-ATPase with other proteins involved in tracheal tube formation (54). The NH2-terminal (NOX) domain of the Na-K-ATPase may have a role in regulating the H+/H2O exchange activity of the Na-K-ATPase (50). Importantly, the NH2-terminal (NOX) domain of the zebrafish Na-K-ATPase is required for the interaction of the Na-K-ATPase with other proteins involved in tracheal tube formation (54). The NH2-terminal (NOX) domain of the Na-K-ATPase may have a role in regulating the H+/H2O exchange activity of the Na-K-ATPase (50).
As discussed in Tingen and others (15), the Na-K-ATPase function and morphogenesis of the intracellular tubes of the H-shaped canal cell that functions as the worm’s kidney (7) (canal cell morphogenesis is reviewed in Ref. 15) (FIGURE 3D). In exc-4 mutants, the intracellular tubes develop cystic enlargements along what should be a long thin lumen. The exact role of EXC-4 in preventing cyst formation is unclear, but EXC-4 is a member of the chloride intracellular (CLIC) family of channels that have the remarkable ability to exist as either soluble cytoplasmic proteins or as transmembrane chloride channels. It is not known whether ion transport by EXC-4 is required for its tubulogenesis function, but the observation that exc-4 mutants have defects in several plasma membrane fusion events combined with evidence that the CLIC family member CLIC5a can participate in cytoskeletal complexes containing actin, ezrin, alphactinin, and gelsolin (8, 15) raises the possibility that EXC-4 may have an ion transport-independent role in lumen morphogenesis.

The Na-K-ATPase and ion transporters in polycystic kidney disease

One of the most common diseases of tubular epithelia is polycystic kidney disease, which affects ~1:400 to 1:1000 people and is characterized by cystic enlargements of renal tubules (reviewed by Refs. 23, 32). Most cases of autosomal dominant PKD result from mutations in either polycystin-1 (PC1) or polycystin-2 (PC2). PC1 and PC2 are transmembrane proteins that can form or regulate Ca2+ channels (Ref. 31 and reviewed in Refs. 23, 32). The prevailing model has PC1 and PC2 as part of a primary cilium-based sensor that detects fluid flow in kidney tubules. Loss of either PC1 or PC2 is thought to disrupt Ca2+-based intracellular signaling, which activates multiple pathways that promote cyst formation. Cyst enlargement results from dedifferentiation and overproliferation of renal epithelial cells and from fluid transport into the cysts mediated by Na-K-ATPase ion transport and CFTR Cl– channels (reviewed in Refs. 23, 32). Critically, inhibitors of the CFTR channel can block cyst enlargement in a mouse model of PKD (82). In addition to driving fluid transport via CFTR, an unexpected association of the Na-K-ATPase with PKD was recently revealed by the work of Zatti et al. (86), who showed in vitro and in vivo that the Na-K-ATPase binds directly to the COOH terminus of PC1. The normal function of this interaction remains to be determined, but PC1 can stimulate Na-K-ATPase ion transport, which could contribute to cyst growth through increased fluid transport.

Ion transport-independent functions of the Na-K-ATPase have also been implicated in PKD. In addition to displaying abnormal fluid transport, the cells in a cyst misexpress the embryonic β2-subunit and have apical/basal polarity defects, with the Na-K-ATPase abnormally localizing to the apical surface (3, 17, 52, 79, 80). Although apical Na-K-ATPase could contribute to fluid transport into cysts analogously to the inflation of the zebrafish ventricles by apical apfata.la Na-K-ATPase (45), the correlation of polarity defects with a change of β-subunit isoform expression is reminiscent of the requirements of a specific β-subunit isoform for Drosophila SJ formation and of the recent finding that the Na-K-ATPase is part of a novel apical/basal polarity pathway (41). It is therefore possible that defects in Na-K-ATPase-mediated adhesion and/or polarity contribute to PKD progression. Further work is required to dissect both the transport-dependent and possible transport-independent roles of the Na-K-ATPase in PKD, but it remains possible that the Na-K-ATPase has functions and influence in PKD that extend beyond simply acting as a workhorse pump.

Unifying principles for the roles of the Na-K-ATPase and ion transporters in tubulogenesis

A common mechanistic basis for the roles of ion transport in tubulogenesis likely arises from organisms using ions to move fluids by osmotic forces. Ion transporters and pumps translocate ions to create hydrostatic pressure or fluid flow that can provide motive force to drive tube morphogenesis (e.g., inflation of zebrafish ventricles [45] or cyst enlargement in PKD [32]) or a pressure or flow that would reflect some status of the tube. Another likely role for ion transport in tubulogenesis is that activity of some channels affects physiology of the cells themselves and thus has regulatory roles distinct from fluid transport. However, the specific physiology and mechanisms of tubulogenesis in different organs are very poorly understood, so in most cases considerable work will be needed to elucidate the particular role of any given transporter.

If fluid transport is a key role for ion transport in tubulogenesis, is there a similar key role for ion transport-independent functions of the Na-K-ATPase and other ion transporters? Cell adhesion, cell junctions, and epithelial polarity are underlying requirements for the integrity of epithelial tubes, and ion transport-independent functions of transporters can have roles of any of these. Thus “tube integrity” could broadly be considered a unifying theme of ion transport-independent functions of ion transporters. However, further refinement of tube integrity is difficult, since the mechanisms of adhesion, polarity, and junctions are diverse, even within a given animal.
and tubulogenesis. It is also clear that there are tissue-specific requirements for these functions. In some cases, tissue specificity reveals the multiplicity of mech-

anisms that can make junctions and tubes. In other cases, tissue specificity results from genetic redundan-
ty. Thus many of the already identified mechanisms may function most widely than currently known, and it is likely that many roles of ion transporters in junctions and tubulogenesis remain to be identified.

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