Proximal Tubular Phosphate Reabsorption: Molecular Mechanisms

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I. Introduction: Overall Mechanism

A. Site of reabsorption

B. Cellular mechanism

II. Physiological Regulation

A. Major factors

B. Other factors

III. Pathophysiological Alterations

A. Genetic aspects

B. "Acquired" alterations

IV. Phosphate Transport Molecules in Proximal Tubular Cells

A. Type I Na-Pi cotransporter

B. Type II Na-Pi cotransporter

C. Type III Na-Pi cotransporter

V. Type IIa Sodium-Phosphate Cotransporter: The Key Player in Brush-Border Membrane Phosphate Flux

A. Transport characteristics

B. Altered expression as the basis for altered Pi reabsorption

C. Cellular mechanisms in the control of type II Na-Pi cotransporter expression

VI. Summary and Outlook

Murer, Heini, Nati Hernando, Ian Forster, and Jürg Biber. Proximal Tubular Phosphate Reabsorption: Molecular Mechanisms. Physiol Rev 80: 1373–1409, 2000.—Renal proximal tubular reabsorption of Pi is a key element in overall Pi homeostasis, and it involves a secondary active Pi transport mechanism. Among the molecularly identified sodium-phosphate (Na/Pi) cotransport systems a brush-border membrane type IIa Na-Pi cotransporter is the key player in proximal tubular Pi reabsorption. Physiological and pathophysiological alterations in renal Pi reabsorption are related to altered brush-border membrane expression/content of the type IIa Na-Pi cotransporter. Complex membrane retrieval/insertion mechanisms are involved in modulating transporter content in the brush-border membrane. In a tissue culture model (OK cells) expressing intrinsically the type IIa Na-Pi cotransporter, the cellular cascades involved in "physiological/pathophysiological" control of Pi reabsorption have been explored. As this cell model offers a "proximal tubular" environment, it is useful for characterization (in heterologous expression studies) of the cellular/molecular requirements for transport regulation. Finally, the oocyte expression system has permitted a thorough characterization of the transport characteristics and of structure/function relationships. Thus the cloning of the type IIa Na-Pi cotransporter (in 1993) provided the tools to study renal brush-border membrane Na-Pi cotransport function/regulation at the cellular/molecular level as well as at the organ level and led to an understanding of cellular mechanisms involved in control of proximal tubular Pi handling and, thus, of overall Pi homeostasis.

I. INTRODUCTION: OVERALL MECHANISM

Renal handling of Pi determines its concentration in the extracellular space, the “traffic” place between the two major body compartments: skeleton and intracellular space (37, 46, 101, 102, 216–218, 374). In cells phosphate participates in energy metabolism and is a constituent of signaling molecules, lipids, and nucleic acids. Under “normal” (“steady-state”) physiological conditions, urinary Pi excretion corresponds roughly to phosphate intake in the alimentary tract, mainly via upper small intestine (37, 94, 101, 218). To fulfill the “homeostatic” function, i.e., keep-
ing extracellular Pi concentration within a narrow range, urinary Pi excretion must be (and is) under strong physiological control (37, 101, 102). In contrast to intestinal Pi absorption, which adjusts rather “slowly” (for review, see Refs. 94, 290), renal Pi excretion can “adjust” very fast to altered physiological conditions.

A. Site of Reabsorption

Renal Pi excretion is the balance between free glomerular filtration and regulated tubular reabsorption. Under normal physiological conditions, ~80–90% of filtered load is reabsorbed; renal tubular reabsorption occurs primarily in proximal tubules, with higher rates at early segments (S1/S2 vs. S3) and in deep nephrons (e.g., Refs. 24, 142, 146, 159, 203, 232, 318; for review, see Refs. 37, 218, 374). A small fraction of filtered Pi seems to be reabsorbed in the distal tubule (13), but the apparent loss of Pi observed after proximal tubular micropuncture sites could be most likely explained by the higher reabsorption in proximal tubules of deep nephrons (for review, see Ref. 37). Therefore, a study/analysis of mechanisms participating at the level of the kidney in control of Pi excretion can be reduced to phenomena occurring in the proximal tubule.

B. Cellular Mechanism

The cellular mechanisms involved in proximal tubular Pi reabsorption have been studied by a variety of techniques including in vivo and in vitro microperfusions and studies on isolated membrane vesicles (e.g., Refs. 24, 49, 99, 100, 142, 144, 402), tissue-culture techniques (e.g., Refs. 41, 43, 64, 66, 67, 116, 261, 264), and studies with isolated brush-border and basolateral membrane vesicles (e.g., Refs. 18, 22, 23, 33–35, 44, 52, 53, 55, 76, 78, 80, 88, 95, 98, 118, 127, 143, 148, 149, 152, 153, 155, 179–182, 204, 239–241, 245, 246, 255, 256, 278, 291, 321, 324, 328, 352, 355, 360, 370–372, 375, 392, 400, 401, 410, 429–434). We and others have written previously several comprehensive reviews on cellular mechanisms participating in renal tubular handling of Pi and summarized the experiments with above-mentioned techniques (e.g., Refs. 37, 46, 100, 101, 138, 149, 278, 282, 283, 291). From these studies a secondary active transport scheme emerged (see Fig. 1, left). Pi is taken up from the tubular fluid by (a) brush-border membrane sodium/phosphate (Na-Pi) cotransporter(s) and leaves the cell via basolateral transport pathways. The brush-border entry step is the rate-limiting step and the target for almost all physiological (and pathophysiological) mechanisms altering Pi reabsorption (see below). Basolateral exit is ill defined, and several Pi transport pathways have been postulated including Na-Pi cotransport, anion exchange, and even an “unspecific” Pi leak (channel?). Basolateral Pi transport has to serve at least two functions: (1) complete transcellular Pi reabsorption in a case where luminal Pi entry exceeds the cellular Pi requirements and (2) guarantee basolateral Pi influx if apical Pi entry is insufficient to satisfy cellular requirements. The second can be considered as a “house-keeping” function and might not be specific for (re)absorptive cells. In this review we sum-

![Fig. 1. Scheme for proximal tubular Pi reabsorption. Left: concept of secondary active transport as evidenced by microperfusion studies and studies on isolated membrane vesicles. Right: Na-Pi cotransporter molecules in the proximal tubular epithelial cell. For further details and references, see text. [Adapted from Murer et al. (288).]](http://physrev.physiology.org/Content/Full/80/7/1374/F1.jpg)
marize the present knowledge on the key transporter molecules involved in proximal tubular transmembrane Pi movement (apical and basolateral; see Fig. 1, right).

II. PHYSIOLOGICAL REGULATION

As already indicated, regulation of proximal tubular Pi reabsorption and thus of brush-border membrane Na-Pi cotransport codetermines overall Pi homeostasis. Again, many reviews summarizing the regulation of proximal tubular Pi reabsorption at the organ, tubule, cell, and membrane levels have been written (37, 46, 138, 282–289, 292). This information is briefly presented here. In this review we focus on the molecular mechanisms underlying these regulations.

For a brief overview on regulatory events, we focus on major factors and other factors, and the latter is subdivided into hormonal and nonhormonal factors controlling proximal tubular Pi reabsorption (see Ref. 37). For each of these regulatory phenomena, a “memory” effect exists, i.e., the changes are induced by adequate pretreatment, and after characterization (e.g., by clearance techniques) in vivo can then be further analyzed in vitro, e.g., in microperturbation studies or in studies with isolated membrane vesicles (for review, see Refs. 37, 46, 138, 282, 283, 287). This memory effect can at present easily be understood, as physiological regulation of Pi reabsorption involves, as far as they have been studied at the molecular level, an altered expression of a brush-border Na-Pi cotransporter protein (type IIa Na-Pi cotransporter; for review, see Refs. 39, 242, 284–289, 292; see below). Therefore, they are in all cases, with the possible exception of “fasting” (204), related to changes in maximum velocity ($V_{\text{max}}$) of brush-border membrane Na-Pi cotransport activity in isolated brush-border membrane vesicles (for review, see Refs. 37, 46, 107, 206, 283).

A. Major Factors

1. Dietary Pi intake

A low dietary Pi intake can lead to an almost 100% reabsorption of filtered Pi, whereas a high dietary Pi intake leads to a decreased proximal tubular Pi reabsorption (for review, see Refs. 37, 218). These changes can occur independent of changes in the plasma concentration of different phosphaturic hormones (for review, see Refs. 37, 218; see also Refs. 7, 9, 316). Thus an “unknown” humoral factor may be involved in the mediation of these effects. However, as evidenced by studies on cultured renal proximal tubular epithelial cells (e.g., OK cells), a direct effect (“intrinsic”) of altered Pi concentration in the extracellular fluid (plasma, glomerular filtrate, culture media) also elicits changes in apical (brush-border) membrane Na-Pi cotransport activity (e.g., Refs. 41, 43, 64, 309, 339).

2. Parathyroid hormone

Parathyroid hormone (PTH) induces phosphaturia by inhibiting brush-border membrane Na-Pi cotransport activity; removal of PTH (parathyroidectomy) leads to an increase in Na-Pi cotransport activity (e.g., Refs. 108, 120, 153; for review, see Refs. 37, 46, 101, 138, 206, 218, 283, 292). These effects can also be analyzed in a tissue-culture model to study cellular/molecular mechanisms involved in proximal tubular Pi handling, in opossum kidney cells (OK cells; Refs. 67, 85, 86, 261–264, 267, 268, 307, 308, 310, 311, 341–345). This in vitro model also provided evidence for cAMP-dependent and cAMP-independent signaling mechanisms in PTH action (see below; see also Refs. 85, 86, 235, 264, 308, 329–333; for review, see Refs. 280, 283, 288, 289).

3. Vitamin D

Vitamin D is suggested to increase/stimulate proximal tubular Pi reabsorption. 1,25-Dihydroxycholecalciferol treatment of rats was found to stimulate brush-border membrane Na-Pi cotransport (226, 227). It is, however, difficult to discriminate between direct versus indirect effects, as in vivo the vitamin D status is closely associated with alterations in plasma calcium and PTH concentrations (for review, see Refs. 37, 46, 101, 107). Thus, at present, it is not clear whether 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$] directly regulates mammalian brush-border membrane Na-Pi cotransport. This is in contrast to the upper small intestine where 1,25(OH)$_2$D$_3$ stimulates brush-border membrane Na-Pi cotransport (for review, see Refs. 94, 290). In chicken tubular preparations, administration of 1,25(OH)$_2$D$_3$ increased Pi uptake, an effect prevented by inhibition of protein synthesis (249, 250). However, in these studies in suspended cells, it is not clear whether the stimulation is related to an increased uptake across the brush-border membrane. It has been suggested that the effects of 1,25(OH)$_2$D$_3$ are related to changes in the lipid characteristics of the membrane (114; for review, see Refs. 21, 37). A stimulatory effect of 1,25(OH)$_2$D$_3$ was also observed in a subclone of OK cells and in studies on promoter activation (see sect. vC; Refs. 8, 380).

B. Other Factors

1. Hormonal factors

There are additional hormonal factors (e.g., insulin, growth hormone/insulin-like growth factor I/other growth factors, thyroid and other lipophilic hormones, calcitonin, glucocorticoids, atrial natriuretic peptide, nerve transm-
ters, prostaglandins, parathyroid hormone-related peptide, phosphatonin, and stanniocalcin) with reported effects on proximal tubular Pi reabsorption, i.e., brush-border membrane Na-Pi cotransport (for review, see Refs. 37, 101, 107, 206, 283).

A) INSULIN. Insulin enhances proximal tubular Pi reabsorption by stimulation of brush-border membrane Na-Pi cotransport and prevents the phosphaturic action of PTH (e.g., Ref. 155; for review, see Refs. 37, 150, 206). Specific binding sites for insulin have been identified in basolateral membranes of proximal tubular epithelial cells (154, 155; for review, see Ref. 150).

B) GROWTH HORMONE/INSULIN-LIKE GROWTH FACTOR OTHER GROWTH FACTORS. Growth hormone, at least in part mediated by insulin-like growth factor I (IGF-I; locally produced in the kidney), stimulates proximal tubular Na-Pi cotransport (e.g., Refs. 65, 153, 281, 335; for review, see Refs. 37, 150, 206), an effect also observed in OK cells (63, 193). Receptors for growth hormone have been identified on the basolateral membrane of proximal tubular cells and appear to activate the phospholipase C pathway (350). Receptors for IGF-I have also been identified in proximal tubular cell membranes, and associated effects may involve tyrosine kinase activity (151, 154; for review, see Ref. 150).

