FXYD proteins: new regulators of Na-K-ATPase

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Geering, Käthe. FXYD proteins: new regulators of Na-K-ATPase. Am J Physiol Renal Physiol 290: F241–F250, 2006; doi:10.1152/ajprenal.00126.2005.—FXYD proteins belong to a family of small-membrane proteins. Recent experimental evidence suggests that at least five of the seven members of this family, FXYD1 (phospholemman), FXYD2 (γ-subunit of Na-K-ATPase), FXYD3 (Mat-8), FXYD4 (CHIF), and FXYD7, are auxiliary subunits of Na-K-ATPase and regulate Na-K-ATPase activity in a tissue- and isoform-specific way. These results highlight the complexity of the regulation of Na⁺ and K⁺ handling by Na-K-ATPase, which is necessary to ensure appropriate tissue functions such as renal Na⁺ reabsorption, muscle contractility, and neuronal excitability. Moreover, a mutation in FXYD2 has been linked to cases of human hypomagnesemia, indicating that perturbations in the regulation of Na-K-ATPase by FXYD proteins may be critically involved in pathophysiological states. A better understanding of this novel regulatory mechanism of Na-K-ATPase should help in learning more about its role in pathophysiological states. This review summarizes the present knowledge of the role of FXYD proteins in the modulation of Na-K-ATPase as well as of other proteins, their regulation, and their structure-function relationship.

ion transport regulation; protein-protein interaction; structure-function relationship

Moreover, in the kidney Na-K-ATPase is exclusively located in the basolateral membrane of epithelial cells and thus becomes the driving force for Na⁺ reabsorption essential to maintain extracellular volume and blood pressure.

In view of its important physiological role, it may be expected that dysfunction or dysregulation of the Na-K-ATPase may have severe pathophysiological consequences. However, only recently have mutations in Na-K-ATPase been associated with genetic diseases. Four different mutations in a single allele of the α₂-isofrom of the Na-K-ATPase, which cause loss of function, are linked to familial hemiplegic migraine type 2 (FHM2), a hereditary form of migraine characterized by aura and some hemiparesis (15, 26, 79, 95). Moreover, missense mutations in the gene for the α₂-isofrom of the Na-K-ATPase have been identified as a cause of rapid-onset dystonia-Parkinsonism (RDP; DYT12) (25). Dysregulation of Na-K-ATPase through a defective production or function of a tissue-specific regulator has been correlated with various disorders, including cardiovascular, neurological, renal, and metabolic diseases, but the direct link between altered pump function and defective regulation remains in most cases obscure (50, 76).

Numerous mechanisms are involved in the regulation of the Na-K-ATPase to adapt its activity and/or expression to changing physiological demands, permitting proper “housekeeping” and specialized tissue functions of Na-K-ATPase. The requirements for Na-K-ATPase modulation are likely to be greatest in response to physiological stimuli such as nerve impulse propagation, exercise, and changes in dietary Na⁺ and K⁺.

Because intracellular Na⁺ is the limiting factor for Na-K-ATPase activity, any change in the intracellular Na⁺ concentration affects its transport rate. Moreover, peptide hormones or neurotransmitters may provoke phosphorylation of Na-K-ATPase by protein kinases, which modulates its cell surface expression (88). Finally, steroid hormones such as aldosterone

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affect α and β gene transcription, leading to an increased number of Na-K-ATPase pump units (30).

In addition to these regulatory effects mediated by hormones and neurotransmitters, recent experimental evidence has revealed a novel regulatory mechanism that involves interaction of the Na-K-ATPase with small-membrane proteins of the FXYD family. In contrast to hormonal regulation, interaction of FXYD proteins does not produce a change in Na-K-ATPase expression but rather modifies the transport properties of the Na-K-ATPase in a tissue- and isoform-specific way.

