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Na,K-ATPase Subunit Heterogeneity as a Mechanism for Tissue-Specific Ion Regulation

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The Na,K-ATPase comprises a family of isozymes that catalyze the active transport of cytoplasmic Na^+ for extracellular K^+ at the plasma membrane of cells. Isozyme diversity for the Na,K-ATPase results from the association of different molecular forms of the α (α1, α2, α3, and α4) and β (β1, β2, and β3) subunits that constitute the enzyme. The various isozymes are characterized by unique enzymatic properties and a highly regulated pattern of expression that depends on cell type, developmental stage, and hormonal stimulation. The molecular complexity of the Na,K-ATPase goes beyond its α and β isoforms and, in certain tissues, other accessory proteins associate with the enzyme. These small membrane-bound polypeptides, known as the FXYD proteins, modulate the kinetic characteristics of the Na,K-ATPase. The experimental evidence available suggests that the molecular and functional heterogeneity of the Na,K-ATPase is a physiologically relevant event that serves the specialized functions of cells. This article focuses on the functional properties, regulation, and the biological relevance of the Na,K-ATPase isozymes as a mechanism for the tissue-specific control of Na^+ and K^+ homeostasis.
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Maintenance of an asymmetric distribution of Na^+ and K^+ between the cytoplasm and cell surroundings is a crucial event in the physiology of animal cells. The typical low Na^+/K^+ ratio of the intracellular space depends on the activity of the Na,K-ATPase or Na pump, a membrane-associated enzyme that uses the energy from the hydrolysis of adenosine triphosphate (ATP) to transport 3 Na^+ out of the cell in exchange for 2 K^+ that are taken in.[1] The ion gradients created by the Na,K-ATPase are necessary for many common, as well as cell-specific, processes. The enzyme is involved in maintaining cell osmotic balance and volume, the resting membrane potential of most cells, the excitability of muscle and neuronal cells, and the Na^+-coupled secondary transport of H^+, Ca^+, glucose, amino acids, and neurotransmitters across the plasma membrane.[1] In addition, the Na pump drives the vectorial movement of water and salt across many epithelia. It plays a primary role in urine formation in the kidney,[2] and is important in maintaining the electrolyte composition of particular fluid compartments, such as the aqueous humor,[3] endolymph,[4] and cerebrospinal fluid.[5] The contribution of the Na,K-ATPase to such diverse processes requires its function to be adjusted specifically to the needs of each tissue. One of the strategies organisms have developed to confer enzymes the functional versatility that is needed to fulfill specific tasks is the expression of different molecular variants or isozymes with distinct functional capabilities. The interesting discovery that not one but multiple forms of the Na,K-ATPase exist in animal cells provided the basis for the heterogeneity of the enzyme (reviewed in [6-10]). This stimulated intense research to unveil the physiologic role of the Na,K-ATPases. The information available at present suggests that the Na,K-ATPase diversity is not a biologically redundant phenomenon.[9] This article reviews the current knowledge on the Na,K-ATPase isozymes and their significance for the maintenance of cell-specific Na^+ and K^+ homeostasis and tissue function.

Na,K-ATPase Molecular Structure and Subunit Composition

The Na,K-ATPase isozymes are oligomers that result from the association of various molecular forms or isoforms of 2 major

