Haim Garty

Roman Aizman
Carol Asher
Shi Hai-Kun
Hedva Latter
Moshit Lindzen
Indranil Sinha

Regulation of ion transport in the kidney

Department of Biological Chemistry

Tel. 972 8 934 2706 **Fax.** 972 8 934 4177
E-mail: h.garty@weizmann.ac.il

Scientific background

Epithelial cells in the kidney and intestine perform vectorial transport of Na^+, K^+, Cl^- and water. These processes are mediated by a number of channels and pumps, and play a vital role in the maintenance of normal blood pressure and ionic composition. A key process is the absorption of Na^+ and secretion of K^+ which takes place in kidney collecting duct and distal colon surface cells (Fig. 1). It involves the basolateral Na^+/K^+ ATPase (or the Na^+ pump) and two apical channels: The Na^+ channel ENaC, and the K^+ channel ROMK. Regulation of these processes is primarily done by the mineralocorticoid aldosterone, the principal hormone controlling salt and water balance in vertebrates (Garty, 2000). Like other steroids, aldosterone functions by altering gene expression, leading to an enhanced synthesis of proteins which mediate its physiological actions. These include subunits of the above channels and pumps, as well as regulatory proteins which activate these transporters. Current work in our laboratory aims to elucidate mechanisms involved in the regulation of ion transport and in particular the function of aldosterone-induced proteins cloned by us.

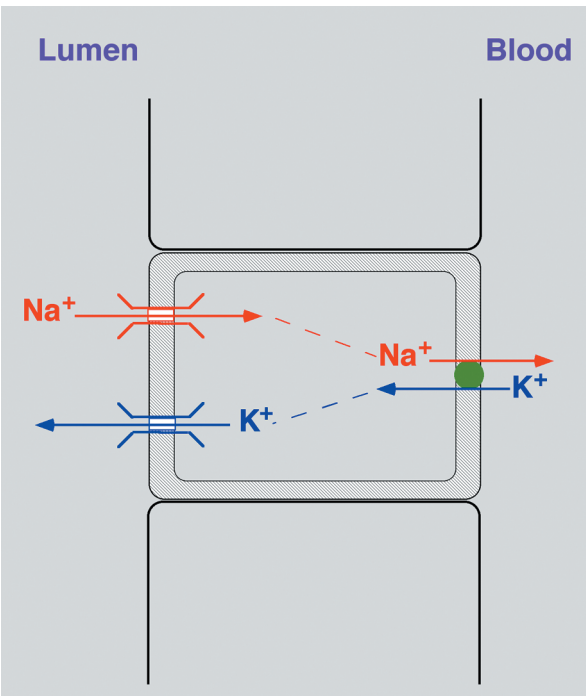

Fig. 1 *A general scheme of ion transport across epithelial cells in kidney collecting duct and distal colon.*

Regulation of the Na^+ channel by phosphorylation.

The apical Na^+ channel ENaC is a major target to the action of aldosterone (Garty and Palmer, 1997; Palmer and Garty, 2000). ENaC is composed of three homologous subunits termed α, β and γ. The C-tails of β and γ play a special role in determining cell surface expression and activity of the channel. Proline-rich motifs on these segments bind to the WW domains on the ubiquitin ligase Nedd4, leading to channel ubiquitination, internalization and degradation (for review see Garty and Palmer, 1997; Palmer and Garty, 2000). We have tested whether C-tail phosphorylation plays a role in channel regulation. *In vitro* phosphorylation of recombinant proteins by fractionated cytosol has identified three kinases phosphorylating conserved serine/threonine residues on β and γ ENaC. Surface plasmon resonance has been used to study ENaC/Nedd4 interactions and their possible modulation by channel phosphorylation (Asher et al., 2001). Recent studies have demonstrated that phosphorylation of one of the above residues substantially increases the rate of ENaC/Nedd4 association, leading to downregulation of channel activity. The up-stream signals mediating this process are under investigation.

Regulation of the Na^+ pump by FXYD proteins.

