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Conserved loci on the X chromosome confer phosphate homeostasis in mice and humans

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Summary

Several genes expressed in kidney and other tissues determine phosphate homeostasis in extracellular fluid. The major form of inherited hypophosphatemia in humans involves an X-linked locus (HPDR, Xp22.31-p21.3). It has two murine homologues (*Hyp* and *Gy*) which map to closely-linked but separate loci (crossover value 0·4%–0·8%). Both murine mutations impair Na^+-phosphate cotransport in renal brush border membrane; an associated renal disorder of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D$) metabolism has been characterized in *Hyp* mice. Whereas experiments with cultured *Hyp* renal epithelium indicate that the gene is expressed in kidney, studies showing the development of the mutant renal phenotype in normal mice parabiosed to *Hyp* mice implicate a circulating factor; these findings can be reconciled if the humoral factor is of renal origin. The gene dose effect of HPDR, *Hyp* and *Gy* on serum phosphorus values is consistently deviant and heterozygotes resemble affected hemizygotes. The deviant effect is also seen on renal phosphate transport; all mutant females (*Hyp/Hyp* and *Hyp/+*) have similar phenotypes. On the other hand, there is a normal gene dose effect of HPDR in mineralized tissue; tooth PRATIO (pulp area/tooth area) values for heterozygotes are distributed between those for affected males and normals. The tooth data imply that the X chromosome locus is expressed in both renal and non-renal cells. The polypeptide product of the X chromosome gene(s) is still unknown.

1. Introduction

This article describes a form of hypophosphatemia with X-linked inheritance. The associated disease in humans is a classic under any of its names – Vitamin D resistant rickets, Familial hypophosphatemic rickets, X-linked hypophosphatemia (Rasmussen & Tenenhouse, 1989), but in the half century since its 'discovery' it has eluded a tidy explanation. The putative gene has not yet been cloned and answers do not yet arrive by 'reverse' genetics. Two developments among several have greatly enhanced knowledge about X-linked hypophosphatemia (XLH). The first was the evidence for Mendelian inheritance of hypophosphatemia (Winters *et al.* 1958). The second was the identification of two strains of laboratory mice (Eicher *et al.* 1976; Lyon *et al.* 1986) which are counterparts of the human disorder. Mary Lyon has influenced everyone who thinks about X-linked traits. She made her own fundamental contributions to one of the mouse 'models' of XLH. The present authors thank her for inspiration (and data!). Patients with XLH are her grateful beneficiaries.

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2. Heritability of rickets

In the early 20th century, rickets was endemic affecting up to 80% of infants and young children living in industrialized Europe and North America (Still, 1907). Living conditions were implicated as important social determinants of the disease (Loomis, 1967) and its heritability was low.

The discovery of Vitamin D (McCollum *et al.* 1922; Mellanby, 1919), the role of ultraviolet radiation in its synthesis (Huldschinsky, 1919; Holick *et al.* 1980), and universal prophylaxis made endemic rickets a thing of the past. Nonetheless rickets did not disappear. The obstinate cases had a different character; they were called 'vitamin D resistant' (Albright *et al.* 1938), they seemed to have a 'hereditary metabolic disorder' (Christensen, 1940–41), and the

Table 1. *Mendelian disorders of phosphate or calcium homeostasis associated with Osteomalacia/rickets in man*

1. Phosphopenic forms (primary)	McKusick number
X-linked hypophosphatemia (XLH)	30780, 30781
Hereditary hypophosphatemic rickets with hypercalciuria (HHRH)	24153
Autosomal dominant hypophosphatemic bone disease (HBD)	14635
Autosomal dominant vitamin D-resistant rickets	19310
Autosomal recessive hypophosphatemic rickets	None assigned (Baker & Stamp, 1989)
Fanconi syndromes (various primary and secondary inherited forms)	Several
2. Calcipenic forms (with secondary hypophosphatemia)	
Vitamin D dependency Type I (defective synthesis of $1,25(OH)_2D$)	26470
Vitamin D dependency Type II (defective $1,25(OH)_2D$ receptor)	27742, 27744

most prevalent form was hypophosphatemic and inherited as an X-linked dominant (Winters *et al.* 1958). Since the majority of cases of childhood rickets (excepting azotemic forms) are now hereditary, it follows that the heritability of the disease has increased in developed countries (Scriver & Tenenhouse, 1981).