THE FXYD PROTEIN FAMILY

Sweadner and Rael (86) have defined the gene family of FXYD proteins based on the invariant amino acids in a signature sequence containing the FXYD motif and two conserved glycine and a serine residue. In mammals, this family contains seven members including FXYD1 (or phospholemman) (71); FXYD2 (or the γ-subunit of Na-K-ATPase) (33, 59); FXYD3 (or mammary tumor marker Mat-8) (67); FXYD4 (or corticosteroid hormone-induced factor CHIF) (5); FXYD5 (or related to ion channel RIC or dysadherin) (34); FXYD6 (or phosphohippolin) (100); and FXYD7 (7) (Fig. 1). Moreover, FXYD2 from *Xenopus laevis* (8) and a phospholemman-like protein from shark (55) have been cloned. FXYD proteins contain 61–95 amino acids, with the exception of FXYD5, which has 178 amino acids due to an NH2-terminal extension. FXYD1 (71), FXYD2 (8), FXYD4 (6), and FXYD7 (7) are type I membrane proteins with a single membrane span and the COOH terminus exposed to the cytosol. FXYD1 and FXYD4 achieve this membrane orientation after cleavage of a signal peptide, whereas FXYD2 and FXYD7 have no signal peptide. Circular dichroism and NMR spectra predict that the transmembrane domains (TMs) of FXYD1, 2, 3, and 4 adopt an α-helical conformation (24, 89). For years after their cloning, the functional role of FXYD proteins remained unknown. Because FXYD1 (63), FXYD3 (67), FXYD4 (5), and FXYD5 (34) induce ion-specific conductances when overexpressed in *X. laevis*, it was long thought that FXYD proteins may be ion channels or regulators of ion channels. However, the physiological significance of these observations remains unclear because induction of ion conductances was often observed only under nonphysiological conditions. Experimental evidence then showed that FXYD2, known for over 20 years to be associated with renal Na-K-ATPase (33), indeed modulates Na-K-ATPase activity (8). This promoted intense research that showed, until now, that at least five of the seven FXYD proteins interact with Na-K-ATPase and regulate its function. Thus, even though it is not known whether Na-K-ATPase regulation is the only function of FXYD proteins, it is likely that all FXYD proteins are tissue-specific auxiliary subunits of Na-K-ATPase. The experimental criteria that have been used to support this hypothesis are 1) coimmunoprecipitation of

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Fig. 1. Structural characteristics of FXYD proteins. A: sequence alignment of FXYD proteins. The transmembrane (TM) domain is indicated, and the FXYD motif is underlined. The 2 conserved glycine residues are highlighted. The position of the predicted signal peptide cleavage site, which is not used. B: membrane topology of FXYD proteins. With the exception of FXYD3, all FXYD proteins are type I membrane proteins. The positions of the 2 conserved glycine residues and the FXYD motif are indicated. Phosphorylation sites in FXYD1 are indicated by P, and O-glycosylation sites in FXYD7 by spirals. FXYD5 has a very long NH2-terminal extension. For details and references, see the text.
FXYD proteins with Na-K-ATPase both in expression systems and in tissues. 2) distinct modulation of Na-K-ATPase activity by different FXYD proteins. 3) Na-K-ATPase isozyme-specific association with and/or modulation by different FXYD proteins, and 4) FXYD protein-deficient mice. Below is a summary of the most recent studies that support that FXYD proteins are auxiliary subunits of Na-K-ATPase and deals with the characterization of each FXYD protein (some relevant structural and functional properties of FXYD proteins are summarized in Table 1). (For further reference, see Refs. 18, 20, 92.)

FXYD1 (PHOSPHOLEMMAN)

FXYD1 is mainly expressed in the heart, skeletal muscle, and liver (10, 71). Detection of FXYD1 in the juxtaglomerular apparatus of the kidney indicates that FXYD1 can also be expressed in particular regions of other tissues (99). Expression of FXYD1 in X. laevis oocytes (63) or addition of FXYD1 to planar bilayers (17, 62) induces a Cl⁻-selective conductance that is activated by hyperpolarizing voltages. Moreover, FXYD1 selectively transports the zwitterionic amino acid taurine (62), an osmolyte of animal cells. In response to cell swelling, taurine efflux and the regulatory decrease in cell volume increase in FXYD1-transfected cells (64). Finally, taurine efflux in astrocytes is inhibited after a decrease in endogenous FXYD1 by antisense oligonucleotides (65). Thus, based on these observations, it is believed that FXYD1 plays a specific role in muscle contractility and cell volume regulation.

The recent experimental evidence that FXYD1 interacts with Na-K-ATPase adds a new perspective to the potential physiological role of FXYD1. FXYD1 interacts specifically with α₁β₁-isozymes in native cardiac and skeletal muscle (19, 35, 85) and with α₁β₁, α₂β₂, and α₃β₁ isoforms in the cerebellum and choroid plexus (32). After expression in X. laevis oocytes, FXYD1 decreases the apparent Na⁺ affinity and, to a lesser extent, the apparent K⁺ affinity of Na-K-ATPase (19). In contractile tissues, the existence of low-Na⁺-affinity Na/K pumps may be necessary to permit efficient extrusion of increased intracellular Na⁺ during action potentials and thus to control appropriate muscle contractility. Alternatively, the existence of a low-Na⁺-affinity Na/K pump associated with FXYD1 could lead to increased intracellular Na⁺ concentrations, an increase in intracellular Ca²⁺ concentrations due to inhibition of Ca²⁺ efflux via the Na/Ca exchanger, and ultimately an increased contractility of heart and skeletal muscles. However, this hypothesis is not supported by recent data, which show that at high extracellular Ca²⁺ concentrations, FXYD1 overexpression in rat myocytes leads to a reduction (102) and downregulation of FXYD1 and thus to increased (60) contractility. Overexpression of FXYD1 was correlated with inhibition of the Na/Ca exchanger of both its forward and reverse exchange activity (102), and downregulation with an enhancement of Na/Ca exchanger function (60). FXYD1 colocalizes with the Na/Ca exchanger, and coinmunoprecipitation experiments suggest a direct interaction between the two proteins (60), indicating that FXYD1 associates not only with Na-K-ATPase but also with the Na/Ca exchanger.

