Regulation of ion transport by FXYD proteins.

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FXYD proteins are a group of seven short single span transmembrane proteins termed after the invariant motif FXYD in their extracellular domain. They have been cloned from different tissues and were thought to be involved in a variety of cellular functions. However, it is clear today that at least six, and probably all, members of this group specifically interact with the Na^+/K^+-ATPase (the Na^+ pump) and alter its kinetic properties (for review see (Garty and Karlish, 2005; Garty and Karlish, 2006)). Each FXYD protein has a different and unique tissue distribution and some of them are also subject to transcriptional and/or post translational regulation. Thus, they appear to be tissue specific regulators or auxiliary subunits of the Na^+/K^+ ATPase which function to tailor its kinetic properties to the needs of one cell type or physiological state without affecting it elsewhere. As such, they provide a new and unique mode of regulation of this ubiquitous protein which maintains Na^+ and K^+ gradients across the plasma membrane of all vertebrate cells.

Our group has cloned one of the first FXYD proteins and together with the group of Steve Karlish studies functional and structural properties of a few members of this family. In particular, we are interested in FXYD proteins that are expressed in the kidney and involved in the regulation of the blood Na^+ and K^+ homeostasis as well as blood pressure and volume. These are the γ subunit of Na^+/K^+-ATPase (γ or FXYD2), Corticosteroid Hormone Induced Factor (CHIF or FXYD4) and Related to Ion Channel (RIC or dysadherin or FXYD5). The three proteins are expressed in the basolateral membrane of different kidney cells. FXYD2 is expressed primarily in the thick ascending limb of Henle's loop (TALH), while FXYD4 and FXYD5 are detected in the distal tubule and collecting duct (Shi et al., 2001; Garty et al., 2002; Pihakaski-Maunsbach et al. 2003; Lubarski et al., 2005). Even though both FXYD4 and FXYD5 are found in the same nephron segment, their expression appears to be mutually exclusive. FXYD4 is abundant in the principal Na^+ absorbing cells, while FXYD5 is detected only in the intercalated acid secreting cells (Fig. 1).

We have characterized functional effects of FXYD proteins expressed in mammalian cells, *Xenopus* oocytes, and the methylotrophic yeast *Pichia Pastoris*. (Garty et al., 2002; Lindzen et al., 2003; Lubarski et al., 2005; Lifshitz et al., 2006). These studies have demonstrated different effects of each FXYD protein on the kinetic properties of the pump. Thus, while FXYD2 reduces the apparent affinity to cell Na^+, FXYD4 increases it by

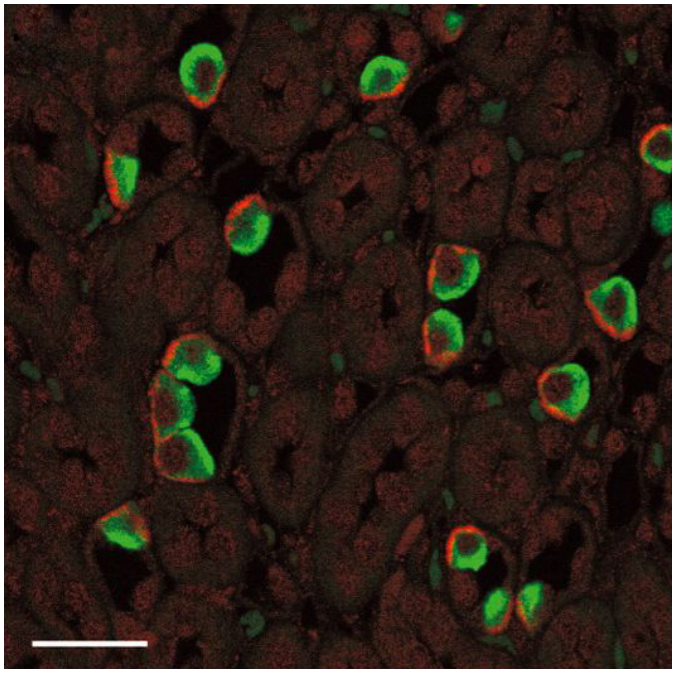

Fig. 1 *Double labeling of a kidney outer medulla section with antibodies to FXYD5 (**red**) and the 56 kDa subunit of vacuolar H^+ ATPase (**green**). Only cells expressing H^+ ATPase in their apical pole also express FXYD5 and this expression is restricted to the basolateral membrane. Bar=20 μm. For further details see (Lubarski et al., 2005). Done in collaboration with A. Maunsbach from the University of Aarhus, Denmark.*

