Phosphate transporters: a tale of two solute carrier families

Leila V. Virkki, Jürg Biber, Heini Murer, and Ian C. Forster
Institute of Physiology and Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland
Submitted 16 May 2007; accepted in final form 14 June 2007

Virkki LV, Biber J, Murer H, Forster IC. Phosphate transporters: a tale of two solute carrier families. Am J Physiol Renal Physiol 293: F643–F654, 2007.—Phosphate is an essential component of life and must be actively transported into cells against its electrochemical gradient. In vertebrates, two unrelated families of Na+/Pi cotransporters (SLC20) transport monovalent H2PO4−, whereas type II Na+/Pi cotransporters (SLC34) prefer divalent HPO42−. The SLC34 family comprises both electrogenic and electroneutral members that are expressed in various epithelia and other polarized cells. Through regulated activity in apical membranes of the gut and kidney, they maintain body P i homeostasis, and in salivary and mammary glands, liver, and testes they play a role in modulating the P i content of luminal fluids. The two SLC20 family members PiT-1 and PiT-2 are electrogenic and ubiquitously expressed and may serve a housekeeping role for cell P i homeostasis; however, also more specific roles are emerging for these transporters in, for example, bone mineralization. In this review, we focus on recent advances in the characterization of the transport kinetics, structure-function relationships, and physiological implications of having two distinct Na+/Pi cotransporter families.

Address for reprint requests and other correspondence: I. C. Forster, Institute of Physiology, Univ. of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland (e-mail: Iforster@access.uzh.ch).

PHOSPHORUS IS AN ESSENTIAL element of all living organisms and fulfills both structural and metabolic roles. Cells obtain phosphorus in the form of negatively charged inorganic phosphate (P i) from the extracellular environment by means of secondary-active transport. In vertebrates, P i transporters use the inwardly directed electrochemical gradient of Na+ ions, established by the Na+-K+-ATPase, to drive P i influx. Because defects in P i homeostasis result in severe pathologies, it is essential to understand the transport mechanisms at the molecular level.

In this review, we focus on two unrelated families of mammalian phosphate transporters with an emphasis on the current state of knowledge about their structure and mechanistic properties at the molecular level and their physiological roles. Both are secondary-active, Na+-coupled, yet their transport mechanisms are different. Proteins of the solute carrier family SLC34 (also called type II Na+/Pi cotransporters) are the most extensively characterized in terms of function, structure, and regulation: its members play essential physiological roles in the kidney and small intestine for maintaining P i homeostasis, and in salivary and mammary glands, liver, and testes they play a role in modulating the P i content of luminal fluids. The two SLC20 family members PiT-1 and PiT-2 are electrogenic and ubiquitously expressed and may serve a housekeeping role for cell P i homeostasis; however, also more specific roles are emerging for these transporters in, for example, bone mineralization. In this review, we focus on recent advances in the characterization of the transport kinetics, structure-function relationships, and physiological implications of having two distinct Na+/Pi cotransporter families.

according to the human SLC34 and SLC20 gene family nomenclature.

A third transporter family, SLC17, is sometimes referred to as the type I Na+-dependent P i transporter family. The first cloned member of this family (NaPi-1) was first thought to be a Na+-dependent P i transporter based on Xenopus laevis oocyte expression studies (117) but was later shown to transport organic anions (14). Another SLC17 protein, BNP1, was also first classified as a Na+/Pi transporter but was later shown to be a vesicular glutamate transporter (6). As no SLC17 family members are known to be strict Na+/Pi cotransporters, they will not be reviewed further here (for a review of SLC17 see Ref. 82).

**SLC34: Type II Na+/Pi Cotransporter Family**

Discovery and tissue distribution. Members of the SLC34 family, designated type II Na+/Pi cotransporters (NaPi-II), are generally found in apical membranes of epithelia and epithelial-like cells. Early transport studies using membrane vesicles from renal and intestinal epithelial tissue documented secondary-active, Na+-dependent P i transport. Further studies used both cloned transporters and native tissue to characterize the kinetics and regulation of Na+/Pi transport (for a review, see Ref. 70). SLC34 proteins belong to one of three phylogenetically distinct branches that are distantly related to the bacterial Vibrio cholerae Na+-dependent P i transporter (Fig. 1A). The first family member (NaPi-IIa/SLC34A1) was identified by expression cloning using X. laevis oocytes (61), and immunohistochemistry confirmed its localization at the apical membrane of renal proximal tubular cells. NaPi-IIa protein was also detected in rat brain (69), osteoclasts (53), and osteoblast-like cells (59), suggesting a wider expression profile for this “renal”

http://www.ajprenal.org
0363-6127/07 $8.00 Copyright © 2007 the American Physiological Society
F643
isofrom. After the discovery of NaPi-IIa, a second isofrom (NaPi-IIb/SLC34A2) was localized to the brush-border membrane of enterocytes and to lung, colon, testes, and liver (42). The third and newest member (NaPi-IIc/SLC34A3), like NaPi-IIa, is apically expressed in the renal proximal tubule (60, 89). The amino acid sequences of all three SLC34 proteins are similar in the predicted transmembrane-spanning regions but diverge in the NH2 and COOH termini and a prominent extracellular loop that separates the protein into two halves (see Topology and structure-function studies).