Epidermal growth factor (EGF) stimulates Pi reabsorption in perfused proximal tubules (336, 337) but inhibits Pi transport in LLC-PK1 and OK cells (15, 140, 314). These effects are independent of cAMP and may involve tyrosine kinase activity and/or phospholipase C activation (see below; for review, see Ref. 206).

Transforming growth factors (e.g., transforming growth factor-α [TGF-α]) decrease Na-Pi cotransport activity in OK cells (233, 314). These effects are independent of cAMP, and the mechanisms might be similar to those in EGF action, sharing the same receptor (TGF-α and EGF; for review, see Refs. 150, 206).

C) THYROID HORMONE/LIPOPHILIC HORMONES. Thyroid hormone stimulates proximal tubular Pi reabsorption via a specific increase in brush-border membrane Na-Pi cotransport (31, 118, 213, 433, 434; for review, see Refs. 37, 107). The effect of thyroid hormone can also be observed in primary cultured chick renal cells and in OK cells and is dependent on protein synthesis (298, 367).

There are additional lipophilic hormones with reported effects on “renal tubular” Pi transport. All-trans-retinoic acid (CatRA) specifically increases Na-Pi cotransport in OK cells (30; for review, see Ref. 107). On the other hand, β-estradiol specifically decreases Na-Pi cotransport in brush-border membranes from adequately pretreated rats (32; for review, see Ref. 107).

D) CALCITONIN. Calcitonin reduces proximal tubular brush-border membrane Na-Pi cotransport in a PTH- and cAMP-independent manner (36, 430, 436; for review, see Refs. 37, 206). This effect might be mediated by a rise in intracellular calcium concentration (for review, see Ref. 37).

E) GLUCOCORTICOIDS. Glucocorticoids increase phosphate excretion by an inhibition of proximal tubular brush-border membrane Na-Pi cotransport (47, 127; see also Ref. 411); this effect can occur independent of an increase in PTH (for review, see Ref. 37). The effects of glucocorticoids are also apparent in vitro, in primary chick proximal tubular cells (299), and in OK cells (192; see also Refs. 156a, 319, 320). An increase in plasma glucocorticoid levels may mediate the phosphaturic response in chronic metabolic acidosis (11, 47, 127; for review, see Ref. 37).

F) ATRIAL NATRIURETIC PEPTIDE. Atrial natriuretic peptide (ANP) also inhibits proximal tubular brush-border membrane Na-Pi cotransport (156, 429). Although a small effect of ANP, mediated by a rise in cGMP, was observed on OK cell Na-Pi cotransport (294), a direct effect on proximal tubular cells is questionable, since receptors for ANP were not identified in proximal tubular epithelial cells (for review, see Ref. 37). An increase in renal dopamine production (see below) could mediate, in the intact organ, the effect of ANP on brush-border membrane Na-Pi cotransport (for review, see Ref. 37; see also Ref. 419).

G) PTH-RELATED PEPTIDE. PTH-related peptide produced by tumors causes phosphaturia. This “PTH analog” causes phosphaturia by mechanisms identical to that involved in PTH action (for review, see Refs. 37, 206; see also Refs. 315, 349).

H) PHOSPHATONIN. Studies in patients with tumor-induced osteomalacia, with associated hypophosphatemia and renal Pi wasting, led to the hypothesis that there is an additional humoral factor controlling serum Pi concentration and renal Pi handling (for review, see Refs. 37, 111, 224, 225). This as yet unidentified factor was named phosphatonin and is suggested to inhibit proximal tubular Pi reabsorption (60). It was observed that conditioned culture media from tumor cells derived from patients inhibited OK cell Na-Pi cotransport. This factor (phosphatonin?) was suggested to have a proteinous nature and a molecular weight between 8,000 and 25,000. The inhibition of Na-Pi cotransport occurred independently of changes in cellular cAMP content. Also, a PTH-receptor antagonist was found (but not identified; PTH related) in these culture media; it interfered with PTH inhibition of OK cell Na-Pi cotransport but not with the inhibitory effect of phosphatonin (for review, see Refs. 37, 111, 224, 225).

I) GLUCAGON. Glucagon administration increases Pi excretion. It was suggested that the effect of pharmacological doses of glucagon is indirect and related to an increase in plasma concentration of liver-derived cAMP (3).

J) STANNIOCALCIN. Two different isoforms of stanniocalcin (STC) were identified and suggested to be involved in calcium and phosphate homeostasis in fish and in mam-
mals. STC-1 was originally identified in fish and later in rat kidney, in more distantly located nephron segments (420). STC-2, ~34% amino acid similarity to STC-1 (189), was identified from an osteosarcoma library, and related transcripts were found in different tissues including kidney (72, 105, 189). STC-1 stimulates proximal tubular brush-border membrane Na-Pi cotransport (409); STC-2 has at least in vitro (OK cells), the opposite effect, by a suppression of the type IIa Na-Pi cotransporter (189). Thus STC-1/2 may serve paracrine modulators of P1 reabsorption.

K) PROSTAGLANDIN. Prostaglandins, produced intrarenally, also modulate renal P1 handling. PGE2, antagonizes the phosphaturia observed under different physiological conditions, e.g., increased PTH levels. This effect is in part, but not fully, explained by effects on the cAMP signaling cascade. The latter is illustrated by the observation that inhibition of renal prostaglandin synthesis (by indomethacin) potentiates the cAMP-independent phosphaturic action of calcitonin (36; for review, see Ref. 37).

L) NERVE TRANSMITTERS. Nerve transmitters also appear to control renal proximal tubular Na-Pi cotransport. Acute renal denervation increases renal P1 excretion, independent of the PTH status (for review, see Ref. 37). These effects can be related to the production of dopamine and/or reduced α- or β-adrenoceptor activity. Dopamine and its precursor L-dopa increase P1 excretion (104, 187, 188) and inhibit Na-dependent P1 transport in OK cells as well as in isolated rabbit proximal tubules (19, 79, 104, 129, 137, 196). Dopamine can be generated from L-dopa after brush-border membrane uptake of γ-glutamyl-L-dopa and leads in an autocrine/paracrine manner via a stimulation of adenyly cyclase to the inhibition of brush-border membrane Na-Pi cotransport (104). Stimulation of α-adrenoceptors might interfere with hormone-dependent stimulation of adenylyl cyclase activity (e.g., by PTH) and might therefore lead to an apparent increase in Na-Pi cotransport activity, and explain a hypophosphaturic action of α-agonists (70, 403, 422, 423; see also Ref. 234). In addition, stimulation of α-adrenoceptors in OK cells blunted the actions of PTH on cAMP production and inhibition of Na-Pi cotransport (77; see also Ref. 103). Serotonin is also synthesized in the proximal tubules and is antiphosphaturic; it stimulates proximal tubular P1 reabsorption (103, 128, 129, 147).

Adenosine infusion in rats stimulates renal P1 reabsorption (312).

2. Nonhormonal factors

In addition to above hormonal factors, there are several nonhormonal factors known to affect proximal tubular Na-Pi cotransport.

A) FASTING. Fasting may result in phosphaturia and reverse the effects of a low-P1 diet (for review, see Ref. 37; see also Ref. 28). This effect relates also to a change in brush-border membrane Na-Pi cotransport (204). In contrast to dietary P1-induced changes and other regulatory conditions, the lowered P1 uptake under fasting conditions might be explained by an increase in the apparent Michaelis constant ($K_m$) value for P1 (204). The effect of fasting may involve, but cannot be explained by, an increase in glucagon levels (for review, see Ref. 37).

B) PLASMA CALCIUM. Changes in plasma calcium lead to changes in renal proximal tubular P1 reabsorption that are primarily associated with the corresponding changes in PTH concentration (12, 421; for review, see Ref. 37). However, in vitro data also suggest a direct cellular effect of extracellular calcium on proximal tubular brush-border membrane Na-Pi cotransport (e.g., Ref. 301). In isolated perfused convoluted rabbit proximal tubules, an increase in bath and perfusate calcium concentration provoked an increase in P1 reabsorption (351). In studies on OK cells, opposite data were obtained: a decrease in medium calcium concentration stimulated Na-Pi cotransport (62). These differences are not understood but might be related to the time scale used in the experiments. The effects in OK cells required prolonged exposure, were dependent on protein synthesis, and may be related to changes in intracellular Ca$^{2+}$ concentration (see sect. vC$/\gamma$; see also Ref. 353).

C) ACID BASE. The influence of changes in systemic acid-base status on renal proximal tubular Na-Pi cotransport are rather complex and are summarized only briefly. The effects on the kinetic properties of the carrier are discussed in section vA$/\delta$; in brief, an alkaline intratubular pH leads to a stimulation of Na-Pi cotransport (14, 328, 334, 352; for review, see Refs. 37, 138, 216–218, 283). Acute metabolic acidosis does not significantly interfere with P1 reabsorption. In contrast, chronic metabolic acidosis leads to a decrease in Na-Pi cotransport, most likely related to the evaluated glucocorticoid levels (11, 47, 127). These effects are also apparent in OK cells following appropriate changes in media pH conditions (192, 194). Respiratory acidosis leads to phosphaturia involving corresponding changes in Na-Pi cotransport. In contrast, respiratory alkalosis stimulates proximal tubular P1 reabsorption (for review, see Ref. 37).

D) VOLUME EXPANSION. Volume expansion of animal increases P1 excretion and decreases Na-Pi cotransport rates in isolated brush-border membrane vesicles and in isolated perfused proximal tubules (74, 313, 317, 323, 324; for review, see Ref. 37). It is assumed that the effect of volume expansion on proximal tubules is indirect (i.e., via some humoral factors, in part ANP and/or dopamine).

III. PATHOPHYSIOLOGICAL ALTERATIONS

In addition to the above briefly discussed physiological regulatory mechanisms, that adjust brush-border...
Na-P$_i$ cotransport to the needs of body P$_i$ homeostasis, there are genetically determined alterations in renal P$_i$ handling and “acquired” alterations in renal P$_i$ reabsorption.

A. Genetic Aspects

The genetic aspects of proximal tubular Na-P$_i$ cotransport have been covered in many reviews (e.g., Refs. 338, 384, 390), and we only mention those disorders that have been characterized at the molecular level. Several genetic defects resulting in isolated renal phosphate wasting have been described, such as X-linked hypophosphatemic rickets (XLH; e.g., Refs. 295, 384, 385), autosomal dominant hypophosphatemic rickets not associated with hypercalcinuria (ADHR, 110, 113), and hereditary hypophosphatemic rickets with hypercalcemia (HHHR; 136, 394). The first is caused by mutations in the PHSEX gene, which has homology to neutral endopeptidase genes and is hypothesized to process or degrade a circulating factor that regulates by an unknown mechanism renal brush-border membrane Na-P$_i$ cotransporter (see below; for review, see Refs. 110, 112, 384, 390). A candidate gene for ADHR and/or HHHR could be the brush-border membrane Na-P$_i$ cotransporter (see below). However, the gene involved in ADHR was recently mapped to chromosome 12p13 (113), a gene locus different from the brush-border Na-P$_i$ cotransporter (5q35; 222, 223; see below).

Although HHHR has the biochemical features of mice with a gene deletion for the brush-border membrane Na-P$_i$ cotransporter (25; see below), recent studies on a bedouin kindred with HHHR do not support the hypothesis of a direct involvement of the transporter gene in HHHR (A. O. Jones, I. Tzenova, T. M. Fujiwara, D. Frapier, M. Tieder, K. Morgan, and H. S. Tenenhouse, unpublished data). An interesting form of a genetically determined reduction in renal P$_i$ handling is in Dent’s disease, where mutations in a chloride channel (CLC5) lead to an apparent P$_i$ transport defect (252; for review, see Ref. 391). How the loss of function of an endosomal chloride channel leads to a decreased brush-border Na-P$_i$ cotransport needs to be determined. Other genetic defects in renal P$_i$ handling are secondary to changes in vitamin D, PTH, or acid/base metabolism or are a consequence of more general metabolic disorders (for review, see Refs. 109, 216, 217, 338, 390).

