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RENAL AND SMALL INTESTINAL SODIUM-DEPENDENT SYMPORTERS OF PHOSPHATE AND SULPHATE

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Summary

Homeostasis of inorganic phosphate (P_i) and sulphate (S_i) is largely achieved by absorption in the mammalian small intestine and by reabsorption in the proximal tubule of the kidney. Under normal physiological conditions, the kidney appears to play the major role in maintaining the extracellular concentration of these anions. In both epithelia, reabsorption of P_i and to some extent also of S_i underlie a variety of regulatory acute and chronic control mechanisms. Acute regulatory mechanisms are predominantly found in renal P_i reabsorption, whereas chronic regulation of transepithelial P_i transport is observed in both tissues. Also, in both epithelia, apically located sodium-dependent transport systems (Na^+/P_i and Na^+/S_i symport) represent major targets for known regulatory factors.

By expression cloning using oocytes of *Xenopus laevis*, renal and small intestinal Na^+-dependent phosphate and sulphate transport systems have been identified. Evidence has been obtained that cloned Na^+/P_i and Na^+/S_i symporters are localized in the apical membrane of proximal tubular or small intestinal epithelial cells respectively. Furthermore, recent results indicate that one of the cloned Na^+/P_i symporters is involved in the physiological and pathophysiological regulation of proximal tubular P_i reabsorption.

Introduction

Renal and small intestinal apical Na^+/P_i symport

In the mammalian kidney, up to 80 % of filtered P_i is reabsorbed in the proximal tubule, with the highest rates occurring in convoluted proximal tubules (for a review, see Berndt and Knox, 1992). In the small intestine of most mammals, the highest rate of P_i reabsorption occurs in the duodenum (for a review, see Danisi and Murer, 1991).

Transepithelial transport of P_i in proximal tubules and small intestine is performed by a unidirectional process involving apical Na^+-dependent symport systems (Na^+/P_i symport) and basolaterally localized anion exchange mechanisms (Murer and Biber, 1992). In both epithelia, the apical entry of P_i strictly depends on the presence of luminal sodium; transcellular transport of P_i is therefore considered to be a secondary active transport (Baumann *et al.* 1975).

Apical Na^+-dependent symport of P_i has been thoroughly characterized by transport

Key words: Na^+/phosphate symport, Na^+/sulphate symport, brush-border membrane, proximal tubule, small intestine.

experiments using brush-border membrane vesicles isolated from the kidney cortex and small intestine of a variety of species. The results obtained from these studies indicated that renal apical Na^+/P_i symport typically exibits an apparent K_m for P_i of around 0.1–0.2 mmol l^{-1} and that sodium ions interact with the transport system with a K_d of around 40–70 mmol l^{-1}. At neutral pH, interaction with sodium is sigmoidal, with a Hill coefficient of approximately 2, suggesting a stoichiometry of $2Na^+/HPO_4^{2-}$ (for a review, see Murer *et al.* 1991).

With respect to the interactions of P_i and Na^+, apical Na^+/P_i symport in small intestine (duodenum) is similar to proximal tubular Na^+/P_i symport. In transport studies with isolated small intestinal brush-border membrane vesicles, a K_m for P_i of the order of 0.2–0.5 mmol l^{-1} and a stoichiometry (Na^+/P_i) of around 2:1 has been found (for a review, see Danisi and Murer, 1991). Proximal tubular and small intestinal apical Na^+/P_i symport differ with respect to their pH-dependence. At physiological concentrations of sodium, the rate of proximal tubular Na^+/P_i transport is higher at alkaline pH values; a difference in rate of about threefold was observed between pH 6.5 and 8 (Amstutz *et al.* 1985). In contrast, small intestinal apical Na^+/P_i symport exhibits the opposite pH-dependence. Enhanced transport rates of phosphate at pH 6, compared with that at pH 7.5, have been described in intact rat jejunum and in isolated brush-border membranes (Danisi *et al.* 1984).

Renal and small intestinal apical Na^+/S_i symport

As for renal P_i handling, most of the filtered sulphate ions are reabsorbed along the proximal tubule (Murer *et al.* 1992). In the small intestine, the highest rate of S_i absorption is observed in the lower tract (ileum) (Langridge-Smith and Field, 1981; Smith *et al.* 1981).

In both epithelia, (re)absorption of S_i is strictly dependent on the presence of sodium and is initiated by Na^+-dependent symport systems localized in the apical membrane. Completion of transcellular transport of sulphate is achieved by anion antiport systems which, in contrast to the apical Na^+/S_i symporter, are sensitive to 4,4-diisothiocyanostilbene-2,2-disulphonic acid (DIDS) (Hagenbuch *et al.* 1985).

