REFRESHER COURSE | Cellular Homeostasis

Ion homeostasis, channels, and transporters: an update on cellular mechanisms

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Dubyak, George R. Ion homeostasis, channels, and transporters: an update on cellular mechanisms. Adv Physiol Educ 28: 143–154, 2004; doi:10.1152/advan.00046.2004.—The steady-state maintenance of highly asymmetric concentrations of the major inorganic cations and anions is a major function of both plasma membranes and the membranes of intracellular organelles. Homeostatic regulation of these ionic gradients is critical for most functions. Due to their charge, the movements of ions across biological membranes necessarily involves facilitation by intrinsic membrane transport proteins. The functional characterization and categorization of membrane transport proteins was a major focus of cell physiological research from the 1950s through the 1980s. On the basis of these functional analyses, ion transport proteins were broadly divided into two classes: channels and carrier-type transporters (which include exchangers, cotransporters, and ATP-driven ion pumps). Beginning in the mid-1980s, these functional analyses of ion transport and homeostasis were complemented by the cloning of genes encoding many ion channels and transporter proteins. Comparison of the predicted primary amino acid sequences and structures of functionally similar ion transport proteins facilitated their grouping within families and superfamilies of structurally related membrane proteins. Postgenomics research in ion transport biology increasingly involves two powerful approaches. One involves elucidation of the molecular structures, at the atomic level in some cases, of model ion transport proteins. The second uses the tools of cell biology to explore the cell-specific function or subcellular localization of ion transport proteins. This review will describe how these approaches have provided new, and sometimes surprising, insights regarding four major questions in current ion transporter research. 1) What are the fundamental differences between ion channels and ion transporters? 2) How does the interaction of an ion transport protein with so-called adapter proteins affect its subcellular localization or regulation by various intracellular signal transduction pathways? 3) How does the specific lipid composition of the local membrane microenvironment modulate the function of an ion transport protein? 4) How can the basic functional properties of a ubiquitously expressed ion transport protein vary depending on the cell type in which it is expressed?

ION TRANSPORT PROTEINS are most broadly categorized as being either channels or transporters. From the functional perspective, transporters have been classically viewed as “vectorial” enzymes whose catalytic cycle involves 1) a relatively selective recognition/binding of the transported ion(s), 2) conformational changes in the transporter protein itself due to binding of the ion(s), and 3) the coupling of these conformational changes to physical movement of the ion(s) across the membrane bilayer. In contrast, channels have been viewed as transport proteins that facilitate the physical translocation of ions by mechanisms that involve relatively little energetic interaction between the channel protein and the transported ion(s). Rather, extrinsic factors, such as changes in membrane potential or the binding of small regulatory molecules (e.g., extracellular neurotransmitters or intracellular second messengers) dictate whether the channel protein is an open or “gated” state (capable of ion transport) or a closed state (incapable of ion transport). Thus the major conformational changes in channels are produced when the various extrinsic factors regulate those domains of the channel protein that act as gates for controlling the accessibility of the transported ion to a pore domain. In turn, the pore domain acts as the pathway or conduit for ions moving from one side of the membrane to the other. Because the actual transport of ions through channels does not require a defined sequence of energetic interactions between the transport protein (the channel) and the transported ions, the rate of ion transport through channel proteins is usually many times faster than the rate of transport through carrier-type proteins. This constitutes one of the major functional criteria classically used to distinguish channels vs. transporters.

Another classic distinction is how the function of an ion transport protein is related to the electrochemical gradient(s) for the ion(s) being transported. The driving force for the transmembrane flux of an ion is dictated by the electrochemical gradient, which reflects the difference in the concentrations of that ion on each side of the membrane as well as any electrical potential across the membrane. All channels mediate the movement of ions down their respective chemical or electrochemical gradients; this is termed passive transport. Thus the major function of ion channels is to facilitate, usually for only very short durations, the movement of ions down the electrochemical gradients previously established across either the plasma membrane or the membranes of intracellular organelles; this transient dissipation of the ionic gradient comprises a perturbation of cell function and underscores the predominant use of ion channels for rapid signal transduction or information transfer. A prototypical example is the role of voltage-gated Na+ channels in rapidly increasing the Na+ permeability of the plasma membrane of excitable cells during the rising phase of the action potential. In contrast, most transporters (but no channels) can catalyze the active transport of certain ions against their electrochemical gradients, ultimately through a net expenditure of cellular energy. Primary active transporters directly couple the movement of the transported molecule to the binding and hydrolysis of ATP. Such ATPase-coupled ion pumps are typified by the Na+-K+-ATPase, which functions to

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actively transport Na\(^{+}\) from the intracellular compartment (with low [Na\(^{+}\)]) into the extracellular compartment (with high [Na\(^{+}\)]) in exchange for the movement of K\(^{+}\) from its low concentration in extracellular fluids into the high K\(^{+}\) compartment of the cytosol. Secondary active transporters couple the movement of one ion against its electrochemical gradient to the movement of another ion down its electrochemical gradient; this mechanism is used in both cotransporters (e.g., Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter) or exchange transporters (e.g., Na\(^{+}\)/H\(^{+}\) exchanger). Thus a major function of carrier-type transporters is to establish and maintain the transmembrane electrochemical gradients of the physiologically significant inorganic cations and anions.