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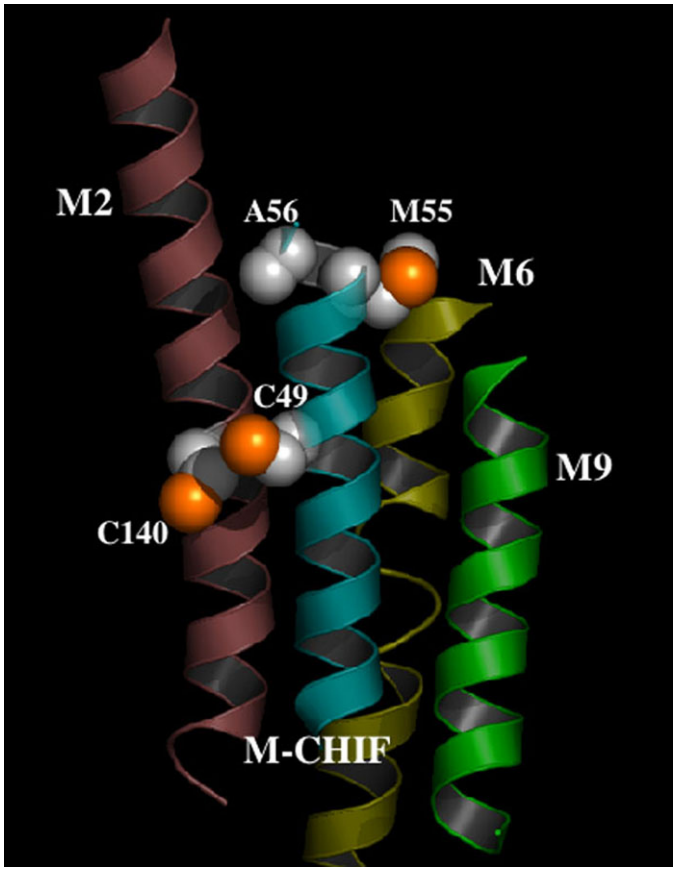

Fig. 2 *Suggested model for the interaction between the transmembrane helix of FXYD4 (M-CHIF, **light blue**) with the 2nd, 6th and 9th transmembrane domains of α1 (**M2**, **M6** and **M9**). The model highlights three FXYD4 residues found to be involved in structural interactions (C49, M55 and A56). For further details see (Lindzen et al., 2006).*

up to 3 fold. FXYD5 on the other hand increases the V_{max} of the pump without affecting its affinity to Na^+ or K^+. These different kinetic effects serve to adjust properties of the Na^+/K^+-ATPase to the specific physiological needs of different nephron segments. Thus, a reduced Na^+ affinity due to an interaction of the α1 subunit of Na^+/K^+-ATPase (α1) with FXYD2, will allow the pump to respond sensibly to an increase of cell Na^+ and better cope with Na^+ loading in the TALH. The FXYD4-dependent increase in Na^+ affinity permits efficient reabsorption of Na^+ from a low Na^+ luminal solution, characteristic of the collecting duct. Some of these predictions where confirmed by the phenotypic analysis of FXYD4 knockout mice generated in our laboratory (Goldschimdt et al., 2004; Aizman et al., 2002).

Structural interactions between FXYD proteins and the Na^+/K^+-ATPase have been studied by chemical crosslinking and mutagenesis analysis (Lindzen et al., 2003; Fuzesi et al., 2005; Lindzen et al., 2006). We have demonstrated that the opposite effects of FXYD2 and FXYD4 on the apparent affinity of the pump to cell Na^+ are mediated by their transmembrane domains. Based on covalent crosslinking, co-immunoprecipitation and mutagenesis studies we have proposed a molecular model which has placed the transmembrane domain of FXYD proteins in a groove formed between the 2nd, 6th, and 9th transmembrane domains of α1 (Fig. 2). It was also demonstrated that the general disposition of the transmembrane domains of different FXYD proteins with respect to α1 is the same, and their different functional effects are mediated by a few non-homologous residues. This model makes a number of structural and functional predictions which are being tested experimentally.

Selected publications

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