Physiological role and pathophysiology. The expression and regulation of SLC34 proteins in their intestinal and renal context have been extensively studied because these organs represent, respectively, the principle entry and exit points for Pi. Renal regulation of NaPi-IIa has been the subject of several recent reviews (30, 66, 70, 98, 100, 116) and is briefly discussed here. The critical role of NaPi-IIa for Pi homeostasis is underscored by the hyperphosphaturia phenotype documented in the NaPi-IIa knockout mouse (5). Dysregulation of NaPi-IIa causes Pi deficiency disorders, such as X-linked hypophosphatemic (XLH) and autosomal-dominant hypophosphatemic rickets (ADHR) (reviewed in Ref. 98). However, no mutation in the NaPi-IIa gene in humans has yet been unequivocally linked to human disease. Prié et al. (78) reported that heterozygous individuals have amino acid substitutions that correspond to functions on the NaPi-IIc protein identified in patients diagnosed with hypercalciuria (HHRH) (7, 47, 57). The effect of mutations on the NaPi-IIc protein identified in patients diagnosed with HHRH is currently undergoing in vitro investigation (C. Bergwitz, personal communication). Interestingly, two of the mutants have amino acid substitutions that correspond to functionally important sites identified in the context of electrogenticity (Ref. 3 and see below). Moreover, the marked pathophysiologocal consequences of NaPi-IIc mutations in human subjects suggest a more important role for this protein than initially suspected from animal data (89).

Whereas the physiological roles of SLC34 proteins, particularly in the kidney, are well characterized, it is obvious that future work must also focus on other organs. For example, regulated Pi transport is crucial for the formation of mineralized bone. NaPi-IIa is expressed in osteoclasts (37, 53) and chondrocytes (62), whereas osteoblasts express both NaPi-IIa and NaPi-IIb (59). NaPi-IIb is responsible for transcellular Pi absorption in the small intestine and is regulated by dietary Pi (80) and metabolic acidosis (91). In the liver, NaPi-IIb is involved in the catabolism of Pi, from primary hepatic bile (35). In salivary glands, NaPi-IIb is involved in secreting Pi into saliva, where a high Pi content is important for remineralization of dental enamel (45). In the brain, NaPi-IIa may play a role in central nervous system regulation of Pi, homeostasis (44, 69). Improper NaPi-IIb expression in the epidydymis is implicated as a possible causative agent in a mouse model of male infertility (120). NaPi-IIb is expressed in alveolar type II cells (101), and mutations in NaPi-IIb have recently been associated with pulmonary microthrombosis (20, 46). In mice, NaPi-IIb is expressed apically in lactating mammary gland but not in virgin mice (67), which suggests a role for NaPi-IIb in milk secretion. Dysfunction of NaPi-IIb may also be involved

In mice with homozygous deletion of the Npt2a gene, ~30% Pi reabsorption remains in the kidney. This fraction can most likely be attributed to NaPi-IIc, which was originally described as a growth-related transporter in weaning rats (89). Recent publications describe mutations of the NPT2c gene in humans as being responsible for hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (7, 47, 57). The effect of mutations on the NaPi-IIc protein identified in patients diagnosed with HHRH is currently undergoing in vitro investigation (C. Bergwitz, personal communication). Interestingly, two of the mutants have amino acid substitutions that correspond to functionally important sites identified in the context of electrogenticity (Ref. 3 and see below). Moreover, the marked pathophysiologocal consequences of NaPi-IIc mutations in human subjects suggest a more important role for this protein than initially suspected from animal data (89).
in the formation of microliths in other organs where the transporter is expressed. Of particular significance is that mammary tissue, microcalcifications containing calcium phosphate, are more commonly associated with malignancy than ones without a phosphate component (34).

Finally, although specific studies are still lacking, it is likely that the newcomer, NaPi-IIc, is not exclusively expressed in the kidney, as originally suggested (71, 89). A query of published microarray data deposited in the Gene Expression Omnibus (GEO) database reveals that the transcript for NaPi-IIc is abundant in several tissues, such as brain, breast, liver, and blood (L. V. Virkki, unpublished observations). Like for NaPi-Ia and NaPi-Ib, it is expected that future studies will reveal important roles for NaPi-IIc also in these extrarenal locations.