The physiological role of FXYD1 has been investigated in generations of FXYD1-deficient mice (45). These mice show an increased ejection fraction, an increased cardiac mass in the absence of hypertension, and a significant reduction in intrinsic Na-K-ATPase activity: with little decrease in total Na-K-ATPase expression. These results were interpreted to indicate that FXYD1 modulates the Na-K-ATPase and that abolishment of FXYD1 expression reduces Na-K-ATPase activity, which leads to compensatory responses. However, because the Na-K-ATPase, Na/Ca exchanger, and FXYD1 are likely to colocalize in the transverse tubules of cardiomyocytes (61) and Na-K-ATPase α₁- and α₂-isoforms interact with the Na/Ca exchanger (28), the relative importance of the Na-K-ATPase or the Na/Ca exchanger, or perhaps other interacting proteins, in the functional effects in FXYD1-deficient mice remains to be elucidated. Moreover, the mechanism by which FXYD1 influences Na-K-ATPase in situ in native tissues is still unclear. The results obtained with FXYD1-deficient mice (45) suggest that FXYD1 stimulates rather than inhibits Na-K-ATPase, and no difference was seen in the apparent Na⁺ affinity of the Na-K-ATPase in wild-type and FXYD1-deficient mice in contrast to the previously described decrease in the Na⁺ affinity of Na-K-ATPase induced by mammalian FXYD1 (19) or by the phospholemman-like shark protein (55).

Table 1. Structural and functional properties of FXYD proteins

<table>
<thead>
<tr>
<th>Membrane Orientation</th>
<th>Signal Peptide</th>
<th>Protein Association (Oocytes)</th>
<th>Association In Situ</th>
<th>Functional Effects Na-K-ATPase (Oocytes)</th>
<th>KO Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXYD1 type I</td>
<td>Yes, cleaved</td>
<td>Na-K-ATPase all isozymes</td>
<td>α₁ Isozymes</td>
<td>K₁/₂ Na ↑</td>
<td>Heart: increased ejection fraction, hypertrophy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K₁/₂ Na ↑</td>
<td>Na-K-ATPase activity: ↓</td>
</tr>
<tr>
<td>FXYD2 type I</td>
<td>No</td>
<td>Na-K-ATPase all isozymes</td>
<td>α₁ Isozymes</td>
<td>K₁/₂ Na ↑</td>
<td>Kidney: no phenotype, Na⁺ affinity Na-K-ATPase ↑</td>
</tr>
<tr>
<td>FXYD3 2 TM domains</td>
<td>Yes, not cleaved</td>
<td>Na-K-ATPase</td>
<td>α₁ Isozymes</td>
<td>K₁/₂ Na ↑</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K₁/₂ Na ↑</td>
<td>ND</td>
</tr>
<tr>
<td>FXYD4 type I</td>
<td>Yes, cleaved</td>
<td>Na-K-ATPase</td>
<td>α₁ Isozymes</td>
<td>K₁/₂ Na ↑</td>
<td>Kidney: no phenotype</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K₁/₂ Na ↓</td>
<td>Colon: Na⁺ reabsorption ↓</td>
</tr>
<tr>
<td>FXYD5 ND</td>
<td>ND</td>
<td>Na-K-ATPase</td>
<td>α₁ Isozymes</td>
<td>K₁/₂ Na ↑</td>
<td>ND</td>
</tr>
<tr>
<td>FXYD6 ND</td>
<td>ND</td>
<td>Na-K-ATPase</td>
<td>α₁ Isozymes</td>
<td>K₁/₂ Na ↑</td>
<td>ND</td>
</tr>
<tr>
<td>FXYD7 type I</td>
<td>No</td>
<td>Na-K-ATPase</td>
<td>α₁ Isozymes</td>
<td>K₁/₂ Na ↑</td>
<td>ND</td>
</tr>
</tbody>
</table>