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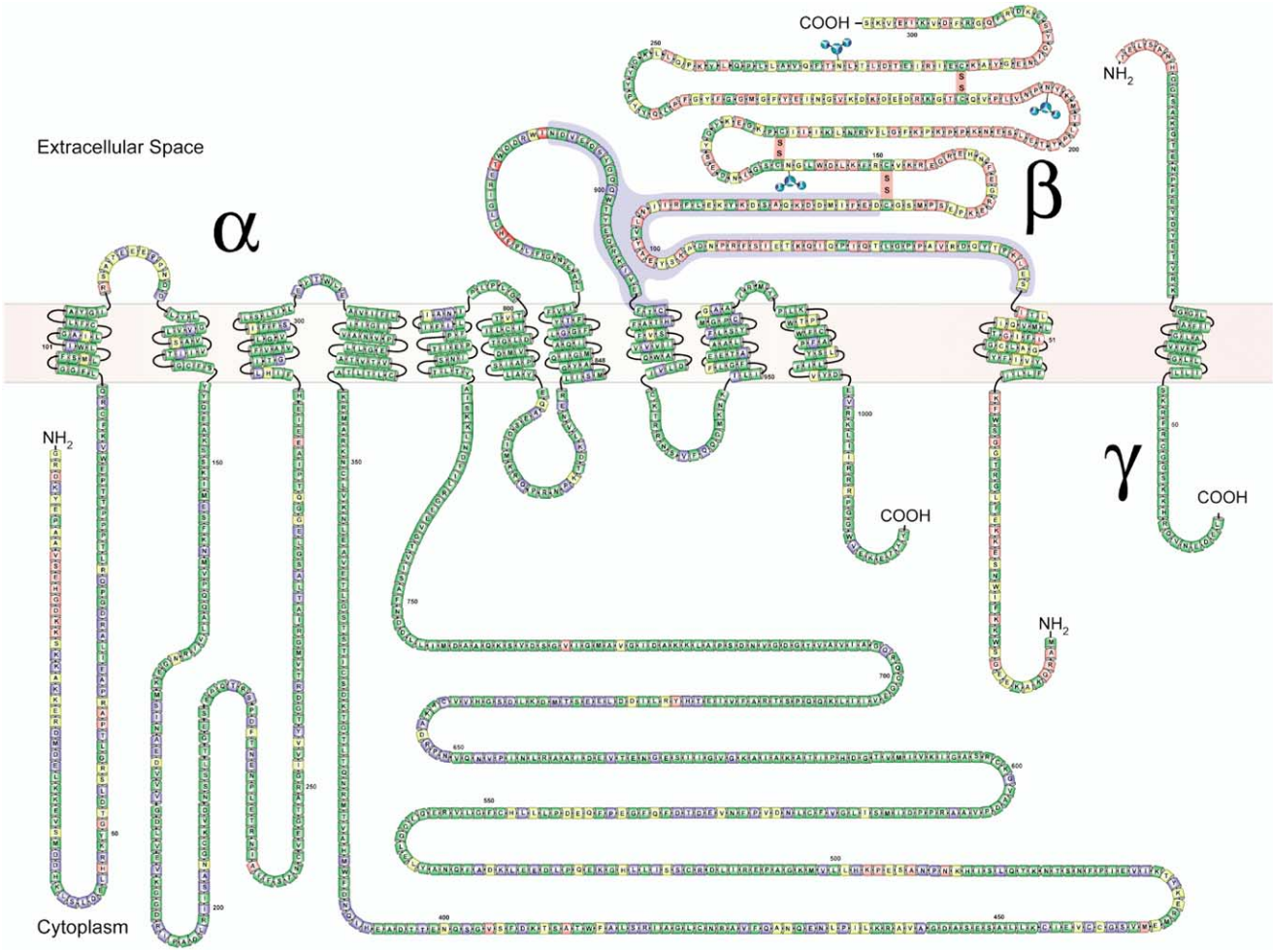

Figure 1 The primary structure and membrane topology of the α (α1, α2, α3, and α4) and β (β1, β2, and β3) isoforms of the Na,K-ATPase and the γ (γa and γb) polypeptides. The sequences of the rat α1 and β1 isoforms and the γa subunit are shown. Amino acid alignment among the 4 α, the 3 β, or the 2 γ polypeptides was performed using DNAstar and Megalign software (DNASTAR, Inc., Madison, WI). Each block represents an amino acid, and residues are colored to indicate the homology among the polypeptides. Green, identical residues among all the α isoforms, β subunits, or the γ polypeptides; blue, residues identical for 3 isoforms; yellow, amino acids identical for 2 isoforms; red, residues different for all isoforms and variants of the γ subunit. The area shaded in purple indicates the association domain between the α and β subunits. The S-S groups shaded in pink represent the disulfide bridges of the β isoforms.

polypeptides: the α and β subunits.[11] A scheme of the primary structure, membrane organization, and amino acid differences among α and β isoforms are shown in Fig. 1. The α polypeptides have a molecular weight between 110 and 112 kd and are arranged in a large cytoplasmic mass, 10 membrane-spanning helices, and a small ectodomain. The α subunit is responsible for the catalytic and transport properties of the Na,K-ATPase and undergoes conformational changes (designated E1 and E2) that are coupled to the binding, occlusion, and translocation of the cations.[11,12] For this, the α subunit contains the binding sites for Na^+, K^+, and ATP. Cardiotonic steroids such as ouabain also bind to the α subunit and inhibit the enzyme. Recently, molecular modeling based on homology to the rabbit sarcoplasmic reticulum Ca-ATPase (SERCA1a) has helped us to understand the structure-function of the enzyme.[13] This shows that the ATP-coupled translocation of ions depends on the relative motion of 3 cytoplasmic domains in the protein. These include the actuator or A domain at the N-terminus and first intracellular loop, and the nucleotide binding (N) and phosphorylation (P) domains both located between transmembrane domains 4 and 5.[11,12]

The β subunits are polypeptides that have molecular weights between 40 and 60 kd, depending on isoform- and tissue-specific differences in glycosylation.[14] The basic structure common to all β isoforms consists of an N-terminal cytoplasmic tail, a single transmembrane region, and a large C-terminal extracellular domain comprising approximately 80% of the protein (Fig. 1). The ectodomain of all β subunits characteristically present 3 disulfide bridges and consensus sequences for glycosylation. The β subunit is essential for normal activity of the Na,K-ATPase and in vertebrates acts as a chaperone protein that assists in membrane insertion, folding, and delivery of the holoenzyme to the plasma membrane (reviewed in[14]). In addition, the β polypeptides also deter-