Studies with isolated brush-border membranes of kidney cortex and small intestine of a variety of animal species have identified a Na^+/S_i symport system exhibiting an apparent K_m for sulphate of the order 0.3–0.5 mmol l^{-1} and an apparent interaction constant for sodium of around 30–50 mmol l^{-1}. Furthermore, these studies demonstrated that this transport system also interacts with other oxyanions, such as thiosulphate, selenate and molybdate (Ahearn and Murer, 1984; Murer *et al.* 1992; Busch *et al.* 1994*b*).

Identification of Na^+-dependent phosphate and sulphate symport systems

In the past, the biochemical approaches used to identify Na^+/P_i and Na^+/S_i symporters have not allowed one to draw unequivocal conclusions about the molecular identity of the protein(s) involved, mainly because of the lack of specific inhibitors. For renal Na^+/P_i symport, two proteins of 70 and 97 kDa have been identified from rat brush-border membranes on the basis of a P_i-protectable reaction with an azido-derivative of NAD (Al-Mahrouq and Kempson, 1991), and four proteins of 31, 53, 105 and 176 kDa have been

identified from a renal epihelial cell line (OK cells) on the basis of a P_i-protectable reaction with the group-specific reagent *N*-acetylimidazole (Wuarin *et al.* 1989). Alternative approaches have used organic solvents to extract hydrophobic proteins from renal brush-border membranes. Proteins that bind P_i have been partially purified, but Na^+-dependent P_i transport could not be demonstrated (Kessler *et al.* 1982; Schaeli *et al.* 1986). Similarly, after organic extraction from rabbit brush-border membranes, a protein of 64 kDa has been purified and shown by reconstitution to transport P_i (Debiec *et al.* 1992). In small intestinal brush-border membranes, a 130 kDa protein has recently been identified which, upon reconstitution into liposomes, displayed Na^+/P_i symport (Peerce, 1989; Peerce *et al.* 1993).

No attempts to identify biochemically proteins involved in renal or small intestinal Na^+/S_i symporters have been reported.

In recent years, it has become possible to express foreign proteins in oocytes of *Xenopus laevis* (Gurdon *et al.* 1971). The first successful use of oocytes from *X. laevis* in identifying a transport protein (the small intestinal Na^+/glucose symporter SGLT-1) was reported by Hediger *et al.* (1987). Since then, a number of transport proteins have been identified in cDNA libraries by expression cloning (Sigel, 1990).

Expression cloning of Na^+/P_i symport

In cDNA libraries of kidney cortex from the rabbit, rat and humans, we recently identified single clones coding for cRNA species which, upon injection into oocytes, specifically express Na^+/P_i symport. Each Na^+/P_i symport system (NaPi) was numbered in the order of its discovery (see Table 1): NaPi-1 from rabbit (Werner *et al.* 1991), NaPi-2 from rat and NaPi-3 from humans (Magagnin *et al.* 1993). Using a NaPi-2 cDNA probe, additional Na^+/P_i symporters have recently been identified in a cDNA library of OK cells (NaPi-4; Sorribas *et al.* 1994) and in a cDNA library of rabbit kidney cortex (NaPi-6; Verri, unpublished data). On the basis of the nucleotide sequence of the NaPi-1

Table 1. *Comparison of cloned renal Na^+/P_i symporters*

Transporter	Source	Identity (%)	Similarity (%)	Number of amino acids encoded
Type I				
NaPi-1	Rabbit kidney	100	100	465
NaPi-1M	Mouse kidney	64	81	465
NaPi-1H	Human kidney	69	84	467
Type II				
NaPi-2	Rat kidney	100	100	637
NaPi-3	Human kidney	91	94	639
NaPi-4	OK cells	81	88	653
NaPi-6	Rabbit kidney	88	95	645

The BESTFIT algorithm of the GCG-program package was used to align the sequences.

Sequence comparisons are relative to the NaPi system in type I symporters and relative to the NaPi-2 system in type II symporters

transporter, additional Na^+/P_i symporters have been identified in cDNA libraries from mouse and human kidney cortex (Chong *et al.* 1993; S. S. Chong, unpublished data). An intestinal apical Na^+/P_i symport system has not yet been identified by expression cloning. However, it has recently been demonstrated that injection of poly(A)+ mRNA isolated from rat duodenum into oocytes of *X. laevis* increases the expression of Na^+/P_i symport, indicating that small intestinal Na^+/P_i symport may be identifiable by expression cloning (Yagci *et al.* 1992).

