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The ABC of Solute Carriers

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The sodium phosphate cotransporter family SLC34

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Abstract This review summarizes the characteristics of the solute carrier family SLC34 that is represented by the type II Na/P_i-cotransporters NaPi-IIa (SLC34A1), NaPi-IIb (SLC34A2) and NaPi-IIc (SLC34A3). Other Na/P_i-cotransporters are described within the SLC17 and SLC20 families. Type II Na/P_i-cotransporters are expressed in several tissues and play a major role in the homeostasis of inorganic phosphate. In kidney and small intestine, type II Na/P_i-cotransporters are located at the apical sites of epithelial cells and represent the rate limiting steps for transepithelial movement of phosphate. Physiological and pathophysiological regulation of renal and small intestinal epithelial transport of phosphate occurs through alterations in the abundance of type II Na/P_i-cotransporters.

Keywords Sodium-coupled phosphate transport · Type II Na/P_i-cotransporters · Endocytosis

Introduction

The SLC34 family comprises three members: NaPi-IIa, NaPi-IIb and NaPi-IIc (Table 1). Non-mammalian family members have been identified in various organisms such as flounder and zebra fish, *Xenopus laevis*, *Caenorhabditis elegans*, and *V. cholerae* [42].

Approximately 0.1% of the total content of phosphate (P_i) in the body is contained in the extracellular space. It is essential that the extracellular concentration of P_i is held constant at around 1.1 mM for proper cellular functions such as DNA formation and signaling reactions, as well as for bone formation. Members of the SLC34 family are expressed in small intestine and in renal proximal tubules, two important sites that control the extracellular concentration of P_i. It should be noted that extracellular P_i can be influenced by other mechanisms such as intra- to extracellular shifts (e.g., from skeletal muscle) which involve other P_i transporters, such as members of the SLC20 family. In proximal tubules and enterocytes type II Na/P_i-cotransporters are located in the apical membrane and represent the rate limiting steps of transepithelial P_i transport. In both tissues the abundance of type II Na/P_i-cotransporters is controlled by many hormones and metabolic factors according to the body's P_i needs. In kidney, several inherited and acquired phosphate wasting disorders affect the abundance of type II Na/P_i-cotransporters.

Besides the kidney, expression of NaPi-IIa has been described in bone and neurons and in addition to small intestine, expression of NaPi-IIb has been described in a number of organs such as lung and mammary glands. In the latter tissues, the detailed physiological and pathophysiological aspects of the type II Na/P_i-cotransporters remain to be defined.

In a physiological environment, all SLC34 family members exclusively transport phosphate ions in a obligatory sodium-dependent manner. With the exception of NaPi-IIc, Na/P_i-cotransport is electrogenic. To date, no specific high-affinity inhibitors are known for this SLC family; phosphonoformic acid (forscanet) at high concentrations (millimolar) inhibits all members. Based on results obtained from hydropathy analysis, cysteine scanning mutagenesis and epitope-tagging studies, a model for the secondary topology of SLC34 transporters has been proposed.

NaPi-IIa (SLC34A1)

This Na/P_i-cotransporter was identified by functional expression cloning using *X. laevis* oocytes and a rat and human kidney cDNA library [30]. The major site of expression is principally the renal proximal tubule and here, this protein (80–90 kDa) is localized in microvilli that constitute the brush border membrane. Under normal physiological conditions, the abundance of NaPi-IIa is highest in S1 proximal tubular segments of jux-

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Table 1 SLC34: sodium phosphate cotransporter (type II) family (*C* cotransporter, *E* exchanger, *O* orphan transporter, *A* acquired defect, *G* genetic defect, *P* pseudogene)

Human gene name	Protein name	Aliases	Predominant substrates	Transport type; coupling ions	Tissue distribution and cellular/subcellular expression	Link to disease	Human gene locus	Sequence accession ID	Splice variants and their specific features
SLC34A1	NaPi-IIa	NaPi-3	Inorganic phosphate	C; Na^+/HPO_4^{2-}	Kidney (proximal tubules; apical) osteoclasts, neurons	G/XLH; G/ ADHR; A/OHO	5q35	NM_003052	Uncertain
SLC34A2	NaPi-IIb		Inorganic phosphate	C; Na^+/HPO_4^{2-}	Small intestine, lung, testis, liver, secreting mammary gland		4p15	NM_006424	Unknown
SLC34A3	NaPi-IIc		Inorganic phosphate	C; Na^+/HPO_4^{2-}	Kidney (proximal tubules, apical)		Not determined	NM_080877	Unknown

Fig. 1 Secondary topology of the NaPi-IIa (SLC34A1) based on current structure-function studies [9]. The protein comprises eight transmembrane domains (*TMD1–8*) with intracellular N- and C-termini and a large extracellular loop (*ECL-2*) containing two N-glycosylation sites (*hexagons*). An essential disulphide bridge in ECL-2 links each complementary part to form the functional unit. Cysteine scanning mutagenesis has revealed functionally important residues at sites indicated (*open squares*). Two reentrant loops (*ICL-1*, *ECL-3*) are predicted to associate and form the transmembrane cotransport pathway. In ECL-3, proline-461 terminates a 2.5 turn α-helix motif (*boxed*) thought to be an essential part of this pathway, while in ICL-1 asparagine-199 is a critical determinant of transport mode. A triad of three adjacent charge residues (*R462*, *E463*, *K464*) in ECL-3 confers part of SLC34 proton sensitivity

tamedullary nephrons [6]. Three different isoforms of NaPi-IIa have been reported to exist in proximal tubules as well, yet their function has not been established [39].