KO, knockout; TM, transmembrane; ND, not determined. For details and references, see the text.
Phosphorylation may be at least one factor that regulates the putative multiple functions of FXYD1. Considered as the major substrate for protein kinase A in the heart (71), FXYD1 is phosphorylated on specific serine residues by PKA and PKC and the protein kinase never in mitosis A (NIMA) (53, 69, 96). PKA but not PKC phosphorylation of FXYD1 increases the FXYD1-mediated currents and its cell surface expression, whereas similar effects by NIMA kinases are independent of FXYD1 phosphorylation (69). On the other hand, phosphorylation by myotonic dystrophy kinase induces degradation of FXYD1 (68). Moreover, it has been suggested that PKC phosphorylation of a phospholemman-like protein from shark induces its dissociation from Na-K-ATPase and an increase in the Na\(^+\) affinity of Na-K-ATPase (54). In contrast, in isolated sarcolemma, ischemia produces a substantial activation of FXYD1 with Na-K-ATPase that was correlated with PKA phosphorylation of FXYD1 without influencing the association efficiency of Na-K-ATPase pump currents measured in forskolin-treated ventricular myocytes (85).

In conclusion, compelling evidence suggests that FXYD1 is an auxiliary subunit of Na-K-ATPase in contractile tissues and in the brain. So far, the intrinsic physiological role in these tissues is not known. In the heart and skeletal muscle, Na-K-ATPase is tightly linked to contractility, and regulation of its kinetic properties by FXYD1 may have distinct effects on this tissue function. Because both \(\alpha_1\) and \(\alpha_2\)-isozymes are implicated in cardiac contractility (28), the specific association of FXYD1 with \(\alpha_1\)-isozymes suggests a subtle difference in the mechanism underlying the function of \(\alpha_1\)-isozymes. The apparently discrepant results obtained regarding the mechanism of action of the regulatory effect of FXYD1 on Na-K-ATPase activity may partly be due to variations in its phosphorylation but could also be a result of an interplay between different functional roles of FXYD1, e.g., interaction with Na/Ca exchanger and channel formation.

**FXYD2 (\(\gamma\)-SUBUNIT OF Na-K-ATPase)**

The FXYD2 gene is located on chromosome 11q23 (87). It encodes two splice variants, FXYD2a and FXYD2b (\(ya\) and \(yb\)), which have been identified by mass spectroscopy and differ only in their most NH\(_2\)-terminal amino acids (49). FXYD2 is predominantly expressed in the kidney (59, 90) with segment specific distribution of FXYD2a and FXYD2b (4, 73) (Table 2). FXYD2a and FXYD2b colocalize with Na-K-ATPase in the basolateral membrane of renal epithelial cells. No FXYD2 was detected in the collecting duct (4, 73).

FXYD2 was the first FXYD protein that was found to be associated with Na-K-ATPase (33, 59) and to produce a functional effect on its transport properties (8, 90). In contrast to FXYD1, no other functional role than that of a Na-K-ATPase regulator has been proposed, although it was reported that FXYD2 expressed in \(X. \text{laevis}\) oocytes activates Ca\(^{2+}\)- and voltage-gated, nonselective pores endogenous to the oocyte (80). Evidence provided from different experimental approaches suggests that FXYD2 may have several concomitant and independent effects on Na-K-ATPase function. FXYD2 was shown to increase the apparent K\(^+\) affinity of Na-K-ATPase at high negative membrane potentials in both the presence and absence of extracellular Na\(^+\) (6). On the other hand, FXYD2 decreases the apparent K\(^+\) affinity at less negative membrane potentials but only in the presence of extracellular Na\(^+\), suggesting a shift in E1-E2 equilibrium toward the E1 conformation (6). FXYD2 also increases the affinity for ATP (90, 91), which is consistent with a shift toward the E1 conformation. Moreover, it has been reported that FXYD2 increases the K\(^+\) antagonism of intracellular Na\(^+\) binding, suggesting an additional effect of FXYD2 on intrinsic binding of K\(^+\) at cytoplasmic sites (73). Finally, FXYD2 decreases the Na\(^+\) activation of Na/K pump currents (6) and produces a parallel decrease in the Na\(^+\) and K\(^+\) activation of Na-K-ATPase activity (3).