We focus here on X-linked hypophosphatemia (XLH). Designated HPDR I (hypophosphatemic vitamin D resistant rickets, type 1) in the McKusick catalogues (number 30780), it has a murine counterpart, gene symbol *Hyp* (Eicher *et al.* 1976). A second phenotype in mouse called gyro (gene symbol *Gy*) is also hypophosphatemic but *Gy* maps to a region of the distal X chromosome adjacent to *Hyp* (Lyon *et al.* 1986). The search for a gyro counterpart in human patients has apparently been successful (Boneh *et al.* 1987) and the human disease is provisionally designated HPDR II (McKusick 30781). These four phenotypes are categorical informants about certain molecular events controlling phosphate homeostasis in man and mouse. The existence of other Mendelian hypophosphatemias (Table 1) implies that the molecular components of phosphate homeostasis are quite numerous.

3. Homeostatic processes reflect genes

Two metabolic axes underlie the pathogenesis of rickets (and the corresponding lesion, osteomalacia). One involves the metabolic homeostasis of calcium, the other of phosphate* (Scriver, 1974; Scriver *et al.* 1982). Impaired homeostasis results in calcipenia or phosphopenia, or both, in body fluids. Normal function of the associated controlling mechanisms (and controlled components) (Neer, 1989) ensures that extracellular calcium is conserved at expense of bone. Since primary disruptions in either phosphate or calcium homeostasis affect phosphate – calcipenia affects it through the controlling loop dependent on parathyroid hormone – it follows that a deviant metrical trait such as hypophosphatemia could have many different causes (Table 1).

The mammalian skeleton contains a crystalline substance which is a variable mixture of phosphorus, calcium, oxygen and hydrogen called hydroxyapatite. Maintenance of hard tissues such as bone involves turnover of mineral content. In simple terms, the moment to moment homeostasis of calcium and phosphorus in extracellular pools is controlled by parathyroid hormone while day to day regulation is a function of vitamin D (Neer, 1989).

The kidney is an important arbiter of extracellular phosphate anion homeostasis in mammals (Scriver & Tenenhouse, 1990). Net conservation of anion in kidney is determined by phosphate-specific transporters in proximal nephron (Gmaj & Murer, 1986). There is evidence of functional diversity in phosphate transporters along the axis of proximal nephron (Dennis *et al.* 1977; Walker *et al.* 1987). There is evidence also for diversity across the epithelial cell (Gmaj & Murer, 1986). Luminal and antiluminal surfaces face different environments and the corresponding transporters serve different functions. The brush border membrane carriers are coupled to a sodium gradient ($Na_o^+ > Na_i^+$). Net reabsorption of phosphate involves uphill movement at the luminal surface and facilitated (Na^+ independent) efflux at the antiluminal pole.

Some Mendelian disorders of phosphate homeostasis are dominantly inherited; others are recessive (Table 1); they provide evidence for the relative

* Phosphate anion is the chemical species metabolized by cells; the chemical assay measures elemental phosphorus.

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importance – expressed as the 'sensitivity coefficient' (Kacser & Burns, 1981) – of the component affected by the allele in question. They also reveal that local reaction rates (phosphate transport in one or other nephron segment, or membrane) affect global properties of the system (phosphate content of extracellular fluids). These principles of physiological genetics (Kacser & Burns, 1981; Kacser & Porteous, 1987) are evident in XLH, its mouse counterparts, and in the other inborn errors of phosphate transport mentioned here.

4. The XLH phenotype

Early on in the history of this disease, one school of thought advocated that it was a consequence of impaired vitamin D action (The Albright hypothesis (Albright *et al.* 1938)), while a second proposed renal wastage of phosphate as the primary mechanism (Robertson *et al.* 1942; Dent, 1952). Pedigree analysis (Winters *et al.* 1958) revealed that serum phosphorus values segregated as an X-linked dominant trait in affected families. Serum phosphorus values were slightly lower in mutant hemizygotes than in heterozygotes (Table 2). Hypophosphatemia was associated with impaired renal reabsorption of filtered phosphate (but of no other solute). The accompanying bone disease was, on average, considerably more severe in male patients than in affected females. It was not evident that the small differences in serum phosphorus between affected males and females explained the greater difference in severity of their bone disease.