B. Acquired Alterations

Disturbances in proximal tubular P$_i$ transport seem to be an early indicator of “nonspecific” proximal tubular alterations, occurring as a consequence of “unphysiological” extrarenal factors (for review, see Refs. 216, 217). This may be explained by the specific kinetic properties of the brush-border membrane Na-P$_i$ cotransporter (see sect. vH). An example of this may be the observed phosphaturia when the filtered load of glucose is augmented (in diabetes mellitus), where a “competition” for driving force will reduce Na-P$_i$ cotransport rate (22, 392). More generally speaking, when driving forces across the brush-border membrane (Na$^+$ gradient and/or membrane potential) are altered, the transport of phosphate will be reduced and thus phosphaturia will occur. Furthermore, as part of its physiological regulation, the transporter protein mediating the rate-limiting Na-P$_i$ cotransport has a high turn-over. Therefore, “damage” to the brush-border membrane or the transporter protein itself will result in a massive reduction in the brush-border membrane content of Na-P$_i$ cotransporters and thus reduce P$_i$ transport leading to phosphaturia. This may explain, for example, the sensitivity of renal P$_i$ reabsorption to heavy metal intoxication (see Refs. 4, 141, 169).

Diuretics may inhibit proximal tubular P$_i$ reabsorption when administered to animals or intact tubular preparations (for review, see Ref. 37). Because the greatest effect is produced by acetazolamide, it is assumed that inhibition is related to an inhibition of carbonic anhydrase; therefore, the effect is also dependent on the presence of bicarbonate. The effect of other diuretics on proximal tubular P$_i$ reabsorption correlates to some extent with their potency to inhibit carbonic anhydrase. Inhibition of carbonic anhydrase leads to acute and/or chronic changes in systemic and/or tubular pH, which in turn causes the changes in P$_i$ reabsorption.

IV. PHOSPHATE TRANSPORT MOLECULES IN PROXIMAL TUBULAR CELLS

The cellular scheme for proximal tubular P$_i$ reabsorption given above includes three Na-P$_i$ cotransporters (Fig. 1). They have been molecularly identified and have been named type I, type II, and type III Na-P$_i$ cotransporters (175; for review, see Refs. 284–289, 377). However, there may be additional pathways in the brush-border and basolateral membranes that have not yet been defined at the molecular level. In heterologous expression systems (e.g., Xenopus laevis oocytes), the corresponding cRNA/proteins augment highly Na$^+$-dependent P$_i$ uptake. The three families of Na-P$_i$ cotransporters share no significant homology at the level of their primary amino acid sequence (Fig. 2 and Table 1). We discuss the structural properties, tissue expression, and functional characteristics of these three families of Na-P$_i$ cotransporters. Because the type IIa Na-P$_i$ cotransporter is the key player (see sect. v), the kinetic properties and the regulatory behavior of the type IIa transporter are then covered separately and in more detail.
A. Type I Na-P₁ Cotransporter

A cDNA related to the type I Na-P₁ cotransporter was initially identified by screening a rabbit kidney cortex library for expression of P₁ transport activity in X. laevis oocytes (416). Homologous cDNA (and in part proteins) were then found in human, mouse, and rat kidney cortex, in cerebellar granular cells, and in Caenorhabditis elegans (81, 82, 247, 276, 296, 297; for review, see Ref. 414). The gene encoding the type I cotransporter (NPT₁) maps in humans to chromosome 6 p21.3-p23 (82, 223), in mouse to chromosome 13 close to the Tcrg locus (81, 437), and in rabbits to chromosome 12p11 (223). The promoter organization of NPT₁ has been characterized; 104 bp upstream of exon 1, a single transcription start site was found and a TATA-like sequence at -41 (378).

Type I transporter mRNA has been detected by in situ hybridization in mouse kidney proximal tubules and to a lesser extent also in distal tubules (81). In rabbits, RT-PCR of microdissected tubular segments localized type I mRNA to the proximal tubules (92). Immunohistochemical experiments and studies with isolated membranes localized the type I transporter protein to the proximal tubular brush-border membrane in rabbits and in mice (40; M. Lötscher, J. Biber, and H. Murer, unpublished).

Table 1. The three families of Na-P₁ cotransporters

<table>
<thead>
<tr>
<th>Family Name</th>
<th>Type I</th>
<th>Type II</th>
<th>Type IIa</th>
<th>Type IIb</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecule name</td>
<td>NaPi-I, rabbit, rat, mouse, or human (NaPi-I, NPT1, Npt1)</td>
<td>NaPi-IIa, mouse, rat, human, rabbit, or opossum (NaPi-2/3/4/6/7)</td>
<td>NaPi-IIb, mouse, human, flounder, or Xenopus (NaPi-5)</td>
<td>Glvr-1 (PiT-1)</td>
<td>Ram-1 (PiT-2)</td>
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<td>4</td>
<td>2 (PiT-1)</td>
<td>8 (PiT-2)</td>
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<td>~690</td>
<td>670, 656</td>
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<td>Predicted transmembrane segments</td>
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<td>8</td>
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<td>Function (in Xenopus oocytes)</td>
<td>Na-P₁ cotransport, Cl channel activity, interaction with organic anions</td>
<td>Na-P₁ cotransport, electrogenic, pH dependent</td>
<td>Na-P₁ cotransport, electrogenic</td>
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<td>0.1–0.2 mM</td>
<td>0.05 mM</td>
<td>0.025 mM</td>
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<tr>
<td>pH dependence</td>
<td>Stimulated at high pH</td>
<td>&quot;Decreased&quot; at high pH</td>
<td>&quot;Decreased&quot; at high pH</td>
<td>Ubiquitous</td>
<td></td>
</tr>
<tr>
<td>Tissue expression (mRNA protein)</td>
<td>Kidney cortex/PT, liver, brain</td>
<td>Kidney cortex/PT</td>
<td>Small intestine, lung, and other tissues</td>
<td>P₁ diet</td>
<td></td>
</tr>
<tr>
<td>Regulated by PTH/P₁ diet</td>
<td>No</td>
<td>PTH and P₁ diet</td>
<td>P₁ diet</td>
<td>(P₁ diet)</td>
<td></td>
</tr>
</tbody>
</table>

PT, parathyroid; PTH, parathyroid hormone.
observations). Studies on brush-border membranes provided evidence for a higher expression in “deep” juxtamedullary compared with superficial nephrons (97).

On the basis of hydropathy predictions, the type I Na-Pi cotransporter protein may contain six to eight transmembrane regions (Fig. 2); it contains three N-glycosylation motifs of which some are used as indicated by immunoblotting studies with isolated brush-border membrane vesicles and by in vitro translation experiments (416; for review, see Refs. 285, 284, 414).

The induction of increased Na-Pi cotransport activity after injection into X. laevis oocytes was the basis for the expression cloning of the type I Na-Pi cotransporter cDNA (416). Stable transfection of type I transporter cDNA into Madin-Darby canine kidney (MDCK) and LLC-PK1 cells resulted also in an increased cellular uptake of Pi (325). Na\(^+\)-dependent Pi uptake, induced after expression of the type I transporter in oocytes, has been extensively characterized (50, 56, 276, 297, 416). The apparent \(K_m\) for Pi was \(0.3\) mM for expression of the human and \(1\) mM for the rabbit type I Na-Pi cotransporter. The apparent \(K_m\) value for Na\(^+\) interaction was \(50\) mM, with a Hill coefficient exceeding unity. Furthermore, no pH dependence of type I transporter-mediated Na\(^+\)-dependent Pi uptake could be observed in oocytes. In electrophysiological studies in oocytes, evidence was obtained that the type I transporter protein might be multifunctional, since evidence for anion channel function with permeability for chloride and different organic anions was obtained (50, 57). In the oocyte experiments it was observed that the induction of chloride conduction by expression of the type I transporter cDNA was time and dose dependent, in contrast to Na\(^+\)-dependent Pi uptake, which was maximally increased at low doses of injected cRNA and after short time periods of expression (50). This could suggest that the type I transporter protein may modulate an intrinsic “oocyte” Na-Pi uptake activity, present not only in oocytes, and that the type I transporter protein may or may not be a Na-Pi cotransporter itself but rather an anion channel protein with expression in renal brush-border membrane. Its role in proximal tubular secretion of anions (e.g., organic anions, xenobiotics) needs to be determined. Certainly, the above-described characteristics of type I transporter-induced Na\(^+\)-dependent Pi uptake does not resemble the characteristics of Na\(^+\)-dependent Pi uptake in brush-border membrane vesicles (e.g., Ref. 14; for review, see Refs. 138, 283). Therefore, the type I transporter is not a major player in mediating or controlling brush-border membrane Na-Pi cotransport.

Yabuuchi et al. (426) have studied in more detail the anion conductive properties of the type I Na-Pi cotransporter (human Npt1). In oocytes, benzylpenicillin, β-lactam antibiotics, probenecid, foscarnet, and melavonic acid were transport substrates. In the hepatocytes, Npt1 was located on the sinusoidal membrane (426).

To establish the physiological role of NPT1 in above anion secretion (as well as in renal Pi handling), gene deletion experiments are required (H. S. Tenenhouse and I. Soummounou, personal communication).

B. Type II Na-Pi Cotransporter

The cDNA encoding the type II (type IIa) Na-Pi cotransporter was identified by expression cloning in X. laevis oocytes, from rat and human kidney cortex libraries, respectively (260). Homology-based approaches then led to the identification of type II-related transporters in kidneys from different species including flounder and zebrafish, in opossum kidney cells (OK cells), and in a bovine epithelial cell line (NBL-1; Refs. 87, 88, 163, 168, 219, 294a, 366, 405, 417; see also Fig. 3). A type II-related Na-Pi cotransporter was identified in apical membranes of mammalian small intestine and type II pneumocytes (121, 175, 396) and has been designated type IIb Na-Pi cotransporter (Table 1). The regions with highest homology be-
between type IIa and type IIb transporters are in transmembrane domains, and regions with no or little homology are at the cytoplasmic NH₂ and COOH termini (Fig. 4; Ref. 175).

Werner et al. (414) compared the sequences of the type II Na-Pᵢ cotransporters (Fig. 5) and three "families" were identified. Interestingly, the type IIa transporter is preferentially expressed in kidney, with a proximal tubular apical location (see sect. V B1). The type IIb transporter can have multiple locations; in mammals, it is expressed in the small intestine, type II pneumocytes, and other tissues, whereas in nonmammalian vertebrates it can be either in the kidney and/or small intestine (175, 190, 219, 294a, 396). A type IIa Na-Pᵢ cotransporter appears to be expressed also in osteoclasts and may play a role in bone resorption (145). A type II Na-Pᵢ cotransporter seems also to be expressed in brain, where the function is not yet established (177). Finally, type II related proteins appeared very early in evolution, and related genes were found in Vibrio cholerae and C. elegans (see Fig. 5; for review, see Ref. 414).

1. Chromosomal location/genomic organization

The human type IIa cotransporter gene (NPT 2) maps to chromosome 5q35 (Fig. 6; Refs. 222, 223, 269, 277) and
The genomic structure of \textit{NPT2} (human IIa) and \textit{Npt2} (murine IIa) has been determined; they are 16 kb in length and consist of 13 exons and 12 introns (Fig. 6; Refs. 162, 379). In the promoter region of the human, murine and OK cell \textit{NPT2}/\textit{Npt2} gene, a TATA box is present 31 bp upstream from the transcription start sites. A GCAAT element and several AP-1 sites may control promoter activity (162, 379). The \textit{NPT2}/\textit{Npt2} promoter is active only in a proximal tubular environment, i.e., in OK cells (162, 174, 176). 5'-Flanking sequences of the OK cell type II \textit{Na-Pi} cotransporter gene contain elements mediating transcriptional control under different bicarbonate/carbon dioxide tensions (194). For the rat \textit{Npt2} promoter, an important role of repeating AP-2 consensus sites in regulating cell-specific expression was documented (359). In reporter gene studies, no physiological regulation (e.g., by low-Pi, medium, PTH, thyroid hormones, and growth factors) was observed using a short promoter (327 bp) fragment (174, 176). However, in COS-7 cells expressing the human vitamin D receptor, a vitamin D response element was observed at \(\sim\) 1 kb upstream of the transcription start site, were identified as binding sites to nuclear proteins upregulated in kidneys of weaning mice fed a low-Pi diet (212a). The corresponding DANN-binding protein could be identified; it corresponds to a known transcription factor (TFE3) that activates transcription through the \(\mu\)E3 site of the immunoglobulin heavy chain enhancer (212a). The mRNA encoding TFE3 was found to be significantly increased in kidney tissues of weaning mice fed a low-Pi diet (212a).