It is important to appreciate that most ion transport proteins exhibit certain common structural features regardless of whether they function as channels or transporters. The vast majority of membrane transport proteins possess several transmembrane-spanning domains (usually \(\alpha\)-helices) that are physically juxtaposed to create the amino acid-lined pore, which acts as the physical conduit for the ion as it passes from one side of the membrane to the other. The composition of this amino acid-lined pore creates a microenvironment, with suitable electrostatic and polar characteristics, that greatly reduces the energetic constraints that effectively prevent the direct passage of an inorganic ion through a lipid bilayer. The relative rates at which ions enter the pore from one side of the membrane and exit the pore at the other side determines the effective net transmembrane flux of the ion. Channels facilitate only the net transmembrane flux of an ion from a “cis” compartment with higher concentration to a “trans” compartment with lower concentration. Thus the major mechanistic issue for channel-type transporters is merely limiting the overall accessibility of the ion to the pore by using a single gate (another protein domain of the channel itself) to prevent entry of cis ions into the pore until an appropriate gating signal is generated by the cell (Fig. 1A). Once ions from the cis compartment have entered the pore domain, it is much more probable that they will exit the pore at the trans side (and remain there) due to the lower ion concentration in that compartment. However, it should be stressed that ion channels also contain particular arrangements of amino acid residues (usually within the permeability pore itself) that favor the entry of ions with particular charges and sizes and will retard ions with the reverse charge or the wrong size. Although channels are most broadly categorized as cation or anion specific, many cation and anion channels exhibit marked selectivity for particular species of ions. For example, certain voltage-gated cation channels select either Na\(^{+}\) over K\(^{+}\), whereas others transport K\(^{+}\) rather than Na\(^{+}\).

In contrast, the effective function of a carrier-type transporter raises a more complex set of mechanistic issues, particularly for transporters, such as the Na\(^{+}\)-K\(^{+}\)-ATPase, that catalyze the active transmembrane flux of an ion from the cis compartment with lower concentration to the trans compartment with higher concentration. In this case, at least two gates are required to sequentially control accessibility to the pore domain of ions within both the trans and cis compartments (Fig. 1B). Although the opening of a single gate at the cis side will permit entry/exit of ions from that compartment with lower concentration into the pore region, a second gate needs to be simultaneously closed to prevent entry/exit of ions from the trans compartment with higher concentration. Once ions have entered the pore domain from the cis compartment, the cis gate must be closed before the trans gate opens to permit accessibility of ion within the pore domain to the trans compartment (and vice versa). This simultaneous closing of both

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**Fig. 1.** Fundamental differences between ion channels and ion transporters in terms of gating and occlusion states [adapted from Gadsby (14)].
accessibility gates effectively traps or “occludes” ions that have entered the pore from the cis compartment. Although subsequent opening of the second gate will allow ions to move between the pore domain and trans compartment, this gating needs to be coupled to some other conformational change within the transporter that effectively favors the exit of the ions previously occluded within the pore domain while minimizing the entry (or reentry) of the same species of ion from the trans compartment. This can be accomplished by changing the affinity and/or selectivity of the pore domain for particular ions depending on whether the pore is opened to the cis or trans compartment. This is accomplished in the Na⁺-K⁺-ATPase by conformational changes that reduce the association of Na⁺ ions with the permeability pore while increasing the affinity of the pore cavity for extracellular K⁺ ions. By thermodynamically coupling the sequential opening and closing of the two accessibility gates to the sequential conformational changes that alter the ionic selectivity of the pore domain, carrier-type transporters can catalyze the net flux of ions against their electrochemical gradients. The required conformational changes that modulate the ionic selectivity and affinity of carriers can be energized by either the direct binding of transported ions to specific sites within the pore domain or parallel chemical reactions (e.g., ATP hydrolysis, protein phosphorylation/dephosphorylation) catalyzed by the carrier-type transporter.

ION TRANSPORT PROTEINS AS CHANNELS VS. TRANSPORTERS: NOT AS DIFFERENT AS WE THINK

Concept: Both channel-like activity and transporter-type activity can be accommodated within the basic structures of most ion transport proteins.

Model Example: The “conversion” of the Na⁺-K⁺-ATPase transporter, an ATP-powered ion exchange pump, into a cation channel by palytoxin (PTX), a marine coral toxin that selectively binds to the Na⁺-K⁺-ATPase.

Most physiologists will readily agree that the Na⁺-K⁺-ATPase pump is one of the most important and extensively characterized ion transporters. The Na⁺-K⁺-ATPase is an archetypal active transporter that catalyzes the net efflux of three Na⁺ ions in exchange for the net accumulation of two K⁺ ion per reaction cycle. Recent analyses of the interactions of the Na⁺-K⁺-ATPase with PTX, a marine coral poison, indicate that this prototypical carrier-type transporter may continue to provide paradigmatic insights regarding the fundamental function and structure of such transport proteins (3, 4, 15, 16, 20, 22). Significantly, these PTX studies suggest that the basic functional and structural features that define ion transporters vs. ion channels may be much more similar than expected. Full appreciation of the insights and implications of these PTX experiments requires a review of the generally accepted mechanism of the Na⁺-K⁺-ATPase as an ion transporter.

Figure 2 illustrates this cyclic interplay of chemical reactions, gating movements, and progressive ion transport as it occurs in the Na⁺-K⁺-ATPase. The initial reactions correspond to the entry of cytosolic Na⁺ into the permeability pore followed by occlusion of now-resident Na⁺ ions within the cavity defined by simultaneous closing of both the cis and trans gates. The initial binding of cytoplasmic Na⁺ ions within the cis-gated open state of the Na⁺-K⁺-ATPase triggers the ATP-driven phosphorylation of a regulatory aspartate within the carrier (i.e., the carrier acts as a Na⁺-activated protein kinase). This autoprophosphorylation drives the conformational change that closes the cis gate to trap the bound Na⁺ ions within the now occluded permeability pore. Closing of the cis gate also enables the opening of the trans gate. This opening of the trans gate is accompanied by yet another conformational change that greatly reduces the Na⁺ affinity of the pore domain while increasing the relative affinity for K⁺. After the sequential exit of Na⁺ ions and entry of K⁺ ions, the binding of the K⁺ ions triggers a conformational switch that results in both reclosing of the trans gate to trap the bound K⁺ ions in an occluded state of the pore and the dephosphorylation of the regulatory aspartate (i.e., the carrier now acts as a K⁺-activated protein phosphatase). Finally, the binding of ATP to low-affinity sites (distinct from the high-affinity sites required for aspartate phosphorylation) accelerates the opening of the cis gate with consequent deocclusion of the trapped K⁺ ions and their release into the cytosolic compartment.