Intriguingly, the two splice variants, FXYD2a and FXYD2b, produce identical effects on the catalytic and transport properties of Na-K-ATPase studied so far (6, 73). However, the functional effects of FXYD2a and FXYD2b differ depending on nonidentified posttranslational modifications, which may be cell specific or depend on the physiological state (2). Posttranslational modifications abolish the effect of FXYD2a on the apparent Na\(^+\) affinity of Na-K-ATPase activity. On the other hand, modifications of FXYD2b do not influence the effect on the apparent Na\(^+\) affinity, whereas modifications are needed for the effect of FXYD2b on the apparent K\(^+\) affinity (2). Finally, FXYD2a, but not FXYD2b, is enriched in caveolae of renal membranes (31), providing another argument for different functional roles of FXYD2 variants.

The physiological relevance of Na-K-ATPase modulation by FXYD2 in the kidney remains speculative. FXYD2 are mainly distributed in renal segments, which reabsorb most of the filtered Na\(^+\) load. Because a major effect of FXYD2 appears to be a decrease in the Na\(^+\) affinity of Na-K-ATPase, it may be speculated that the existence of low-Na\(^+\)-affinity Na-K-ATPase may be favorable for an efficient reabsorption of Na\(^+\) in renal segments with high cellular Na\(^+\) load (6). The physiological importance of the modulation of the Na\(^+\) affinity of Na-K-ATPase by FXYD2 is also supported by the observation that FXYD2 transfectants in culture, which reduce Na\(^+\) affinity, decrease cellular growth (2). On the other hand, in view of the increased affinity for ATP of Na-K-ATPase associated with FXYD2, it has been suggested that FXYD2 may be important in preserving the Na-K-ATPase activity in renal segments such as the outer medulla, which are highly prone to anoxia (73, 91). Significantly, renal Na-K-ATPase of FXYD2-deficient mice

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**Table 2. Distribution of FXYD2 variants and FXDY4 in the renal nephron**

<table>
<thead>
<tr>
<th>Nephron Segment</th>
<th>Predominant FXYD2 Variant</th>
<th>FXDY4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal tubule</td>
<td>FXYD2a (+FXYD2b)</td>
<td>None</td>
</tr>
<tr>
<td>mTAL</td>
<td>FXYD2a + FXYD2b</td>
<td>None</td>
</tr>
<tr>
<td>cTAL</td>
<td>FXYD2b</td>
<td>None</td>
</tr>
<tr>
<td>Macula densa</td>
<td>FXYD2a (+FXYD2b)</td>
<td>None</td>
</tr>
<tr>
<td>CCD</td>
<td>None (FXYD2a)</td>
<td>+</td>
</tr>
<tr>
<td>OMCD</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>IMCD</td>
<td>None (FXYD2a + b)</td>
<td>+</td>
</tr>
</tbody>
</table>

Data for FXYD2 variants are from Ref. 73 and Ref. 2. Data in brackets reflect differences in the expression patterns in the 2 reports. Data for FXDY4 are from Ref. 81. cTAL, medullary thick ascending limb; cTAL, cortical thick ascending limb; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct.
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Invited Review

FXYD3 (Mat-8)

FXYD3 has initially been identified in murine breast tumors induced by Neu or Ras oncogenes (66) and was then also found to be present in primary human breast tumors and in human breast tumor cell lines (67), in colorectal cancer cell lines (101), and in prostate tumors (41). In normal tissue, FXYD3 is mainly expressed in the uterus, stomach, colon, and skin (67). In adult mice, FXYD3 and Lgi4 genes have been mapped to the same locus on chromosome 7 and produce partially overlapping transcripts of opposite orientation, which potentially may impact on their respective expression (77).

When expressed in X. laevis oocytes, FXYD3 induces a hyperpolarization-activated chloride conductance similar to that observed with FXYD1 (67). Moreover, FXYD3 can associate with Na-K-ATPase and produces a decrease in both the apparent K⁺ and Na⁺ affinity of Na-K-ATPase (22). Interestingly, in addition to these functions shared with other FXYD proteins, FXYD3 exhibits some uncommon characteristics. First, in contrast to other FXYD proteins, FXYD3 may have two TMs because its signal sequence is not cleaved. Second, FXYD3 not only colocalizes with Na-K-ATPase in basolateral membranes of IMCD3 cells lacking FXYD2, the expression of both FXYD2a and FXYD2b is induced by hypertonicity depending on c-Jun kinase activation and phosphatidylinositol 3-kinase (12). Upregulated FXYD2 is routed to the basolateral membrane of IMCD3 cells, consistent with its localization in inner medullary collecting duct cells in vivo (72). The osmotic regulation of FXYD2 is also observed in mouse inner renal medulla in response to changes in hydration (12). In IMCD3 cells, failure to synthesize FXYD2 in response to hypertonicity leads to decreased cell viability, suggesting a potential role of the regulation of Na-K-ATPase by FXYD2 in cell survival under anisotonic conditions (12). Upregulation of FXYD2 and c-Jun kinase activation during hypertonicity is mediated by chloride but not by sodium, in contrast to upregulation of the Na-K-ATPase α-subunit induced by sodium (14). In IMCD3 cells, activation of c-Jun kinase regulates FXYD2 at the transcriptional level, whereas activation of PI3-kinase influences the translation of FXYD2 (13). Finally, FXYD2a but not FXYD2b expression is induced in renal cells of proximal origin as well as in several cell lines of other than renal origin by hypertonicity, heat shock, and chemical stress (98). Moreover, injury-induced NF-κB activation induces FXYD2 expression in the hippocampus (48). FXYD2a induction in cells of renal origin is accompanied by a reduction in Na-K-ATPase activity and in the rate of cell division (98). Both of these effects are reduced when FXYD2 expression was knocked down by small interfering RNA (siRNA), suggesting that induction of FXYD2a may be part of a cellular response to genotoxic stress (98).

