Disorders of Renal Tubular Phosphate Transport

HARRIET S. TENENHOUSE* and HEINI MURER†
*Departments of Pediatrics and Human Genetics, McGill University, Montreal Children's Hospital Research Institute, Montreal, Canada; and †Department of Physiology, University of Zurich, Zurich, Switzerland.

The kidney plays a major role in the maintenance of inorganic phosphate (Pi) homeostasis and, as such, ensures that an adequate supply of this ubiquitous anion is available for proper cellular function and skeletal mineralization. Physiologic studies have revealed that the bulk of filtered Pi is reabsorbed in the proximal tubule, with higher rates of reabsorption in the early segments and in deep nephrons. This review will summarize the progress that has been made in the molecular identification and regulation of renal Pi transporters. In addition, it will focus on the pathophysiology of inherited (X-linked hypophosphatemia [XLH], autosomal dominant hypophosphatemic rickets [ADHR], and hereditary hypophosphatemic rickets with hypercalcemia [HHRH]) and acquired (oncogenic hyperphosphatemia [OHO]) renal Pi wasting disorders, and discuss the role of two novel Pi-regulating genes, PHEX and FGF-23, in the regulation of Pi homeostasis. For recent reviews see references 1–5.

Cellular Mechanism of Proximal Tubular Pi Reabsorption

Pi reabsorption in the proximal tubule is mediated by Na+-dependent, secondary-active transport mechanisms. Influx of Pi from the tubular lumen into the epithelial cell involves brush border membrane-associated Na/Pi cotransporter(s) which are rate-limiting and targets for physiologic/pathophysiologic regulation. Efflux of Pi across the basolateral membrane may involve an anion exchange mechanism and/or a "Pi leak" to complete transcellular reabsorptive flux, and a Na+-dependent Pi uptake mechanism to guarantee Pi uptake from the interstitium if apical influx is insufficient to maintain cellular metabolism [for review see (1)].

Na/Pi Cotransporters

Three distinct and unrelated families of mammalian Na/Pi cotransporters have been identified: type I, type II, and type III (solute carrier series SLC17, SLC34, and SLC20, respectively, in the human gene nomenclature database [http://www.gene.ucl.ac.uk/nomenclature]). All three types are expressed in proximal tubular cells and have the capacity to induce an increase in Na-dependent Pi uptake in heterologous expression systems (e.g. Xenopus laevis oocytes) (for review, see references 1 and 6).

The type I Na/Pi cotransporter is localized in the brush border membrane of proximal tubular cells (7). It is unlikely, however, that this transporter is an important player in proximal tubular Pi flux. Heterologous expression studies demonstrated that type I-mediated Na/Pi cotransport does not have the characteristics and regulatory features of brush border membrane Na/Pi cotransport, and suggested a channel function, mediating the flux of chloride and organic anionic compounds (1,8,9).

Three closely related type II Na/Pi cotransporters are expressed in the apical membrane of absorptive/reabsorptive epithelia: type IIa (10), type IIb (11), and type IIc (12). Type IIa is more abundant in kidneys of adult animals and type IIc in kidneys of young animals and neither is detected in the intestine (12). Type IIb is expressed in small intestine and type II alveolar cells, but not in kidney (11).

A key role for the type IIa transporter in renal Pi handling in adult animals is supported by the following observations: (1) exclusive proximal tubular brush border membrane localization (13); (2) disruption of the gene encoding type IIa in mice (Npt2−/−) leads to impaired renal Pi reabsorption and a 70 to 80% loss of brush border membrane Na/Pi cotransport (14); (3) abundance of type IIa protein in the brush border membrane correlates with Na/Pi cotransport activity under a variety of physiologic/pathophysiologic conditions (for review, see reference 1); (4) residual brush border membrane Na/Pi cotransport in Npt2−/− mice is not responsive to regulatory phenomena (e.g., parathyroid hormone [PTH] and dietary Pi intake [15,16]; see below). A candidate for residual Na/Pi cotransport activity in brush border membranes of type IIa knockout mice is the type IIc cotransporter (12). In favor of this interpretation is the similarity between type IIa and type IIc with regard to inter- and intra-nephron expression, namely higher in deep/juxtamedullary nephrons as compared with superficial/cortical nephrons, and proximal tubular segment localization, namely higher in S1/S2 segments as compared with S3 segments of proximal tubules ([12,13]; unpublished observations). In addition, the demonstration that type IIc protein abundance is 2.7-fold higher in renal brush border membranes of Npt2−/− mice, relative to wild-type littermates, provides support for this hypothesis (17).
The type III Na/Pi cotransporters are cell-surface viral receptors (18) and appear to exhibit ubiquitous renal (and extrarenal) expression. Type III mRNA expression is detected in all nephron segments (19). To date, precise information on membrane localization of type III protein (e.g., apical versus basolateral) is not available. It has been suggested that type III Na/Pi cotransporters are responsible for basolateral Pi influx in all tubular cells to maintain cell metabolism as well as in proximal tubular cells under conditions of limited apical influx (for review, see reference 1).