Cloned Na^+/P_i symporters seem not to belong to a currently described family of membrane-transport proteins, such as the SGLT-1 or Na^+/Cl symport families (Wright *et al.* 1992). Also, no significant overall identity was found with other cloned mammalian and non-mammalian membrane Na^+-dependent and Na^+-independent transport systems deposited in current databanks. Comparison of the newly cloned renal Na^+/P_i symporters revealed that NaPi-1 has only 20 % overall identity to NaPi-2, NaPi-3, NaPi-4 and NaPi-6 which, however, are about 80 % identical to each other. Most of the highly conserved portions among NaPi-2, NaPi-3, NaPi-4 and NaPi-6 were found in the putative transmembrane regions (homology of 95 %), including the loops between these regions (see Fig. 1), whereas less conserved portions (50–70 % homology) were found in the N-terminal regions and in the extracellular loops between the putative transmembrane domains M3 and M4.

We propose that the cloned Na^+/P_i symport systems belong to two different types of renal Na^+/P_i symporters (see Table 1): a system type I, based on the originally described system NaPi-1 (Werner *et al.* 1991); and a system type II, based on the originally described systems NaPi-2 and NaPi-3 (Magagnin *et al.* 1993).

The open reading frames of the two types of renal Na^+/Pi symporters predict proteins of the following lengths and (unglycosylated) relative molecular masses respectively: NaPi-1, 465 amino acids and 51 797; and NaPi-2, 637 amino acids and 68 703. Using polyclonal antibodies raised against N- and C-terminal peptides of NaPi-1 and NaPi-2, the following apparent molecular masses were observed on Western blots using isolated brush-border membrane vesicles: 60–64 kDa for NaPi-1 (type I) and 80–90 kDa for NaPi-2 (type II) (Biber *et al.* 1993; Custer *et al.* 1994).

Expression cloning of renal and small intestinal Na^+/S_i symporters

Using *X. laevis* oocytes as an expression system, a Na^+/S_i symporter has recently been cloned and identified (renal NaSi-1; Markovich *et al.* 1993) from a cDNA library of rat kidney cortex. Using a renal NaSi-1-derived cDNA probe, a Na^+/S_i symport system has also been identified in a cDNA library from rat ileum (ileal NaSi-1; Norbis *et al.* 1994).

The open reading frames of both renal NaSi-1 and ileal NaSi-1 cDNAs encode proteins of 595 amino acids in length and of a predicted (unglycosylated) molecular mass of 66 kDa. Interestingly, the predicted amino acid sequences of the two cloned Na^+/S_i symporters (renal NaSi-1 and ileal NaSi-1) are 100 % identical.

The amino acid sequences of renal NaSi-1 and ileal NaSi-1 showed no significant overall similarity to other cloned Na^+/solute symport systems deposited in current databanks, including the Na/P_i symporters described above.