Untreated XLH patients have essentially normal serum calcium and parathyroid hormone (PTH) values (Arnaud *et al.* 1971) indicating that hypophosphatemia in XLH is not secondary to hyperparathyroidism. Renal T_mPi values (tubular maximum reabsorption rate for phosphate) in XLH patients are depressed in both sexes, apparently more so in males (Glorieux & Scriver, 1972). This evidence hints at a gene dose effect on renal phosphate transport in XLH. A gene dose effect is not consistently apparent (Winters *et al.* 1958).

Table 2. *Adjusted serum phosphorus values (mM) for normals and XLH patients*

	Normals		XLH
Males			
Mean	1·64	Mean	1·0
−2SD	1·19	+2SD	1·30
Females			
Mean	1·68	Mean	1·15
−2SD	1·22	+2SD	1·33

Adapted from Winters *et al.* (1958).
Values have been adjusted to age 2 years. Differences between normals and XLH are highly significant; differences between XLH males and females, although small, are significant ($P < 0·01$).

Serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) values are depressed in XLH patients (Scriver *et al.* 1978). One expects the values to be elevated in the hypophosphatemic state (Tanaka & DeLuca, 1973; Baxter & DeLuca, 1976; Gray *et al.* 1977; Neer, 1989). This paradox provoked an interesting question: Is XLH a primary disorder of phosphate homeostasis with secondary impairment of vitamin D metabolism; or vice versa? The Albright hypothesis had reappeared from limbo! Meanwhile, it was shown that the bone disease in XLH healed best when vitamin D (in the form of $1,25(OH)_2D$) was combined with supplements of phosphate in the diet (Glorieux *et al.* 1972; Drezner *et al.* 1980; Glorieux *et al.* 1980; Costa *et al.* 1981).

5. The *Hyp* mouse

A laboratory mouse strain with an X-linked mutation (*Hyp*) (Eicher *et al.* 1976), conferring a phenotype similar to that of XLH in man, has greatly advanced our understanding of the corresponding pathophysiology in the human counterpart (Smith & Riordan, 1987). Corresponding genes, particularly those on the X chromosomes of humans and mice, are presumed to encode polypeptides with homologous functions (Ohno, 1967; ibid. 1973). The *Hyp* mouse has hypophosphatemia, bony changes resembling rickets (without overt hypocalcemia), dwarfism and elevated fractional excretion of phosphate into urine (Eicher *et al.* 1976).

(i) *Renal phosphate transport in the* Hyp *mouse*

Micropuncture studies localized the phosphate transport defect to the proximal tubule of *Hyp* kidney (Giasson *et al.* 1977; Cowgill *et al.* 1979). The phosphate leak persisted after parathyroidectomy (Cowgill *et al.* 1979; Kiebzak *et al.* 1981). Two approaches were taken to establish which renal epithelial cell membrane, basolateral or brush border, expressed the transport defect. Renal cortical slices were used in the first. They preferentially expose the basolateral membrane (Arthus *et al.* 1982) and net uptake and efflux of phosphate by cortical slices was similar in *Hyp* and control mice implying that the *Hyp* gene is not expressed at that membrane (Tenenhouse *et al.* 1978).

In the second approach, phosphate transport was measured directly at the renal brush border membrane. The sealed vesicle preparation, derived primarily from the proximal tubule, has a right-side-out orientation, and permits the measurement of transport processes without the complication of cellular metabolism (Haase *et al.* 1978). The Na^+-dependent component of phosphate transport was about half normal in the brush border membrane of *Hyp* kidney (Tenenhouse *et al.* 1978; Tenenhouse & Scriver, 1978). Na^+-dependent transports of D-glucose (Tenenhouse *et al.* 1978; Tenenhouse & Scriver, 1978), L-alanine (Lyon

et al. 1986) and L-proline (Kiebzak & Dousa, 1985) were all normal implying that the transport defect is specific for phosphate.

Two distinct phosphate transport systems have been identified in renal brush border membrane (Walker *et al.* 1987; Tenenhouse *et al.* 1989) (Figure 1). One system has high capacity and low affinity and is located in proximal convolutions where it can reabsorb the bulk of filtered phosphate. The other has low capacity and high affinity and is located both in proximal convolutions and in proximal straight segment where it reabsorbs residual filtered phosphate. Kinetic studies established which of these processes is impaired in *Hyp* kidney (Table 3 and Figure 1). V_{max} for the low capacity, high affinity phosphate transport process is half normal in vesicles from mutant mice; the Km value is unaltered. The system with high capacity and low affinity shows no change. These findings in the mutant phenotype indicate the relative importance of the high affinity phosphate transport process in the maintenance of phosphate homeostasis in extracellular fluids.