The Type IIa Na/Pi Cotransporter: Structure and Function

Type IIa-mediated Na/Pi cotransport is electrogenic — inward flux of a positive charge — and involves the cotransport of three Na⁺-ions and one P₇-anion (preferentially divalent) (20). The binding of one Na⁺-ion to the negatively charged carrier is followed by the interaction of P₇ and two Na⁺-ions with the carrier (20,21). The transfer of the fully-loaded carrier is electroneutral and electrogenicity is achieved by the reorientation of the empty carrier after the discharge of Na⁺ and P₇ ions to the cell interior (21). Similar to other Na/solute cotransporters, there is a significant Na⁺ leak after interaction with the first Na⁺-ion (20). The latter is of minimal physiologic significance because the transporter is preferentially in its fully loaded transport cycle in the presence of P₇ (20).

Considerable information is available on the structure of the type IIa Na/Pi cotransporter. This is summarized in Figure 1 and is derived from different analytical approaches: (1) hydrophobicity predictions (10); (2) antibody accessibility together with epitope insertion (22); (3) cysteine insertion and accessibility of permeant/impermeant reagents (23,24); and (4) glycosylation studies (25). These studies demonstrated several important features of the type IIa transporter (Figure 1): (1) the transporter has a large extracellular loop, which separates it into two domains (10,25); (2) there is intramolecular homology within two domains (ICL1 and ECL3) (24); (3) the NH₂- and COOH-termini are oriented intracellularly (22).

The functional significance of specific domains of the type IIa Na/Pi cotransporter has also been addressed (Figure 1): (1) cysteine-insertion studies suggested that ICL1 and ECL3 comprise an important part of a "permeation pore" participating in both "cotransport" and "Na⁺ leak" function (23,24); (2) chimera construction — based on different transport properties of type IIa and IIb cotransporters (10,11) — suggested the involvement of three amino acid residues in determining the pH-dependence of type IIa (increased transport at higher pH) (26,27); (3) the chimera approach also suggested the importance of two basic amino acid residues in ICL3 for PTH-dependent internalization (28) (see below); (4) deletion studies documented that the COOH-terminus contains information for brush border membrane expression, i.e., a terminal PDZ-binding motif and an internal signal (29).

Both the NH₂- and COOH-termini portions of type IIa protein are required for transport activity. However, functional studies revealed that cleavage of the type IIa protein backbone, between the two glycosylation sites in the large extracellular loop, does not interfere with transport function (30,31). It is assumed that under this condition a disulfide bridge within this large extracellular loop stabilizes the transporter (Figure 1). Although the type IIa transporter might be part of a multimeric complex (see below), one transporter unit mediates Na/Pi cotransport (32).

The Type IIa Na/Pi Cotransporter as a Target for Physiologic/Pathophysiologic Regulation