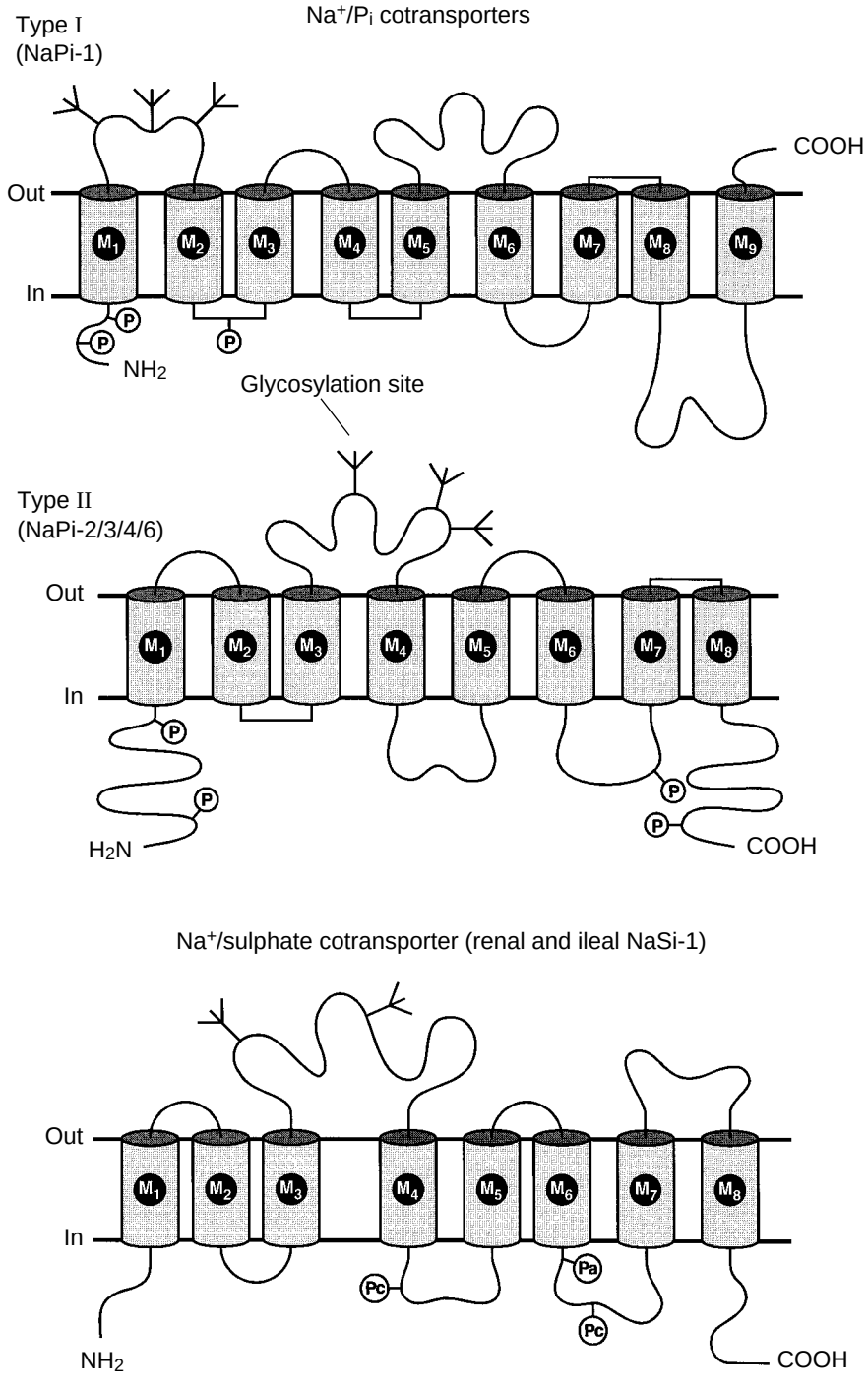

Fig. 1. Proposed secondary structures of type I and type II renal Na^+/P_i symporters and Na^+/S_i symporters. P stands for consensus phosphorylation sites of protein kinases C (Pc) or protein kinase A (Pa). Potential N-glycosylation sites located in extracellular loops are indicated.

Structural aspects of Na^+/P_i and Na^+/S_i symporters

Hydropathy analysis of the deduced amino acid sequences suggests that both types of cloned renal Na^+/P_i symporters and the renal and small intestinal Na^+/S_i symporters may contain at least eight transmembrane domains. Proposed secondary structures are shown in Fig. 1.

N-glycosylation sites

The deduced amino acid sequences of both types of cloned Na^+/P_i symport systems and of renal and ileal NaSi-1 contain several putative N-glycosylation sites (N-*X*-T/S); the sites which, according to the proposed models, would be located extracellularly are indicated in Fig. 1. In NaPi type II symporters, and also in the renal and ileal NaSi-1 symporter, an additional potential hydrophilic N-glycosylation site is found at position 5 at the C-terminal end. In contrast to renal and ileal NaSi-1, both types of Na^+/P_i symporters contain an additional potential N-glycosylation site located in a hydrophobic environment, in M8 of type I and in M6 of type II symporters (Magagnin *et al.* 1993; Werner *et al.* 1991).

In vitro translation experiments in the presence of pancreatic microsomes revealed that in both types of cloned renal Na^+/P_i symport systems and in the renal and ileal NaSi-1 symporter, certain potential N-glycosylation sites are in fact used (Werner *et al.* 1991; Magagnin *et al.* 1993; Markovich *et al.* 1993). At present, more detailed information about the N-glycosylation sites likely to be used is only available for the type II Na^+/P_i symporter NaPi-2 from the rat. Site-directed mutagenesis has shown that amino acid residues Asn-298 and Asn-328 (located within the extracellular loop between M3 and M4) are N-glycosylated in the mature protein (G. Hayes, in preparation).

No such studies have yet been performed with the Na^+/S_i symporter NaSi-1.

Potential phosphorylation sites for protein kinases

The deduced amino acid sequences of the types I and II Na^+/P_i symporters and the Na^+/S_i symporter contain several potential phosphorylation sites for protein kinase C; the sites which, according to the proposed secondary structures, would face the cytoplasmic surface are indicated in Fig. 1. In addition to protein kinase C sites, a potential protein kinase A site can also be recognized in Na^+/S_i symporters.

Phosphorylation reactions are thought to be involved in the regulation of proximal P_i reabsorption, for example by parathyroid hormone (Murer *et al.* 1991). It remains to be demonstrated whether phosphorylation of the Na^+/P_i symport system is a determinant for alterations in the rate of transmembrane transport of P_i or whether phosphorylation reactions really are determinants for a change of the number of Na^+/P_i symporters within the brush-border membrane; e.g. by endocytosis (Murer *et al.* 1991). Preliminary studies revealed that the NaPi-2 protein is a phosphoprotein; phosphorylation of the NaPi-2 protein has been observed in isolated brush-border membranes of rat kidney cortex and also in *X. laevis* oocytes after injection with NaPi-2 cRNA (G. Hayes, unpublished results).

