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Phosphate transport: Molecular basis, regulation and pathophysiology

Harriet S. Tenenhouse *

Department of Human Genetics, McGill University, Montreal, Quebec, Canada

Abstract

Inorganic phosphate (Pi) is fundamental to cellular metabolism and skeletal mineralization. Ingested Pi is absorbed by the small intestine, deposited in bone, and filtered by the kidney where it is reabsorbed and excreted in amounts determined by the specific needs of the organism. Two distinct renal Na-dependent Pi transporters, type IIa (*NPT2a*, *SLC34A1*) and type IIc (*NPT2c*, *SLC34A3*), are expressed in brush border membrane of proximal tubular cells where the bulk of filtered Pi is reabsorbed. Both are regulated by dietary Pi intake and parathyroid hormone. Regulation is achieved by changes in transporter protein abundance in the brush border membrane and requires the interaction of the transporter with scaffolding and signaling proteins. The demonstration of hypophosphatemia secondary to decreased renal Pi reabsorption in mice homozygous for the disrupted type IIa gene underscores its crucial role in the maintenance of Pi homeostasis. Moreover, the recent identification of mutations in the type IIc gene in patients with hereditary hypophosphatemic rickets with hypercalciuria attests to the importance of this transporter in Pi conservation and subsequent skeletal mineralization. Two novel Pi regulating genes, *PHEX* and *FGF*23, play a role in the pathophysiology of inherited and acquired hypophosphatemic skeletal disorders and studies are underway to define their mechanism of action on renal Pi handling in health and disease.

Keywords: Kidney; Proximal tubule; NaPi-IIa; NaPi-IIc; Hypophosphatemia; Pi wasting; Parathyroid hormone; PHEX; FGF-23; 1,25-dihydroxyvitamin D

1. Introduction

Inorganic phosphate (Pi) is an essential nutrient in terms of both cellular metabolism and skeletal mineralization. To accomplish these functions, transport systems have evolved to permit the efficient transfer of Pi anions across hydrophobic membrane barriers. Ingested Pi is absorbed by the small intestine, deposited in bone, and filtered by the kidney where it is reabsorbed and excreted in amounts determined by the specific requirements of the organism. The kidney is a major regulator of Pi homeostasis and can increase or decrease its Pi reabsorptive capacity to accommodate Pi need. Up to 70% of filtered Pi is reabsorbed in the proximal tubule where sodium (Na)-dependent Pi transport systems in the brush-border membrane (BBM) mediate the rate-limiting step in the overall Pi reabsorptive process. Three classes of Na/Pi cotransporters have been identified in mammalian kidney and considerable progress has been made in our understanding of their function, regulation, and role in the maintenance of Pi homeostasis. In addition, recent studies of Mendelian [X-linked hypophosphatemia (XLH) and autosomal dominant hypophosphatemic rickets (ADHR)] and acquired [oncogenic hypophosphatemic osteomalacia (OHO)] renal Pi wasting disorders have uncovered novel genes, *PHEX* and *FGF*23, which play a role in the regulation of renal Pi handling. This review will briefly summarize the current state of our knowledge of Na/Pi cotransporters expressed in the kidney and the molecular mechanisms involved in their regulation. The impact of mutations in genes encoding type IIa (*NPT*2*a*/*Npt*2*a* or *SLC34A1*/*Slc34a1*—upper case italics refer to genes of human origin and lower case to italics to genes of murine origin) and type IIc (*NPT*2*c*/*Npt*2*c* or *SLC34A3*/*Slc34a3*) renal Na/Pi cotransporters on human and murine phenotypes will be described. In addition, advances in our understanding of the mechanisms for renal Pi wasting in XLH, ADHR and OHO will be discussed. For

* Correspondence address: PH5-35 Boardwalk Drive, Toronto, ON, M4L 3Y8 Canada.
E-mail address: harriet.tenenhouse@mcgill.ca.

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a more comprehensive discussion of renal epithelial Na/Pi cotransport in health and disease states, see Refs. [1–9].

2. Renal phosphate transport

2.1. Cellular aspects

The rate limiting step in renal Pi reabsorption occurs in the proximal tubule and involves the transport of Pi from the tubular lumen across the apical BBM. Pi then moves across the cell and effluxes at the basolateral membrane. Pi transport across the BBM is saturable, Na-dependent and driven by a Na-gradient (outside > inside) that is maintained by the basolateral membrane-associated Na,K-ATPase. Na/Pi cotransport across the BBM is electrogenic, sensitive to pH, with 10–20-fold increases documented when the pH is increased from 6 to 8.5, and the target for physiologic/pathophysiologic regulation. Little is known about the precise mechanisms involved in the translocation of Pi across the cell and the efflux of Pi across the basolateral membrane.