Proximal tubular Na/Pi cotransport is regulated by a variety of conditions/factors, which elicit either an increase (e.g., increased P₇ demand, P₇ deprivation) or a decrease (e.g., PTH) in P₇ reabsorption. Regulation is achieved primarily by an alteration in the amount of type IIa protein in the brush border membrane (for review, see reference 1). Accordingly, increased and decreased P₇ reabsorption are, respectively, the result of increased and decreased abundance of type IIa protein in the membrane. Changes in Na/Pi cotransport and type IIa protein expression occur primarily in the absence of changes in type IIa mRNA levels, except perhaps after prolonged treatment with 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), thyroid hormone (T₃), PTH, or prolonged changes in dietary P₇. Thus, changes in Na/Pi cotransport are attributable to either membrane insertion of type IIa protein, which may be preceded by de novo synthesis of the protein, or to membrane retrieval of type IIa protein, followed by its lysosomal degradation (for review, see reference 1). Thus, membrane trafficking of type IIa protein is an important element in the regulation of Na/Pi cotransport. The microtubular network does not participate in the initial internalization step, but it is crucial for the delivery of type IIa protein to the lysosomes (33). In contrast, (rapid) insertion of the type IIa transporter depends on an intact microtubular network (33).
The intracellular signaling mechanism involved in insertion/retrieval of type IIa protein is not completely understood. We postulate that synthesis/insertion of type IIa protein is always high and “driven” by the high renal content of type IIa mRNA (34) and that the internalization of type IIa protein is the regulated process. Several studies have examined the signals for type IIa internalization.PTH action is initiated by binding to receptors that activate protein kinase A (PK-A) and/or protein kinase C (PK-C) signaling pathways, depending on their membrane localization (basolateral PTH receptors activate PK-A and PK-C; apical PTH receptors activate PK-C (35)). For atrial natriuretic protein (and nitrous oxide), type IIa protein internalization is mediated by PK-G activation (36). Studies in opossum kidney (OK) cells demonstrated that the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway also participates in PTH-induced signaling (37). In addition, FGF-23, a factor contributing to renal Pi wasting in autosomal dominant hypophosphatemic rickets and oncogenic hypophosphatemic osteomalacia (see below), inhibits type IIa-mediated Na/Pi cotransport in OK cells via activation of MAPK (38). Moreover, it was recently documented in isolated perfused cortical slices that the different signaling pathways (PK-A, PK-C, PK-G) converge on the ERK/MAPK cascade pathway (Pribanic, H. Murer; submitted manuscript). The downstream targets for ERK/MAPK (PK-A, PK-C, PK-G) converge on the ERK/MAPK pathway to internalize type IIa protein (D. Bacic, C. Wagner, J. Biber, H. Murer; submitted). The downstream targets for ERK/MAPK-mediated phosphorylation remain unknown. Although the type IIa Na/Pi cotransporter is a phosphoprotein, changes in its phosphorylation state are not associated with PTH-induced internalization, thereby excluding it as a downstream target (39). As an alternative, phosphorylation of proteins that associate with type IIa may play a role in its regulation (see below).

Endocytosis/retrieval of type IIa Na/Pi cotransporters occurs at intermicrovillar clefts and seems to involve clathrin (40). Internalized transporters are then routed to the lysosomes for degradation (41,42). There is no evidence for recycling of the type IIa protein, although it cannot be excluded as a fine-tuning system in response to small fluctuations in PTH levels. Although retrieval of type IIa protein appears to occur for most phosphaturic conditions (1,43), it is unclear whether internalization is preceded by transporter inhibition, as is the case for PTH-dependent inhibition of the Na\(^+\)/H\(^+\)-exchanger (NHE-3) (44) (for review, see 2).

The internalization of the type IIa protein in response to phosphaturic signals is a highly specific process. Other brush border membrane proteins, e.g., the Na/sulfate cotransporter, are not internalized, whereas others that are internalized are recycled back to the brush border membrane (e.g., NHE-3 [2,44]). Specificity of retrieval can be achieved at the level of the transporter and/or at the level of the cellular machinery involved in recognition and internalization of the transporter (see below). One of these processes has to be under regulatory control. Internalization of the type IIa transporter is independent of “endocytosis motifs,” e.g., tyrosine, dileucine, or diacidic motifs (45). A chimera approach (type IIa versus type IIb) identified a dibasic amino acid motif (R/K-R) in ICL-3 (Figure 1) that participate in the PTH-responsiveness of the type IIa Na/Pi cotransporter (28).