**Transport kinetics.** The transport kinetics of SLC34 proteins have been extensively studied (for a review, see Refs. 30 and 31) by means of heterologous expression in *X. laevis* oocytes. In essence, all three SLC34 proteins exhibit a strict dependence on external Na\(^+\) as the driving substrate, with an apparent affinity for Na\(^+\) of \(\sim 50 \text{ mM}\), a preference for divalent Pi, (HPO\(_4^{2-}\)) as the driven substrate with an apparent affinity of \(\leq 0.1 \text{ mM}\), and cotransport activity inhibitable by phosphonoformic acid (PFA) (15, 119; G. Stange, I. C. Forster, unpublished observations). These common features indicate that the basic transport mechanism is the same for all SLC34 members. Nevertheless, two important, interrelated differences in the kinetic properties have been documented, namely, electrogenericity and stoichiometry. NaPi-IIa and NaPi-IIb are electrogenic, and transport Pi with a 3:1 Na\(^+\):HPO\(_4^{2-}\) stoichiometry, whereas NaPi-IIc is electroneutral and operates with a 2:1 Na\(^+\):HPO\(_4^{2-}\) stoichiometry.

**ELECTROGENIC ISOFORMS NaPi-Ia/b.** Tracer uptake assays on brush-border membrane vesicles first suggested that Na\(^+\)-coupled Pi cotransport was electrogenic (12). However, this property could not be unequivocally defined without determination of the Na\(^+\)-Pi stoichiometry, identification of the preferred Pi species, and transmembrane electrical measurements. More direct evidence for electrogenic Na\(^+\)-dependent Pi cotransport was obtained by recording the transmembrane potential of isolated, perfused proximal tubule cells (88), later confirmed by heterologous expression of cloned NaPi-IIa in *X. laevis* oocytes (13, 39). These studies established that Pi induced an inward current in the presence of external Na\(^+\) when the cell was hyperpolarized (Fig. 2, A and B). By assuming that the Pi-induced change in cell holding current under voltage clamp (\(I_{\text{h}}\)) directly reflected the cotransport activity, it was inferred that NaPi-IIa operated with a 3:1 Na\(^+\):Pi stoichiometry at physiological pH (7.4), where divalent Pi predominates. Nevertheless, there was uncertainty about the selectivity of the transporter for divalent vs. monovalent Pi and the influence of external pH, which itself determines the availability of the Pi species. Simultaneous measurement of net charge transfer and substrate flux (32, 110) and surface pH measurements (81) (see Fig. 6C) on the same oocyte have resolved this issue unam-

![Fig. 2. Basic transport properties of SLC34 proteins expressed in X. laevis oocytes. A: original current recordings from an oocyte expressing flounder NaPi-Ib in solution containing 100 mM Na\(^+\) (left) and 100 mM Na\(^+\) 1 mM Pi, (right). The voltage was stepped from \(-160\) to \(+80 \text{ mV}\) in 40-mV increments. B: characteristic current-voltage curve for Pi-dependent currents (\(I_{\text{p}}\)) with different driving cations (at 100 mM) for an oocyte expressing the flounder NaPi-Ib isoform. Each data point is the difference between the oocyte holding current at the indicated membrane potential with and without 1 mM Pi, and in 100 mM cholineCl, relaxations are still resolvable. These are thought to arise from molecular rearrangements within the NaPi-Ib protein. D: determination of the stoichiometry of SLC34 proteins. Top: individual oocytes expressing the electrogenic rat NaPi-IIa isoform were voltage clamped, and the charge (\(Q\)) and amount of tracer substrate (\(^{32}\text{P}\) or \(^{32}\text{Na}\)) taken up was assayed. The ratio \(Q_{\text{P}}\), (filled symbols) is \(\sim 1:1\); the ratio \(Q_{\text{Na}}\) (open symbols) is \(\sim 1:3\). These data indicate that the Na:Pi stoichiometry is 3:1, with one net charge translocated per Pi (redrawn and modified from Ref. 32). The \(Q_{\text{P}}:\text{Na}\) ratio remains unchanged at lower pH, indicating that divalent Pi is the preferred species (not shown). Bottom: for electroneutral NaPi-IIc, data indicate a ratio Na:Pi, of 2:1, consistent with a preference for divalent Pi, and 2 Na\(^+\) ions translocated per cycle (redrawn and modified from Ref. 3).
biguously. Moreover, these studies confirmed that NaPi-IIa and NaPi-IIb preferentially transport divalent Pi, independently of the external pH (Fig. 2D) (32). External acidification affects both the intrinsic voltage dependence and Na\textsuperscript{+} interaction of electrogenic NaPi-II cotransporters (29, 110), which results in a decrease in maximum transport rate and reduced apparent affinity for divalent Pi (see Fig. 6A).

The electrogenicity of NaPi-IIa/b implies that net charge is transported across the membrane. Moreover, \( I_{\text{Pi}} \) shows rate-limiting, voltage-independent behavior at hyperpolarizing and depolarizing extremes (Fig. 2B). This indicates that the transport cycle involves both voltage-dependent and voltage-independent partial reactions. The former have been identified from analysis of pre-steady-state charge movements evoked by voltage steps (for a review, see Ref. 31) (Fig. 2C). For carrier proteins like NaPi-IIa/b, the charge movements in the absence of substrate are proposed to reflect two events: 1) a major reorientation of the carrier that alternately exposes the substrate binding sites to the external or internal medium and 2) movement of Na\textsuperscript{+} ions from the external milieu to and from their binding site(s) within the transmembrane field (30, 31).