2.2. Molecular characterization

Three classes of Na/Pi cotransporters have been identified by expression and homology cloning. The type I Na/Pi cotransporter is expressed predominantly in the BBM of proximal tubular cells [10] and mediates fluxes of chloride and organic anions as well as Pi. Recent studies suggest that expression of the type I gene (*Npt*1 or *Slc*17*a*1) is transcriptionally regulated [11] and that Npt1 may function as a modulator of intrinsic cellular Pi transport rather than a Na/Pi cotransporter per se [12]. The type III Na/Pi cotransporters, encoded by *Glvr*-1 (*Pit*-1 or *Slc*20*a*1) and *Ram*-1 (*Pit*-2 or *Slc*20*a*2) genes, are cell surface retroviral receptors that are ubiquitously expressed [13] and, in the kidney, appear to be localized to the basolateral membrane where they serve a housekeeping function. The types I and III Na/Pi cotransporters have no sequence similarity.

The type II family of Na/Pi cotransporters, which exhibit 25% homology with Npt1, is comprised of three highly homologous isoforms; type IIa (Npt2a) and type IIc (Npt2c), which are expressed almost exclusively in the BBM of the renal proximal tubule [14,15], and type IIb, which exhibits a broader tissue distribution, is likely responsible for intestinal absorption of Pi, and is not expressed in the kidney [16]. The abundance of Npt2a is an order of magnitude greater than Npt2c in mouse kidney [17] and functional studies reveal that Npt2a- and Npt2c-mediated transport exhibits the characteristics of Na-dependent Pi transport across the proximal tubular BBM.

Npt2a-mediated Na/Pi cotransport is electrogenic and involves the inward flux of three Na-ions and one divalent Pi anion. In this case, membrane voltage becomes a kinetic determinant of the transport mechanism and under physiological conditions serves to enhance the concentrating ability of the Npt2a protein [18,19]. The extra-renal type IIb Na/Pi cotransporter is also electrogenic whereas the Npt2c isoform mediates the electroneutral transport of two Na-ions with one divalent Pi anion.

2.3. Regulation

Many hormonal and non-hormonal factors are known to regulate renal Pi handling although parathyroid hormone (PTH) and dietary Pi intake are the major regulators of this process [1]. Both Npt2a and Npt2c are targets for regulation. PTH and high Pi intake inhibit Na/Pi cotransport across the BBM by triggering the endocytic retrieval of Npt2a and Npt2c proteins from the BBM to the subapical compartment. The internalized transport proteins are then routed to the lysosomal compartment where they are degraded. On the other hand, Pi deprivation elicits an increase in BBM Na/Pi cotransport that is associated with microtubule-dependent recruitment of existing Npt2a and Npt2c proteins to the apical membrane. The post-transcriptional mechanisms underlying BBM retrieval and recruitment/insertion of Npt2a and Npt2c proteins are complex and involve the interaction of the transporters with various scaffolding and signalling proteins in the apical and subapical compartments [6]. FGF-23, a novel regulator of renal Pi handling, inhibits both types IIa- and IIc-mediated Na/Pi cotransport [8] (see Section 5).

2.4. Structure/function of type II Na/Pi cotransporters

Several approaches were used to define the topology and functional significance of specific domains within the type II isoforms [3]. These include hydrophobicity predictions, antibody accessibility combined with epitope insertion, cysteine insertion and accessibility of permeant and impermeant reagents, functional expression of chimeric transport proteins and amino acid replacement. The studies demonstrated that the type II Na/Pi transporters are comprised of eight transmembrane domains, with the N- and C-terminal ends oriented intracellularly. The first intracellular loop (ICL1) and third extracellular loop (ECL3) comprise an important part of a 'permeation pore' which participates in both 'cotransport' and 'Na^+ leak' function [20,21]. Three amino acid residues in ECL3 determine the pH-dependence of Npt2a [22]. Two basic amino acid residues in ICL3 are required for PTH-dependent internalization of Npt2a [23]. The COOH-terminus contains a terminal PDZ-binding motif which is essential for interaction with PDZK1 and NHERF-1, essential for Npt2a stabilization in the BBM [24]. The third transmembrane domain contains amino acid residues which confer electrogenicity [18]. Both the NH_2- and COOH-termini are required for transport activity. However, cleavage of the Npt2a protein backbone, between the two glycosylation sites in the large extracellular loop, does not interfere with transport function [25,26]. It is assumed that under this condition a disulfide bridge within this large extracellular loop stabilizes the transporter. Although Npt2a might be part of a multimeric complex, one