Disorders of Renal Tubular Phosphate Transport

The Type IIa Transporter as Part of a Heteromultimeric Complex

Specificity in apical retention (e.g., positioning, polarized expression) and retrieval of type IIa protein suggest a role for other proteins that interact with this Na/Pi cotransporter. Indeed, several type IIa-interacting proteins were identified and localized in the proximal tubular epithelial cell (47). PDZK1 (NaPi-Cap1), NaPi-Cap2, and NHERF-1 are PDZ-proteins and interact with the last three amino acids (TRL) at the C-terminus of the type IIa transporter. NaPi-Cap1 and NHERF-1 are both associated with the brush border membrane and may be part of a scaffold that is comprised of several transporters and regulatory proteins, such as kinase anchoring proteins. Furthermore, NaPi-Cap1 and NHERF-1 may be involved in the interaction of type IIa protein with the cytoskeleton (S. Gisler, S. Pribanic, H. Murer, J. Biber; submitted manuscript). Experiments in OK cells have suggested a role for NHERF-1 and NaPi-Cap1 in apical positioning of the type IIa Na/Pi cotransporter (48), and a similar conclusion was reached from studies in mice homozygous for the disrupted NHERF-1 gene (49). Thus, these type IIa interacting proteins might build a local regulatory complex and provide regulatory specificity. The interaction of the type IIa transporter with NaPi-Cap1 or NHERF-1, which occurs via one of multiple PDZ-domains (four in NaPi-Cap1; two in NHERF-1), may be specifically and (dynamically) altered under a given regulatory condition (e.g., by posttranslational modification) and result in the release of the transporter from the apical scaffold and available for internalization. In the subapical compartment, binding of the type IIa transporter to yet another PDZ-protein (NaPi-Cap2) (47), via a specific interaction with one of the four PDZ-domains, may prevent the cotransporter from recycling and facilitate its lysosomal delivery and degradation. Altered protein/protein interactions might also participate in abnormal tubular Pi reabsorption. In support of this hypothesis is the demonstration that Pi wasting in NHERF-1 knockout mice is associated with a decrease in type IIa protein abundance in the renal brush border membrane (49).

Disorders of Renal Tubular Phosphate Transport

Pi is sufficiently abundant in the diet; therefore, Pi deficiency is unlikely to develop except under unusual circumstances (for review, see reference 50). The present discussion will focus on four well-characterized hypophosphatemic disorders associated with renal Pi wasting: X-linked hypophosphataemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), oncogenic hypophosphatemic osteomalacia (OHO), and hereditary hypophosphatemic rickets with hypercalciuria (HHRH). All are characterized by bone disease and, with the exception of HHRH, abnormal regulation of renal vitamin D metabolism (Table 1). Although recent advances have led to an improved understanding of the skeletal phenotype in these disorders (for reviews, see references 3–5), we will focus on the renal Pi transport defect and describe how studies in mouse models have contributed to our understanding of its underlying pathophysiology.
Table 1. Summary of renal phosphate wasting disorders in humans and mice

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Hypophosphatemia</th>
<th>Elevated Serum 1,25-(OH)₂D</th>
<th>Hypercalciuria</th>
<th>Primary Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>XLH</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>PHEX mutations</td>
</tr>
<tr>
<td>Hyp</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>3’Phex deletion</td>
</tr>
<tr>
<td>Gy</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>5’Phex deletion</td>
</tr>
<tr>
<td>ADHR</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>FGF23 mutations</td>
</tr>
<tr>
<td>OHO</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>tumour-mediated</td>
</tr>
<tr>
<td>HHRH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>unknown</td>
</tr>
<tr>
<td>Npt2−/−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Npt2 deletion</td>
</tr>
</tbody>
</table>

X-Linked Hypophosphatemia

XLH is characterized by growth retardation, rachitic and osteomalacic bone disease, hypophosphatemia, and renal defects in the reabsorption of filtered Pᵢ and the metabolism of vitamin D (Table 1) (3). The features that distinguish XLH from other hypophosphatemic disorders are its X-linked dominant mode of inheritance, its higher prevalence (1 in 20,000), and the availability of two murine homologues, Hyp and Gy, which have served as valuable models to examine the mechanism for renal Pᵢ wasting in XLH (51).