The mechanism by which phosphate transport on the high affinity system is impaired is not yet understood. Half-normal V_{max} values are compatible either with fewer phosphate transport units or with malfunction of a normal number of carriers. It has not yet been shown that the mutant *Hyp* gene actually encodes a component of a phosphate transporter; it could be a separate regulator of the transporter's function.

Hyp mice retain the ability to adapt to dietary restriction of phosphate (Tenenhouse & Scriver, 1979). Renal brush border membrane vesicles prepared from phosphate-deprived mice exhibit a 3-fold increase in V_{max} of the high affinity phosphate transport system (Tenenhouse *et al.* 1989). It is not yet known whether this increase represents a recruitment of transporters from an intracellular pool, *de novo* synthesis of new transport units, or activation of existing transporters within the brush border membrane.

(ii) *Intestinal transport*

Phosphate transport at the intestinal brush border membrane is not impaired in the *Hyp* mouse (Tenenhouse *et al.* 1981); the finding corresponds to evidence for normal intestinal phosphate absorption in XLH patients (Glorieux *et al.* 1976). $1,25(OH)_2D$ improves phosphate homeostasis in the mutant phenotype by stimulating intestinal phosphate transport in both *Hyp* mice (Tenenhouse & Scriver, 1981) and in XLH patients (Glorieux *et al.* 1980). The hormone does not stimulate renal phosphate reabsorption nor does it correct the defect in renal phosphate conservation in *Hyp* or XLH individuals (Tenenhouse & Scriver, 1981; Glorieux *et al.* 1980). These findings imply that phosphate transport in intestine is not served or influenced by the *Hyp*/HPDR gene product and that phosphate transport processes are dissimilar in kidney and intestine.

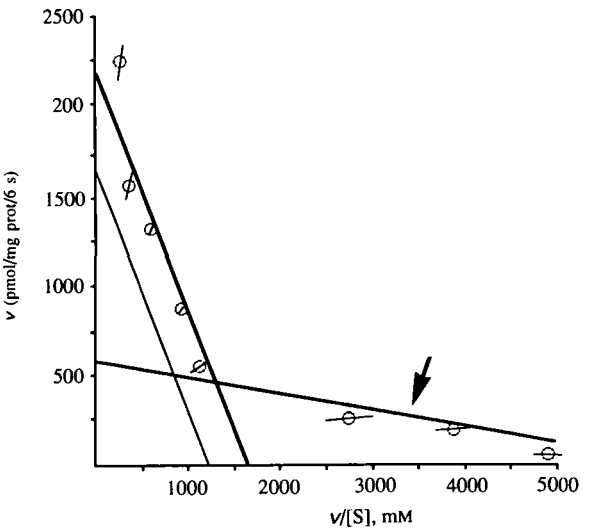

Fig. 1. Eadie-Hofstee plot depicting two kinetically distinct Na^+-dependent phosphate transport systems in mouse renal brush border membrane vesicles. Renal phosphate transport was measured at 6 s at phosphate concentrations between 0·01 mM and 10 mM. Uptake measured in the presence of KCl was subtracted from that in the presence of NaCl to obtain the Na^+-dependent component of phosphate flux. Computer transformation of the data was used to determine the regression lines and calculate kinetic parameters (see Table 3). Data were derived from 10 different brush border membrane preparations. The corrected line depicting the low affinity, high capacity phosphate transport process is shown. The arrow depicts the phosphate transport process which is impaired in brush border membrane vesicles prepared from mice bearing the X-linked *Hyp* mutation (from Tenenhouse *et al.* 1989).