2. Tissue-specific expression

In situ hybridization of renal sections (Fig. 3) and nephron microdissection, followed by RT-PCR, documented that type IIa mRNA expression is restricted to the kidney proximal tubule (87, 91, 348, 388). Therefore, in mouse kidney, the type IIa is by far the most abundant of known \textit{Na-Pi} cotransporters (388). Type IIa \textit{Na-Pi} cotransporter protein is found in the brush-border membrane of proximal tubules (see Fig. 3; Refs. 91, 348). Inter- and intranephron distribution type of IIa \textit{Na-Pi} cotransporter highly depends on the physiological requirements within overall Pi homeostasis, (see Figs. 3 and 11; Refs. 91, 210, 243, 348; for review, see Refs. 242, 284–287). The type IIa \textit{Na-Pi} cotransporter is also expressed in OK cells but not in other renal cell lines (310, 311, 366, 386, 424; and J. Forgo, G. Strange, J. Biber, and H. Murer, unpublished observations). Recently, a type IIa transporter protein-related immunoreactivity was observed in membrane fractions isolated from nontransformed immortalized mouse kidney cortex epithelial cells (71). There is no evidence that the type IIa \textit{Na-Pi} cotransporter is expressed in primary renal proximal tubular epithelial cell cultures (Forgo et al., unpublished observations). Its expression in OK cells is the basis for the use of this cell line as an in vitro model for the study of cellular mechanisms involved in regulation of type IIa \textit{Na-Pi} cotransport activity (see Refs. 192–194, 234–236, 263, 307–311; for review, see Refs. 283–288).

The related type IIb \textit{Na-Pi} cotransporter is found in the apical membrane of upper small intestinal enterocytes and type II pneumocytes (see Refs. 175, 396); type IIb transcripts have been found in a variety of other tissues (175).
3. Structural aspects

Hydropathy analysis predicted eight transmembrane segments for the type IIa cotransporter protein (Fig. 2; Ref. 260; for review, see Refs. 284, 285). This membrane topology was supported by several experimental findings: (1) insertion of FLAG epitopes and accessibility of the epitope to antibodies (231); (2) lack of accessibility of antibodies directed against COOH- and NH₂-specific amino acid sequences (231); (3) identification of two glycosylation sites in the second “suggested” extracellular loop (166); and (4) accessibility to membrane-impermeant sulfhydryl reagents after insertion of cysteine residues at specific sites of the protein (228, 229). Two regions may penetrate partially into the lipid bilayer (Fig. 7).

Type IIa Na-P₅ cotransporters contain numerous potential phosphorylation sites for protein kinase C and casein II kinases (165, 260). The role of these sites in physiological control of transport activity is not clear (see sect. vC4).

On immunoblots of brush-border membrane proteins performed under nonreducing conditions, the type IIa Na-P₅ cotransporter shows an apparent molecular mass of 80–90 kDa; under reducing conditions two bands of ~45–50 kDa appear (39, 48, 91, 425). The latter suggests that the transporter might be proteolytically cleaved between the two glycosylation sites at positions N298 and N328 (39, 48, 228, 229, 305). It is not known whether this proteolytic cleavage occurs in situ or whether it is experimentally induced. Site-directed mutagenesis studies documented an S-S bridge in the second extracellular loop (Fig. 7; Refs. 228, 229). It is of interest that separate oocyte injections of cRNA encoding NH₂- and COOH-terminal fragments of the flounder type IIb Na-P₅ cotransporter resulted in induction of P₅ uptake activity only if both “parts” of the proteins were “present” (220).

The question of a multimeric structure of the type IIa cotransporter has been addressed mainly in radiation inactivation studies (33, 98, 195). The size of the functional unit of brush-border membrane Na-P₅ cotransport (mostly type IIa Na-P₅ cotransporter mediated) was found to be between 170 and 200 kDa, suggesting a multimeric structure. Recent experiments in oocytes expressing wild-type and mutant (inactivatable, cysteine insertion) type IIa Na-P₅ cotransporters suggested that each individual wild-type cotransporter molecule within an assumed homomultimeric complex is functional (220a). The apparent high functional molecular mass observed in brush-border membranes could also be due to a heteromultimeric complex (see below). The experiments in different heterologous expression systems (e.g., in SF9 cells, Refs. 134, 135; in MDCK cells and LLC-PK₁ cells, Refs. 325, 326; and in oocytes, Ref. 260) suggest that an unknown additional protein within the functional complex is not an obligatory requirement for the type IIa Na-P₅ cotransporter-mediated P₅ uptake activity or, rather unlikely, is present as an intrinsic protein (to serve as a transporter subunit) in different expression systems.
Tatsumi et al. (381) have identified type IIa Na-Pi cotransporter-related cDNA (named NaPi-2α, NaPi-2β, and NaPi-2γ). The NaPi-2α-encoded protein (355 amino acids) has a high homology to the NH₂-terminal half of the type IIa cotransporter, NaPi-2β encodes for 327 amino acids identical to the NH₂-terminal part of type IIa cotransporter with a completely different 146-amino acid COOH-terminal end, and NaPi-2γ encodes a 268-amino acid protein from the COOH-terminal end of the molecule (381). It seems that the related mRNA are formed by alternative splicing of the type IIa cotransporter gene (381). Isoform specific mRNA were found on Northern blots of rat kidney cortex mRNA. With the use of a full-length type IIa Na-Pi cotransporter cDNA probe, the major transcript detected was ~2.6 kb (260, 381). Additional bands (9.5, 4.6, and 1.2 kb) were seen, although in our experience, these bands are not abundant (260, 381). The NaPi-2α probe hybridizes with transcripts of 9.5 and 4.6 kb, the NaPi-2β probe with a transcript of 1.2 kb, and the NaPi-2γ probe with transcripts of 9.5 and 2.6 kb (381). In Western blots, with the use of NH₂- or COOH-terminal type IIa Na-Pi cotransporter specific antibodies, proteins of 45, 40, and 37 kDa were observed, corresponding approximately to the size of the in vitro translated proteins (NaPi-2α, NaPi-2β, NaPi-2γ, Ref. 381). The full-length type IIa Na-Pi cotransporter protein is recognized in Western blots from brush-border membrane as a 80- to 90-kDa protein in its glycosylated form (87). In our hands, the lower molecular mass bands are not detected in the absence of reducing agents (see Refs. 39, 91). Because they

![Diagram of secondary structure](http://physrev.physiology.org/images/diagram.png)

**Fig. 7.** Secondary structure (membrane topology) of type IIa Na-Pi cotransporter (rat, NaPi-2). The model is derived from hydropathy predictions (Fig. 2; Ref. 260) and is experimentally supported by studies on N-glycosylation (166), accessibilities of specific antibodies to either the NH₂ or COOH terminus (231), FLAG-epitope insertion (231), and cysteine insertions/deletions (228, 229). For further details, see text and included references.
are only visible under reducing conditions, type 2α, 2β, and/or 2γ related proteins might be linked to the full size type IIa Na-Pi cotransporter via S-S bridges. Alternatively, the possibility exists that the smaller proteins, apparent after reduction of S-S bridges, are a product of proteolytic cleavage of the full size type IIa Na-Pi cotransporter protein (see above). Based on coexpression experiments in oocytes, Tatsumi et al. (381) postulated that the smaller isoforms might regulate, in a dominant negative manner, the function of the type IIa Na-Pi cotransporter protein. However, this interpretation requires further studies to, for example, document the coexistence, within a “heterologous” complex, of the different proteins at the brush-border membrane. Furthermore, quantitative aspects are crucial, since in our experience NaPi-2α, -2β, and -2γ can only be present in rather small amounts relative to the full size type IIa cotransporter. Thus the role of the small type IIa Na-Pi cotransporter-related proteins in brush-border membrane Na-Pi cotransport in vivo is not clear.

An antisense type IIb Na-Pi cotransporter transcript was detected in different nonmammalian tissues. It was postulated that it might be involved in the control of cotransporter protein expression (physiological control; tissue specificity; Ref. 184).

When expressed in X. laevis oocytes, the type IIa Na-Pi cotransporter mediates Na-Pi cotransport activity with functional characteristics identical to those observed in isolated brush-border vesicles (87, 163, 260, 366, 405). A 3:1 stoichiometry (Na\(^+:\)Pi\(^-\)) is the basis for its membrane potential sensitivity (electrogenicity; e.g., Refs. 59, 123). As discussed in section V, the transport characteristics and kinetic behavior of the type IIa transporter have been studied in great detail. Similar transport characteristics were also observed in different other heterologous expression systems such as insect Sf9 cells, fibroblasts, and MDCK cells (134, 135, 326, 385).

C. Type III Na-P\(_i\) Cotransporter

Surprisingly, the receptor for gibbon ape leukemia virus (Glvr-1) and the receptor for the mouse amphotropic retrovirus (Ram-1) have been shown to mediate Na-Pi cotransport activity after their expression in X. laevis oocytes (201, 202, 302). The transporter proteins have been named Pit-1 and Pit-2 and are now classified as type III Na-Pi cotransporters (Table 1).

Expression of type III Na-P\(_i\) cotransporters seems to be ubiquitous, and related mRNA have been identified in kidney, parathyroid glands, bone, liver, lung, striated muscle, heart, and brain (Table 1; Refs. 84, 201, 202, 302, 303, 362, 382). In mouse kidney, transcripts of type III cotransporters are found throughout the different structures (362, 388). Immunofluorescence studies showed in the proximal tubule a basolateral location (C. Silve, personal communication). Based on mRNA levels, type III Na-Pi cotransporters are two orders of magnitude less abundant than type IIa transporters (388). Its role in the proximal tubule seems not to be in transcellular P\(_i\) transport but rather in cell P\(_i\) uptake if luminal P\(_i\) entry is insufficient for cell metabolic functions. Type III transporter expression seems not to be altered by PTH (386).

The type III transporters show some homology to a Neurospora crassa gene (Pho-4+) involved in transmembrane P\(_i\) movements (302). Hydropathy analysis suggests 10 transmembrane regions (Fig. 2; Refs. 201, 202).

Pit-1- and Pit-2-mediated Na-Pi cotransport has been studied by expression in X. laevis oocytes or in fibroblast transfection (201, 202, 302). Transport is characterized by a \(K_m\) for P\(_i\) in the order of 20–30 μM and a \(K_m\) for Na of 40–50 mM. pH dependence of type III Na-Pi cotransporter is opposite to the type IIa cotransporter, i.e., decreased activity by increasing pH. Similar to the type IIa, type III-mediated transport of P\(_i\) is electronegic with a net influx of a positive charge during the transport cycle, suggesting also a 3:1 stoichiometry.

V. Type IIa Sodium-Phosphate Cotransporter: The Key Player in Brush-Border Membrane Phosphate Flux

The tissue expression, the relative renal abundance, and overall transport characteristics of type I, II (IIa), and III Na-P\(_i\) cotransporters suggest that the type IIa transporter plays a key role in brush-border membrane P\(_i\) flux. As discussed in this section, changes in expression of the type IIa Na-P\(_i\) cotransporter protein parallel alterations in proximal tubular P\(_i\) handling, documenting its physiological importance (for review, see Refs. 242, 284–289). In addition, experiments on molecular (genetic) suppression of the type IIa Na-P\(_i\) cotransporter support its role in mediating brush-border membrane Na-P\(_i\) cotransport. 1) Intravenous injection of specific antisense oligonucleotides led to reduced brush-border membrane Na-P\(_i\) cotransport activity that was associated with a decrease in type IIa cotransporter protein (300). 2) Disruption of the type IIa Na-P\(_i\) cotransporter gene (Npt 2) in mice led to an ~70% reduction in brush-border Na-P\(_i\) cotransport rate and complete loss of the protein (25, 178; see also below). The molecular basis for the remaining brush-border membrane Na-P\(_i\) cotransport after Npt 2 gene disruption is unclear. Either the type I transporter protein or another not yet identified Na-P\(_i\) cotransporter could account for residual transport activity. 3) Injection of type IIa antisense oligonucleotides in oocytes completely inhibited Na-P\(_i\) cotransport mediated by kidney cortex mRNA, confirming its major role in brush-border membrane Na-P\(_i\) cotransport (275, 276, 389, 415).
A. Transport Characteristics

As already indicated, the transport characteristics of the type IIa cotransporter heterologously expressed in different cellular systems (mainly X. laevis oocytes) resembles closely those of Na-Pi cotransport activity observed in isolated brush-border membranes (e.g., Refs. 87, 88, 163, 168, 219, 260, 366, 405, 417). In particular, in all expression systems studied thus far, type IIa-mediated Na-Pi cotransport activity increased with increasing media pH values, a “signature” for proximal tubular brush-border membrane Na-Pi cotransport (e.g., Refs. 14, 260).

