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Proximal tubular handling of phosphate: A molecular perspective

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Members of the SLC34 gene family of solute carriers encode for three Na^+-dependent phosphate (P_i) cotransporter proteins, two of which (NaPi-IIa/*SLC34A1* and NaPi-IIc/*SLC34A3*) control renal reabsorption of P_i in the proximal tubule of mammals, whereas NaPi-IIb/*SCLC34A2* mediates P_i transport in organs other than the kidney. The P_i transport mechanism has been extensively studied in heterologous expression systems and structure–function studies have begun to reveal the intricacies of the transport cycle at the molecular level using techniques such as cysteine scanning mutagenesis, and voltage clamp fluorometry. Moreover, sequence differences between the three types of cotransporters have been exploited to obtain information about the molecular determinants of hormonal sensitivity and electrogenicity. Renal handling of P_i is regulated by hormonal and non-hormonal factors. Changes in urinary excretion of P_i are almost invariably mirrored by changes in the apical expression of NaPi-IIa and NaPi-IIc in proximal tubules. Therefore, understanding the mechanisms that control the apical expression of NaPi-IIa and NaPi-IIc as well as their functional properties is critical to understanding how an organism achieves P_i homeostasis.

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Homeostasis of P_i in higher organisms depends on the coordinated transport of P_i across intestinal and renal epithelia. Transport of P_i across the apical membrane is mediated by the three members of the SLC34 family of solute carriers.[1] NaPi-IIa (*SLC34A1*) and NaPi-IIc (*SLC34A3*) are specifically expressed in the brush border membrane (BBM) of renal proximal tubules. NaPi-IIb (*SLC34A2*) has a broader pattern of expression and it is highly abundant in the BBM of small intestine. In both epithelia, the basolateral exit of P_i is mediated by a transporter that remains unidentified. In the proximal tubule it has been proposed that a Na^+-dependent electroneutral anion exchanger is at least partially responsible for P_i exit.[2]

NaPi-IIa and NaPi-IIc mediate the reabsorption of P_i from the primary urine by using the free energy provided by the electrochemical gradient for Na^+. NaPi-IIa is electrogenic and transports divalent P_i preferentially. It functions with a strict Na^+:P_i stoichiometry of 3:1, which results in the net inward movement of one positive charge per cotransport cycle.[3] NaPi-IIc, in contrast, is electroneutral and exhibits a 2:1 stoichiometry[4,5] (Figure 1). In mice, NaPi-IIa is the protein mainly responsible for P_i reabsorption in the adult kidney, whereas NaPi-IIc appears to be more important in weaning animals. Indeed, the phenotype of NaPi-IIa knockout mice first suggested that this cotransporter is responsible for the bulk of renal P_i reabsorption with a very small percentage potentially attributed to NaPi-IIc.[6] However, recent data indicate that in humans, NaPi-IIc may have a previously unpredicted importance. The expression of NaPi-IIa and NaPi-IIc is regulated to adapt the renal reabsorption of P_i to the organism needs. Thus, the phosphaturic effect associated with parathyroid hormone (PTH) is due to the membrane retrieval of both cotransporters, whereas in conditions of P_i deprivation their expression is increased.[4,7–9]

The following sections summarize our present state of knowledge of the regulatory and pathophysiological roles of NaPi-IIa in renal P_i handling as well as its mechanism and structure–function relations.

REGULATION OF NaPi-IIa EXPRESSION

Many hormonal and non-hormonal factors regulate renal reabsorption of P_i (for review, see Murer *et al.*[10]). The effect of PTH and dietary P_i on NaPi-IIa has been the subject of detailed investigation. These studies suggest that NaPi-IIa