Although much has been learned about the functional effects of FXYD2 on Na-K-ATPase and on its regulation, we are far from understanding the physiological relevance of this protein and its potential role in pathophysiological states such as human primary hypomagnesemia.
Na-K-ATPase in expression systems and in situ, as well as modulates its transport properties (6, 37).

In the absence of extracellular Na\(^+\), a situation in which K\(^+\) kinetics most closely reflect the intrinsic affinity for extracellular K\(^+\), FXYD4 has no effect on the K\(^+\) activation of Na-K-ATPase. On the other hand, in the presence of extracellular Na\(^+\), a condition in which the K\(^+\) kinetics depend on the competition by extracellular Na\(^+\), FXYD4 produces a large increase in the K\(_{1/2}\) value for K\(^+\) of the Na-K-ATPase (6). Moreover, FXYD4 induces a two- to threefold increase in the apparent affinity for intracellular Na\(^+\) (6, 37). This Na\(^+\) effect of FXYD4 on Na-K-ATPase is likely to be of physiological relevance for the Na\(^+\) reabsorption process in the collecting duct, which is the ultimate site of electrolyte conservation. An increase in the Na\(^+\) affinity of Na-K-ATPase produced by the association with FXYD4 in this renal segment is favorable because it permits efficient Na\(^+\) reabsorption even at low intracellular Na\(^+\) concentrations. At physiologically low intracellular Na\(^+\) concentrations (e.g., 5 mM), the Na-K-ATPase transport rate of Na-K-ATPase associated with FXYD4 should be about four times higher than that of Na-K-ATPase lacking FXYD4 (6).

Results from FXYD4 knockout mice (1, 40) at least partially confirm that a major if not unique effect of FXYD4 is the activation of Na-K-ATPase by increasing its apparent Na\(^+\) affinity. Abolition of FXYD4 expression appears to be fully compensated for in the kidney because fractional excretions of Na\(^-\) and K\(^+\) are normal under resting conditions, and animals have no deficit in the adaptation to low Na\(^+\) and K\(^+\) intake. However, in the distal colon, amiloride-inhibitable Na\(^+\) reabsorption is reduced under control conditions, glucocorticoid treatment, and low Na\(^+\) intake (40).

Regulation of FXYD4 expression in the kidney and colon is different. Both distal colon and the renal collecting duct, where FXYD4 is expressed, are aldosterone target sites, but aldosterone induces FXYD4 cRNA expression only in the colon but not in the kidney (11, 97). On the other hand, low Na\(^+\) intake increases FXYD4 mRNA and protein expression in the colon, but only FXYD4 protein and not mRNA expression in the kidney (16, 81). Moreover, in experimental ischemic acute renal failure (39, 75) and in acute tubular necrosis (84), which are characterized by hyperkalemia, FXYD4 cRNA expression is decreased in kidney and increased in colon. Possibly, suppression of FXYD4 expression in kidney could lead to decreased renal K\(^+\) secretion, and the enhanced FXYD4 expression in colon might be an adaptive response.

Although compelling evidence suggests that an important biological activity of FXYD4 is mediated through its interaction with Na-K-ATPase, it remains open whether this is the only function of FXYD4 or whether FXYD4 interacts with and regulates other, as yet unidentified, partner proteins.

**FXYD5 (Ric) AND FXYD6 (PHOSPOHIPPOLIN)**

FXYD5 (34) and FXYD6 (100) are poorly characterized, and interaction with and modulation of Na-K-ATPase have so far not been reported.

Compared with other FXYD proteins, FXYD5 has an unusual long NH\(_2\)-terminal extension (86). The so-called IUW-1 protein, which may be an isoform of FXYD5, has a shorter NH\(_2\) terminus and shows 61% sequence identity with the cytoplasmic domain of the angiotensin II type 1 receptor (70).