The transport turnover rate of NaPi-IIa/b can be estimated from pre-steady-state and steady-state data. Rates in the range 4–10 s\(^{-1}\) at 20°C are predicted (33) and should be about threefold faster under normal physiological conditions, when the temperature dependence of the kinetics is taken into account (2). Combining steady-state and pre-steady-state data has led to the proposal of an ordered kinetic scheme for the NaPi-IIa/b transport cycle (28, 33, 110, 113) (see Fig. 7A). Voltage dependence is conferred by the empty carrier and one Na\textsuperscript{+} binding transition. Similar schemes have been proposed for other Na\textsuperscript{+}-coupled solute carriers [e.g., Na\textsuperscript{+}/glucose transporter SGLT1 (76) and Na\textsuperscript{+}/iodide transporter NIS (23)] and are consistent with the alternating access mechanism for transmembrane transport. Previously, a single Na\textsuperscript{+} ion was assumed to interact with the protein before Pi binding (28). The model was revised to incorporate new evidence obtained by simultaneous whole-oocyte electrophysiology and time-resolved fluorescence of a site-directed fluorescent label (voltage clamp fluorometry; VCF) (113) that identified an additional, electrically silent, Na\textsuperscript{+} binding transition preceding Pi binding.

**Electroneutral NaPi-IIc.** The electroneutrality of NaPi-IIc (71, 89) comes as a surprising contrast to the voltage dependence of NaPi-IIa/b, and this fact emphasizes how small differences in amino acid composition can drastically alter specific kinetic properties. Oocytes expressing functional NaPi-IIc (confirmed by \( ^{32}\text{P} \) uptake) are electrically silent (3, 89). For other kinetic properties, such as apparent substrate

---

Fig. 3. Topological model for rat NaPi-IIa. Transmembrane domains were assigned according to Ref. 79. The model was drawn with the aid of TOPO2 software (http://www.sacs.ucsf.edu/TOPO/topo.html). Enlarged symbols indicate sites of significant structure-function importance. Pink, identical residues in the NH\textsubscript{2}- and COOH-terminal repeated regions (see text); green, sites that are, when mutated to a cysteine, accessible to the extracellular milieu; blue, sites that are, when mutated to a cysteine, accessible to the intracellular milieu; red, sites important for electrogenicity; orange, sites important for regulation and targeting; yellow, sites of naturally occurring SLC34A1 (human NaPi-IIa) mutants shown not to affect function; purple, equivalent sites of reported naturally occurring SLC34A3 (human NaPi-IIc) mutations. An essential disulfide bridge in the large extracellular loop is indicated (dashed line). Light blue regions are reentrant loops, proposed to form a putative transport pathway through the protein. Two N-glycosylation sites have been identified in the large extracellular loop.
affinity and pH dependence, NaPi-IIc is indistinguishable from its electrogenic cousins. Moreover, its Na\(^{+}\):Pi stoichiometry of 2:1 is consistent with a preference for divalent P\(_i\), and is thus similar to NaPi-IIa and IIb (Fig. 2D) (3). Thus NaPi-IIc lacks one of the three Na\(^{+}\) interaction steps proposed for NaPi-IIa/b. Simulations of the transport steady-state and pre-steady-state kinetics also support the notion that the first Na\(^{+}\) interaction may be electrogenic, followed by an electroneutral or weakly electrogenic second Na\(^{+}\) interaction (see Fig. 7A). The latter would correspond to the first Na\(^{+}\) interaction for NaPi-IIc (I. C. Forster, unpublished observations).

**Topology and structure-function studies.** The current topological model of SLC34 has been developed mainly using cysteine scanning and in vitro transcription/translation on NaPi-IIa but is probably applicable also to NaPi-IIb and NaPi-IIc (for a review, see Refs. 30 and 31). The model comprises 12 transmembrane-spanning domains, intracellularly located NH\(_{2}\) IIc (for a review, see Refs. 30 and 31). The model comprises 12 cysteine scanning and in vitro transcription/translation on (I. C. Forster, unpublished observations).

The current topological model of SLC34 has been developed mainly using cysteine scanning and in vitro transcription/translation on NaPi-IIa but is probably applicable also to NaPi-IIb and NaPi-IIc (for a review, see Refs. 30 and 31). The model comprises 12 transmembrane-spanning domains, intracellularly located NH\(_{2}\) IIc (for a review, see Refs. 30 and 31). The model comprises 12 cysteine scanning and in vitro transcription/translation on (I. C. Forster, unpublished observations).
cantly, the $\text{Na}^+:\text{Pi}$ stoichiometry of the electrogenic mutant NaPi-IIc is 3:1; however, its apparent substrate affinities and voltage dependence are altered compared with wild-type transporters. Furthermore, replacing the conserved Asp-224 in NaPi-IIa with NaPi-IIc’s glycine resulted in electroneutral Pi transport, thus confirming that Asp-224 is necessary for a 3:1 $\text{Na}^+:\text{Pi}$ stoichiometry (109).

