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Ion channels and pumps mediating Na^+ and K^+ transport in the kidney

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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 this process is primarily done by the mineralocorticoid aldosterone, the principal hormone controlling salt and water balance in vertebrates.

The apical Na^+ channel ENaC is a major target to the action of aldosterone (Garty, 2000; Palmer and Garty, 2000). The C-termini of ENaC's three subunits contain proline-rich (PY) motifs which bind to WW domains on Nedd4 type ubiquitin ligases and thereby evoke channel ubiquitination, internalization and degradation. We have used surface plasmon resonance to characterize interactions between the PY motifs of ENaC and the WW domains of Nedd4 and Nedd4-2 (Asher et al., 2001; Asher et al., 2003). These studies have determined the association and dissociation constants of these interactions and demonstrated that phosphorylation of conserved S/T residues may alter these constants. Subsequent studies have identified three protein kinases phosphorylating conserved S/T residues in the carboxy termini of beta and gamma ENaC (Shi et al., 2002a; Shi et al., 2002b). In particular, it was shown that beta T613 and gamma T623 are phosphorylated by ERK, leading to enhanced association with Nedd4 and down regulation of channel activity. The upstream signals mediating this process are under investigation. Other studies focused on the phosphorylation of Nedd4-2 by the aldosterone-induced protein kinase SGK, as well as by other protein kinases acting on the same target residues and affect ENaC/Nedd4 interactions.

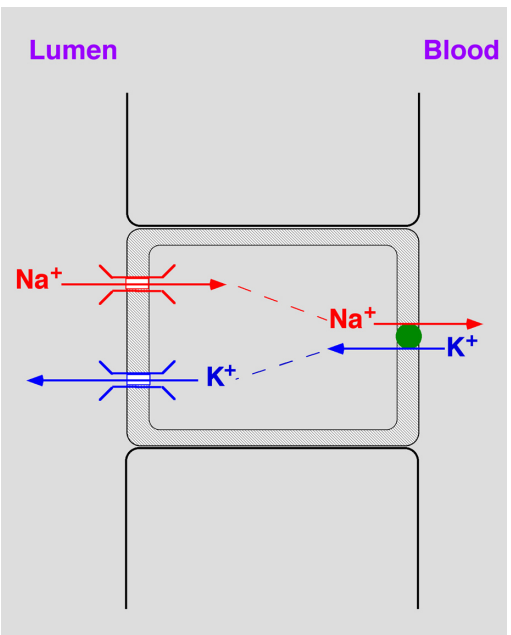

Fig. 1 *A general scheme of ion transport across epithelial cells in kidney collecting duct and distal colon.*

Another 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 aldosterone and high K^+ intake, and is exclusively expressed in kidney collecting duct and distal colon surface cells. CHIF knockout mice were generated in our laboratory and found to exhibit mild defects in water absorption that are secondary to impaired, collecting duct specific, electrolyte transport (Aizman et al., 2002; Goldschimdt et al., 2004). CHIF shares 30%-50% sequence homology with six other transmembrane proteins collectively termed the 'FXYD family' (Sweadner et al. Genomics 68, 41, 2000). Work

in collaboration with the group of Steve Karlish and others has demonstrated that at least four members of this group interact with the Na^+ pump and alters its kinetic properties (Béguin et al., 2001; Beguin et al., 2002; Crambert et al., 2002; Garty et al., 2002; Lindzen et al., 2003). Each one of these FXYD proteins has a unique tissue distribution and different functional effects. Thus, FXYD proteins act as tissue specific regulators of the pump which adapt its kinetic properties to specific needs of one target tissue or physiological state without affecting the pump elsewhere. In addition, a number of FXYD proteins were reported to alter channel activity in expression systems. This raises the possibility of an additional role for FXYD proteins in maintaining pump/channel synchronization. Together with Steve Karlish and his co-workers, we are defining the functional interactions between four FXYD proteins (CHIF, phospholemman, RIC and gamma) and the Na^+/K^+ ATPase.

Selected Publications

Aizman, R., Asher, C., Fuzesi, M., Latter, H., Lonai, P., Karlish, S.J. and Garty, H. (2002) Generation and phenotypic analysis of CHIF knockout mice. Am J Physiol Renal Fluid Electrolyte Physiol, 283, F569-F577.

Asher, C., Chigaev, A. and Garty, H. (2001) Characterization of Interactions between Nedd4 and beta and gammaENaC Using Surface Plasmon Resonance. Biochem Biophys Res Commun, 286, 1228-1231.

Asher, C., Sinha, I. and Garty, H. (2003) Characterization of the interactions between Nedd4-2, ENaC, and sgk-1 using surface plasmon resonance. Biochim. Biophys. Acta, 1612, 59-64.

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.

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.

Beguin, P., Crambert, G., Monnet-Tschudi, F., Uldry, M., Horisberger, J.D., Garty, H. and Geering, K. (2002) FXYD7 is a brain-specific regulator of Na,K-ATPase alpha 1-beta isozymes. EMBO J, 21, 3264-3273.

Crambert, G., Fuzesi, M., Garty, H., Karlish, S. and Geering, K. (2002) Phospholemman (FXYD1) associates with Na,K-ATPase and regulates its transport properties. Proc Natl Acad Sci U S A, 99, 11476-11481.

Garty, H. (2000) Regulation of the Epithelial Na+ Channel by Aldosterone: Open Questions and Emerging Answers. Kidney Int., 57, 1270-1276.

Garty, H., Lindzen, M., Scanfano, R., Aizman, R., Fuzesi, M., Goldshleger, R., Farman, N., Blostein, R. and Karlish, S.J.D. (2002) A functional interaction between CHIF and Na,K-ATPase. Implication for regulation by FXYD proteins. Am J Physiol Renal Fluid Electrolyte Physiol, 283, F607-F615.

Goldschimdt, I., Grahammer, F., Warth, R., Schulz-Baldes, A., Garty, H., Greger, R. and Bleich, M. (2004) Kidney and colon electrolyte transport in CHIF knockout mice. Cell. Physiol. Biochem., 14, 113-120.

Lindzen, M., Aizman, R., Lifshitz, Y., Lubarski, I., Karlish, S.J.D. and Garty, H. (2003) Structure-function relations of interactions between Na, K ATPase, the gamma subunit and CHIF. J. Biol. Chem, 278, 18738-18743.

Palmer, L.G. and Garty, H. (2000) Epithelial Na+ channels. In Seldin, D.W. and Giebisch, G. (eds.), The Kidney, Physiology and Pathophysiology. Lippincott Williams and Wilkins, Philadelphia, PA, pp. 251-276.

Shi, H., Asher, C., Chigaev, A., Yung, Y., Reuveny, E., Seger, R. and Garty, H. (2002a) Interactions of beta and gamma ENaC with Nedd4 can be facilitated by an ERK-mediated phosphorylation. J. Biol. Chem., 277, 13539-13547.

Shi, H.-k., Yung, Y., Kligman, L., Reuveny, E., Seger, R. and Garty, H. (2002b) CK2 specifically binds to and phosphorylates the carboxy termini of ENaC subunits. Eur. J. Biochem., 269, 4551-4558.

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