FXYD5 expression is induced in cells transformed by the oncogene E2a-Pbx1 (34). FXYD5 is also expressed in several cancer tissues but only in a few normal cell types (44). A role of FXYD5 in tumor progression and metastasis has been suggested, based on the observations that transfection of FXYD5, called dysadherin, into liver cancer cells results in decreased E-cadherin-mediated cell-cell adhesion (44), implicating O-glycosylation of FXYD5 (94). Moreover, dysadherin positivity was correlated with poor prognosis in various human cancers (82, 83).

FXYD6 is expressed in several tissues (86). In the brain, expression studies during development suggest a role in neuronal excitability during postnatal development and in the adult brain (47, 78).

**FXYD7**

FXYD7 has been found exclusively in the brain, where it is expressed in neurons and to a lesser extent in glial cells (7).

FXYD7 is subjected to O-glycosylation, which appears to be necessary for the stability of the protein (7).

Mutational analysis of FXYD7-specific regions revealed a COOH-terminal, cytoplasmic valine residue that is involved in rapid endoplasmic reticulum export and controls the rate of its cell surface expression (23). After coexpression in *X. laevis* oocytes, FXYD7 associates posttranslationally with Na-K-ATPase (23), probably due to the different rates of endoplasmic reticulum exit of the two proteins. Association of FXYD7 occurs with \(\alpha_1\beta_1\), \(\alpha_2\beta_1\), and \(\alpha_3\beta_1\) isoforms but not with Na-K-ATPase isoforms containing \(\beta_2\)-isoforms (7). On the other hand, in the brain, FXYD7 exclusively associates with Na-K-ATPase containing \(\alpha_1\)-isoforms (7). These data suggest that in the brain, FXYD7 is specifically associated with Na-K-ATPase \(\alpha_1\)-isoforms.

Electrophysiological analysis of the modulatory effect of FXYD7 on Na-K-ATPase in *X. laevis* oocytes revealed that FXYD7 modulates the transport properties of \(\alpha_1\beta_1\)-isoforms in a specific way, distinct from other FXYD proteins (7). The apparent affinity for K\(^+\) is increased over a wide range of membrane potentials in both the presence and absence of extracellular Na\(^+\), which suggests a modification of the intrinsic affinity of the external K\(^+\)-binding site. On the other hand, FXYD7 does not influence the apparent affinity for intracellular Na\(^+\) (7). It can be speculated that in the brain, the existence of Na-K-ATPase \(\alpha_1\beta_1\)-isoforms with low K\(^+\) affinity, acquired by association with FXYD7, may be necessary for efficient clearance of extracellular K\(^+\) during neuronal activity to ensure neuronal excitability.

Future studies should be directed to determine whether the endoplasmic reticulum export control of FXYD7 dependent on the COOH-terminal valine residue may be linked to the specific expression of FXYD7 in neurons and glial cells and/or to the particular requirements of the regulation of Na-K-ATPase expression and function in the brain.

**STRUCTURE-FUNCTION RELATIONSHIP**

In addition to the elucidation of the functional effects on Na-K-ATPase of several FXYD proteins, recent studies have revealed details on the molecular basis of these effects and on the structural and functional interaction sites in Na-K-ATPase...
and FXYD proteins. It is becoming clear that multiple sites of interaction exist that involve both the transmembrane and extramembrane domains.

The role of conserved amino acids in the structural and functional interaction with Na-K-ATPase has been studied in some FXYD proteins. Replacement of Gly-41 with Arg in the TM domain of FXYD2 (103), which is associated with a form of renal hypomagnesemia (58), or replacement of the analogous Gly-40 with Arg in FXYD7 (23) abolishes the interaction of these FXYD proteins with Na-K-ATPase. Replacements with Leu of Gly-41 in FXYD2 (103) or with Ala or Trp of Gly-40 in FXYD7 (23) permit partial association with Na-K-ATPase, which is paralleled by a partial loss of the effect of FXYD7 on the apparent K⁺ affinity of Na-K-ATPase (23). A similar reduction of the association efficiency and the K⁺ effect is also observed when the other conserved Gly-29 in FXYD7 is replaced by Ala. Gly-40 and Gly-29 are on the same side of the TM α-helix of FXYD proteins and form a groove that could be important as an interaction site between Na-K-ATPase and FXYD proteins. On the other hand, experiments using synthetic transmembrane mimetic peptides suggest that Gly-41 in FXYD2 is important for the effect of FXYD2 on the apparent Na⁺ affinity of Na-K-ATPase and not primarily on the association of FXYD2 with Na-K-ATPase (103). Finally, experiments using perfluorooctanoate gel electrophoresis indicate that FXYD2 and FXYD1 (56) and peptides corresponding to the TM domain of FXYD2 (89) may form oligomers. Gly-41 is necessary for oligomerization of FXYD2 peptides (89).