The simplest experimental technique to analyze the transport characteristics of a Na-substrate cotransporter is by studying Na\(^+\) gradient-driven tracer substrate influx under different conditions. For the type IIa cotransporter, this has been done already in 1976 in isolated rat brush-border membrane vesicles (179); obviously, it was then not known that the brush-border Na-Pi cotransport activity is mostly associated with the type IIa cotransporter protein (25, 260). The studies with isolated membrane vesicles provided, however, significant insights into the mechanism/kinetic of brush-border membrane Na-Pi cotransport (e.g., Refs. 14, 34, 35, 55, 80, 352). A detailed kinetic characterization of type IIa-mediated Na-Pi cotransport activity was performed after its expression in X. laevis oocytes (e.g., Ref. 260). The first characterization, performed using tracer techniques, suggested a Na\(^+\):Pi stoichiometry exceeding unity (see sect. 1A1; Ref. 260). These data and the evidence for electrogenicity of Na-Pi cotransport across the brush-border membrane from microperfusion experiments in vivo (133) and from studies with isolated vesicles (35, 55) were the rationale for an electrophysiological characterization of the type IIa Na-Pi cotransporter after its expression in oocytes. The electrophysiological studies, performed under steady-state conditions, complemented the tracer uptake study, whereas pre-steady-state measurements provided new insights into individual steps within the transport cycle (see below).

1. Steady-state electrophysiological characteristics

Under voltage-clamp conditions, superfusion of oocytes expressing the rat type IIa cotransporter with 1 mM P\(_i\) in the presence of Na\(^+\) (100 mM) elicits an inward current, the magnitude of which depends on the holding potential (Fig. 8, A and B; Refs. 56, 58, 59, 122, 123, 125, 126, 407, 408). This observation indicates that a P\(_i\)-induced inward movement of positive charge(s) occurs during the transport cycle. At a given membrane potential, dose-response relationships can then be obtained for both Na\(^+\) and P\(_i\). Furthermore, the interdependence of the apparent affinity for either Na\(^+\) or P\(_i\) and/or the applied membrane potential was studied (e.g., Ref. 122). For P\(_i\), a hyperbolic saturation curve is observed, whereas for Na\(^+\), the saturation curve is sigmoidal (Fig. 8, C and D). At 100 mM Na\(^+\), the apparent \(K_m\) for P\(_i\) interaction is \(~0.1\) mM and shows little dependency on the holding potential (Fig. 8E). At 1 mM P\(_i\), the apparent \(K_m\) for Na\(^+\) interaction is \(~50\) mM (Fig. 8F). The concentration dependence of P\(_i\)-induced current depends on the external Na\(^+\) concentration with a dual effect: increasing Na\(^+\) leads to a decrease in the apparent \(K_m\) for P\(_i\) and to an increase in the apparent \(V_{max}\) (Fig. 8C). On the other hand, increasing P\(_i\) also leads to an increase in affinity for Na\(^+\) (Fig. 8D). At the lower Na\(^+\) concentrations, the apparent \(K_m\) for P\(_i\) interaction shows a marked dependence on the holding membrane potential (Fig. 8E); this is not observed for the \(K_m\) for Na\(^+\) interaction (Fig. 8F). Finally, these saturation experiments provide some information with respect to the stoichiometry (Pi : Na\(^+\)). Hill coefficients calculated on the basis of the P\(_i\) saturation curves were always close to 1, whereas those calculated for Na\(^+\) saturation were always close to 3 (e.g., Refs. 59, 122, 123). A 3.1 stoichiometry explains the positive inward current (59, 122, 123). The stoichiometry (Na\(^+\):Pi) has been determined more directly by simultaneous measurements of substrate flux and charge movement under voltage-clamp conditions in the same oocytes (123). It was found that translocation of a positive charge into the oocyte is associated with the transfer of 1 P\(_i\) and 3 Na\(^+\). These experiments also provided evidence for the preferential transport of divalent P\(_i\) anions (123).

The antiviral agent foscarin (phosphonoformic acid, PFA) is a known competitive inhibitor of brush-border membrane Na-Pi cotransport (e.g., Refs. 10, 205, 256, 375, 404). In electrophysiological studies, PFA inhibited P\(_i\)-induced inward currents but did not elicit PFA-induced currents (59, 122). Thus PFA interferes with P\(_i\) binding but is not a transported substrate. In addition, arsenate is a competitive inhibitor of brush-border membrane and oocyte type IIa Na-Pi cotransporter-mediated P\(_i\) uptake (179). In contrast to PFA, arsenate induces inward currents and is thus a transported substrate (e.g., Ref. 163). Recently, a “slippage current” associated with the transfer of the partially loaded type IIa cotransporter was identified (only with Na\(^+\), see below; Fig. 9; Ref. 122). This current was blocked by PFA and showed a dose dependence suggesting interaction with only one Na\(^+\). The slippage current accounts for \(~10\)% of maximally induced current of the fully loaded carrier (122). Although this slippage current is of little functional significance, it is important in our understanding of the transporter cycle (see sect. 1A3).

2. Pre-steady-state electrophysiological characteristics

Pre-steady-state relaxation’s resulting from the application of voltage steps to the voltage-clamped cell have been
first reported for the cloned Na\(^{+}\)-glucose cotransporter (SGLT-1; Refs. 45, 75, 167) expressed in oocytes and subsequently for many other Na\(^{+}\)-solute cotransport systems (e.g., Ref. 117). They permit an identification of partial reactions within the transport cycle. Such measurements were also performed with the rat (types IIa and IIb) and flounder isoforms (type IIb) of the type II Na-P\(_i\) cotransporters expressed in oocytes (122, 124, 125, 126). Figure 9 provides an example from a study on the rat type IIa cotransporter (122). Application of a voltage step in the presence of 96 mM Na\(^{+}\), and in the presence or absence of saturating P\(_i\), leads to current transients that are primarily due to charging oocyte membrane capacitance (Fig. 9A). Recording at a higher gain results in a slower relaxation to the steady state in the absence of Pi (Fig. 9B). Subtraction of the curves obtained in the presence/absence of Pi shows transporter cycle-dependent relaxation currents (Fig. 9C), whose magnitude could be directly related to the magnitude of P\(_i\)-induced steady-state currents in oocytes expressing different amounts of cotransporters at their surface (122). Furthermore, the P\(_i\)-induced effects on the pre-steady-state relaxation shows the same saturation characteristics (apparent K\(_{m}\)) as that ob-

![Diagram](http://example.com/diagram.png)
served for $\Pi_1$ interaction in steady-state measurements (122). These current transients are consistent with the translocation of charged entities within the transmembrane electric field. The voltage dependence of the time constants of relaxation and equivalent charge associated with the relaxation can be obtained from measurements after voltage jumps of different magnitudes from the same holding potential. With the Boltzmann equation, the transporter number, turnover number, and the apparent valency for the charge movement can be calculated, taking also into account $\Pi_1$-induced steady-state currents obtained in the same oocytes as the pre-steady-state measurements. A value of 25 s$^{-1}$ was estimated for the turnover of the rat type IIa Na-$\Pi_1$ cotransporter at −100 mV, in agreement with that measured for other Na-solute transporters (e.g., Refs. 45, 75, 117, 167); the apparent valency of the charge translocated within the relaxation cycle is −1 (122).

3. Kinetic scheme

Above steady-state measurements (electrophysiological and tracer studies) as well as electrophysiological pre-steady-state measurements led to the formulation of a kinetic scheme as shown in Figure 10 (122). The empty carrier has a valency of −1 and can interact at the extracellular surface with one Na$^+$. Translocation of the empty carrier as well as interaction with one Na$^+$ are voltage-dependent partial reactions within the transporter cycle. The carrier loaded with only 1 Na$^+$ can translocate (“slippage”); this is an electroneutral process. The Na$^+$ interaction then allows interaction with $\Pi_1$ (or with the inhibitor PFA) at the extracellular surface. Finally, the fully loaded carrier is formed by interaction with two additional Na$^+$. Translocation of the fully loaded carrier is again an electroneutral process. We have no information for distinct steps occurring at the cytoplasmic surface but assume a transition from the fully loaded to the empty state (mirror symmetry). A net inward movement of one positive charge occurs per transport cycle, due to the reorientation of the charged (−1) empty carrier. Although the carrier might interact with mono- or divalent $\Pi_1$, a preferential transport of divalent $\Pi_1$ prevails under most physiological and experimental conditions.

![Figure 9](http://physrev.physiology.org/)
4. pH dependence of transport

Proximal tubular brush-border membrane Na-P$_i$ cotransport is increased by increasing pH (e.g., Refs. 14, 52, 55, 80, 327, 328, 334, 352). Studies with brush-border membrane vesicles provided evidence that this phenomenon is to a significant extent explained by a competition of protons with sodium for an interaction with the carrier (14). Preferential transport of divalent Pi also contributes to the observed pH dependence (352). Steady-state electrophysiological measurements also suggested a competitive interaction of H$^+$ with the Na$^+$-binding site(s) (59). Pre-steady-state measurements provided evidence for an additional direct effect of H$^+$ on the carrier, on the reorientation of the empty transporter (124). As indicated in Figure 10, the pH dependence of the carrier includes a kinetic effect on the reorientation of the free carrier as well as competition for Na$^+$ binding. Thus the pH dependence of type IIa Na-P$_i$ cotransport activity is in part but cannot be fully explained by preferential transport of divalent Pi. More recent studies indicated that this pH dependence is determined by basic amino acid residues in the third extracellular loop (96).

B. Altered Expression as the Basis for Altered Pi Reabsorption

With the cloning of the type IIa Na-P$_i$ cotransporter (Npt 2), the key player in brush-border membrane Na-P$_i$ cotransport (see above and below), the tools were available for a more detailed analysis of cellular/molecular mechanisms involved in physiological/pathophysiological alterations of proximal tubular Na-P$_i$ cotransport.

The Npt 2 knockout mice (25, 178) documented clearly the importance of the type IIa Na-P$_i$ cotransporter in renal Pi handling and in the overall maintenance of Pi homeostasis. In Npt 2 knockout mice, other Na-P$_i$ cotransporters (e.g., Npt 1, Glvr-1, and Ram-1) are not upregulated (178). Furthermore, renal handling of Pi is in Npt 2 knockout mice unaffected by PTH and Pi diet (178; N.-X. Zhao and H. S. Tenenhouse, personal communication). In addition to the renal defects, Npt 2 knockout mice have intrinsic skeletal abnormalities most likely related to the lack of type IIa Na-P$_i$ cotransporter in osteoclasts (145a).

Alterations in proximal tubular Na-P$_i$ cotransport activity after induction of altered renal Pi handling in the intact organism or in the OK cell tissue-culture model are always associated with an altered apical membrane expression of the type II Na-P$_i$ cotransporter protein (fasting as an exception). In the following sections, we might first describe situations of altered expression and discuss then cellular mechanisms leading to altered brush-border expression.

1. Ontogeny/aging

Pi reabsorption in the kidney shows a strong ontogenetic/developmental as well as age-dependent behavior
(e.g., Refs. 20, 66, 160, 161, 383). Age dependence was also observed at the level of the type IIa Na-P\textsubscript{i} cotransporter protein expression (365). In kidneys of newborn rats, expression of the type IIa Na-P\textsubscript{i} cotransporter was observed in differentiated juxtamedullary and intermediate nephrons only and was absent in nephron “Anlagen” in the outer cortex (S-shaped bodies; Ref. 397). After completion of nephron formation, during suckling, expression of the transporter was similarly high in the brush-border membrane of all nephron generations. In weaning, the expression pattern resembled that in adults (238, 397), i.e., type IIa abundance decreased in the brush-border membrane of superficial and midcortical nephrons. The immunohistochemical data suggest that, as soon as nephrogenesis is completed, the type IIa transporter in the kidney is functional. As indicated above, in weaning mice, a specific transcription factor (TFE3) might contribute to the expression of the Npt2 gene, especially in low P\textsubscript{i} conditions (212a).