Na/P_i cotransport by NaPi-IIa is electrogenic, involving the inward transfer of one net positive charge per transport cycle. Divalent P_i is the preferred species and is transported together with three Na^+ ions [9, 10]. The substrates (three Na^+ ions and one HPO_4^{2-} ion) bind in an ordered manner: $Na/P_i/2Na$. Typical apparent substrate affinities are: K_m^{Pi} ~0.1 mM; K_m^{Na} ~70 mM. In the absence of P_i, the transporter operates in a uniport mode, whereby Na^+ ions leak according to the electrochemical gradient with a probable stoichiometry of 1 Na^+ ion. Protons interact with the empty carrier and the final Na-binding steps. Higher transport rates are observed at more basic external pH values [10]. Dependency on pH has partially been conferred to the charged amino acid motif (REK) contained in the putative extracellular loop ECL3 [7] (Fig. 1).

NaPi-IIb (SLC34A2)

NaPi-IIb was identified based on EST clones derived from lung tissue [8, 16]. Expression of NaPi-IIb mRNA has been detected in a number of tissues such as small intestine, lung, mammary glands, testis, and liver [43]. By immunofluorescence, NaPi-IIb was localized in brush borders of enterocytes, in the apical pole of alveolar type

ll cells as well as in apical membranes of mammary secretory cells [16, 31, 41]. On Western blots, fully glycosylated NaPi-llb is observed as a band of approximately 108 kDa [16]. Interestingly, in weaning animals NaPi-llb was reported to be only partially glycosylated [1].

At the primary sequence level NaPi-llb differs from NaPi-lla mainly in the C-terminus, which is rich in Cys residues and longer by approximately 50 amino acids. Na/P_i-cotransport is also electrogenic with a likely stoichiometry of 3Na:1P_i and with K_m^{Pi}<50 μM and K_m^{Na}=40 mM. Dependence on pH is moderate, slightly higher cotransport is observed at more acidic pH [16].

NaPi-IIc (SLC34A3)

This member was identified based on human EST clones. Expression of NaPi-llc was found exclusively in kidney and was described as being growth related. The protein (75 kDa) was localized in apical membranes of proximal tubules of deep nephrons. Na/P_i-cotransport by NaPi-llc is electroneutral (K_m^{Pi}=70 μM; K_m^{Na}=50 mM) and is highly pH dependent, exhibiting higher transport at more alkaline pH [36].

Structural aspects of type II Na/P_i-cotransporters

As predicted from structure-function studies [10, 26], each type ll Na/P_i-cotransporter protein most likely spans the membrane eight times. Both N- and C-termini are located cytoplasmatically and there are multiple N-glycosylation sites in a large extracellular loop. Furthermore, two short (extracellular and intracellular) loops have been postulated to be important functional regions for the transport pathway [10, 24]. A model of the secondary structure of SLC34 Na/P_i-cotransporters is depicted in Fig. 1. This model is based on data obtained with NaPi-lla (SLC34A1), but appears to be valid for the other family members as well, since in all members, the transmembranous regions are over 80% identical and exhibit the same hydropathy profile [10]. The largest dissimilarities are found in the N- and C-termini and in the large extracellular loops. Based on results obtained with tandem constructs of NaPi-lla, it is assumed that the monomeric form is sufficient for full type ll-mediated Na/P_i cotransport [23].

Physiological, pathological and pharmaceutical aspects

The concentration of the extracellular P_i is controlled by the body's need, and in adults it is kept constant at around 1.1 mM. This is achieved largely by a control of the renal capacity to reabsorb P_i from the primary urine and, to a lesser extent, by a control of small intestinal absorption of P_i (see below). Expression of SLC34 members was also