Fig. 2. Cyclic interplay of chemical reactions, gating movements, and progressive ion transport as it occurs in the Na⁺-K⁺-ATPase [adapted from Artigas and Gadsby (3)].

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It is easy to appreciate how this stepwise and highly coupled sequence of gating movements, conformational changes, and enzymatic reactions contributes to the much slower rates of net ionic transport facilitated by transporters relative to channels. Indeed, the rate of ion flux through an open channel can be up to $10^6$ faster than the flux catalyzed by transporter/pumps. A corollary of this difference in flux rate is that there must be an exceedingly low probability (on the order of $1/10^6$ to $10^6$) that both gates of a transporter/pump might open at the same time (3, 14). These significant differences in physiological function might also portend major differences in the structures of the pore domains and gating domains in channels vs. transporters. Structures of the pore and gating domains of several different ion channels have now been described at high resolution through extensive functional characterization of mutant proteins and X-ray crystallographic analysis (10, 12). In addition to providing detailed molecular information regarding the crystallized channels per se, these structures have provided a framework for directing new functional analyses of related channel proteins that have not yet been crystallized. For example, Estevez et al. (13) used the crystal structure of the bacterial Ec-CIC and St-CIC Cl$^-$ transporters (see below for why these are called “transporters” rather than channels), to direct mutagenesis studies of the mammalian CIC-1 Cl$^-$ channel. Based on the Cl$^-$-coordinating sites of Ec-CLC and St-CIC, these investigators mutated relevant residues of CIC-1 to map the interaction site of 9-anthracene carboxylic acid (9-AC), a known blocker of Cl$^-$ flux through CIC-1.

Crystal structures have also been described for several transporters, including the Ca$^{2+}$-bound and Ca$^{2+}$-free states of the sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA) pump, another extensively studied ion transporter protein (37, 38) Although transmembrane (TM) helices 4, 5, and 6 contain the key residues for binding the two Ca$^{2+}$ ions transported per each reaction cycle of a SERCA pump, the precise structures of the entire permeability pathway and the gating domains still need to be established. At present, there are no crystal structures of the Na$^{+}$-K$^{+}$-ATPase. However, modeling of the Na$^{+}$-K$^{+}$-ATPase based on the SERCA1 pump lattices suggests that the homologous TM4, 5, and 6 of the Na$^{+}$-K$^{+}$-ATPase contain key residues suitable for binding two of the three Na$^{+}$ ions and the two K$^+$ ions (30). The third Na$^+$ may be coordinated by a pocket formed by apposition of TM 6, 8, and 9. However, the nature of the ion translocation pore domain and the structure/function of the regulatory gates remain key issues for a more complete mechanistic description the Na$^{+}$-K$^{+}$-ATPase. In this regard, the recent studies of PTX-modified Na$^{+}$-K$^{+}$-ATPase transporters are likely to provide a valuable starting point for generating new and experimentally testable hypotheses.

PTX is a marine toxin first isolated 33 years ago from the Palythoa toxica coral (27). It is a large (~3,000 Da) nonpeptide molecule that consists of a chain of >100 carbons with a complex variety of organic side groups (20). PTX was identified on the basis of its striking lethality when injected into mice; this lethality in intact organisms is due to rapid disruption of cardiac function together with severe vasoconstriction. However, studies with isolated cells have revealed that PTX readily affects most cell types by depolarizing the plasma membrane potential as a secondary consequence of inducing a small-conductance, nonselective cation channel activity. In excitable cells, such as neurons or myocytes, this is manifested as an increase in the resting Na$^{+}$ conductance (23, 28). In osmotically fragile cells, such as erythrocytes, PTX elicits Na$^+$ accumulation, K$^+$ loss, and swelling that can culminate in lysis (2, 15). Early studies by Habermann et al. (15) revealed that these effects of PTX on erythrocyte homeostasis were substantially attenuated by the presence of ouabain, the classical and highly selective inhibitor of the Na$^{+}$-K$^{+}$-ATPase. Subsequent biochemical and physiological analyses over the past 20 years have identified the Na$^{+}$-K$^{+}$-ATPase as the major molecular target of PTX (20).

The take-home message from these studies indicates that bound PTX directly affects the Na$^{+}$-K$^{+}$-ATPase by converting this archetypal active ion transporter into a non-selective cation channel that acts to dissipate the transmembrane Na$^+$ and K$^+$ gradients which are generated by the Na$^{+}$-K$^{+}$-ATPase acting in its normal (i.e., non-PTX occupied) transporter mode. PTX induces this remarkable functional transformation by overriding the fundamental mechanism that distinguishes pumps and transporters from channels: the stringent maintenance of at least one gate in the closed position at every phase of the catalytic transport cycle. Thus PTX binding appears to greatly increase the probability for simultaneous opening of both gates of the Na$^{+}$-K$^{+}$-ATPase protein. Recent electrophysiological analyses, together with the mutagenesis studies, have begun to define the mechanistic characteristics of the PTX-ligated Na$^{+}$-K$^{+}$-ATPase protein.

Earlier studies of yeast cells expressing normal or mutated versions of mammalian Na$^{+}$-K$^{+}$-ATPase pumps revealed that PTX can induce ouabain-sensitive cation fluxes even when the heterologous Na$^{+}$-K$^{+}$-ATPase protein lacked residues critical for ATP hydrolysis and autophosphorylation (34). This indicated that PTX could independently stabilize the open gates that regulate ionic access to the permeability pathway or pore in the absence of the coupled chemical reactions that regulate gating in the normal transport protein. However, these studies left open the reverse mechanistic question: how is the interaction of PTX with the Na$^{+}$-K$^{+}$-ATPase “channel” modulated quantitatively or qualitatively by the ligands, such as intracellular ATP or extracellular K$^+$, that regulate conformational changes in the native transporter? A converse set of questions is concerned with how the binding of PTX to the Na$^{+}$-K$^{+}$-ATPase may modulate the ability of the protein to bind ATP and to be autophosphorylated.