A specific type IIa-related Na-P\textsubscript{i} cotransporter protein was postulated to account for high P\textsubscript{i} transport rates in weaning rats (363, 364). Evidence for this postulate was obtained by antisense experiments and transport expression in X. laevis oocytes. When mRNA isolated from kidney cortex of rapidly growing rats was treated with type IIa transporter antisense oligonucleotides, or depleted of type IIa specific mRNA by a subtractive hybridization procedure, Na\textsuperscript{+}-dependent P\textsubscript{i} uptake was still detected in injected oocytes. The type IIa transporter-depleted mRNA contained a mRNA species that has some sequence homology to the type IIa transporter encoding message. This interesting observation was not followed up, and at present, the identity of this growth-related transporter is not clear and/or remains hypothetical.

Tubular P\textsubscript{i} reabsorption decreases during aging as has been indicated by metabolic balance studies, clearance studies, and studies with isolated vesicles (66, 160, 161, 214). This decrease is due to a reduction in the \( V_{\text{max}} \) without a change in the apparent \( K_m \) for P\textsubscript{i} of brush-border membrane Na-P\textsubscript{i} cotransport. The type IIa Na-P\textsubscript{i} cotransporter brush-border membrane protein mRNA abundance decreased approximatively twofold when 3-mo-old rats are compared with 12- to 16-mo-old rats, in parallel to the decrease in brush-border membrane Na-P\textsubscript{i} cotransport activity (365).

2. Regulatory control

As already indicated, physiological (and pathophysiological) alterations in renal handling of P\textsubscript{i} are related to an altered brush-border content of the type IIa Na-P\textsubscript{i} cotransporter protein.

This was observed for altered brush-border membrane Na-P\textsubscript{i} cotransport activity as observed in response to altered dietary P\textsubscript{i} intake (e.g., Refs. 48, 53, 61, 76, 78, 204, 243, 245, 246, 348, 370–372, 387, 405, 415, 434; for review, see Ref. 283). An increase in brush-border membrane Na-P\textsubscript{i} cotransporter activity in response to a low-P\textsubscript{i} diet correlated with an increase in type IIa transporter protein in Western blots and in immunohistochemistry (e.g., Refs. 48, 243, 258). The histochemical analysis suggests a “recruitment” phenomenon (Fig. 11; Ref. 348). In animals fed a high (or normal)-P\textsubscript{i} diet, expression of transporter is mostly in deep (juxtamedullary) nephrons, and in animals fed a low-P\textsubscript{i} diet, the transporter is also highly expressed in superficial nephrons. The diet-induced changes are observed at the functional and protein levels within the first 2 h, but feeding a low-P\textsubscript{i} diet for prolonged time periods also leads to a change in type IIa Na-P\textsubscript{i} cotransporter mRNA that is not observed after short time periods. Refeeding high-P\textsubscript{i} diets to animals adapted chronically to low-P\textsubscript{i} diet results in a reversal of this phenomenon, first a decrease in brush-border expression of the transporter protein without a decrease in specific mRNA content (243; see also Refs. 27, 199, 376, 387, 415). The type I Na-P\textsubscript{i} cotransporter does not show such alterations (e.g., Refs. 48, 405). Similar findings could also be obtained in OK cells, i.e., an increase in type IIa protein content in response to a low-P\textsubscript{i} media occurred within hours, in parallel with an increase in Na-P\textsubscript{i} cotransport activity (e.g., Refs. 266, 309). The findings on the response of OK cell-specific type IIa transporter mRNA to low-P\textsubscript{i} diet are controversial (266, 309, 353, 354). In our laboratory, the low-P\textsubscript{i} diet-induced changes in specific mRNA content were rather small, and transport adaptation was not prevented by actinomycin D (41, 266, 309). Furthermore, the adaptive phenomena were also observed in OK cells with the transfected rat type IIa cotransporter on the protein but not mRNA level (309).

An altered PTH status in the animal is associated with an altered brush-border expression of the type IIa Na-P\textsubscript{i} cotransporter as analyzed by Western blots of isolated brush-border membrane vesicles or by immunohistochemical staining on kidney sections (210). Injection of PTH in rats or mice leads within minutes to a reduction in brush-border membrane transporter content without a concomitant loss of other brush-border proteins, e.g., the Na-sulfate cotransporter protein (Fig. 12; Refs. 210, 257, 259; see also Ref. 265). A prolonged increase in PTH can also lead to a decrease in type II Na-P\textsubscript{i} cotransporter mRNA content (210). Also in OK cells, PTH leads to a decrease in the apical expression of the intrinsic and transfected rat type IIa Na-P\textsubscript{i} cotransporters (235, 308–311).

A role for insulin has been postulated for the adaptive response of the proximal tubule to changes in dietary P\textsubscript{i} intake (247). In vitro and in vivo experiments provided evidence that insulin stimulates brush-border membrane Na-P\textsubscript{i} cotransport. Streptozotocin-induced diabetes is associated with a reduced proximal tubular P\textsubscript{i} reabsorption,
a decreased type I Na-P_i cotransporter content, and no change in either type IIa or III Na-P_i cotransporter content (247). It is, however, questionable whether phosphaturia in diabetes can be explained by a reduced type I Na-P_i cotransporter expression. Phosphaturia could rather be explained by an inhibition of type IIa Na-P_i cotransport activity related to a competition for driving forces (increase in glucose reabsorption; see Refs. 22, 392). In streptozotocin-induced diabetes, the adaptive response (dietary intake) of the type IIa Na-P_i cotransporter was prevented (1, 7, 358).

Thyroid hormone (3,3’,5-triiodothyronine; T_3) increases proximal tubular brush-border membrane and OK cell apical Na-P_i cotransport (e.g., Refs. 31, 107, 118, 213, 433); a parallel increase in PFA binding sites suggested an increased brush-border membrane content of transporters (433, 434). Euzet et al. (119) have shown an important role for T_3 in the maturation of the type IIa Na-P_i cotransporter (119). It was shown that physiological doses of T_3 lead in rats to an increase in brush-border membrane Na-P_i cotransport activity, type IIa transporter protein content, and specific mRNA content (6). The stimulatory effect of T_3 is less evident in aging animals (6). Nuclear run-on experiments provided evidence that these effects are due to an increased transcription of the transporter gene (6). In OK cells, the stimulatory effect of T_3 was completely prevented by the transcriptional inhibitor actinomycin D (367).

IGF-I directly stimulated OK cell Na-P_i cotransport.
associated with an increase in specific type IIa Na-P\textsubscript{i} cotransporter content, an effect most likely involving activation of tyrosine kinase activity (193).

EGF caused a time- and dose-dependent decrease in OK cell Na-P\textsubscript{i} cotransport rate, an effect associated with a decrease in type IIa Na-P\textsubscript{i} cotransporter mRNA abundance (15). This observation is in agreement with findings on weaned and suckling rats but in disagreement with observations on isolated perfused rabbit proximal tubules where EGF stimulates Na-P\textsubscript{i} cotransport (17, 336).

Glucocorticoid administration to rats and neonatal rabbits decreased rat brush-border membrane Na-P\textsubscript{i} cotransport activity and kidney cortex type IIa transporter content (16, 244, 254, 320). Also in OK cells glucocorticoids led to a reduction in Na-P\textsubscript{i} cotransport activity and associated transporter protein content (192; see also Ref. 156a).

Fasting-induced phosphaturia leads to decreased expression of type I Na-P\textsubscript{i} cotransporter mRNA but to no change in type IIa mRNA content (247). However, it is unlikely that a decreased type I Na-P\textsubscript{i} cotransporter content accounts for the phosphaturia observed in fasting conditions (28). As indicated above, the type IIa Na-P\textsubscript{i} cotransporter determines overall renal P\textsubscript{i} handling.

Acid-base changes also induce alterations in the expression of the type IIa Na-P\textsubscript{i} cotransporter. Chronic metabolic acidosis in rats significantly decreased brush-border membrane Na-P\textsubscript{i} cotransport activity, associated with a decrease in type IIa cotransporter protein and mRNA content (11). At the onset of acute (6 h) metabolic acidosis, changes in transport activity and brush-border transporter protein content were not paralleled by a change in cortical tissue type IIa Na-P\textsubscript{i} cotransporter mRNA or protein (11). This may indicate that in acute situations changes in membrane trafficking of the type IIa Na-P\textsubscript{i} cotransporter (see below) contribute to the acid-induced changes. Interestingly, in OK cells, we have found an opposite effect of exposure to an acid medium (192, 194). Transport activity, transporter protein, and mRNA content were all increased; the increase was, however, pre-

![FIG. 12. Parathyroid hormone (PTH)-dependent retrieval of type IIa Na-P\textsubscript{i} cotransporters from the brush-border membrane. Rats with removed parathyroid glands (PTX) were injected with PTH, and kidney slices were then stained for the type IIa Na-P\textsubscript{i} cotransporter (NaPi-2) or for the Na-SO\textsubscript{4} cotransporter (NaSi-1; Ref. 265). It was seen that the type IIa Na-P\textsubscript{i} cotransporter is removed from the brush-border membrane in response to PTH administration. At early time periods (35 min), a subapical appearance of the transporter is visible. The Na-SO\textsubscript{4} cotransporter is not removed from the brush-border membrane in response to PTH administration. For further reading, see text and included references.](http://physrev.physiology.org/DownloadedFrom/Paper/10.220.33.6/1392-MURER-HERNANDO-FORSTER-AND-BIBER-Volume-80)
vented or reversed by adding glucocorticoids (192). This indicates that the above observations in rat might be the result of acid base-induced changes in glucocorticoid levels rather than of direct cellular effects (see also Refs. 47, 127).

3. Acquired alterations

Proximal tubular brush-border membrane Na-P\textsubscript{i} cotransport is sensitive to intoxication by heavy metals (5, 141, 255, 321). A direct acute inhibitory effect of Hg\textsuperscript{2+}, Pb\textsuperscript{2+}, and Cd\textsuperscript{2+} has been shown in X. laevis oocytes expressing the type IIa Na-P\textsubscript{i} cotransporter (408). Hg\textsuperscript{2+} and Cd\textsuperscript{2+} effects were mostly on $V_{\text{max}}$ effects, but Pb\textsuperscript{2+} also increased the apparent $K_m$ for P\textsubscript{i} (408). It will be of interest to determine whether the inhibitory effect can be assigned to specific sulphydryl groups, which would then also explain the observations in brush-border membranes (Refs. 255, 321; e.g., also in studies with cisplatin, Ref. 90). In vivo intoxication of rats with cadmium reduced brush-border membrane Na-P\textsubscript{i} cotransport rate accompanied by a loss of the type IIa Na-P\textsubscript{i} cotransporter protein (169).

4. Genetic abnormalities

Among the different genetically determined alterations in renal P\textsubscript{i} handling, only X-linked defects have been characterized at the level of brush-border membrane P\textsubscript{i} transporters. Studies on the murine Hyp and Gy homologs of the human disease X-linked hypophosphatemia have identified a specific reduction in brush-border membrane Na-P\textsubscript{i} cotransporter activity that was associated with a reduction in type IIa Na-P\textsubscript{i} cotransporter protein and specific mRNA content, thereby accounting for the P\textsubscript{i} leak in affected mice (27, 384, 385, 389, 393; see also Refs. 87–89). Because the Npt 2 gene does not map to the X-chromosome (see above and Refs. 222, 223, 437), a defect in a gene on the X-chromosome must influence the renal brush-border expression of the type IIa Na-P\textsubscript{i} cotransporter (for review, see Ref. 384). This mutant gene has been identified in affected humans (106, 110, 112, 185) by linkage/positional cloning and subsequently in PEX (formerly PEX/Pex) to signify phosphate regulating gene homology to endopeptidases on the X-chromosome. The PHEX/Phex protein is not expressed in the kidney but rather in bones (26). On the basis of its homology to a membrane-bound endopeptidases, it is postulated that PHEX/Phex is involved in the processing of humoral factor(s) (e.g., phosphatonin and stanniocalcin 1/2; see also Refs. 72, 105, 111, 189, 224, 225, 272) which regulate renal P\textsubscript{i} handling by altering the type IIa Na-P\textsubscript{i} cotransporter expression, and P\textsubscript{i} metabolism in general (for review, see Refs. 110, 112, 295, 384). In Hyp mice, a decreased transcription rate of the type IIa Na-P\textsubscript{i} cotransporter gene was observed (89). In Hyp and Gy mice, different gene deletions were identified: a 3′-deletion in Hyp and a 5′-deletion in Gy (26, 273).