**SLC20: Type III Na$^+/\text{Pi}$ Cotransporter Family**

*Discovery.* Type III Na$^+/\text{Pi}$ cotransporters were first identified as receptors for retroviruses, which may enter a cell following a specific interaction with the receptor. The first hint that the retroviral receptor Glvr-1, which renders cells susceptible to infection by gibbon ape leukemia virus, is a phosphate transporter was revealed by its homology to a phosphate permease from *Neurospora crassa*, a filamentous fungi (48). A related protein, which renders cells susceptible to infection by amphotropic murine retrovirus (Ram-1) was subsequently identified (65, 104). Experimental evidence showing that retroviral receptors Glvr-1 and Ram-1 are electrogenic Na$^+/\text{Pi}$ symporters then followed (51, 52, 72), and the receptors were renamed PiT-1 and PiT-2, respectively.

PiT-1- and PiT-2-related proteins are present in all phyla (Fig. 1B). In prokaryotes and in plants, Pi transport is coupled to the $\text{H}^+$ gradient (21, 38, 103), whereas in animals and fungi, the driving cation is Na$^+$ (19, 64, 106). In the bacterium *Bacillus subtilis*, the related protein CysP mediates $\text{H}^+/\text{SO}_4^{2-}$ cotransport (63), but so far no other PiT family member has been shown to transport $\text{SO}_4^{2-}$.

*Physiological role and pathophysiology.* PiT proteins have a broad tissue distribution, and it was proposed that they serve a housekeeping function in cells. At the mRNA level, PiT-1 and PiT-2 are ubiquitously but differentially expressed in different tissues (4, 97, 102). However, recently more specific roles for PiT proteins in various physiological and pathophysiological processes have emerged. For example, PiT-mediated $\text{Pi}$ transport appears to play an important role in providing $\text{Pi}$ for the formation of mineralized bone (16, 36, 75, 93, 121, 123). Accordingly, it has been shown that in cultured bone-derived cells $\text{Pi}$ transport and PiT mRNA levels are regulated by various factors, such as $\text{Pi}$, epinephrine, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1), and basic fibroblast growth factor (bFGF) in osteoblast-like cells (18, 94, 95, 122, 123) and in chondrogenic cells by transforming growth factor-$\beta$ (TGF-$\beta$) (74) as well as $\text{Pi}$ levels (114). Interestingly, $\text{Pi}$ regulation of PiT-2 transport function may result from posttranslational modification of the transporter (83), and $\text{Pi}$ may regulate the formation of PiT-2 oligomers, implying that PiT proteins play a role in phosphate sensing (84). In addition to playing a role in normal calcification, PiT

![Fig. 5. Topological model for human PiT-2. Transmembrane domains were assigned according to Ref. 86. The model was drawn with the aid of TOPO2 software (http://www.sacs.ucsf.edu/TOPO/topo.html). Residues important to PiT function investigated by mutagenesis (10, 11, 85) are indicated. Light blue, PD1131 homology domains identified in Ref. 86; pink, region that can be removed without compromising retroviral receptor function of PiT-2 (9). Red, acidic residues important for transport function (D28, E55, D506, E575) and not critical for transport function (E68, D78, E91). The protein is N-glycosylated at Asp-81. Numbers designate predicted transmembrane domains and loops (italic) referred to in the text.](http://ajprenal.physiology.org/)

_Fig. 5._ Topological model for human PiT-2. Transmembrane domains were assigned according to Ref. 86. The model was drawn with the aid of TOPO2 software (http://www.sacs.ucsf.edu/TOPO/topo.html). Residues important to PiT function investigated by mutagenesis (10, 11, 85) are indicated. Light blue, PD1131 homology domains identified in Ref. 86; pink, region that can be removed without compromising retroviral receptor function of PiT-2 (9). Red, acidic residues important for transport function (D28, E55, D506, E575) and not critical for transport function (E68, D78, E91). The protein is N-glycosylated at Asp-81. Numbers designate predicted transmembrane domains and loops (italic) referred to in the text._
Table 1. Properties of Na+/Pi cotransporters

