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Minireview Volume on Na^+-Coupled Cotransporters: A Brief Editorial Overview

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Na^+-coupled cotransporters are a class of membrane proteins that use the energetically "downhill" movement of Na^+ across the membrane as a driving force to catalyze the simultaneous "uphill" translocation of their specific substrate or substrates, either in the opposite or the same direction as Na^+. When Na^+ and the coupled substrate(s) move in opposite directions, transporters are exchangers or antiporters, whereas symporters translocate substrate(s) in the same direction as Na^+. The Na^+ gradient is maintained by the Na^+/K^+ ATPase. Thus, a key concept associated with Na^+-coupled cotransporters is that of ion gradients as valuable sources of energy, distinct from hydrolysis of ATP, for crucial cell functions.

The articles included in this volume on eukaryotic Na^+-coupled cotransporters cover just a sample of molecules in this particular subclass, but even this sample is sufficient to provide a clear picture both of the widespread impact of these transporters on virtually all facets of cell function, and of the considerable strides made in their study. As this volume shows, a new level of understanding of Na^+-coupled cotransporters has undoubtedly been reached as a result of the use of molecular biology tools. The biochemical characterization of membrane proteins like those described in this volume has long been made difficult by their hydrophobicity. As a result, the elucidation of their properties and structure/function relations has lagged behind that of soluble proteins. Moreover, revealing experimental approaches such as electrophysiological recordings have only relatively recently started to be used in the study of Na^+-coupled cotransporters, as it became possible to obtain high-efficiency expression of some of these transporters in *Xenopus laevis* oocytes. Prior to this development, electrophysiological recordings were mostly applied to the study of channel proteins that, unlike Na^+-coupled cotransporters, exhibit very high turnover numbers (10^6–10^7 sec^{-1}). Yet presently, both steady-state and pre-steady-state currents have been measured in *Xenopus laevis* oocytes expressing some Na^+-coupled cotransporters, notwithstanding turnover numbers that are much lower than those of channels. Thus, it is noteworthy that electrophysiological information has been included for all the transporters discussed in this volume, with the obvious exception of the electroneutral $Na^+/K^+/Cl^-$ cotransporters.

The molecules examined in this issue include the Na^+/Ca^{2+} exchanger, a crucial link in cardiac contractility and Ca^{2+} homeostasis; the electroneutral $Na^+/K^+/Cl^-$ cotransporters, central players in a wide variety of secretory and absorptive epithelia; the neurotransmitter transporter family, which are central to brain physiology; the renal type II Na^+/phosphate cotransporter, a major target of renal function regulation by parathyroid hormone; and the Na^+/I^- symporter, the mediator of the first step in thyroidal hormone biosynthesis. John P. Reeves examines with lucidity and thoroughness the topic of Na^+/Ca^{2+} exchange and Ca^{2+} homeostasis, and brings into focus such exciting developments as *in vitro* studies of exchanger regulation in excised patches, which have revealed how changes in the function of the exchanger are brought about by the substrates themselves (Na^+-dependent inactivation and regulatory Ca^{2+} activation). In addition, he also discusses at length the finding that the exchanger can be activated by phosphatidylinositol-*bis*phosphate and other negatively charged amphipathic compounds, as well as the intriguing possibility of a functional link between the activity of the exchanger and cellular tension through the cytoskeleton and the cross talk between the exchanger and Ca^{2+} sequestering organelles.