C. Cellular Mechanisms in the Control of Type II Na-P\textsubscript{i} Cotransporter Expression

As discussed in section vB, changes in renal P\textsubscript{i} handling are attributable, for the most part, to altered brush-border membrane expression of the type IIa Na-P\textsubscript{i} cotransporter. It is apparent that alterations in the type IIa Na-P\textsubscript{i} cotransporter expression occur independently of transcriptionally regulated changes in mRNA, perhaps with the exception of T\textsubscript{g} (6, 367), 1,25(OH)\textsubscript{2}D\textsubscript{3}-mediated effects (8, 380), or prolonged feeding of low-P\textsubscript{i} diets in weaning mice (212a). If changes in type IIa Na-P\textsubscript{i} cotransporter mRNA are observed, they are either rather small or they occur only after prolonged stimulation (e.g., Refs. 210, 243), i.e., after changes in the specific transporter protein content, suggesting that changes in mRNA represent a phenomenon secondary to the primary event, i.e., downregulation or upregulation of brush-border expression of type IIa Na-P\textsubscript{i} cotransporter protein. In agreement with this apparent lack of transcriptional control mechanisms are the negative observations with promoter/reporter gene studies in OK cells (e.g., Refs. 174, 176), where with the above-mentioned exceptions of alterations in 1,25(OH)\textsubscript{2}D\textsubscript{3} (380), of the low-P\textsubscript{i} diet-dependent increase in TFE3 in weaning mice (212a), or in ambient bicarbonate/carbon dioxide tension (194) of the media, no activation of the NPT 2/Npt 2 promoter could be detected. Therefore, it is appropriate to restrict the discussion primarily to mechanisms leading to altered membrane expression, i.e., mainly to membrane retrieval and membrane insertion of the type IIa Na-P\textsubscript{i} cotransporter (for review, see Refs. 286, 288). The crucial role of “membrane trafficking” in the control of brush-border membrane expression of the type IIa Na-P\textsubscript{i} cotransporter offers the potential for physiological/pathophysiological control of Na-P\textsubscript{i} cotransport involving alterations in the participating complex machinery. In this way, for example, an endosomal chloride channel defect in Dent’s disease may affect membrane insertion and thus lead to phosphaturia (252, 253, 391).

1. Membrane retrieval

The phenomenon of membrane retrieval of the transporter can be best studied in PTH-induced or high-P\textsubscript{i} diet-induced downregulation of brush-border membrane Na-P\textsubscript{i} cotransporter activity and type IIa Na-P\textsubscript{i} cotransporter protein abundance (see Figs. 11 and 12 and Refs. 208, 210, 257, 259; see also Refs. 180, 209, 211, 306). The data from immunohistochemical (immunogold) studies on PTH-induced internalization are summarized in Figure 13 (398).
In rats, infusion of PTH or feeding high-Pi diets leads to a transient accumulation of type IIa Na-Pi cotransporters in the so-called subapical vacuolar apparatus (83, 164). Internalization can occur at intermicrovillar clefts via clathrin and adapter protein (AP-2) containing membrane cargos (398). The same endocytic structures can be filled by horseradish peroxidase injected into the animals before PTH treatment (398). Thus type IIa Na-Pi cotransporters are internalized via the same pathway as soluble proteins.

Evidence from experiments with cholchicine-pretreated rats indicates that there might be an additional “unknown” internalization step, separate from the intermicrovillar clefts (259; Fig. 13). Morphological and biochemical data suggest that internalized type IIa Na-Pi cotransporters are directed to the lysosomes for degradation (212, 311, 398). Although recycling of transporters back to the membrane cannot be entirely excluded, under the experimental conditions applied, no evidence for such a reutilization of internalized transporters could be obtained in studies on OK cells (262, 311). Surface biotinylation experiments performed in OK cells provided direct evidence that the increase in intracellularly located transporters after PTH treatment is related to a decrease of transporters at the cell surface (191). An alternate mechanism that would lead to reduced transporter expression at the surface would have been degradation at the membrane site and/or a reduced insertion rate (see sect. V C2). For further details, see text and included references. The NaPi cotransporter may also be internalized via a non-clathrin-mediated pathway (dashed line; see text). [Adapted from Traebert et al. (398).]

2. Membrane insertion

This phenomenon can be best analyzed in rats adapted to high-Pi diet and fed “acutely” a low-Pi diet (e.g., Fig. 11; e.g., Refs. 243, 258). The increase in brush-border type IIa Na-Pi cotransporter content is observed within 1–2 h and can also be observed in OK cells after exposure to media with adjusted Pi contents (309). This rapid increase occurs independently of any change in specific type IIa Na-Pi cotransporter mRNA content (243, 309). In OK cells, this adaptive response could be prevented by blocking protein synthesis at the translational level (e.g., Ref. 41). In contrast, in animal models, pretreatment with
cycloheximide did not prevent upregulation of brush-border membrane type IIa Na-Pi cotransport activity and protein (242, 245, 246, 258). However, it has not been shown whether protein synthesis is indeed blocked in proximal tubular cells after intraperitoneal injection of cycloheximide. Upregulation of Na-Pi cotransporter activity and type IIa transporter protein content is obviously also observed after recovery from PTH inhibition. In OK cells it was found that the recovery of Na-Pi cotransport activity and type II Na-Pi transporter protein is entirely dependent on protein synthesis (262, 311). In chronic parathyroidectomized rats, a specific upregulation of type IIa Na-Pi cotransporter protein was also observed in the absence of changes in specific mRNA content (210).

3. Involvement of microtubules

In contrast to the known dependence of the endocytosis of soluble proteins on an intact microtubular network (115), the internalization of type IIa Na-Pi cotransporters in response to either PTH or acute high-Pi diet feeding was not impaired (258, 259) by agents disturbing microtubules. Similar observations were made in OK cells (158). In contrast, the intracellular routing of the transporter to the lysosomes depends on an intact microtubular network (258), in agreement with the important role of microtubules for apical routing of post-Golgi vesicles and for the maintenance of the dense tubular subapical network (115, 270). It is of interest that PTH and altered medium Pi content were found to have profound effects on the cytoskeleton of cultured proximal tubular cells (139, 258, 259, 304).

4. Signaling pathways

A) CAMP AND DIACYLGLYCEROL/INOSITOL TRISPHOSPHATE (CALCIUM). cAMP- and diacylglycerol (DAG)/inositol trisphosphate (IP₃)-related signaling pathways in the regulation of brush-border membrane type II Na-Pi cotransporter activity and expression have been studied in relation to PTH-dependent regulation of proximal tubular Pi reabsorption. PTH and PTH-related peptide receptors, respectively, are located in both apical and basolateral membranes of proximal tubular epithelial cells and of OK cells (e.g., Refs. 68–70, 73, 85, 86, 186, 200, 215, 261–264, 331–333, 341, 342, 346; for review, see Refs. 280, 283). The PTH receptor can signal through activation of both adenylate cyclase and phospholipase C, generating the second messengers cAMP, IP₃, a rise in intracellular Ca²⁺, and DAG (e.g., Refs. 2, 183, 271). The PTH-(1–34) analog activates both signaling pathways, whereas the PTH-(3–34) analog only activates the cAMP-independent signaling pathway (e.g., Refs. 85, 86, 235, 308). Although pharmacological activation of either pathway separately leads to an inhibition of apical Na-Pi cotransport activity associated with a decrease in brush-border membrane type IIa Na-Pi cotransporter protein content (e.g., Refs. 235, 264, 308), there seems to be an interdependence of the two regulatory pathways. In OK cells, abolition of the cAMP/protein kinase A pathway prevents PTH inhibition (267, 268, 357); similarly, inhibition of the DAG/protein kinase C pathway also reduces PTH inhibition of the Na-Pi cotransporter (330, 333). Furthermore, in OK cells, the maximal inhibition induced by PTH-(3–34) is only ~50% of the maximal PTH-(1–34)-induced inhibition (85, 86, 235, 308).

Although most observations on PTH-dependent signaling mechanisms were derived from studies with OK cells, similar mechanisms may be operative in the proximal tubule; PTH-(3–34) similar to PTH-(1–34) leads to a reduction in apical type IIa Na-Pi cotransporter content in intact rats and in vitro perfused mice proximal tubules (399). Furthermore, the experiments with the isolated perfused tubules showed that PTH-(1–34) leads to a reduction in brush-border transporter content after perfusion either through the lumen or basolateral side, whereas PTH-(3–34) only induces this reduction after apical perfusion only (399). It is of interest that earlier studies on OK cells have also provided evidence for asymmetrical signaling mechanisms in PTH control of apical Na-Pi cotransport (341, 342). The experiments with isolated perfused tubules also documented the involvement of protein kinases A and C in internalization of the apical Na-Pi cotransporter (399).

The events after second messenger generation leading to inhibition and/or retrieval of the type IIa Na-Pi cotransporter are not clear. Obviously, phosphorylation events do play a role, but there is no direct evidence that altered phosphorylation of the type IIa Na-Pi cotransporter protein is essential in the regulation (42, 152, 182). Recently, in studies in OK cells, it has been shown that the type IIa transporter is a phosphoprotein. However, PTH-induced alterations in the type IIa Na-Pi cotransporter phosphorylation are difficult to document (M. Jankowski, H. Hiefiker, J. Biber, and H. Murer, unpublished observations). Because there are several potential phosphorylation sites within the protein sequence, changes at individual sites need to be analyzed. It is of interest that in X. laevis oocytes, activation of protein kinase C leads to an internalization of the transporter and to an associated inhibition of transport (125). This inhibition/internalization is not prevented after mutagenesis of known kinase consensus sites, indicating that at least in this experimental model, the phosphorylation consensus sites are not involved in this regulation (165). Finally, it is reasonable to assume that PTH-induced regulatory phosphorylation events are not at the level of the transporter but rather at interacting proteins required for its regulation (see sect. V.C.4.b). In this respect it is of interest that regulation by PTH and/or pharmacological activation of protein kinases...
A/C requires the correct cellular context; transfection experiments showed that only the OK cells provide the correct cellular environment for this regulation of the type IIa Na-P\textsubscript{i} cotransporter (Z. Karim-Jimenez, N. Hernandez, H. Murer, and J. Biber, unpublished data). Such dependence on an interacting protein has also been shown for brush-border membrane Na/H exchange (NHE-3) and its kinase A-induced inhibition (412, 413, 428; see also Ref. 293).

Extracellular cAMP (intratubular) is known to inhibit proximal tubular and OK cell apical Na-P\textsubscript{i} cotransport. This effect can be related to specific receptor interactions and intracellular signaling or more likely to luminal hydrolysis of cAMP followed by uptake of adenosine, which might after incorporation into ATP be the source for additional intracellular cAMP (130, 131, 132; see also Refs. 274, 361). A role of intratubular cAMP, generated in the liver after stimulation by glucagon, has been suggested for in vivo control of P\textsubscript{i} reabsorption (3). It is unclear at this moment how these observations and interpretations can be reconciled with the stimulatory effect of adenosine on tubular P\textsubscript{i} reabsorption in rats (312).