In a recent series of elegant studies, Artigas and Gadsby (3, 4) have addressed these questions by using classic patch-clamp electrophysiological methods to analyze the interactions of PTX with native Na$^{+}$-K$^{+}$-ATPase transporters in excised outside-out patches from HEK293 cells or guinea pig ventricular myocytes. High concentrations (2 nM) of the toxin could induce channel activity, albeit with a low open-state probability ($P_o$), in the absence of ATP (at the cytosolic side). Conversely, in the presence of internal ATP, even low concentrations (25 pM) of PTX rapidly induced Na$^+$ channels with high $P_o$. Other experiments showed that poorly hydrolyzable analogs of ATP were equally effective in potentiating the $P_o$ of PTX-induced channels. These findings indicated that the PTX-ligated channels can be allosterically modulated by the simultaneous binding of ATP, which is a principal physiological regulator and substrate of Na$^{+}$-K$^{+}$-ATPase conformation. Other experiments showed that the presence of extracellular K$^+$ markedly altered the kinetics of channel opening/closing.
by the PTX-modified Na\(^{+}\)-K\(^{+}\)-ATPase by increasing the rate and probability of channel closure. Recall that the entry of extracellular K\(^{+}\) ions into sites of the Na\(^{+}\)-K\(^{+}\)-ATPase permeability pore acts to close the trans (extracellular) gate and so induce occlusion of the bound K\(^{+}\). Thus these various electrophysiological data indicated that PTX-liganded Na\(^{+}\)-K\(^{+}\)-ATPase channels retain this sensitivity to allosteric modulation by extracellular K\(^{+}\), another physiological regulator and substrate of the Na\(^{+}\)-K\(^{+}\)-ATPase.

Interestingly, other recent studies by Horisberger and colleagues (15, 16, 22) have used cysteine-scanning mutagenesis of the Na\(^{+}\)-K\(^{+}\)-ATPase to determine sites in the transmembrane segments of the PTX-modified protein that are accessible to small hydrophilic sulfhydryl reagents. This approach, commonly used by channel biophysicists, relies on the ability of the small sulfhydryl reagent to enter the permeability pore of an open-gated channel, covalently react with any cysteine residues (native or purposely substituted for the native, noncysteine amino acids at particular positions) that line the pore, and thereby attenuate ionic flux through the permeability pore. Significantly, cysteine substitution of residues in the TM4, TM5, and TM6 segments of the Na\(^{+}\)-K\(^{+}\)-ATPase become accessible in the PTX-bound state. Recall that these TM segments contain the likely binding sites for the three Na\(^{+}\) ions and two K\(^{+}\) ions that are normally transported by the Na\(^{+}\)-K\(^{+}\)-ATPase in its physiological transporter mode (30).

Thus the observations of Artigas and Gadsby (3, 4), together with those of Horisberger and colleagues (15, 16, 22), suggest that PTX acts to stabilize intermediate conformational states, which are normally extremely short lived, of the Na\(^{+}\)-K\(^{+}\)-ATPase-mediated ion transport process (Fig. 2). This indicates that PTX may prove to be a highly useful tool for defining the structures and regulation of the gates that control the entry, occlusion, and release of Na\(^{+}\) and K\(^{+}\) during the physiological cycling of the Na\(^{+}\)-K\(^{+}\)-ATPase. Moreover, these findings indicate that the fundamental structures of channels and transporters may share a highly related molecular architecture derived from common ancestral membrane proteins (1, 14). In this regard, Accardi and Miller (1) have recently demonstrated that bacterial (Escherichia coli) homologs (12) of the mammalian ClC-family Cl\(^{-}\) channels actually function as Cl\(^{-}\)/H\(^{+}\) exchange transporters rather than high-flux Cl\(^{-}\) channels. For channels, the ancestral protein architecture has evolved to retain only one major gating domain so as to favor rapid ionic flux when the gate is the open position. Conversely, in transporters, this ancestral structure may have evolved to retain (or possibly to gain) at least two gating domains whose movements are allosterically regulated by the ion substrate(s) of the transport reaction and/or biochemical reactions directly catalyzed by the transport protein.

**INTERACTIONS OF ION TRANSPORT PROTEINS WITH ADAPTER PROTEINS: NO TRANSPORTER IS AN ISLAND**

**Concept:** Many channels and transporters physically associate with adapter proteins that regulate the subcellular localization of the transport protein. Additionally, the associated adapter protein may control the direct interaction of the transport protein with signal transduction complexes that include receptors, second messenger-producing effector enzymes, and protein kinases.

**Model Example:** The role of NHERF-family adapter proteins in the localization and acute regulation of the type 2 Na-phosphate cotransporter (NPT2) within apical signaling complexes of renal epithelial cells.

Over the past decade or so, the study of ion transport proteins has increasingly utilized the tools of biochemistry and cell biology to describe the subcellular localization of ion transport proteins and their regulation by various intracellular signaling cascades. Such analyses have been facilitated by two types of reagents: 1) specific antibodies directed against native ion transport proteins and 2) cDNA expression vectors (plasmids or viruses) encoding chimeric versions of ion transport proteins with various protein tags, such as green fluorescent...
proteins and smaller peptide epitopes. The use of these reagents with standard immunoprecipitation and immunohistochemical methods has demonstrated that many transport proteins interact, via direct protein-protein association, with a variety of regulatory proteins that have been generically termed “adapter domains.” By definition, adapter proteins contain several different types of highly conserved modular protein-protein interaction domains (e.g., the src homology 2 domains that recognize phosphotyrosine motifs) that allow adapters to act as molecular bridges between two or more different proteins; these can include signaling proteins, membrane transport proteins, and cytoskeletal elements. The number and variety of adapters continues to increase and includes both soluble and intrinsic membrane proteins. As schematically depicted in Fig. 4, adapter proteins affect the function of ion transport proteins in two, nonmutually exclusive ways. 1) Adapters can modulate the steady-state levels of an ion transport protein at the plasma membrane by affecting trafficking to, or retention within, the plasma membrane compartment. 2) Adapters can concentrate an ion transport protein within subdomains (“punctate patches,” “hot spots,” “clusters”) of the plasma membrane that are also enriched in the receptors or other signaling proteins known to acutely regulate the trafficking or activity of the transport protein. In these ways, adapter proteins can comprise the central nexus of multiprotein complexes that greatly increase the selectivity, subcellular localization, and temporal control of critical ion transport responses to extrinsic stimuli.