Table 3. *Effect of* Hyp *mutation on kinetically distinct phosphate transport systems in mouse renal brush-border membrane vesicles*

	Normal	*Hyp*
High affinity system		
V_{max} (pmol/mg protein per 6 s)	539 ± 50	253 ± 30*
K_m (mM)	0·09 ± 0·02	0·08 ± 0·02
Low-affinity system		
V_{max} (pmol/mg protein per 6 s)	1677 ± 193	1868 ± 352
K_m (mM)	1·28 ± 0·35	3·45 ± 1·1·15

Brush border membrane vesicles were prepared from *Hyp* mice and normal littermates. Uptake studies were performed as described in the legend to Figure 1. Values are means ± S.E. of four separate brush-border membrane vesicle preparations derived from *Hyp* mouse kidney and eight membrane vesicle preparations derived from kidneys of normal littermates. * $P < 0·001$. Normal vs *Hyp* (from Tenenhouse *et al.* 1989).

(iii) *Vitamin D metabolism*

Renal metabolism of 25-hydroxyvitamin D (25(OH)D) is abnormal in the *Hyp* mouse; synthesis, catabolism

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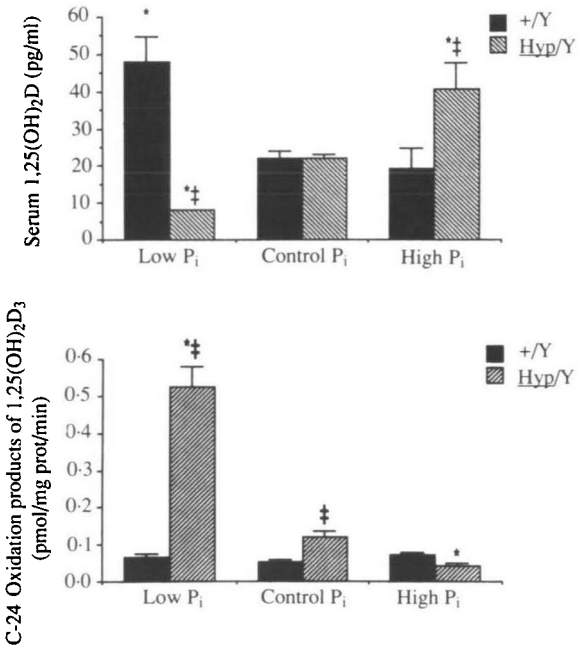

Fig. 2. Effect of dietary phosphate on serum $1,25(OH)_2D$ concentration (A) and renal mitochondrial $1,25(OH)_2D$ catabolism by the C-24 oxidation pathway (B) in normal (+/Y) and mutant (*Hyp*/Y) littermates. ‡ Effect of genotype, $P < 0{\cdot}05$; * Effect of diet, $P < 0{\cdot}05$ (from Tenenhouse & Jones, 1990, with permission).

and regulation are each affected. *Hyp* mice exhibit blunted renal synthesis of the vitamin D hormone, in response to activators of its production, such as phosphate deprivation (Meyer *et al.* 1980; Lobaugh & Drezner, 1983; Yamaoka *et al.* 1986), PTH infusion (Nesbitt *et al.* 1986), calcium restriction (Nesbitt *et al.* 1986; Tenenhouse, 1984*a*) and vitamin D deficiency (Tenenhouse, 1983; Tenenhouse, 1984*b*). *Hyp* mice also exhibit accelerated renal catabolism of $1,25-(OH)_2D$, and of its precursor 25(OH)D, via the C-24 oxidation pathway (Tenenhouse *et al.* 1988) which degrades hormone to calcitroic acid, the major excretory metabolite of $1,25(OH)_2D$ (Makin *et al.* 1989). Regulation of vitamin D metabolism, particularly in response to dietary phosphate, is also abnormal in the mutant strain. *Hyp* mice respond to phosphate deprivation with a fall in serum $1,25(OH)_2D$; and to phosphate supplementation with a rise in hormone (Tenenhouse & Jones, 1990); control mice have the opposite responses (Fig. 2*a*). The exaggerated catabolism of $1,25(OH)_2D$ in *Hyp* mice is further accelerated by phosphate depletion and corrected by phosphate supplementation (Tenenhouse & Jones, 1990) (Fig. 2*b*). These results have two implications. First, the disorder in vitamin D metabolism in the mutant strain is likely to be secondary to the perturbation in phosphate homeostasis. Second, the catabolic pathway for hormone is an important determinant of $1,25(OH)_2D$ levels in *Hyp* mice. Together these interpretations explain the efficacy of combined therapy (supplements of phosphate and $1,25(OH)_2D$) in XLH patients.

