

Studies on the Membrane Topology of the
(Na,K)ATPase

Kyunglim Lee Yoon (이경림)

College of Pharmacy, Ewha Woman's University

The (Na,K)ATPase is a membrane ion transporting ATPase composed of an α catalytic subunit and a β glycoprotein subunit. The topology of the rat $\alpha 1$ and $\beta 1$ subunits has been studied by insertion of epitope(s) : at the NH₂-terminus and COOH-terminus and between Glu117 and Glu118, Lys828 and Arg829, Gln900 and Trp901, and Val939 and Phe940 of the α subunit; and at the NH₂-terminus and COOH-terminus and between Glu228 and Tyr229 of the β subunit. The epitope-tagged $\alpha 1$ constructs were expressed in HeLa cells to select for stable cell lines expressing a functional (Na,K)ATPase. All constructs, except for the one tagged between Gln900 and Trp901, resulted in ouabain-resistant colonies indicating that modified proteins retained functional integrity. The epitope-tagged β constructs were transiently expressed in Cos-7 cells. The orientation of the epitopes with respect to the cell membrane was revealed by indirect immunofluorescence performed on permeabilized and non-permeabilized cells expressing the (Na,K)ATPase chains. The results indicate that the α subunit has 4 transmembrane segments in the COOH terminal membrane bound domain between residues 760 and 938, and that both the NH₂-terminus and the COOH-terminus are in the cytosol; it was not determined whether there are more transmembrane segments between residue 938 and the COOH-terminus. The β subunit has only one transmembrane spanning region with the NH₂-terminus in the cytosol and the COOH-terminus on the extracytoplasmic surface of the plasma membrane.