The defect in Pᵢ reabsorption in Hyp and Gy mice resides in the proximal tubule, persists after parathyroidectomy, is specific for Pᵢ, and involves a decrease in the maximal velocity (Vmax) of a high affinity, low capacity Na/Pᵢ cotransport system in the BBM (51). The Vmax decrease is secondary to a corresponding decrement in the renal abundance of type IIa Na/Pᵢ cotransporter (Npt2) mRNA and protein in kidneys of Hyp and Gy mice (52,53), consistent with the central role of the type IIa transporter in renal Pᵢ reabsorption (14). In addition, type IIc protein abundance is decreased in renal brush border membranes of Hyp mice (<10% of normal) (17). Finally, parabiosis and renal transplantation studies indicated that the renal Pᵢ transport defect in Hyp mice is not intrinsic to the kidney but rather is mediated by a circulating factor (54–56) that is produced in and/or modified by osteoblasts (57).

The gene responsible for XLH was identified by positional cloning (58) and designated PHEX (formerly PEX) to depict a PHosphate regulating gene with homology to ndopeptidases on the X chromosome. The PHEX gene encodes a 749 amino acid protein (58) that exhibits significant homology to the M13 family of zinc metallopeptidases, which includes the well-characterized enzyme, neutral endopeptidase (NEP). These are type II membrane glycoproteins with a large extracellular domain containing ten highly conserved cysteine residues and a zinc-binding motif, essential for conformational integrity and catalytic activity, respectively (59).

The 166 mutations in the PHEX gene identified to date are catalogued in a locus-specific database (60) available online (http://data.mch.mcgill.ca/phexdb). The mutations are scattered throughout the gene and include deletions, splice junction and frameshift mutations, as well as duplications, insertions, and missense and nonsense mutations, and are consistent with loss of PHEX function. Recent studies showed that in contrast to the wild-type recombinant PHEX protein, which is targeted to the plasma membrane, some disease-causing missense mutations in the PHEX gene result in proteins that remain trapped in the endoplasmic reticulum, where they are degraded (61). Of interest is the finding that some mutant proteins can be rescued from the endoplasmic reticulum to the cell surface (61). Thus, for patients carrying trafficking mutations, these results provide a mechanism for loss of PHEX function and a basis for the development of novel therapeutic approaches.

Mutations in the Phex gene have also been identified in the Hyp and Gy models of the human disease. Hyp mice harbor a large 3’ deletion in the Phex gene (62,63), whereas Gy mice have a deletion in the 5’ region that includes the upstream gene, spermine synthase (63). Gy is thus a contiguous gene deletion syndrome, which may explain why these mutants exhibit phenotypic features that are not apparent in Hyp mice (see reference 51).

Several groups demonstrated that PHEX is expressed predominantly in osteoblasts, osteocytes, and odontoblasts, but not in kidney (see reference 51). The pattern of PHEX expression is consistent with the skeletal and dental abnormalities evident in XLH and Hyp, and the notion that the renal Pᵢ leak in Hyp mice is dependent on a circulating factor (54–57). Although it is not immediately apparent how loss of PHEX function leads to a decrease in renal Pᵢ reabsorption, it has been suggested that PHEX is involved in the inactivation of a phosphaturic hormone or the activation of a Pᵢ conserving hormone (58,62,64) and that loss of PHEX function is associated with either an excess of phosphaturic hormone or a deficiency in a Pᵢ conserving hormone. In either case, renal type IIa, type IIc, and perhaps other Na/Pᵢ cotransporters would be downregulated and Pᵢ wasting would ensue. However, endogenous PHEX substrates have not yet been identified.

The characterization of PHEX-mediated endopeptidase activity is complicated by its plasma membrane localization. To prevent interference by contaminating membrane endopeptidases and facilitate its purification, a soluble and secreted form of PHEX (secPHEX) was genetically engineered (65). The rationale for this approach was based on previous studies demonstrating that a soluble and secreted form of NEP retained full catalytic activity with kinetic parameters that were similar to that of the native enzyme (66). Of the peptides tested as potential secPHEX substrates, only PTH-related peptide...
(PTHrP<sub>107–139</sub>) was degraded by the recombinant enzyme (65).
However, the precise function of PTHrP<sub>107–139</sub> and its role in the regulation of brush border membrane Na/P<sub>i</sub> cotransporters and renal P<sub>i</sub> handling, and the pathophysiology of XLH remain to be established.