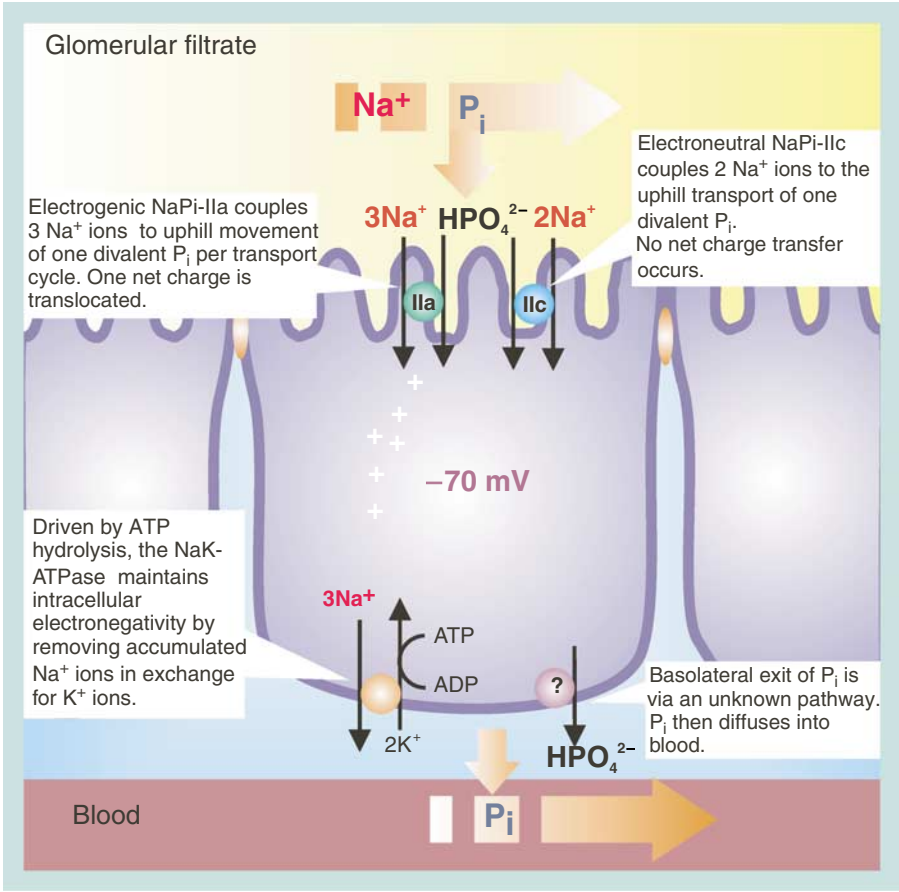

Figure 1 | Energetics of P_i reabsorption. In the BBM of proximal tubule epithelia, two Na^+-coupled transporters, designated as NaPi-IIa and NaPi-IIc, mediate apical uptake of P_i from the glomerular filtrate. Both are secondary active and drive P_i inward using the electrochemical free energy difference across the membrane for Na^+ ions. NaPi-IIa is electrogenic and NaPi-IIc is electroneutral. With a typical transmembrane Na^+ concentration ratio of 10:1, the theoretical P_i concentrating capacity of NaPi-IIc is ~100:1, whereas that for NaPi-IIa is ~10 000:1 because of its 3:1 Na^+:P_i stoichiometry and the additional driving force contributed by the transmembrane potential difference.

regulation depends on its shuttling to/from the BBM. This contrasts with many other transporters, which activity is modulated by modification of the transport protein itself (e.g. phosphorylation, dimerization etc). This means that the body's requirements for a higher P_i reabsorption (i.e. after low P_i-diet) are met by increasing the expression of NaPi-IIa[7,11,12] and NaPi-IIc[4] at the BBM. For NaPi-IIa, acute upregulation is independent of changes in transcription or translation. Therefore, the increased expression of NaPi-IIa must be owing to either the stabilization of the transporter at the BBM or to an increased rate of insertion at the membrane. Experimental data supports this dual mechanism. Thus, dietary-induced upregulation depends on the presence of scaffolding proteins,[13] suggesting a stabilization action, and on the microtubule network,[11] suggesting an increased rate of insertion. This latter mechanism requires the presence of an intracellular pool of NaPi-IIa ready to be shuttled to the membrane. Immunostainings of kidneys from rats fed acutely a low P_i-diet have indeed revealed the presence of NaPi-IIa in the Golgi apparatus, although this pool is not detected with all immunostaining protocols.[11]

In contrast, reduced reabsorption of P_i (i.e. upon PTH release or high P_i-diet) is achieved via downregulation of NaPi-IIa[8,11,14] and NaPi-IIc[9] at the BBM. PTH-induced downregulation of NaPi-IIa has been extensively studied and the identifiable steps are summarized in Figure 2. Because endocytosed cotransporters do not recycle to the BBM but instead are degraded in lysosomes, recovery of NaPi-IIa basal levels upon PTH removal depends on *de novo* synthesis. It is therefore clear that apical retention/removal of NaPi-IIa must be a regulated process, beyond the control of protein turnover. We will now describe in detail the steps summarized in Figure 2, integrating what is known about the mechanisms that regulate NaPi-IIa expression with the role of protein complexes.

Regulation of apical expression (step 1)

Apical expression of NaPi-IIa is dependent on its last three residues (TRL, see Figure 4a). Truncation of these residues leads to intracellular accumulation of the cotransporter, suggesting an impaired sorting and/or stability of the mutated protein.[15] The TRL sequence represents a PDZ