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Stoichiometry Na+/Pi</th>
<th>Electrogenic</th>
<th>Concentrating Capacity</th>
<th>PFA Block</th>
<th>Mutations Associated with Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaPi-IIa</td>
<td>Na(^{+}), HPO(_4^{2-}), Arsenate</td>
<td>3:1</td>
<td>Yes</td>
<td>10,000</td>
<td>Yes</td>
</tr>
<tr>
<td>NaPi-Iib</td>
<td>Na(^{+}), HPO(_4^{2-}), Arsenate</td>
<td>3:1</td>
<td>Yes</td>
<td>10,000</td>
<td>Yes</td>
</tr>
<tr>
<td>NaPi-Iic</td>
<td>Na(^{+}), HPO(_4^{2-}), Arsenate</td>
<td>2:1</td>
<td>No</td>
<td>100</td>
<td>Yes</td>
</tr>
<tr>
<td>PiT-1</td>
<td>Na(^{+}), H(_2)PO(_4^{2-}), Li(^{+}), Arsenate</td>
<td>2:1</td>
<td>Yes</td>
<td>1,000</td>
<td>No</td>
</tr>
<tr>
<td>PiT-2</td>
<td>Na(^{+}), H(_2)PO(_4^{2-}), Li(^{+}), Arsenate</td>
<td>2:1</td>
<td>Yes</td>
<td>1,000</td>
<td>No</td>
</tr>
</tbody>
</table>

Concentration capacity is shown for a membrane potential of \(-60\) mV and a 10-fold Na\(^{+}\) gradient (outside-inside). HHRH, hereditary hypophosphatemic rickets with hypercalciuria. References are shown in parentheses.

proteins were recently implicated in pathological processes, such as hyperphosphatemia-induced calcification of vascular tissue (50, 56, 68) and osteoarthritis (17).

Regulation of PiT function has also been shown for extraskeletal cells and tissues. For example, in human embryonic kidney cells (HEK-293), PiT-1-mediated Pi transport is regulated by Pi levels and PTH (25). In rat parathyroid glands, PiT-1 mRNA levels are regulated by plasma vitamin D and Pi levels (97).

Unfortunately, at the protein level only limited data are available regarding the tissue and subcellular distribution of PiT-1 and PiT-2. Khadeer et al. (53) detected PiT-1 mRNA in osteoclasts and macrophages and showed that transfected proteins were recently implicated in pathological processes, such as hyperphosphatemia-induced calcification of vascular tissue (50, 56, 68) and osteoarthritis (17). PiT proteins may also play a role in Pi transport in the distal segments of the kidney. Tenenhouse et al. (99) showed that both PiT-1 and PiT-2 mRNAs are present in immortalized mouse distal convoluted tubule (MDCT) cells and that the pH dependence of Pi transport is consistent with PiT-mediated transport.

Finally, PiT proteins may play an important role in basolateral as well as apical Pi uptake in polarized epithelia. In the liver, PiT-1 and PiT-2 localize to the basolateral membrane in hepatocytes (35), whereas in airway epithelial cells PiT-2 is expressed both apically and basolaterally (115). In Pi-secreting glands, such as the lactating mammary gland (90) and ruminant parotid gland (105), PiT proteins may provide basolateral uptake of Pi from the blood for subsequent secretion into the lumen through an as yet unknown mechanism.

Recently, Saliba et al. (87) showed that the malaria parasite Plasmodium falciparum expresses a PiT protein (PfPiT) in its plasma membrane that is instrumental for supplying Pi to the intraerythrocytic parasite. PfPiT-mediated Pi transport is energized by the Na\(^{+}\) gradient and, because infection causes a gradual increase in intracellular Na\(^{+}\), there is adequate driving force for Pi uptake via PfPiT.

Transport kinetics. After Kavanaugh et al. (52) in 1994 showed that the retroviral receptors Glvr-1 and Ram-1 are electrogenic Na\(^{+}/Pi\) cotransporters, very limited characterization of the transport kinetics of either PiT-1 or PiT-2 has been reported (4, 8, 97, 99, 107). None of these more recent studies used electrophysiology, which is essential for the kinetic characterization of electrogenic transporters. Furthermore, these studies suffered from low transport activities, possibly because of low expression. To close this knowledge gap, we recently characterized PiT transport kinetics using the X. laevis oocyte expression system and two-electrode voltage clamp as well as radiotracer uptake (81). We performed most of our kinetic studies on a X. laevis PiT-1 clone (XIPiT-1), which gave much...
higher Pi transport in *X. laevis* oocytes than mammalian PiT-1 or PiT-2.

In XIPiT-1-expressing oocytes, applying Pi, in the presence of Na\(^+\) induces an inward current (*I*\(_N\)) with no reversal potential over the voltage range applicable to oocytes (−180 to +80 mV; Fig. 4B). PiT-dependent Pi transport is Na\(^+\) dependent, but Li\(^+\) supports Pi transport to a small extent (11, 81, 107). Interestingly, two studies have shown that in the absence of Na\(^+\), lowering pH from 7.5 to 6.0 induced significant Pi uptake in oocytes expressing PiT-2, indicating that in this isoform H\(^+\) could substitute for Na\(^+\) (8, 107). Li\(^+\) may support cotransport in some Na\(^+\)-driven transporters, such as the Na\(^+/\)dicarboxylate transporter (73), Na\(^+\)-driven Cl\(^−/\)HCO\(_3\)^− exchanger (108), and the Na\(^+\)/glucose cotransporter (43), but not in type II Na\(^+\)/Pi cotransporters, although Li\(^+\) may interact with their transport cycle (112, 113).