A possible role for phosphorylation of the Na^+/S_i symporter is less clear, and demonstration of direct phosphorylation of the Na^+/S_i symporter is lacking.

Tissue and nephron localization of cloned Na^+/P_i and Na^+/S_i symporters

Tissue specificity

Northern blot analysis of mRNA isolated from various tissues of the rabbit, rat and humans indicated that mRNA corresponding to the cloned Na^+/P_i symport systems NaPi-1 (1.9 kb), NaPi-2 (Fig. 2) and NaPi-3 (2.7 kb) is expressed almost exclusively in kidney cortex. In addition, NaPi-1-related mRNA was also observed in mRNA from rabbit liver and NaPi-3-related mRNA was detected in mRNA from human lung tissue (Werner *et al.* 1991; Magagnin *et al.* 1993). Notably, no hybridization signal related to either type I or type II Na^+/P_i symporters was observed in mRNA isolated from the mucosa of small intestine, suggesting that small intestinal apical Na^+/P_i symport might represent another type of apical Na^+/P_i symport which remains to be identified. Furthermore, the absence of a hybridization signal in most other tissues suggests that ubiquitous ('housekeeping') Na^+/P_i symport systems, expected to be expressed in most (if not all) cells, might also belong to a different class of Na^+/P_i symporter from those described for renal apical Na^+/P_i symporters.

In contrast, as indicated by Northern blot analysis, the originally cloned renal Na^+/S_i symporter renal NaSi-1 is also present in the small intestine, with highest levels of expression being in the ileum (Fig. 2). Northern blot analysis suggested that NaSi-1 is absent in other tissues, such as muscle, lung and liver. It also revealed evidence for the existence of two Na^+/S_i-symporter-related mRNA species. In mRNA isolated from rat kidney cortex and from rat small intestine, two mRNA species of 2.3 and 2.9 kb were observed (Fig. 2). As demonstrated by sequencing, the two trancripts differ only in the 3′ untranslated region and code for the same protein (Norbis *et al.* 1994).

Nephron localization

Proteins and mRNA species related to the cloned Na^+/P_i symporters have been localized in the nephron by the use of the reverse transcription/polymerase chain reaction (RT-PCR) (performed with microdissected nephron segments) and by immunohistochemistry using polyclonal antibodies raised against N- and C-terminal peptides of the respective deduced amino acid sequences. In rabbit kidney cortex, expression of NaPi-1-related mRNA and protein was found exclusively in proximal tubules (Biber *et al.* 1993; Custer *et al.* 1993). Homogeneous expression of both NaPi-1-related mRNA and protein was observed throughout the whole proximal tubule of the rabbit; no differences in expression of the NaPi-1 system between the proximal tubules of superficial and juxtamedullary nephrons could be detected. Immunohistochemistry revealed that the NaPi-1-related protein is associated with the brush border. As demonstrated recently, the NaPi-2-related protein is predominantly expressed in convoluted proximal tubules and to a lesser extent in straight proximal tubules of rat kidney; again, immunofluorescence was restricted to the brush borders (see Fig. 3). In agreement with these results, NaPi-2-related mRNA was detected in proximal tubular segments by RT-PCR (Custer *et al.* 1994).

Similarly, analysis of the expression of renal NaSi-1-related mRNA in microdissected nephron segments of rat kidney showed that renal NaSi-1 mRNA is expressed in