(iv) *Protein kinases*

Protein kinases A and C are both implicated in the regulation of renal phosphate transport (Hammerman & Hruska, 1982; Boneh *et al.* 1989), but should not play a primary role in XLH since the kinase genes are autosomal. Genotype differences in renal protein kinase C (Tenenhouse & Henry, 1985; Boneh & Tenenhouse, 1990) but not protein kinase A (Brunette *et al.* 1984; Hammerman & Chase, 1983; Tenenhouse *et al.* 1980; Tenenhouse & Henry, 1985) have been reported.

6. Contrary findings in *Hyp* renal cell cultures and parabiotic (normal to *Hyp*) pairs

Primary cultures of mouse renal epithelial cells can be established (Bell *et al.* 1988*a*) and they show that the abnormalities of phosphate transport and vitamin D metabolism in *Hyp* kidney are intrinsic renal defects. After 6 days in culture in a hormonally defined medium, the mean phosphate/α-methylglucose uptake ratio in *Hyp* cultures is significantly lower than in normal cultures (Bell *et al.* 1988*b*); and 25(OH)D-24-hydroxylase (the first enzyme in the C-24 oxidation pathway) is significantly elevated. The evidence implies that the *Hyp* gene is expressed in kidney epithelial cells.

Studies of parabiotic mouse pairs indicate the contrary; a circulating factor may indeed play a role in the renal phenotype. After surgical joining of normal and *Hyp* mice, the former develop renal wastage of phosphate, and hypophosphatemia (Meyer *et al.* 1989*a*). Moreover, there is impaired transport of phosphate in the corresponding brush border membrane vesicles (Meyer *et al.* 1989*b*). This response is not seen after surgical joining of normal mice. The evidence implicates a circulating phosphaturic factor in *Hyp* mice. The factor is not PTH (Meyer *et al.* 1989*b*) since parathyroidectomy of both animals in the pair does not alter the response in normal mice joined to *Hyp* mice.

The findings in parabiotic mice and cultured renal epithelial cells can be reconciled if the putative factor is actually renal in origin. When the factor is isolated, cloned and mapped, it will become apparent whether it is the product of the X chromosome locus, or of another.

7. The gyro mutation

A second locus on the mouse X chromosome is involved in phosphate homeostasis and it was discerned in the gyro mouse (Lyon *et al.* 1986). The gyro phenotype is X-linked dominant. It is characterized by hypophosphatemia, increased renal loss of phosphate and skeletal changes of rickets/osteomalacia. Although *Hyp* and gyro mice have similar phenotypes, their differences became apparent after *Hyp* and *Gy*

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genes were put on the same genetic background. Gyro males weigh less than *Hyp* males but have equivalent shortening of tail length. Gyro males circle strongly, show extreme hyperactivity, have abnormalities of inner ear and head shape, are deaf, sterile and less viable. Both mutants have half normal Na^+-dependent phosphate transport function in renal brush border membrane vesicles; the impairment is selective for phosphate (Lyon *et al.* 1986). The different phenotypes of *Gy* and *Hyp* are explained by non-allelic mutations (see below).

8. Locations of HPDR, *Hyp* and *Gy* on the X chromosome

(i) *HPDR*

The human gene (HPDR – now called HYP) maps to a region on the short arm of the human X chromosome (Xp22.31-p21.3) that Lyonizes. It is in one of five conserved segments (Buckle *et al.* 1985; Lyon, 1988) which have different arrangements on human and mouse X chromosomes (Searle *et al.* 1989) (Figure 3). The human gene was mapped by multilocus linkage analysis in well-characterized families with polymorphic anonymous cDNA probes derived from defined regions of the human X chromosome (Read *et al.* 1986, Machler *et al.* 1986). Flanking markers located the HPDR locus distal to the DXS41 (99·6) and proximal to DXS43 (D2) (Thakker *et al.* 1987). This placed HPDR distal to Duchenne muscular dystrophy (DMD) and proximal to steroid sulfatase (STS) on Xp. A hypophosphatemic patient with a contiguous gene deletion syndrome affecting this region of the X chromosome has not yet been reported but such a person would be a valuable resource to clone HPDR.