An aldosterone-induced gene cloned in our laboratory codes for a 6.5 kDa transmembrane protein termed CHIF (Attali et al., 1995). It is strongly and independently induced by both aldosterone and by a high K^+ diet and is exclusively expressed in kidney collecting duct and distal colon (Shi et al., 2001; Wald et al., 1997). CHIF shares 30%-50% sequence homology with six other proteins, most of which have not been studied yet. Together, they are termed the FXYD family [Sweadner et al. Genomics 68, 41 (2000)]. The best studied FXYD protein is the γ subunit of Na^+/K^+ ATPase, a kidney-specific subunit of the pump which modulates its apparent affinity to ATP and cytoplasmic Na^+ [Therien et al. JBC 274, 12252 (1999)]. Recent

work in collaboration with the group of Steve Karlish has provided evidence that CHIF too is a regulator of the pump whose functional effects different from those of γ. CHIF could be immunoprecipitated with the α subunit of the pump from transfected cells and native epithelia. Co-precipitation was apparent only under conditions that preserve active pump conformation (Na^+ plus oligomycin, or Rb^+ plus ouabain) (Garty et al., 2001). Measurements in transfected HeLa cells showed that CHIF decreases the $K_{0.5}$ value for activation by cytosolic Na^+ from 6.3±2.0 mM to 1.9±0.4 mM. Similar observations have been made in *Xenopus* oocytes injected with CHIF cRNA (Beguin et al., 2001). Other studies have demonstrated complementary expression of CHIF and γ along the nephron. i.e. the medullary and cortical segments of the ascending loop of Henle have γ but no CHIF, while the collecting duct has CHIF but no γ (Fig. 2). CHIF knockout mice have been generated in our laboratory and are been analyzed for abnormalities in ion transport. The null mutated mice exhibit mild defects in concentrating urine and K^+ secretion which are consistent with impaired electrolyte metabolism in the inner medullary collecting duct, the major site of CHIF expression. Taken together the above data indicate that CHIF and possibly other FXYD proteins are regulators of the Na^+/K^+ ATPase. We suggest that members of this family modulate pump characteristics in different tissues and tailor its response to various stimuli. Current work characterizes CHIF structure-function relationships as well as interactions of other FXYD proteins with the pump.

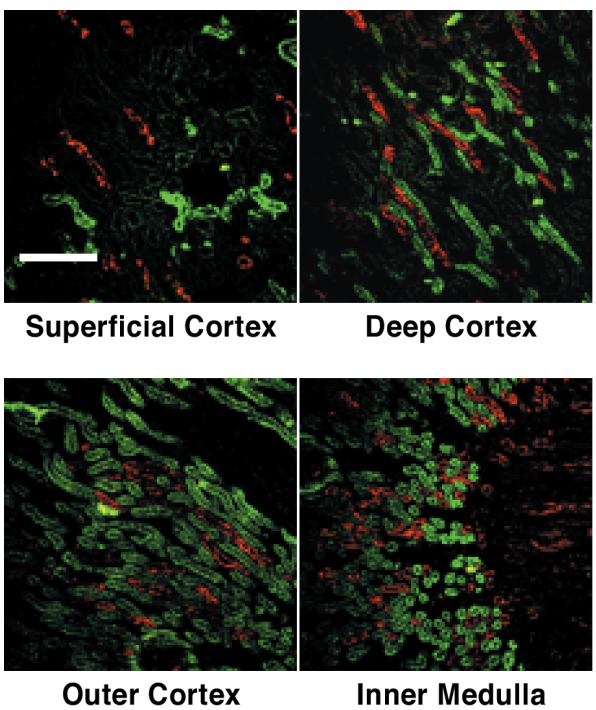

Fig. 2 *Double labelling of kidney slices with antibodies to CHIF (red) and Gamma (green) The bar corresponds to 150 μM. Experiments were done in collaboration with Dr. N. Farman, INSERM, Paris.*

Selected Publications

Attali, B., Latter, H., Rachamim, N. and Garty, H. (1995) A corticosteroid-induced gene expressing an "IsK-like" K+ channel activity in Xenopus oocytes. Proc. Natl. Acad. Sci. USA, 92, 6092-6096.

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Béguin, P., Crambert, G., Guennoun, S., Garty, H., Horisberger, J.-D. and Geering, K. (2001) CHIF, a member of the FXYD protein family, is a regulator of Na,K-ATPase distinct from the Gamma subunit. EMBO J, 20, 3993-4002.

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