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Structure, function and regulation of the cell membrane Na,K-pump

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Scientific background

The Na,K-ATPase or Na, K-pump couples ATP hydrolysis to active transport of Na and K ions and is essential for the life of mammalian cells. The Na,K-ATPase is one of a family of P-type cation pumps. The field has been transformed by the publication of the crystal structure of sarcoplasmic reticulum Ca-ATPase (Toyoshima et al, Nature 405, 647-55, 2000). Nevertheless, additional information is required, particularly for homologous proteins such as Na,K-ATPase and gastric H,K-ATPase which contain α (112k Da) and β (35k Da) subunits.

Transition metal catalysed oxidative cleavage of Na,K-ATPase

We have developed a novel technique for selective cleavage of renal Na^+,K^+-ATPase, catalysed by bound Fe^{2+} or Cu^{2+} ions, which provides information on the spatial organization of the protein. Cleavage of renal Na,K-ATPase and gastric H,K-ATPases by bound Fe^{2+} ions or ATP-Fe^{2+} complexes has been described extensively (Goldshleger and Karlish, 1997, 1999; Patchornik et al, 2000; Shin et al, 2001). Fe^{2+} substitute for Mg^{2+} ions in the ATP-Fe^{2+} complex. Thus cleavages reveal details of Mg^{2+} sites. The results indicate that E1-E2 conformational changes are associated with large movements of cytoplasmic domains, in agreement with the Ca-ATPase structure (see Fig. 1), and presumably, common to all P-type pumps. The cleavages also reveal important features of ATP and Mg^{2+} sites not seen in the crystal structure. Cleavages catalyzed by Cu^{2+} ions at the extracellular surface reveal regions of subunit interactions (Bar Shimon et al., 1998). Cleavages catalysed by a Cu^{2+}-diphenyl phenanthroline complex at the membrane-water interface indicates proximity of trans-membrane segments M1 and M3 (Tal et al., 2001). We are synthesizing cleavage reagents based on cardiac glycosides and using mass spectrometry to analyse fragments (with Alla Shainskaya).

Expression of Na,K-ATPase in Pichia Pastoris (collaboration with Eitan Bibi)

We are expressing Na,K-ATPase in the methylotrophic yeast *P. pastoris* which grows to high densities, permitting isolation of recombinant enzyme in large quantities. Expression has been optimized and Fe^{2+}-catalysed cleavage is being applied to identify Fe^{2+} sites and engineer new sites. A His-tagged labeled enzyme has been expressed.

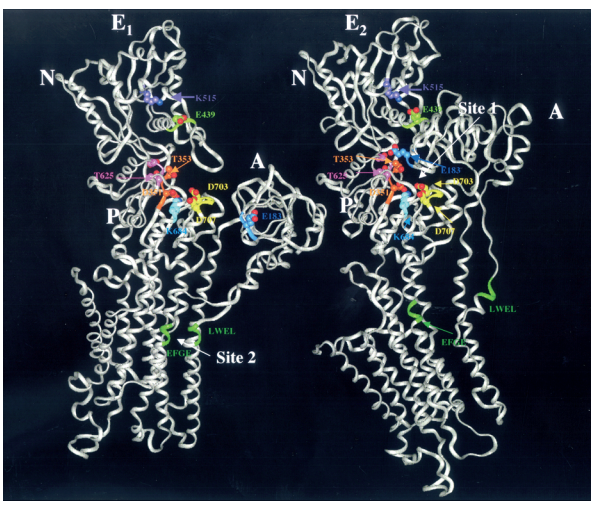

Fig. 1 *Structure of Ca^{2+}-ATPase in an E1 conformation (left) and a model in an E2 conformation (right).*

Regulation of the Na,K-pump by FXYD proteins.

Activity and expression of Na,K-ATPase are tightly regulated. We are investigating a novel mechanism of tissue-specific regulation by small single trans-membrane proteins. The FXYD family consists of seven members, including a γ- subunit (FXYD 2) of renal Na,K-ATPase, phospholemman (FXYD1) expressed in heart, and CHIF (FXYD4) an aldosterone-induced gene expressed in kidney and colon (Attali et al., 1995). When expressed in mammalian cells gamma raises the apparent affinity for ATP and reduces that for cytosolic Na (Therein et al., 1997, 1999; Pu et al., 2001). As shown by mass spectrometry gamma exists as two splice variants (Kuster et al., 2000). Functional differences have not been found but the variants are expressed differentially in nephron segments (Pu et al., 2001).

Recent work, in collaboration with Haim Garty, indicates that CHIF too is a Na/K-pump regulator which differs from gamma. CHIF is expressed exclusively in kidney collecting duct

and distal colon. CHIF and gamma display complementary expression along the nephron. CHIF is specifically immunoprecipitated with the α subunit from transfected cells and native epithelia (Garty et al., 2001) and in transfected cells CHIF increases the apparent affinity for cytosolic Na^+. CHIF, and possibly other FXYD proteins, may tailor Na/K-pump kinetics to tissue-specific requirements. We are characterizing CHIF structure-function relationships and interactions of gamma and phospholemman with the pump.

Selected Publications

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