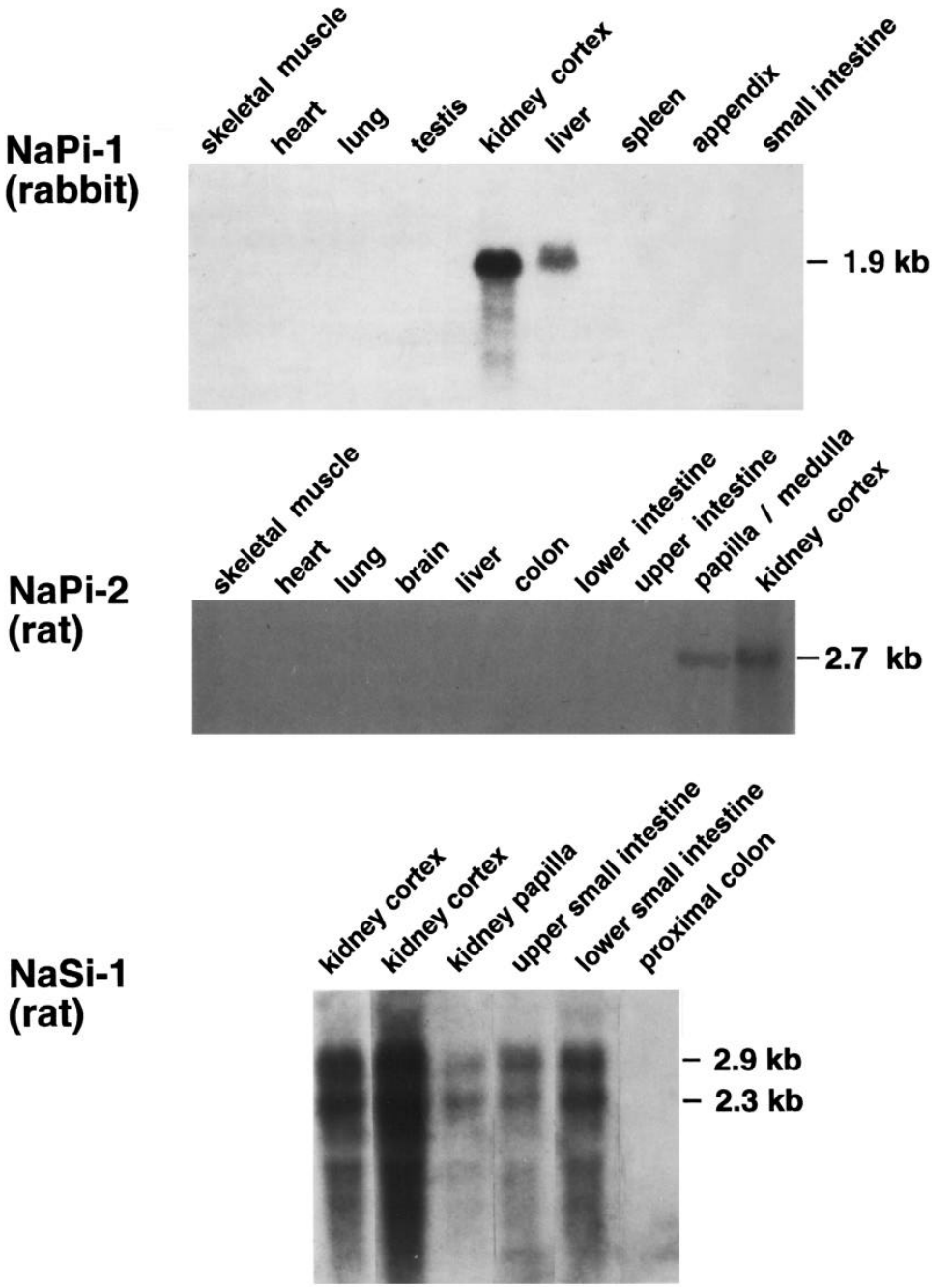

Fig. 2. Tissue distribution of Na^+/P_i and Na^+/S_i symporters as revealed by Northern blots. mRNAs isolated from various tissues of the rabbit and rat were hybridized with probes derived from symporters NaPi-1, NaPi-2 and NaSi-1 (Magagnin *et al.* 1993; Markovich *et al.* 1993; Werner *et al.* 1991).

Fig. 3. Intrarenal distribution of Na^+/P_i symport (NaPi-2) as revealed by immunofluorescence. A cryostat section (5 μm) of perfusion-fixed rat kidney was stained with an antiserum raised against a synthetic N-terminal peptide of NaPi-2. The photograph includes the cortex (*c*) and the outer medulla (*m*) and demonstrates strong immunostaining in the proximal tubules of deep nephrons (Custer *et al.* 1994).

proximal tubules but that renal NaSi-1-related mRNA is also expressed to a significant level in collecting ducts (M. Custer, unpublished data). Western blots, using antibodies raised against a synthetic C-terminal peptide, suggest that a renal NaSi-1-related protein is expressed both in proximal tubules and in collecting ducts. At present, there is no explanation for the expression of a renal NaSi-1-related protein in collecting ducts.