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Mark Haas and Bliss Forbush III describe in their excellent review two $Na^+/K^+/Cl^-$ cotransporter isoforms: NKCC1, which is widely distributed, and NKCC2, found exclusively in the kidney. These transporters are electroneutral with a stoichiometry of 1 Na^+:1 K^+:2 Cl^-, and participate in ion transport across secretory and absorptive epithelia, as well as in maintenance and regulation of cell volume and ion gradients. Significantly, $Na^+/K^+/Cl^-$ cotransport activity is inhibited by commonly used diuretics. The authors review recent evidence indicating that an autosomal recessive hereditary condition known as Bartter's syndrome, characterized by several systemic ionic imbalances, results from mutations in the NKCC2 gene. They also review studies on the ion and bumetanide affinities of various chimeras of human colonic NKCC1 and shark rectal gland NKCC1, in which it was possible to identify specific regions involved differentially in cation and chloride affinities. These studies have successfully narrowed down the candidate molecular domains in which substrate binding sites are located. It is interesting that the $Na^+/K^+/Cl^-$ cotransporters, like the Na^+/Ca^{2+} exchanger, appear to be regulated by the cytoskeleton and by cell shrinkage.

For his part, Gary Rudnick examines with notable clarity the bioenergetics of neurotransmitter transport, in which various plasma membrane transporters use cation gradients as well as the $\Delta\Psi$ as driving forces for "uphill" neurotransmitter reuptake into the nerve cell that released the neurotransmitter or into neighboring neurons or glial cells. He also discusses the interesting and novel notion of the ability of many neurotransmitter transporters to catalyze uncoupled ion flux, i.e., to function as channels, and explores the possible physiological significance of this phenomenon. The author explains that the existence of uncoupled currents, which reflect this channel activity, suggests that the transporter in question is capable of forming an aqueous membrane pore. Interestingly, uncoupled ion flux is stimulated by substrate transport, suggesting that the conformational changes that take place during the transport cycle activate the channel.

J. Biber, H. Murer, and I. Forster have contributed a richly informative review on the renal type II Na^+/Pi cotransporter, in which they summarize current knowledge on this recently cloned transporter. Strikingly, the type II proximal tubular apical Na^+/Pi cotransporter catalyzes approximately 50% of proximal tubular Pi reabsorption. Among other aspects, the authors review the determination of the Na^+:Pi 2:1 stoichiometry, and the electrophysiological characterization of this transporter in *Xenopus laevis* oocytes. Of special interest is their discussion of the role of the type II Na^+/Pi cotransporter as a target for regulation of renal proximal Pi reabsortion by parathyroid hormone and the dietary content of phosphate. They discuss evidence supporting a mechanism in which dietary Pi leads to the recruitment of existing type II Na^+/Pi cotransporter molecules from an intracellular pool, a process independent of protein synthesis. Parathyroid hormone, on the other hand, inhibits Na^+/Pi cotransport by reducing the number of type II Na^+/Pi cotransporter molecules in the brush border membrane. Parathyroid hormone seems to mediate the lysosomal degradation of type II Na^+/Pi cotransporter molecules.

In our article on the recent advances in Na^+/I^- symporter (NIS) research we focus, among other issues, on the latest structure/function data, the electrophysiological analysis, regulation, the impact that this research is having on thyroid disease, and the remarkable finding that NIS itself seems to catalyze Na^+/I^- symport activity not only in the thyroid but also extrathyroidally in such tissues as the lactating mammary gland and gastric mucosa.

A common thread in the research on Na^+-coupled cotransporters is that a combination of molecular biology, cell biology, immunology, biochemistry, and biophysics is beginning to reveal structure/function relations that provide a solid basis for mechanistic models to explain the function of these proteins. These models, in turn, will continue to be experimentally tested and revised. It is clear that the partial reactions catalyzed by all these molecules are in the process of being determined, and that we are in a much better position than ever before to examine the mechanisms regulating the activity of these transporters. As we learn more about the properties of these molecules, and expression systems and purification and reconstitution procedures are optimized, we hope to eventually obtain structural information at atomic resolution. Meanwhile, the accomplishments on hand already include the discovery of the genetic basis of congenital defects due to mutations in Na^+-coupled cotransporters, including Bartter's syndrome and lack of iodide accumulation leading to congenital hypothyroidism. We have clearly gained an intimate appreciation of the myriad roles played by this class of molecules in virtually every tissue, and of the manner in which these roles are fulfilled.