Another difference between type II and type III Na\(^+\)/Pi cotransporters is that whereas Na\(^+\)-independent pre-steady-state currents are readily measured in oocytes expressing NaPi-IIa or NaPi-IIb in the absence of Pi, analogous charge movement in oocytes expressing XIPiT-1 were undetectable, although *I*\(_Pi\) was of similar magnitude as in NaPi-II-expressing cells (Fig. 4, A and B). PiT-1 pre-steady-state relaxations may be too fast to detect and remain buried in the capacitive transient of the oocyte or indicate that other models of electrogenic transport should be considered.

Neither PiT-1 nor PiT-2 transports sulfate; however, the toxic phosphate mimetic arsenate interacts with PiT-1 (4, 52, 81). Arsenate competes with Pi, and reduces Pi transport in both PiT-1 and PiT-2 and also induces inward currents similar to Pi, but has a lower affinity (4, 81). The effects of arsenate are very similar in type II Na\(^+\)/Pi cotransporters (15, 39).

The transport stoichiometry of XIPiT-1 was determined by combining electrophysiology and \(^{22}\)Na and \(^{32}\)Pi tracer uptake experiments. PiT-1 transports two Na\(^+\) ions for each monovalent H\(_2\)PO\(_4\)^− with one positive charge (81) (Fig. 4, C and D). We confirmed that H\(_2\)PO\(_4\)^− is preferred over HPO\(_4\)^− in PiT-1, using surface pH measurements. Because removal of either H\(_2\)PO\(_4\)^− or HPO\(_4\)^− from the medium by a transporter causes a shift in H\(_2\)PO\(_4\)^−:HPO\(_4\)^− equilibrium, a measurable change in H\(^+\) concentration occurs. For oocytes expressing PiT-1, application of 1 mM Pi, caused alkalinization at the oocyte surface, consistent with transport of H\(_2\)PO\(_4\)^− into the cell (see Fig. 6C). In contrast, expressing the type II Na\(^+\)/Pi cotransporter resulted in acidification, in agreement with transport of HPO\(_4\)^− (see Fig. 6, C and D).

PiT proteins are less sensitive to H\(^+\) than members of the SLC34 family. The maximum attainable Pi transport rate (*V*\(_{\text{max}}\)) is not influenced by changes in pH between 5.0 and 7.4 (see Fig. 6B). Whereas the apparent affinity for total Pi shows a biphasic change with pH with a minimum ~pH 6.8, the apparent affinity for the preferred substrate HPO\(_4\)^− is reasonably constant between pH 6.2 and 8.0 and decreases at more acidic pH. In contrast, Pi transport mediated by members of the type II cotransporter family (see Fig. 6A) is strongly reduced at acidic pH due to the inhibitory effect of protons on the transport cycle as well as reduced availability of divalent HPO\(_4\)^− (29, 110). Thus PiT proteins can maintain high Pi transport capacity even at the highly acidic pH of 5.0, where SLC34 transporters no longer function.

Presently, there are no known inhibitors of PiT-mediated Pi transport. Phosphonoformic acid (PFA) is a well-known inhibitor of Na\(^+\)-dependent Pi transport mediated by type II Na\(^+\)/Pi cotransporters (15, 96), but it does not inhibit PiT-1 or PiT-2 when expressed in oocytes (81). Although inhibition by PFA of PiT-mediated Pi transport has been reported (4, 99, 107), only high concentrations (~10 mM) were effective and membrane potential was not controlled, so the effect of PFA may have been nonspecific. In addition, it has been reported that PFA blocks Pi-induced calcification in smooth muscle cells (50, 56) as well as matrix calcification in osteoblast-like cells (93) and osteoblast subcultures (121). As PFA does not block PiT-mediated Pi transport, it is likely that this effect is due to the ability of phosphonates and bisphosphonates to inhibit calcium crystal formation (26, 27, 118). Alternatively, a member of the PFA-sensitive type II Na/Pi cotransporter family may play a role in the calcification process.
Invited Review

PHOSPHATE TRANSPORT BY PIT AND NaPi-II

Topology. The topology of PiT proteins has been partially determined (Fig. 5). Topology prediction algorithms usually report 9 or 11 transmembrane domains for Pit-1 and Pit-2, which would place the NH2 and COOH termini on opposite sides of the membrane. However, tags inserted at the NH2 and COOH termini are accessible from the external side, indicating that both termini are extracellular (24, 83, 86). A homological search (86) revealed the presence of a conserved domain in Pit proteins that is duplicated in most family members (light blue shading in Fig. 5). Reasoning that such duplicated sequences probably have similar but inverted topologies, Salaun et al. (86) proposed a secondary structure comprising extracellular NH2 and COOH termini and 12 putative transmembrane domains (Fig. 5). They confirmed that loop 2 is extracellular by showing that Pit-2 is N-glycosylated at Asp-81. The intracellular localization of the large loop 7 was confirmed using an antibody raised against it (18). Similar topologies have been suggested for the malaria parasite transporter PIPIT (87) and for the related H+ /P transporter Ph72;1 from the plant Arabidopsis (21). Interestingly, the plant protein is lacking the large intracellular loop present in animal PiTs. Furthermore, Bottger and Pedersen (9) showed that Pit2 retains its retroviral receptor function after deletion of loop 7 as well as transmembrane domains 6–7 (pink shading in Fig. 5). They did not investigate the transport function of their truncated protein, and thus it is unknown whether the large loop is essential for substrate transport.