Functional characteristics of cloned Na^+/P_i and Na^+/S_i symporters

Specificity

Expression of cRNAs of cloned Na^+/P_i and Na^+/S_i symporters in *Xenopus laevis* oocytes resulted in an approximately 20-fold stimulation of Na^+-dependent phosphate or sulphate transport compared with that in water-injected oocytes. Transport of phosphate mediated by expressed NaPi type II proteins and transport of sulphate mediated by expressed NaSi transporters is highly specific, since no transport of other substrates, such as amino acids or sugars, has been observed (Magagnin *et al.* 1993; Markovich *et al.* 1993). Furthermore, the Na^+/P_i and Na^+/S_i symport systems seem to be highly anion-specific, since the expressed Na^+/P_i symport and expressed Na^+/S_i symport could not be inhibited by S_i or P_i, respectively (Markovich *et al.* 1993). Therefore, the cloned Na^+/P_i and Na^+/S_i symport systems represent distinct transport proteins which, under physiological conditions, have no overlapping specificity. Recent electrophysiological studies (Busch *et al.* 1994*b*) have shown that the Na^+/S_i symporter not only transports sulphate but also facilitates transport of thiosulphate and selenate; no substrate other than P_i has yet been described for Na^+/P_i symporters.

Kinetic characterization: tracers studies and electrophysiological analysis

In brush-border membrane vesicles, such as those isolated from rat kidney cortex, Na^+/P_i symport is characterized by an apparent K_m for P_i of the order of 0.1–0.3 mmol l^{-1}, by a sigmoidal dependence on $[Na^+]$ with a Hill coefficient of approximately 2 and by pH-dependence (Murer *et al.* 1991). These functional characteristics of renal proximal apical Na^+/P_i symport have been verified in tracer studies with the cloned type II Na^+/P_i cotransporters after expression in *X. laevis* oocytes (Magagnin *et al.* 1993). Expression of NaPi-1-related Na^+/P_i symport exhibited an apparent K_m for P_i of around 0.2 mmol l^{-1}, but the dependence on $[Na^+]$ was less sigmoidal and P_i transport was not dependent on pH (Werner *et al.* 1991; A. Werner, unpublished results).

After expression in oocytes, renal and ileal NaSi-1-related Na^+-dependent transport of sulphate exhibited similar kinetic characteristics to those described in brush-border membranes isolated from rat kidney cortex and rat ileum respectively. Studies performed by isotope flux measurements in oocytes injected with renal NaSi-1 and ileal NaSi-1 cRNA demonstrated a sigmoidal dependence on extracellular sodium concentration (K_m 22 mmol l^{-1}, Hill coefficient around 2) and simple Michaelis–Menten kinetics for the dependence on sulphate concentration (K_m 0.3 mmol l^{-1}) (Markovich *et al.* 1993; Norbis *et al.* 1994).

As demonstrated recently, both the NaPi type II and NaSi symporters operate in an

electrogenic manner. In voltage-clamped oocytes that have been injected with the cRNAs of these cotransporters, superfusion with phosphate or sulphate, respectively, induced an inward current that depended on the concentration of both sodium and phosphate or sulphate (Busch *et al.* 1994*a*,*b*). On the basis of phosphate- and sulphate-induced currents, a Hill coefficient of 3 for the interaction with sodium was calculated for both the NaPi-2 and the renal NaSi-1 symporters, indicating that at neutral pH, P_i and S_i are transported as divalent ions. However, at low pH, the net charge carried per transported phosphate anion seems to increase, suggesting that monovalent phosphate may also interact to some extent with type II Na^+/P_i symporters (Busch *et al.* 1994*a*).

Involvement of cloned Na^+/P_i and Na^+/S_i symporters in physiological and pathophysiological regulatory mechanisms

From an energetic point of view, apical Na^+/P_i and Na^+/S_i symport represent rate-limiting steps in the transepithelial transport of phosphate and sulphate. Therefore, these transport systems serve as ideal targets for the regulatory control mechanisms involved in the maintenance of phosphate and sulphate homeostasis (Gmaj and Murer, 1986).

Regulation of proximal tubular Na^+/P_i symport

Many hormonal and non-hormonal factors controlling P_i homeostasis have been shown to modulate the rate of apical-to-proximal Na^+/P_i symport (for reviews, see Berndt and Knox, 1992; Hammermann, 1989; Murer *et al.* 1991). The acute and chronic regulatory mechanisms affecting proximal tubular apical Na^+/P_i symport described so far can be grouped according to an involvement of protein synthesis. Protein-synthesis-independent regulation is best described for the inhibitory action of parathyroid hormone, which is due to a cascade of reactions involving protein kinase activities (Cole *et al.* 1987; Murer *et al.* 1991). A direct relationship between phosphorylation of the transporter and a reduced rate of Na^+/P_i symport remains to be demonstrated. *De novo* protein synthesis is involved in most *in vivo* and *in vitro* manoeuvres to stimulate proximal tubular P_i reabsorption. Evidence for protein-synthesis-dependent mechanisms was obtained for the effects of insulin-like growth factors, for thyroid hormone, for the reversal of parathyroid hormone action and for the adaptive response observed under conditions of chronic dietary P_i restriction (for reviews, see Berndt and Knox, 1992; Murer *et al.* 1991).