XLH patients are treated with oral P<sub>i</sub> supplements, in four to five divided doses per day, and 1,25(OH)<sub>2</sub>D (3). This form of therapy is far from ideal; long-term compliance is difficult, complete healing of rickets rarely occurs, and careful monitoring is essential to ensure a correct balance of P<sub>i</sub>, and 1,25(OH)<sub>2</sub>D. Too much P<sub>i</sub> leads to secondary hyperparathyroidism and the exacerbation of the skeletal defect, and an excess of 1,25(OH)<sub>2</sub>D to hypercalcemia, hypercalciuria, and nephrocalcinosis. Novel therapeutic options, based on a knowledge of the mechanism of action of the PHEX gene product, are clearly necessary. In this regard, the <i>H<sub>yp</sub></i> mouse will serve as an invaluable model to test their efficacy.

**Autosomal Dominant Hypophosphatemic Rickets**

The features of ADHR are similar to those of XLH (Table 1). However, ADHR is far less common than XLH, it exhibits male-to-male transmission, consistent with autosomal dominant inheritance, and is characterized by incomplete penetrance and variable age of onset (3). The gene responsible for ADHR was identified by positional cloning and encodes a new member of the fibroblast growth factor (FGF) family, FGF-23, a 251-amino-acid peptide that is secreted and processed to amino- and carboxy-terminal peptides at a consensus pro-protein convertase (furin) site, RHRTR (ArgHisThrArg) (67). Missense mutations in FGF-23, identified in four unrelated ADHR families, involve the two R residues in this proteolytic cleavage site (67) and abrogate peptide processing (68–70). FGF-23 expression is not readily detectable in normal tissues, with the exception of brain and thymus (71). However, it is abundantly expressed in tumors removed from patients with OHO, an acquired renal P<sub>i</sub> wasting disorder with features of XLH and ADHR (72,73) (see Oncogenic Hypophosphatemic Osteomalacia below).

To elucidate the mechanism whereby mutations in the FGF23 gene elicit P<sub>i</sub> wasting, the effect of FGF-23 on renal P<sub>i</sub> handling was examined in intact mice. Transplantation of CHO cells, stably expressing either wild-type FGF-23 or FGF-23 harboring the ADHR mutations in the furin cleavage site, into nude mice led to the development of significant hypophosphatemia (70,72) and phosphaturia (72). In addition, the injection of both wild-type and mutant forms of FGF-23 in mice elicited a significant decrease in serum P<sub>i</sub> (70,72). FGF-23–infused mice also exhibited a decrease in renal P<sub>i</sub> reabsorption that was associated with a decrease in type IIa protein expression in the brush border membrane (74), indicating that this major renal Na/P<sub>i</sub> cotransporter is a target for regulation by FGF-23. In contrast, injection of the processed N- and C-terminal FGF-23 peptide fragments had no effect on P<sub>i</sub> homeostasis in mice (70). Although these studies attribute the phosphaturic action of FGF-23 to the intact protein, differences in the pharmacokinetics of the intact and processed FGF-23 peptides may have contributed to these findings (70).

Recent demonstration that targeted ablation of the FGF23 gene causes hyperphosphatemia in mice provides evidence for a physiologic role for FGF-23 in the maintenance of P<sub>i</sub> homeostasis (46).

In vitro studies of the effect of FGF-23 on renal Na/P<sub>i</sub> cotransport are not as clear. Recombinant FGF-23 either had no effect (72) or inhibited (38,68) Na/P<sub>i</sub> cotransport in OK cells. Moreover, the inhibition that was observed was either prevented (68) or dependent (38) on the presence of heparin in the incubation medium. In the latter study, it was demonstrated that FGF-23 bound to FGF receptor 3c and that inhibition of Na/P<sub>i</sub> cotransport was mediated by a MAPK signaling pathway (38).

Taken together, the data are consistent with the notion that FGF-23, at least in high doses, can inhibit renal P<sub>i</sub> reabsorption and that FGF-23 contributes to renal P<sub>i</sub> wasting in ADHR. Additional work is necessary to elucidate the physiologic role of FGF-23, to define the relative phosphaturic potency of the wild-type, mutant, and processed FGF-23 peptides, and to determine whether FGF-23 is a PHEX substrate. Although there are conflicting data with regard to FGF-23 cleavage by PHEX (68,75), it is tempting to speculate that ADHR and XLH are caused by mutations in autosomal and X-linked genes, respectively, that function in the same metabolic pathway (Figure 2). (See A Unifying Hypothesis for Renal Phosphate Wasting in XLH, ADHR, and OHO below).