The role of the conserved FXYD motif has been studied in several FXYD proteins, but so far no preserved function could be revealed. The FXYD motif is important for the stable interaction of Na-K-ATPase with FXYD2 and FXYD4 (6) but not with FXYD7 (23).

Because an antibody against the COOH terminus of FXYD2 (73, 91) and truncations of the COOH and the NH₂ termini of FXYD2 (74) abolish the effect of FXYD2 on the apparent affinity for ATP of Na-K-ATPase, it was concluded that extramembrane interactions may be important for this functional effect of FXYD2. Interestingly, in FXYD2-deficient mice, the ATP affinity of Na-K-ATPase was not decreased as expected, but slightly increased, suggesting that extramembrane interactions may be context specific (46). On the other hand, by examining FXYD2/FXYD4 chimera, it was found that TM interactions of FXYD proteins determine both the stability of FXYD proteins in detergents and the effects of these FXYD proteins on the Na⁺ affinity of Na-K-ATPase (52). Interestingly, different amino acids in the TM appear to mediate the stability and the functional effects of these FXYD proteins. The functional role of TM interactions in the Na⁺ effect of FXYD2 was confirmed by showing that peptides corresponding to the TM domain of FXYD2 decrease the Na⁺ affinity of Na-K-ATPase similarly to full-length FXYD2 (103).

Recent experimental evidence also shed some light on the sites where FXYD proteins interact with Na-K-ATPase αβ complexes. Based on experiments using thermal denaturation, it has been suggested that FXYD2 interacts with TM8–10 of the Na-K-ATPase α-subunit (27). Moreover, electron crystallographic analysis of renal Na-K-ATPase at 9.5 Å resolution (42) and taking as a basis the high-resolution structure of the Ca-ATPase (93) suggest that FXYD2 is located in a binding pocket made up of TM9, TM6, TM4, and TM2. A role of TM9 in the structural and functional interaction with FXYD proteins could be confirmed by mutational analysis (51). Interestingly, Leu964 and Phe967 of TM9 of the rat Na-K-ATPase contribute to the stable interaction with FXYD2, FXYD4, and FXYD7 but do not influence the functional effect of these FXYD proteins on the apparent K⁺ affinity of Na-K-ATPase. On the other hand, Phe956 and Glu960 do not contribute to the efficient association of FXYD proteins but transmit the K⁺ effect of FXYD proteins to Na-K-ATPase. The relative contribution of Phe956 and Glu960 in the K⁺ effect of different FXYD proteins is different, probably reflecting the intrinsic variations in the modulation of the apparent K⁺ affinity of Na-K-ATPase by different FXYD proteins. In contrast to the K⁺ effect, the modulation of the apparent Na⁺ affinity of Na-K-ATPase by FXYD4 is not mediated by Phe956 and Glu960. The mutational analysis is in good agreement with a docking model of the Na-K-ATPase α-subunit/FXYD7 complex (51). Thus the structural and functional interaction with FXYD proteins involve TM9 of the Na-K-ATPase α-subunit but probably also other TM domains that remain to be determined. Based on cross-linking data, interactions of FXYD2 with the intracellular loop L6/7 in the Na-K-ATPase α-subunit and with the extracellular domain of the Na-K-ATPase β-subunit can also be predicted (36). Moreover, modeling of Na-K-ATPase/FXYD2 complexes is in agreement with the position of FXYD2 in a binding pocket composed of TM9, TM6, TM4, and TM2.

In conclusion, recent experimental evidence strongly supports that at least one functional role of FXYD proteins is the regulation of Na-K-ATPase in a tissue- and isoform-specific way. Although the functional effects of FXYD proteins on the transport and kinetic properties of Na-K-ATPase have been well characterized, much still remains to be learned about the physiological relevance of these effects and the potential implication of a loss of FXYD regulation of Na-K-ATPase in pathophysiological states. For a better understanding of these issues, more studies are needed on the functional roles of each FXYD protein, on their biosynthesis and processing, their tissue and cellular distribution, and their structure-function relationship.

GRANTS

This work was supported by Swiss National Fund Grant 3100A0–107513/1 (to K. Geering).

REFERENCES

6. Béguin P, Crambert G, Guennoun S, Garty H, Horisberger JD, and Geering K. CHIF, a member of the FXYD protein family, is a regulator