Alterations in cytoplasmic calcium concentrations per se are not sufficient/important in PTH-induced signaling of OK cell apical Na-P\textsubscript{i} cotransport; this has been documented using Ca\textsuperscript{2+}-clamping protocols (332). Saxena and Allon (353) have postulated that an increase in steady-state cytosolic calcium plays a role in “chronic” adaptation of OK cell Na-P\textsubscript{i} cotransport to low-P\textsubscript{i} media.

b) \( \text{P}_\text{i} \) Sensing. As discussed above, brush-border membrane type IIa Na-P\textsubscript{i} cotransporter protein content, and thus proximal tubular P\textsubscript{i} reabsorption, responds within hours to alterations in dietary P\textsubscript{i} intake (242, 243, 258). This phenomenon is also observed in OK cells, induced by alteration in media P\textsubscript{i} content (41, 309). In the animal models the adaptive phenomena might be in part related to alterations in different humoral factors and local auto/paracrine factors, although PTH can be excluded as a major factor (see above; for review, see Ref. 37). In this regard, it is interesting that intravenous injections of P\textsubscript{i} are sufficient to induce alterations in brush-border membrane Na-P\textsubscript{i} cotransport rate in thyroparathyroidectomized rats (76; for review, see Refs. 46, 283). Because alterations in dietary P\textsubscript{i} intake are paralleled by rapid changes in plasma P\textsubscript{i} concentration and thus of filtered P\textsubscript{i} load (61, 316), the question of direct P\textsubscript{i} sensing by the cells has to be considered, as indicated by the experiments on OK cells. Experiments with OK cells provided an interesting clue on the potential mechanisms in P\textsubscript{i} sensing by the proximal tubular cell. In cells grown on permeant filters, depletion of P\textsubscript{i} at the apical site was sufficient to provoke an adaptive increase of apical Na-P\textsubscript{i} cotransport rate, whereas removal of P\textsubscript{i} only from the basolateral cell surface was without effect (339, 340). Thus there is the possibility of a P\textsubscript{i} sensing mechanism at the apical surface. Because the Ca\textsuperscript{2+} sensor (Ca\textsuperscript{2+} receptor CaR) is expressed at the apical cell surface of proximal tubular cells, it may “indirectly” be involved in tubular P\textsubscript{i} sensing, because changes in P\textsubscript{i} and Ca\textsuperscript{2+} concentrations are interdependent (51, 347). Another possibility is that the rate of P\textsubscript{i} entry at the apical cell surface contributes to P\textsubscript{i} sensing. It was observed that inhibition of OK cell Na-P\textsubscript{i} cotransport after prolonged exposure to PFA results after PFA removal in an “adapted” state of Na-dependent P\textsubscript{i} uptake (J. Forgo and H. Murer, unpublished observations). Similarly, reduced P\textsubscript{i} entry in response to PTH inhibition could provide a signal for the preferred resynthesis of the type IIa cotransporter resulting in recovery of Na-P\textsubscript{i} cotransport rates after removal of PTH. In this case, the higher transport rates after recovery would then serve as feedback to again slow down the rate of transporter resynthesis. Finally, the adaptive response of Na-P\textsubscript{i} cotransport rate to low P\textsubscript{i} intake (or low-P\textsubscript{i} media) may also involve a more general signaling that is related to changes in intracellular P\textsubscript{i} metabolism after lowering of apical (and/or basolateral) P\textsubscript{i} (20, 102; see also Ref. 38). In addition, alterations in cytosolic Ca\textsuperscript{2+} concentrations were postulated to be part of the P\textsubscript{i}-sensing mechanisms (353).

c) Ubiquitinylation. Membrane retrieval is involved in regulation of the type IIa Na-P\textsubscript{i} cotransporter. Membrane retrieval of a variety of membrane proteins, including membrane receptors and perhaps also transporter proteins, e.g., the epithelial Na\textsuperscript{+} channel, can depend on ubiquitinylation, which can be a signal for endocytosis followed by lysosomal and/or proteosomal degradation (368, 369; see also Ref. 173). In OK cells, lysosomal degradation, but not proteosomal degradation of the type II Na-P\textsubscript{i} cotransporter protein, is involved in its PTH-dependent downregulation (311). Proteosomal (and lysosomal) degradation seems also to be involved in the basic turnover of the OK cell type IIa cotransporter (311). Ubiquitinylation of the transporter itself or of interacting proteins can be involved in membrane retrieval and/or targeting the type IIa transporter to the degradation machinery.

d) Intracrine Regulation. An intracrine regulation pathway was postulated to control apical expression of type IIa Na-P\textsubscript{i} cotransporter (107; see also Refs. 29, 30, 207, 427). Different metabolic and/or hormonal stimuli may lead to the synthesis of cyclic ADP-ribose, which in turn may initiate an intracellular regulatory cascade, with the release of Ca\textsuperscript{2+}, activation of a Ca\textsuperscript{2+}/calmodulin-dependent protein kinase, and changes in the turnover of the transporter at the apical membrane (107).

e) Phosphatidylinositol 3-Kinase. Phosphatidylinositide 3-kinase (PI 3-kinase) seems to be involved in various membrane trafficking processes. In OK cells, inhibition of PI 3-kinase leads to a reduction of endocytosis of albumin (54). However, endocytosis of type IIa Na-P\textsubscript{i} cotransporter in OK cells and its degradation by PTH are not
prevented by inhibition of PI 3-kinase (307). On the other hand, inhibition of PI 3-kinase retards the recovery from PTH inhibition and reduces transport activity and transporter protein content in control cells (307). Thus PTH-induced endocytosis of the type IIa Na-P_i cotransporter occurs via a different pathway than albumin endocytosis. PI 3-kinase activity may be involved in the resynthesis/reinsertion of apically located transporters.

F) Tyrosine Kinase Activity. In OK cells, vanadate, a potent inhibitor of protein tyrosine phosphatases, mimicks the stimulatory effect of IGF-I on Na-P_i cotransport activity (63, 65). Similarly, IGF-I and vanadate increase the membrane abundance of type IIa Na-P_i cotransporter protein content (193). The effects on transporter protein content could not be prevented by actinomycin D and cycloheximide (193). The latter observation led to the conclusion that tyrosine kinase-dependent mechanisms may control the membrane stability (i.e., reduce the retrieval rate) of the type IIa Na-P_i cotransporter (193). Tyrosine kinase activity may also be involved in TGF-α and EGF effects on apical Na-P_i cotransport activity (15, 314). However, the participation of phospholipase C/protein kinase C has also been proposed for the TGF-α- and/or EGF-mediated effects (335–337).

5. Endocytic motifs

Tyrosine based and dileucine signals within the type IIa Na-P_i cotransporter sequence might be important motifs for the endocytic removal and/or membrane targeting, in a manner similar to some “model” proteins in polarized epithelial cells (270). In mutagenesis experiments on the type IIa Na-P_i cotransporter, various tyrosine-based motifs and dileucine motifs located at predicted intracellular sites were found not to be essential for internalization in oocytes and transfected OK cells (170–172). Because the type IIb transporter (intestinal isoform) transfected in OK cells is also apically targeted but not internalized by PTH (171, 197, 198), endocytic motifs/domains can be identified on studies of type IIa/IIb chimeras; these studies suggested a critical role of a dibasic motif located in the last intracellular loop (198; see Fig. 7).

6. Lipids

Alterations in brush-border membrane lipid composition have been associated with altered brush-border membrane Na-P_i cotransport (e.g., Refs. 240, 241, 246, 279, 406). Changes in membrane fluidity and a role of a sphingomyelin-ceramide regulatory pathways were postulated (157, 221, 237, 251, 435). Chronic adaptation to low-P_i diet was reported to alter cholesterol and glycosphingolipid content and thus membrane fluidity (240, 241). However, alterations in Na-P_i cotransport induced by acute changes in low-P_i diets occur before the changes in brush-border lipid composition and thus are not essential for the adaptive response (245, 246). In rat studies, evidence was provided that the dexamethasone-induced inhibition of brush-border membrane Na-P_i cotransport is associated with a decrease in transporter protein content and an increase in glucosylceramide (244). Taken together, it seems unlikely that transport alterations are directly related to changes in membrane fluidity, but alterations in lipid composition, e.g., in microdomains such as glycolipid-cholesterol rafts, may facilitate internalization of the type IIa Na-P_i cotransporter.

Recently, it was shown that different inhibitors of P-glycoprotein functions stimulate proximal tubular P_i reabsorption related to an increased expression of type IIa Na-P_i cotransporter protein. It is speculated that this effect is associated with drug-induced changes in the sphingomyelinceramide pathway and associated changes in the membrane traffic of the transporter protein (D. Prié, S. Couette, I. Fernandes, C. Sieve, and G. Friedlander, personal communication).

7. Reduction of S-S bridge

In Western blots of isolated brush-border membranes, a significant proportion of the type IIa cotransporter appears to be cleaved under reducing conditions, suggesting that the transporter is stabilized by a S-S bridge (e.g., Refs. 39, 48, 91, 229, 305, 425). The degree of transport inhibition under reducing conditions correlated with the appearance of cleaved moieties (425). Internalization and cleavage of the transporter can also be induced in X. laevis oocytes by a treatment with reducing agents (230). On the basis of these findings, we suggest that a reduction of S-S bridge within the type IIa Na-P_i cotransporter may contribute to its basal turnover and perhaps regulation. Reduction of the S-S bridge within the second extracellular loop (Fig. 7) may destabilize the secondary structure of the type IIa transporter protein followed by a degradation at the cell surface or after internalization. Reducing agents might enter the proximal tubular lumen by the secretory mechanism for organic anions.

8. Interacting proteins

Type IIa Na-P_i cotransporters are located among many other membrane proteins along the microvilli. However, in physiological regulation they are specifically internalized at intermicrovillar clefts (210, 257, 259, 398). Conceptionally there should be “anchoring” mechanisms keeping proteins within the microvilli “en place.” In regulation, the interaction between specific anchor (interacting) proteins and specific membrane proteins (e.g., the type IIa Na-P_i cotransporter) could be controlled, permitting via lateral mobility the protein to enter into clefts where it is removed. Alternatively, unrestricted lateral mobility could allow the transporter protein to enter al-
ways into the cleft region and transporters to be removed would then be posttranscriptionally modified (e.g., phosphorylation, ubiquitinylation; see above) providing a signal for membrane retrieval of the transporters to be downregulated. Also, this latter mechanism would require specific proteins to recognize (“interact” with) the transporter protein. PDZ-domain containing proteins have been described to interact with the brush-border membrane Na/H exchange NHE-3 and to be required for its kinase-mediated regulation (293, 412, 413, 428); such regulation also involves the internalization of the NHE-3 exchanger. Interestingly, we recently identified a PDZ-domain containing protein (diphor-1) that colocalized with the type IIa Na-Pi cotransporter in brush borders and interacting with the COOH-terminal cytosolic portion of the molecule (93, 418, 418a). When coexpressed in oocytes with the type IIa Na-Pi cotransporter protein, a diphor-1-induced specific increase in Na-Pi cotransport activity was observed without a stimulatory effect on Na-SO₄ cotransport after coexpression with a cloned Na-SO₄ cotransporter (93). Interacting proteins might be required for brush-border expression of type II Na-Pi cotransporter and/or for its regulation. However, the precise role of above-mentioned PDZ-domain containing proteins (e.g., diphor-1) in controlling the apical expression of the transporter is not yet established.

VI. SUMMARY AND OUTLOOK

The type IIa Na-Pi cotransporter located in the renal proximal tubular brush-border membrane is the key player in renal Pi reabsorption and thus also in overall Pi homeostasis. Physiological regulation of the type IIa Na-Pi cotransporter is responsible for altered renal Pi handling and is mostly directly related to its altered brush-border expression. The latter involves complex membrane retrieval and reinsertion mechanisms. Its expression can also be abnormally regulated in different disease states (e.g., in X-linked hypophosphatemia). Transport activity mediated by the type IIa transporters in the brush-border membrane can also be controlled by changes in intratubular/intracellular pH, in transmembrane potential difference, and posttranslational modification (e.g., protein phosphorylation, reduction of S-S bridge). Finally, the type IIa Na-Pi cotransporter gene might also be directly affected in autosomal disorders in renal Pi handling.

With the cloning of the type IIa cotransporter, tools and information (gene location and structure) become available to dissect physiological regulation and pathophysiological alterations at the molecular level. In the future, studies on the structure/function relationship will provide new insights into the mechanisms of cotransporter regulation/function. Several novel approaches to understand the mechanisms determining the specific retrieval of the transporter and the specific steps within the transport cycle were discussed. Finally, for an understanding of renal Pi handling at a molecular level, the in vitro molecular studies (e.g., in oocytes) have to be reintegrated into the function of the entire cell, nephron, organ, and animal. Tissue-culture experiments, studies on isolated perfused tubules, and experiments on animals (rats and/or mice; normal and/or genetically modified) are essential to develop a more detailed understanding of renal Pi handling.

This review is devoted to many co-workers and collaborators contributing to this work over the last 10 years. The excellent secretarial help of Denise Neukom-Rossi and the excellent art work done by Christian Gasser are gratefully acknowledged. We also specifically acknowledge the excellent collaborations with the laboratories of Dr. M. Levi (Dallas), Dr. H. S. Tenenhouse (Montreal), Dr. S. Kempson (Indianapolis), and Dr. B. Kaissling (Zürich). A special thank goes to H. S. Tenenhouse and M. Levi for critical reading and correction of the manuscript.

This review is in memory of our dear colleague, Thomas P. Douya (10/01/96), a leading investigator in the field of renal phosphate handling.

Major financial support by the Swiss National Science Foundation is gratefully acknowledged.

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