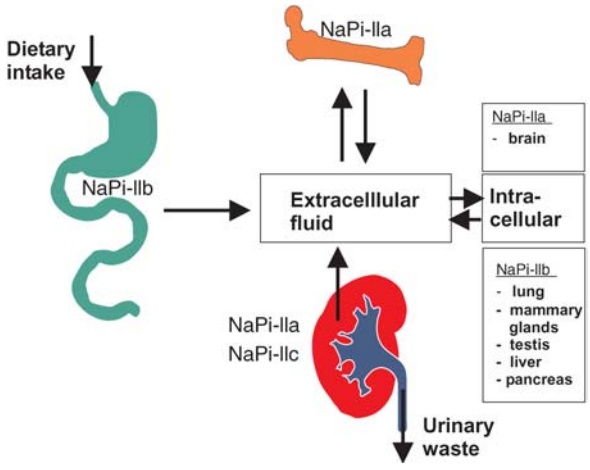

Fig. 2 Sites of expression of type ll Na/P_i-cotransporters (SLC34). Whereas the roles of NaPi-lla and NaPi-llb in epithelia of renal proximal tubules and small intestine are well described (see text for references) the roles of SLC34 cotransporters in other tissues (*indicated in boxes*) are less well defined

described in other tissues such as bone [13], brain [17], mammary glands [31], and lung [8, 41] (Fig. 2); however, the physiological roles of type ll Na/P_i-cotransporters described at these sites have not yet been entirely defined. In type ll alveolar cells, NaPi-llb was localized in the apical membrane and may be involved in the reabsorption of P_i contained in the surfactant. In secreting mammary glands, a role of NaPi-llb could be envisaged in delivering P_i into milk during lactation.

SLC34A1 and SLC34A3 and renal reabsorption of phosphate

The importance of NaPi-lla in the renal handling of P_i was demonstrated by a Npt2 knock out mouse [4]. $Npt2^{-/-}$ mice exhibit severe hypophosphatemia that is explained by an approximately 70% decrease of brush-border membrane Na/P_i cotransport. The remaining Na/P_i cotransport activity has been attributed to NaPi-llc.

Both NaPi-lla and NaPi-llc are targets for the adjustment of renal reabsorption of P_i and are regulated by many different hormones (e.g., parathyroid hormone) and metabolic factors (e.g., P_i diet or acidosis) [33, 36, 38]. Furthermore, factors derived from tumors associated with osteomalacia (e.g., FGF23) have been implicated to regulate NaPi-lla in proximal tubules [11, 25, 40]. Regulation of proximal tubular reabsorption of P_i was described as being due to an alteration of the abundance of NaPi-lla or NaPi-llc proteins residing in the brush-border membrane. It is assumed that at a constant rate of the de-novo synthesis, the amount of NaPi-lla is altered by regulated endocytosis [33]; so far, no such mechanism has been reported for NaPi-llc. In contrast to other regulated membrane transport processes, internalized NaPi-lla proteins do not recycle, but undergo lysosomal degradation [20, 28, 34]. Although many aspects of the

signaling cascades, such as the involvement of cAMP and cGMP and different protein kinases have been described [2, 3, 33], neither the precise mechanisms of the internalization of NaPi-lla nor the mechanisms involved in the vesicular trafficking of internalized NaPi-lla proteins have been defined. With respect to hormone-mediated endocytosis of NaPi-lla, a dibasic amino acid motif (RK) within the putative intracellular loop ICL3 was shown to be important as after mutations at this site NaPi-lla was no longer responsive to parathyroid hormone [18].

Acute (2–4 h) and chronic (days to weeks) P_i depletion has been shown to increase the level of NaPi-lla [21, 27]. In case of chronic P_i depletion a posttranscriptional mechanism has been suggested involving $–P_i$ renal proteins to stabilize NaPi-lla mRNA [32].

Recent data have demonstrated that in renal proximal cells, NaPi-lla interacts with the PDZ proteins NHERF-1 and PDZK1 [12]. Such interactions appear to be important for correct apical sorting and or positioning of NaPi-lla, as shown by a NHERF-1 knock out model [37] and by studies in OK cells [15]. On the other hand, PDZK1$^{-/-}$ mice do not show any changes of NaPi-lla content, suggesting a possible redundancy of other PDZ interactions [22].

Several renal wasting disorders have been described that are based on changes of the expression of NaPi-lla [40]. Studies related to the inherited disorders XLH (X-linked hypophosphatemia) and ADHR (autosomal dominant hypophosphatemic rickets) and studies related to the acquired disorder OHO (oncogenic hypophosphatemic osteomalacia) revealed evidence that the two genes PHEX and FGF23 play important roles in determining the abundance of NaPi-lla in renal proximal tubules [11, 25, 40]. Recently, two naturally occurring mutants of NaPi-lla (positions 48 and 147) have been identified and linked to two cases of renal wasting disorders of phosphate [35].

SLC34A2 and intestinal absorption of phosphate

Several observations provided evidence that NaPi-llb is involved in transcellular flux of phosphate in small intestine [14, 16, 19, 44]. Therefore small intestinal NaPi-llb could represent a possible target for treatment of hyperphosphatemia as often observed in dialysis patients. The most prominent regulators of NaPi-llb in small intestine are 1,25-$(OH)_2$-vitaminD3 and low-phosphate diet. Upregulation of NaPi-llb induced by these factors is manifested by an increase of the protein abundance and appears, in adults, to be nontranscriptional [14, 19, 44]. Furthermore, regulation of NaPi-llb in small intestine by EGF [43], glucocorticoids [1], thyroid hormone [5], and stanniocalcin 1 [29] has been reported.

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