Regulation of epithelial ion transport by FXYD proteins and aldosterone

Department of
Biological Chemistry

Prof. Haim Garty

Carol Asher, Irina Lubarski,
Shiri Moshitzky,
Ekaterina Petrovich

☎ 972 8 934 3924
FAX 972 8 934 4100
@ h.garty@weizmann.ac.il
www.weizmann.ac.il

FXYD proteins are a group of seven short single span transmembrane proteins termed after an invariant motif in their extracellular domain (Fig. 1). 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 all members of this group specifically interact with the Na^+/K^+-ATPase (the Na^+ pump) and alter its kinetics properties (for review see (Garty and Karlish, 2006; Garty and Karlish, 2005)). 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 function as tissue specific regulators or auxiliary subunits of the Na^+/K^+ ATPase and 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. Accumulating data suggest additional roles of FXYD proteins in the regulation or mediation of ion transport through other pathways (Garty and Karlish, 2006).

Together with the group of Steve Karlish we are studying functional and structural properties of several members of this family. In particular, we are interested in: Phospholemman (PLM or FXYD1), 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). FXYD1 is the major phosphoprotein in cardiac myocytes, phosphorylated at two serine residues (S63 and S68) in response to β adrenergic stimulation. The three other FXYD proteins are expressed in several epithelia, and in particular in the basolateral membrane of different kidney segments. Functional and structural interactions of the above FXYD proteins with the Na^+/K^+-ATPase have been characterized in stably transfected mammalian cells, *Xenopus oocytes*, and the methylotrophic yeast *Pichia Pastoris*. These studies have demonstrated different effects of each FXYD protein on the kinetic properties of the pump. Thus, while FXYD2 reduces its apparent affinity to cell Na^+, FXYD4 increases it by up to 3 fold, and FXYD1 can either increase or decrease the Na^+ affinity, depending on its phosphorylation state (Lindzen et al. 2003; Lifshitz et al. 2006). FXYD5 on the other hand, increases the Vmax of the pump without affecting its affinity to Na^+ or K^+ (Lubarski et al. 2005; Lubarski et al. 2007). Thus, the differential expression of these FXYD proteins provides means to adjust properties of the Na^+/K^+-ATPase to the specific physiological needs in different nephron segments. e.g. a reduced Na^+ affinity due to an interaction of the Na^+/K^+-ATPase with FXYD2, will allow the pump to respond sensibly to an increase of cell Na^+ and better cope with Na^+ loading in Henle's loop. The FXYD4-dependent increase in Na^+ affinity will permit 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 (Aizman et al., 2002; Goldschimdt et al., 2004).

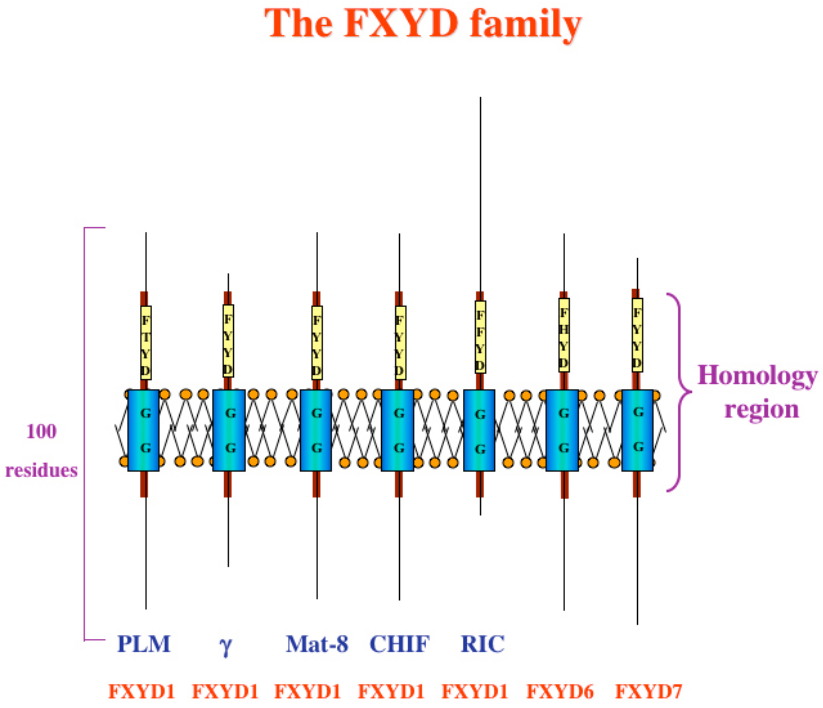

Fig. 1 *Schematic illustration of the FXYD protein family.*

Structural interactions between FXYD proteins and the Na^+/K^+-ATPase have been studied by a variety of methods (Fuzesi et al., 2005; Lindzen et al., 2003; Lindzen et al., 2006; Lubarski et al. 2007). We have demonstrated that both the structural and functional interactions of FXYD proteins with the Na^+/K^+-ATPase are primarily 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 the α subunit of the Na^+/K^+-ATPase. It was also demonstrated that the general disposition of the transmembrane domain of different FXYD proteins with respect to the α subunit is the same, and their different functional effects are mediated by a few non-homologous residues.

A related project addresses mechanisms by which epithelial ion transport is regulated by the steroid aldosterone. This corticosteroid is the principal mineralocorticoid maintaining salt and water balance in all vertebrates. It functions by altering gene expression leading to the induction of the epithelial Na^+ channel ENaC, the Na^+/K^+-ATPase, FXYD4 and several other proteins which mediate or regulate ion transport in the kidney and intestine. We have performed a micro RNA analysis of aldosterone-induced changes in the epithelial transcriptosome. The analysis has identified the previously known aldosterone-dependent transcripts, as well as a number of new and potentially important aldosterone-induced and suppressed genes. In addition, several putative aldosterone-dependent alternative splicing events have been identified. These findings are under further investigation and may provide new insight into mechanisms of aldosterone action.

Selected publications

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