Therapy for ADHR is similar to that of XLH and consists of a combination of oral P<sub>i</sub> and vitamin D (3). However, once the mechanism of FGF-23 processing, action, and degradation are well understood, novel therapeutic approaches will likely be developed.

**Oncogenic Hypophosphatemic Osteomalacia**

OHO, also known as tumor-induced osteomalacia, is an acquired and rare form of renal P<sub>i</sub> wasting, with clinical and biochemical features of XLH and ADHR (Table 1). In contrast to XLH and ADHR, OHO patients also exhibit muscle weakness, fatigue, and fractures. Several types of tumors have been associated with this syndrome, but the majority appears to arise...
from mesenchymal elements (76). Evidence suggests that the phenotypic features of OHO result from the secretion of a humoral factor, designated phosphatonin (77), since removal of the offending tumor results in complete correction of the clinical and biochemical phenotype. Consistent with this hypothesis is the demonstration that extracts from OHO tumors also inhibit Na/phosphate cotransport in OK cells (78).

There is little agreement about the number, nature, stability, or signaling mechanisms of the putative phosphaturic OHO tumor factors (see reference 4). Molecular weights ranging from >3 kD to 55 to 58 kD were reported, both heat lability and heat stability documented, and susceptibility as well as resistance to papain digestion demonstrated. Moreover, the tumor factors were shown to either stimulate or have no effect on the production of cAMP, a second messenger for PTH-tumor factors were shown to either stimulate or have no effect on the production of cAMP, a second messenger for PTH.

These data suggest that several phosphaturic factors may be secreted by OHO tumors.

Recent studies on the molecular analysis of abundantly and differentially expressed genes in OHO tumors led to the identification of phosphatonin candidates, including FGF-23 (68,72,73,79–81), frizzled-related protein 4 (FRP-4) (81), and mepe (matrix extracellular phosphoglycoprotein) (79,82). As discussed above (see Autosomal Dominant Hypophosphatemic Rickets), mutations in the FGF-23 gene (67) that prevent processing of the peptide (68–70) are responsible for ADHR. Moreover, FGF-23 can elicit the renal and skeletal phenotypic features of ADHR and OHO when administered to mice (70,72). At present, there is no information on the effect of mepe or FRP-4 on renal P\textsubscript{i} handling. Thus, although there is evidence to suggest that FGF-23 contributes to the pathogenesis of OHO, the role of mepe and FRP-4 in this disorder remains to be determined. Further work is also necessary to establish whether the OHO tumor factor is related to the circulating phosphaturic factor responsible for the renal P\textsubscript{i} wasting in the Hyp mouse model of XLH (see X-linked Hypophosphatemia above).

The obvious therapy for patients with OHO is surgical removal of the tumor, the source of the offending phosphaturic factor(s). However, the tumors are small, present in obscure areas, difficult to locate without sophisticated imaging procedures, and often inaccessible. If the tumor cannot be found or excised, treatment with P\textsubscript{i} in combination with 1,25(OH)\textsubscript{2}D\textsubscript{3} can be initiated and continued until the tumor is removed (3).

Unifying Hypothesis for Renal P\textsubscript{i} Wasting in XLH, ADHR, and OHO

Figure 2 depicts a model that invokes a common pathway to explain the underlying basis for renal P\textsubscript{i} wasting in XLH, ADHR, and OHO. The hypothesis requires that FGF-23 is phosphaturic, either directly or indirectly, that FGF-23 and/or its N-terminal and C-terminal fragments are degraded by PHEX, and that mutant FGF-23, which retains phosphaturic activity, is not degraded by PHEX. The PHEX substrate pocket can accommodate acidic amino acidic residues (65), the PHEX cleavage site is most likely distinct from the furin-processing motif in FGF-23. In the case of XLH, we speculate that loss of PHEX function results in the accumulation of FGF-23 in the circulation and that this in turn leads to the inhibition of renal P\textsubscript{i} reabsorption. In the case of ADHR, we propose that mutations in the furin cleavage site, which prevent the processing of FGF-23 into N-terminal and C-terminal fragments and their subsequent clearance by PHEX, lead to the accumulation of a “stable” circulating form of the peptide, which also inhibits renal P\textsubscript{i} reabsorption. In the case of OHO, we suggest that ectopic overproduction of FGF-23 overwhelms its processing and degradation by pro-protein convertase and PHEX, respectively, leading to its accumulation in the circulation and inhibition of renal P\textsubscript{i} reabsorption. Consistent with this model is the recent demonstration that the concentration of FGF-23 is significantly elevated in the serum of patients with OHO and XLH when compared with normal subjects (83, 83a).