Adapter proteins that interact with ion transport proteins (channels or transporters) often contain so-called “PDZ” interaction domains (reviewed in Ref. 29). The PDZ domain was first recognized: PSD-95 (postsynaptic density-95, a synaptic protein),Dlg (Discs-large, a protein in septate junctions), and ZO-1 (zona occludins-1, a protein in the tight junctions of epithelia). PDZ-containing proteins are widespread, with over 400 identified in humans alone. NHERFs comprise a family of PDZ-containing adapter proteins that play important roles in the regulation of several ion transport proteins (36). Indeed, the term NHERF is an acronym for Na+/H+ exchanger (NHE) regulatory factor. Two distinct NHERF proteins, NHERF-1 and NHERF-2, have been extensively characterized at the biochemical, physiological, and cell biological levels. NHERFs are 55-kDa proteins that contain two adjacent PDZ domains: PDZ-I near the NH2 terminus and PDZ-II in the COOH-terminal to these PDZ domains is an ERM (ezrin, radixin, moesin, and merlin) motif, a protein-protein interaction domain found in adapter proteins that bridge membrane and cytoskeletal elements. Given these three distinct protein interaction domains, it is not surprising that NHERFs have been shown to bind about 50 different proteins (39) including the 16 illustrated in Fig. 4. In addition to the cytoskeletal proteins mentioned previously, these encompass G protein-coupled receptors (GPCR) such as the β2-adrenergic receptor, P2Y1-nucleotide receptor, and parathyroid hormone (PTH) receptor; ion transport proteins, such as the cystic fibrosis transmembrane regulator (CFTR) Cl- channel, the H+/ATPase pump, the type 3 Na+/H+ exchanger (NHE3), and the type 2 Na-phosphate cotransporter (NPT2); and second messenger-generating enzymes (phospholipase C-β1). Identifying and characterizing the physiological consequences of these various NHERF-based interactions has become an active and exciting area of study in ion homeostasis biology (18, 36, 39, 41). A particularly salient and informative example is provided by the role of NHERFs in orchestrating the regulation of NPT2-mediated phosphate transport by G protein-coupled PTH receptors in the kidney.

NPT2 in the proximal tubules is responsible for most reuptake of phosphate from the glomerular filtrate. Increases in serum phosphate (due to diet and other factors) elevate PTH levels, which, in turn, act to decrease the NPT2-mediated recovery of filtered phosphate. This PTH-induced decrease in NPT2 transport activity involves a rapid decrease in the density of NPT2 transporters in the apical membranes due to acute changes in the steady-state trafficking of NPT2 between cell surface and intracellular membrane pools (26). Thus activation of PTH receptors predominantly acts to alter the subcellular localization of NPT2 rather than to decrease the total cellular content of NPT2. The G protein-dependent signaling cascades that trigger these changes in steady-state NPT2 trafficking involve both the phospholipase C (PLC) and adenylyl cyclase (AC) pathways. In this regard, it is important to note that the PTH receptor belongs to a subgroup of GPCR that can efficiently couple to the activation of PLC and/or AC depending on cellular context. Segre and colleagues (24–26) have dissected the mechanisms by which PTH triggers these changes in NPT2 in the recent series of studies that underscores the critical role of NHERFs in the directly coordinating regulation of both NPT2 transporters and PTH receptors. Their studies utilized an established cell line (OK) from opossum kidney that retains high expression of NPT2 and PTH receptors similar to those in the parental cells. OKH cells, a clonal variant of the OK cell line, exhibited little change in phosphate accumulation response to PTH. In contrast, OKH cells, a clonal variant of the OK cell line, exhibited little change in phosphate accumulation during PTH stimulation despite expressing levels of functional NPT2 and PTH receptors similar to those in the parental cells. This inability of PTH to appropriately regulate NPT2 was correlated with a hypoexpression of NHERF1 in the OKH cells, and stable expression of NHERF1 in these cells (OKH-N1 clone) completely rescued the ability of PTH to repress phosphate accumulation (24). Conversely, expression of a truncated version of NHERF1 lacking the ERM domain

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**Fig. 4.** Three major protein-protein interaction domains of Na+/H+ exchanger-regulatory factor (NHERF) adapter proteins and some of the proteins that can interact with each domain [adapted from Shenolikar and Weinman (36)]. See text for definitions.
for cytoskeletal interaction was unable to restore regulation of phosphate transport. Confocal microscopic analysis of NPT2 subcellular localization revealed similar levels of NPT2 at the apical cell surface of both OKH-N1 and OKH cells in the absence of PTH stimulation. However, the NPT2 was diffusely distributed over the apical surface in OKH cells but clustered into punctate patches on the OKH-N1 cell surface. Significantly, activation of PTH receptors in the OKH-N1 cells triggered redistribution of NPT2 out of the apical plasma membrane and into an intracellular membrane pool while NPT2 remained at the apical surface of PTH-treated OKH cells. Other experiments (25, 26) showed that the presence of NHERF is necessary for effective coupling of PTH receptors to G
proteins (33, 41). In the absence of NHERF, PTH receptors couple only to the AC effector enzyme, which, by itself, appears insufficient to generate optimal signals for the redistribution of cell surface NPT2 into intracellular pools. These findings of Segre and colleagues (24–26) strongly suggest that direct interaction with NHERF proteins appears mandatory for the coordinated physiological functions of both NPT2, a key phosphate transport protein, and the PTH receptor, a critical endocrine element for homeostatic regulation of phosphate.