The ability of a retrovirus to infect cells via its receptor has been used extensively to investigate which amino acids are crucial for infection with different viruses and thus also serves as a tool for probing the secondary structure of PiTs. Most of the attention has been directed towards loop 8 (Fig. 5). This shows large sequence variability between different species, and it appears to play a role in determining virus binding (22, 49, 77). Therefore, it is likely to be extracellular, although an opposing view has also been presented (24). The issue has been extensively discussed (8) in favor of the model proposed by Salaun et al. (86).

So far, only a few features of Pit topology have been experimentally confirmed, such as the location of the termini and loops 2 and 7. Salaun et al. (86) conducted in vitro translation experiments using Pit-2 constructs truncated at the COOH terminus. These data support the extracellular location of the four extracellular loops and the COOH terminus, whereas the other loops are intracellular. However, it is clear that further experiments are needed to establish a definitive membrane topology for Pit-1 and Pit-2, for example, by epitope tagging of putative loops or probing the accessibility of introduced cysteines to membrane-impermeant reagents, as done for the SLC34 protein.

Summary

In vertebrates, the two unrelated solute transport families SLC20 and SLC34 mediate Na+/Pi cotransport through different molecular mechanisms. Their main functional properties are summarized in Table 1. It is remarkable that the two families have evolved a preference for one of the two Pi species that are most abundant at physiological pH, and thus both monovalent and divalent Pi species have their own dedicated carriers. Whether this functional difference between the two families has a strong impact on physiology is unclear. Note that transport of either Pi species across a membrane will affect pH, since it shifts the equilibrium of the reaction H2PO4-/HPO42- + H+. An additional consequence is that the two families differ in their optimum pH range, overlapping around the pKs of the above reaction (pH ~ 6.8). Thus SLC20, which prefers H2PO4-, and SLC34, which prefers HPO42-, transport most efficiently in the acidic and alkaline ranges, respectively (Fig. 6). This difference in preferred Pi species may explain why PFA inhibits Pi transport by SLC34, but not by SLC20.

The two families also differ in their substrate stoichiometry. NaPi-Ia and NaPi-Ib transport three Na+ ions per transport cycle, concomitant with the translocation of one net positive charge, whereas NaPi-Ic only takes two Na+ ions, resulting in electroneutral transport. Like NaPi-Ic also Pit transports two Na+ ions per completed cycle, but because Pi is transported in its monovalent form, net positive charge is also translocated. These differences in stoichiometry and preferred Pi species result in a 100-fold difference in Pi concentration capacity from the lowest (NaPi-Ic, 100-fold) to the highest (NaPi-Ia and Ib, 10,000-fold; see Table 1). What are the physiological consequences of these differences in stoichiometry? Because NaPi-Ic transports one less Na+ ion (resulting in electroneutral transport) than its electrogenic counterparts, the energetic cost of transporting Pi is smaller. Less Na+ is loaded into the cell, and less energy is needed to maintain the cell’s negative membrane potential.

With respect to substrate binding order (Fig. 7), SLC34 proteins appear to exhibit a strict Na+/Pi antiport (Fig. 7A), whereas the available evidence for SLC20 is less clear and random Na+/Pi interactions cannot be excluded (81) (Fig. 7B). Moreover, the lack of detectable pre-steady-state currents in SLC20 prevents assigning voltage dependence to any particular step in the transport cycle, unlike for NaPi-Ia and Ib. This type of analysis assumes that pre-steady-state currents report on events that are associated with significant conformational changes related to the transport cycle; however, other scenarios are possible that would lead to the same macroscopic behavior. It is also conceivable that some of the pre-steady-state charge represents a conventional gating charge that precedes channel opening. In contrast to the canonical alternating access model, a channel-like “hopping” model, in which substrates move single file through a pore, successively occupying binding sites according to availability, was proposed, whereby the salient kinetic features of Na+-coupled transport systems were predicted (92). Although several studies (40, 58) have argued against the validity of such a scheme in its purest form, the recent finding of a Cl- channel that acts like a H+/Cl- antipot (1) indicates that channels and carriers may indeed share common mechanistic features.

ACKNOWLEDGMENTS

The authors acknowledge the valuable contributions made by past and present members of our laboratory.

GRANTS

Financial support for work cited from our laboratory was generously provided by the Swiss National Science Foundation and Gebert Rüf Foundation (www.grstiftung.ch).
REFERENCES