Hereditary Hypophosphatemic Rickets with Hypercalciuria

HHRH shares many of the features of XLH and ADHR, including growth retardation, bone deformities, renal P\textsubscript{i} wasting, and hypophosphatemia, but it can be distinguished from the latter by the appropriately increased serum levels of 1,25(OH)\textsubscript{2}D and associated hypercalciuria (Table 1). HHRH was first identified in a large Bedouin kindred (84,85), and only a few sporadic cases have been reported (see reference 86). P\textsubscript{i} supplementation alone will correct all the clinical and biochemical abnormalities in HHRH, with the exception of the renal P\textsubscript{i} leak, and it was suggested on the basis of these findings that HHRH is a primary disorder of renal P\textsubscript{i} reabsorption (85). Given that the renal type IIa Na/P\textsubscript{i} cotransporter (Npt2) is an important determinant of P\textsubscript{i} homeostasis and a target for its regulation (see reference 1) and that mice homozygous for the disrupted type IIa gene exhibit a biochemical phenotype that resembles HHRH, namely renal P\textsubscript{i} wasting, hypophosphatemia, and an adaptive increase in the serum concentration of 1,25(OH)\textsubscript{2}D, with associated hypercalciuria (14) (Table 1), it was suggested that mutations in the human orthologue NPT2 may be responsible for HHRH.

To test this hypothesis, the NPT2 coding region and a 120-bp fragment of the NPT2 promoter in two affected individuals from the Bedouin kindred and in unrelated HHRH patients from four small families were sequenced (86). No putative disease-causing mutations were found (86). Two single nucleotide polymorphisms (SNP), a silent substitution in exon 7, and a nucleotide substitution in intron 4 were identified; neither segregated with HHRH in the Bedouin kindred (86). Furthermore, linkage analysis demonstrated that these SNP, as well as five microsatellite markers flanking NPT2 in the chromosome 5q35 region, were not linked to HHRH in the Bedouin kindred (86). These data excluded NPT2 as a candidate gene for HHRH and suggest that mutations in another renal Na/P\textsubscript{i} cotransporter, or a regulator thereof, is responsible for the disorder. A genome scan is currently underway to localize and identify the gene that is mutated in the Bedouin kindred with HHRH.

It is of interest that heterozygous mutations in the NPT2a gene (same as NPT2 discussed above) have recently been
identified in two individuals with renal P₇ wasting and hyperphosphatemia, associated with either urolithiasis or bone demineralization (87). Although the authors suggest that one copy of the mutant NPT2a gene is sufficient for the expression of the respective phenotypic abnormalities, other genes or environmental conditions may contribute to the phenotype and explain why a different clinical presentation is observed in each case (87,88). In contrast, inbred mice heterozygous for the disrupted type IIa gene (Npt2+/−) exhibit neither hypercalciuria nor renal calcification, features that are evident in mutant homozygous counterparts (Npt2−/−) (89).

Acknowledgments

Work in the authors’ laboratories was supported by grants from the Canadian Institutes of Health Research and Roche Bioscience (to T.-S.K., M.L., and S.K.). The laboratories was supported by grants from the Canadian Institutes of Health Research and Roche Bioscience (to T.-S.K., M.L., and S.K.). The authors would like to acknowledge the support of the Swiss National Science Foundation (to M.H., N.H., and I.F.), the Swiss National Science Foundation (to M.H., N.H., and I.F.), the Swiss National Science Foundation (to M.H., N.H., and I.F.), the Swiss National Science Foundation (to M.H., N.H., and I.F.), and the Swiss National Science Foundation (to M.H., N.H., and I.F.).

References


60. Sabbagh Y, Jones AO, Tenenhouse HS: PHEXdb, a locus-specific database for mutations causing X-linked hypophosphatemia. *Hum Mut* 16: 1–6, 2000