A physiological role for NHERFs in the regulation of NPT2 function is also consistent with the phenotype of knockout mice, in which the NHERF1 gene has been deleted in collaborative research directed by Shenolikar and Weinman and their colleagues (9, 35). Although total NPT2 protein content is similar in the kidneys of NHERF1−/− and NHERF1+/+ mice, the basal steady-state level of NPT2 at the apical surface of the proximal tubules is much lower in the knockout animals. This reduced apical content of NPT2 is matched by elevated levels of internalized NPT2. Moreover, the NHERF1−/− animals exhibit 1) significant phosphate wasting into the urine under normal steady-state conditions and 2) an inability to adequately match renal phosphate transport to diet-induced changes in total phosphate intake. The altered NPT2 phenotype of the NHERF1 knockout mouse is more complex and nuanced than might be expected from the tissue culture analyses of NHERF1-NPT2 interactions described above. Recall that NPT2 is present mainly in the plasma membrane of the (NHERF1-low) OKH cells under basal conditions. This contrasts with the predominantly intracellular localization of NPT2 in the NHERF1-null proximal tubule cells under basal conditions. This disparity likely reflects differences in the relative rates of steady-state NHERF1 trafficking to, and retention within, apical membranes under in vivo conditions, with the complex contribution of multiple endocrine and other regulatory factors, vs. the more rigidly controlled cell culture environment. Nonetheless, both the in vivo and ex vivo observations support the same fundamental implication: that autonomous expression and function of NPT2 as a “transporter island,” disconnected from the contextual “whole” of proximal tubule cells wherein it is natively expressed, fails to reveal the true physiology of this membrane protein. This theme is becoming the rule, rather than the exception, as the functions of cloned channels and transporters are increasingly analyzed in a similar way.

It should be stressed that the particular interactions between NHERFs, the PTH receptor, and the NPT2 transporter described above represent only a subset of the various ways in which adapter proteins have been shown to regulate ion transport processes. First, interaction of the β3-adrenergic receptor with NHERF allows that receptor to modulate Na+/H+ exchange activity without the classical mediation by G protein-dependent processes (18). Second, in addition to NHERFs, two other PDZ-containing proteins, i.e., PDZK1 and PDZK2, play important roles in regulated various ion transporters in the proximal tubule (19). Third, dimeric PDZ proteins can effectively “cross-link” and thereby induce dimerization of some ion transport proteins with significant functional consequences (33, 40). Fourth, because some cytoskeletal proteins, e.g., ezrin, also act as AKAPs (protein kinase A-anchoring proteins), they can colocalize such protein kinases in the immediate vicinity of ion transporters within NHERF-based protein ensembles; this can facilitate the highly localized phosphorylation and functional modification of the transporter (11, 41).

INTERACTIONS OF ION TRANSPORT PROTEINS WITH LOCAL LIPIDS: THE BILAYER AS MORE THAN A LOW DIELECTRIC PERMEABILITY BARRIER

**Concept:** The function of many channels and transporters is directly modulated by the specific binding of phosphatidylinositol (IP3) to NHERF1. This binding permits rapid modulation of ion transport activity by highly localized changes in IP3 synthesis or degradation.

**Model Example:** Hypersensitization of the activity of nociceptive vanilloid receptor channels (VR1) by inflammatory mediators that activate PLC and IP3 degradation. The detailed biophysical study of ion channels how routinely involves the use of patch clamp electrophysiological methods to analyze channel current in excised samples of plasma membrane. This allows investigators to systematically and precisely manipulate the composition of the solutions bathing either side of the membrane in which the channel resides. A common observation in such studies is that one or more basic indexes of the ion channel’s function (open or closed probability, single-channel conductance, ion selectivity, relative stability or lability) can be markedly changed by the presence or absence of ATP at the cytoplasmic surface of the membrane patch. This ATP-dependent perturbation of channel function can reflect at least three distinct types of regulation. ATP binding can exert direct allosteric effects on channel conformation and function; in these cases, the modulatory actions of ATP can readily be mimicked by nonhydrolyzable analogs of ATP (i.e., there is no requirement for a transfer of phosphate from ATP). Alternatively, ATP can act as a substrate for a protein kinase colocalized with the channel in a membrane-associated signaling complex such as those organized by the PDZ adapter proteins described above. Here, the modulatory effects of ATP cannot be mimicked by nonhydrolyzable analogs but can be attenuated by pharmacological inhibitors of the colocalized protein kinase (if known). The kinase may directly phosphorylate the channel or a distinct modulatory protein that directly associates with the channel. However, regulation of channel activity by ATP can often be unequivocally dissociated from either a direct allosteric action of the nucleotide or the phosphorylation of the channel (or channel-associated protein) by a protein kinase. Rather, the effects of ATP reflect its ability to also act as a substrate for a variety of phosphatidylinositol kinases (i.e., lipid kinases) that can seri-
ally phosphorylate phosphatidylinositol (the most abundant inositol lipid) to phosphatidylinositol 4-monophosphate (PIP) to phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). In turn, the higher-order phosphoinositides (PIP2 in particular) act as the allosteric effectors that hydrolyze PIP2 to generate the second messengers IP3 and diacylglycerol (DAG). PLC enzymes include the PLC-β isoforms that are regulated by certain trimeric G proteins and their upstream GPCR, and the PLC-γ enzymes that can be regulated by the receptor tyrosine kinases (RTK) targeted by growth factors, or nonreceptor tyrosine kinases. Thus exposure of cells to neurotransmitters, local mediators, or growth factors that couple to PLC enzymes can trigger very rapid decreases in PIP2 levels in the immediate vicinity of an ion transport protein. This negative action on PIP2 levels is opposed by the inositol lipid kinases that replenish the PIP2 pool (Fig. 5). As discussed in the previous section, PLC enzymes can be intimately colocalized with ion transport proteins via adapter proteins such as NHERF. Exactly how local PIP2 modulates the activity of particular ion transport proteins remains an area of active investigation. In some cases, this reflects direct binding of PIP2 to intracellular domains of the transport protein (21, 32). In other cases, PIP2 seems to act indirectly by affecting the interactions of the transport protein with other regulatory proteins.