Involvement of cloned Na^+/P_i symporters in the adaptive response of renal P_i reabsorption to a low-P_i diet

Dietary P_i restriction is associated with an increase in the proximal tubular capacity to reabsorb P_i that is manifested by an increased rate of apical Na^+/P_i symport. To a large extent, this phenomenon is independent of extrarenal factors, such as parathyroid hormone, vitamin D and growth factors, and therefore seems to represent an intrinsic adaptive response of the proximal tubular cell to a low-P_i diet (Berndt and Knox, 1992). *In vivo* and cell culture studies have provided evidence that adaptation to P_i restriction is dependent on *de novo* protein synthesis, possibly involving altered rates of transcription

or an altered stability of mRNA species related to a Na^+/P_i symport system(s) (Murer and Biber, 1992; Murer *et al.* 1991).

Analysis of mRNA isolated from kidney cortex of rats fed a normal-P_i diet or a low-P_i diet is consistent with the working hypothesis that a low-P_i diet induces an increased content of Na^+/P_i symporter mRNA. Using Northern blot analysis, a twofold increase in NaPi-2-related mRNA was observed in the total mRNA isolated from the kidney cortex of rats fed on a low-P_i diet compared with the total mRNA isolated from animals fed on a high-P_i diet. In agreement with these results, mRNA from adapted rats caused higher levels of expression of Na^+/P_i symport when injected into oocytes (Werner *et al.* 1994).

By contrast, no correlation could be demonstrated between diet and the symporter NaPi-1 (Biber *et al.* 1994). The latter observation raises the possibility that the two types of Na^+/P_i symport systems discussed above might be involved in the adaptive up-regulation of renal P_i reabsorption in different ways.

Role of type II Na^+/P_i symporters in X-linked hypophosphataemia

The *Hyp*-mouse model is representative of human X-linked hypophosphataemia (Tenenhouse and Scriver, 1979). In studies with the *Hyp*-mouse model, we recently demonstrated that mRNA related to NaPi-2 is reduced by a factor of two in affected animals. This observed decrease in the amount of NaPi-2-related mRNA correlated with reduced Na^+/P_i symport in brush-border membrane vesicles of *Hyp*-mice and, in addition, with a reduced content of NaPi-2-related protein measured by Western blot analysis (Tenenhouse *et al.* 1994). These observations imply that the genetic defect underlying X-linked hypophosphataemia is probably not at the level of the structure of the Na^+/P_i symporter (NaPi-2), but instead could be related to a factor controlling the transcription and/or the stability of Na^+/P_i symporter mRNA. The recent localization of the NaPi-2/3 gene on human chromosome number 5 (Kos *et al.* 1994) suggests that, in X-linked hypophosphataemia, an X-linked factor could be involved in the control of the transcription and/or stability of Na^+/P_i symporter (NaPi-2) mRNA.

Regulation of small intestinal Na^+/P_i symport

An important factor modulating small intestinal phosphate absorption is the hormonal form of vitamin D, $1,25(OH)_2D3$ (for a review, see Danisi and Murer, 1991). The effect of calcitriol on intestinal apical transport of phosphate is characterized by an increase in the maximal transport rate and by the requirement for an intact machinery for protein synthesis. This suggests that the number of transport systems within the apical membrane is controlled by calcitriol. Similarly, as described for proximal tubular P_i reabsorption, dietary phosphate deprivation enhances small intestinal P_i reabsorption. Increased intestinal reabsorption of P_i caused by a low-P_i diet correlates in time with the rise in plasma $1,25(OH)_2D3$ level, suggesting that the hormonal form of vitamin D is implicated in the intestinal adaptive response.

Owing to the lack of molecular information on small intestinal apical Na^+/P_i symport, no detailed information about the regulation of this transporter is yet available.

Regulation of renal and small intestinal Na^+/S_i symport

In contrast to renal and small intestinal apical Na^+/P_i symport, very little information is available about possible regulatory control mechanisms for renal and small intestinal apical Na^+/S_i symport. Glucocorticoids have been shown to be inhibitory for renal reabsorption of sulphate (Renfro *et al.* 1981) and thyroid hormone has been shown to stimulate Na^+/S_i symport activity (Tenenhouse *et al.* 1991). Furthermore, a reduced dietary intake of sulphate seems to modulate renal brush-border membrane Na^+/S_i symport (Neiberger and Gomez, 1992). The intestinal transport of sulphate seems to be regulated by some secretory stimuli, such as theophylline or the heat-stable enterotoxin (Smith *et al.* 1981).

It remains to be shown whether the Na^+/S_i symport system (renal and ileal NaSi-1), described above, is involved in the regulation of apical renal and small intestinal Na^+/S_i symport.

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