The role of PIP2 as a physiological modulator of an important ion transport protein is provided by the elegant work of Julius and colleagues (5, 6, 32) on the vannilanoid receptor channel VR1. VR1, which is highly expressed in sensory nerve endings, belongs to the TRP (transient receptor potential) superfamily of nonselective cation channels (31). VR1 was originally identified as the receptor for capsaicins, the active ingredients in hot peppers and other “spicy” foods. The binding of lipid-permeable capsaicins, or endogenous lipids, such as arachidonic acid, can gate VR1 to the open state to produce depolarization of the sensory neuron and consequent perception (by the organism) of a noxious or painful sensation. Significantly, gating of VR1 to its open conformation is directly produced by elevated temperature or an elevated concentration of extracellular H⁺. Thus VR1 also mediates the sensation of pain-producing burns elicited by high heat or exposure to acid (Fig. 6).

As anyone who has experienced a wound, skin cut, or other tissue damage appreciates, the area in the immediate vicinity of damage is usually characterized by a much heightened sensitivity to touch, heat, cold, and tissue swelling. The Julius group (6, 32) was able to show that, at the cellular level, this...
“sensitization” reflected the actions of extracellular mediators, such as bradykinin, that accumulate at sites of tissue damage as part of the protective inflammatory response. Significantly, many of these local inflammatory mediators act as agonists for GPCR that activate PLC-β effector enzymes. Chuang et al. (6) demonstrated that VR1 channel activity is negatively regulated by the ambient PIP2 levels present in the membranes of nonstimulated cells. Thus only small membrane currents were elicited by extracellular acid or capsaicin pulses in a voltage-clamped HEK293 cell expressing recombinant VR1. However, in cells briefly pretreated with bradykinin, the same pulses of acid or capsaicin triggered very large depolarizing inward currents. In an extensive series of experiments, Chiang et al. conclusively showed that this effect was due to the ability of bradykinin to transiently decrease PIP2 levels and thereby “derepress” the direct, tonic effects of the lipid on VR1 gating by the primary noxious stimuli. Prescott and Julius (32) further demonstrated that mutation of selected amino acids on the
intracellular COOH-terminal tail of the VR1 significantly increased the sensitivity of the channels to gating by low concentrations of acid or capsaicin, even in the absence of bradykinin, while reducing the potentiation of VR1 gating by bradykinin and other stimuli that activate PI2 breakdown. This identified the VR1 COOH terminus as the likely site of high-affinity PI2 interaction.

**INTERACTIONS OF ION TRANSPORT PROTEINS AND MODULATOR PROTEINS: CELL-SPECIFIC CONTEXT EXPLAINS ALL**

**Concept:** An ion transport protein can exhibit tissue-specific differences in function that reflect its direct association with modulator proteins that are expressed in a tissue-specific or stimulus-specific fashion.

**Model Example:** The role of FXYD-family membrane proteins in the tissue-specific modulation of Na⁺-K⁺-ATPase pump activity.

Detailed structure-function analysis of ion channels or transporters routinely involves heterologous expression of cDNAs (or cRNAs) encoding the normal or purposefully altered sequence of the transport protein in various recipient cell types (COS, CHO, HEK293, Xenopus oocytes). Sometimes, significant differences are observed in the fundamental transport properties of the expressed channel or transporter depending on the cell type used for heterologous expression. In other cases, properties of the ion transport protein are similar when heterologously expressed in various cell types but are quite different from the functional properties of the transporter as natively expressed in specialized cells or tissues. Additionally, the properties of ion transport proteins in native tissues may be altered (sometimes in unexpected or surprising ways) when the expression of other proteins is genetically ablated in knockout animal models. Thus the detailed function or phenotype of a given transport protein, even one that is widely or ubiquitously expressed, may be significantly altered by tissue-specific or cell-specific context due to the presence or absence of particular modulator proteins (Fig. 7). A particularly striking example of how modulator proteins can direct the phenotypic characteristics of an ion transport protein is provided by the interactions of the Na⁺-K⁺-ATPase with members of the FXYD protein family (reviewed in Refs. 7 and 8).

Figure 8 illustrates the overall cell biology of the Na⁺-K⁺-ATPase in terms of synthesis, trafficking, and association between its α- and β-subunits. As already noted, Na⁺-K⁺-ATPase is one of the extensively studied and characterized ion transport proteins. Although its basic function and catalytic cycle is invariant regardless of tissue source, significant differences in the kinetic “details” of the Na⁺ pump from different tissues have been widely reported. These differences include variations in its turnover rate or apparent affinity (Kₐ) for its

<table>
<thead>
<tr>
<th>FXYD Subtype (alternative name)</th>
<th>Tissue Distribution</th>
<th>Interacting α-Subunit</th>
<th>V_max</th>
<th>K'_Na</th>
<th>K'_K</th>
<th>K'_ATP</th>
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<tbody>
<tr>
<td>FXYD1 (PLM)</td>
<td>Heart and muscle</td>
<td>α₁ and α₂</td>
<td>⇑</td>
<td>↑</td>
<td>↑</td>
<td>ND</td>
</tr>
<tr>
<td>FXYD2</td>
<td>Kidney</td>
<td>α₁</td>
<td>⇑</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>FXYD4 (CHIF)</td>
<td>Kidney and colon</td>
<td>α₁</td>
<td>⇑</td>
<td>↓</td>
<td>↑ or ⇑</td>
<td>⇑</td>
</tr>
<tr>
<td>FXYD7</td>
<td>Brain</td>
<td>α₁</td>
<td>⇑</td>
<td>⇑</td>
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<td>ND</td>
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Information is adapted from Cornelius and Mahmoud (7) to show the differential tissue distribution of selected FXYD proteins, their association with Na⁺ pump α-subunit subtypes, and their effects on kinetic constants of associated Na⁺ pump, V_max, maximal velocity; K'_Na, affinity constant for intracellular Na⁺; K'_K, affinity constant for extracellular K⁺; K'_ATP, affinity constant for intracellular ATP; ND, not determined.
physiological substrates (Na\(^+\), K\(^+\), and ATP). Although these differences have sometimes been linked to the tissue-specific expression of the four different isoforms of the α-subunit or three distinct β-subunits, there remain examples of tissue-specific differences in function despite the similar expression of α- and β-subunit isotypes. Recent studies have indicated that these latter differences reflect the presence of particular modulator proteins from the so-called FXYD family.

The FXYD family comprises a group of seven, relatively small (66–178 residues) intrinsic membrane proteins that share a common structure of 1) an extracellular amino terminus containing a juxtamembrane stretch of four amino acids with the sequence (-F-X-Y-D-) that gives the family its name, 2) a single transmembrane domain, and 3) a short cytosolic COOH terminus that is quite divergent except for a conserved juxtamembrane domain containing a serine adjacent (or almost adjacent) to positively charged amino acids. Most of the FXYD proteins were first identified and characterized as small plasma membrane proteins of uncertain function but distinctive tissue expression. For example, FYXD1 was originally described as plasmalemman, a muscle-derived protein that induced an unusual channel-like activity when ectopically expressed in Xenopus oocytes. FXD4 was initially characterized as CHIF (corticosteroid hormone-induced factor) a gene predominantly expressed in Na\(^+\)-conserving segments of the colon and kidney (medullary collecting duct). However, subsequent research has demonstrated that the various FXYD proteins predominantly act by directly associating with the Na\(^+\)-K\(^+\)-ATPase and thereby modulating the kinetic properties of this vectorial enzymes. Table 1 summarizes the tissue-specific expression and functional consequences of the best characterized FXYD proteins. The take-home message is that FXYD proteins modulate Na\(^+\)-K\(^+\)-ATPase pump activity in a way that matches the particular rates of Na\(^+\) removal and K\(^+\) reaccumulation that are dictated by the specific functions of a tissue or cell. This is reasonable from a purely teleological view, given the very significant quantitative variations in Na\(^+\)-K\(^+\)-ATPase activity among different tissues (i.e., reflect on the differences in overall ionic fluxes in an actively firing neuron, an epithelial cell specialized for recovery of Na\(^+\) from the urine, or an erythrocyte).

Consideration of how a particular FXYD subtype is integrated into the specialized ion transport requirements of a particular tissue is exemplified by the role of FXD4/CHIF in the medullary collecting duct (MCD) of the kidney. In times of whole body Na\(^+\) deficit, the kidney needs to efficiently salvage Na\(^+\) from the glomerular filtrate. A major mechanism involves the increased secretion of the mineralocorticoid hormone aldosterone that acts to increase expression of ENaC (epithelial Na\(^+\) channels) in the apical membranes of MCD epithelial cells. Although this greatly increases the rate of Na\(^+\) influx into the MCD cell, net Na\(^+\) recovery requires an equivalent increase in the rate of Na\(^+\) efflux at the basolateral MCD plasma membrane. In this regard, aldosterone also increases expression of FXD4 which then acts to increase the Na\(^+\) affinity of Na\(^+\)-K\(^+\)-ATPase and, thereby, the overall rate of transepithelial Na\(^+\) flux. It is likely that additional families of transport modulator proteins will be identified among the many “orphan” genes present in the various completed genomes.

**SUMMARY**

The past 10 years have witnessed major advances in identification and characterization of ion transport proteins at the molecular level. With the routine use of molecular biological approaches for the study of ion transport, we now understand the basic structural features and patterns of tissue expression for hundreds of different ion channels, ion exchangers, ion cotransporters, and ATP-driven ion pumps. Indeed, there are nearly complete “ion transporter-omes” for several of the favorite model organisms of transport physiologists. The challenge for future transport physiologists is to understand how the members of the various superfamilies of structurally related ion transport proteins are selectively used (or co-opted?) for tissue- or cell-specific ion homeostasis. This brief survey of new perspectives on ion transport indicates several take-home lessons that should guide such future studies.

1) Precise homeostasis of the major inorganic cations (Na\(^+\), K\(^+\), Ca\(^2+\), H\(^+\)) and anions (Cl\(^-\), phosphate, bicarbonate) is fundamental to all cells.

2) However, cell-specific expression of different membrane transport proteins and regulatory factors permits wide variations in the absolute rates of transmembrane flux of these ions.

3) These cell-specific differences in ionic flux are exploited for highly contextual and tissue-specific differences in function, such as solute flow (e.g., transepithelial movements of metabolites) or information transfer.

4) These tissue-specific differences in ionic flux are regulated at multiple levels: via increased/decreased expression of membrane transport protein genes, via changes in the steady-state trafficking of membrane transport protein to and from the plasma membrane, via direct posttranslational modification (e.g., phosphorylation) of the membrane transport proteins, via direct association with tissue-specific adapter or modulator proteins, and via the local lipid composition of the membrane bilayer.

Undoubtedly, such studies will uncover additional, as-yet-unappreciated nuances for the regulation of the ion transport proteins. If findings from the past few years are an indicator, one can be certain there will be plenty of new “new perspectives” for the next Refresher Course on Cell Homeostasis.

**REFERENCES**