(ii) Hyp *and* Gy

The *Hyp* gene maps to the distal end of the mouse X chromosome (Eicher *et al.* 1976). Linkage analysis placed *Gy* and *Hyp* in the same region of the chromosome (Lyon *et al.* 1986). It then became important to know whether *Gy* and *Hyp* were allelic or closely linked loci. Crossing over between *Gy* and *Hyp* was sought in offspring of *Gy*+/*Hyp*+ females mated to tabby (Ta/Y) males. Among 239 male offspring, 238 had low serum phosphorus, 66 of which were classified as *Gy*/Y on the basis of circling behaviour. The single male with normal serum phosphorus and normal behaviour was a presumed non-*Hyp*, non-*Gy* (++/Y) crossover. This male was mated with a tabby ($X^{Ta}O$) female. All five non-tabby XO female offspring of this mating had normal serum phosphorus values, implying that the father's X chromosome had neither the *Hyp* or *Gy* allele and was a true crossover; thus *Gy* and *Hyp* are not allelic but are closely linked loci (Lyon *et al.* 1986). The crossover value between the loci was estimated to be 0·4–0·8%

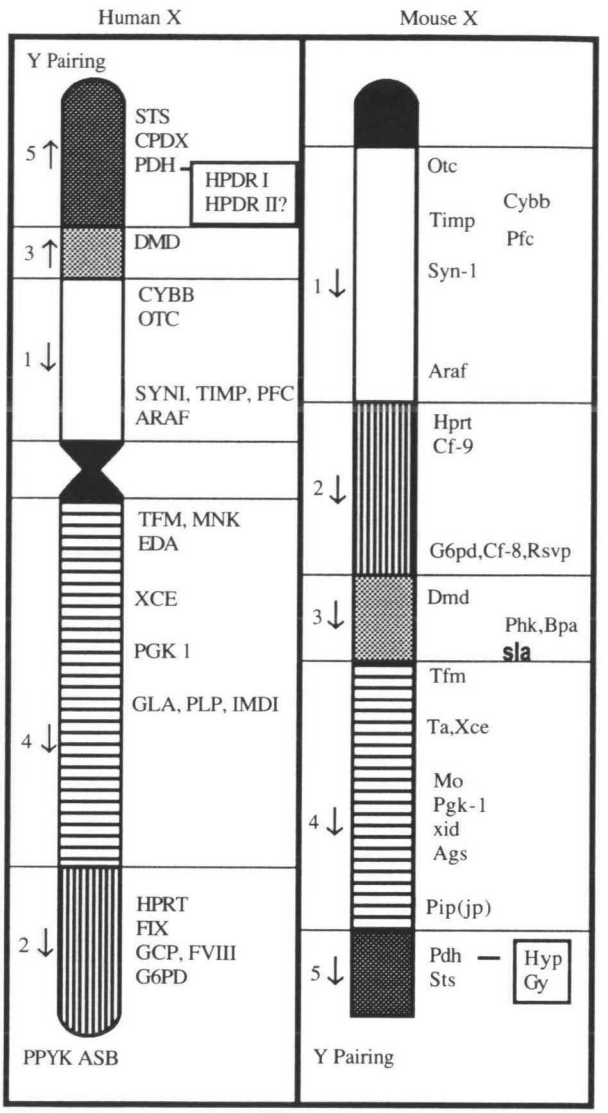

Fig. 3. Homologous linkage groups and rearrangements on human and mouse X chromosomes. HPDR-I and HPDR-II are putative homologues of *Hyp* and *Gy*, respectively. Mutations in these genes cause hypophosphatemia and are associated with bone disease (rickets/osteomalacia) and other abnormalities [Redrawn from Searle *et al.* 1989].

taking into account all male and female offspring of the original *Gy*+/*Hyp*+ × Ta/Y crosses. The possibility of one very large gene tolerating different mutant phenotypes (the Duchenne/Becker muscular dystrophy paradigm) seems unlikely.

9. Gene dose effects

The gene dose effects on serum phosphorus and mineralized tissues are interesting in the X-linked hypophosphatemias. There appears to be a deviant effect on serum phosphorus values and renal phosphate transport and a predicted effect on mineralized tissue. By deviant we mean that the average value in the heterozygote is *not* midway between the values for the mutant hemizygote and for the wild type.

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(i) *Serum phosphorus values*

Four sets of observations appear to show a deviant gene dose effect on serum phosphorus concentration. First, the age-specific values in untreated HPDR heterozygotes and mutant hemizygotes (Table 2) are more alike than they are different (Winters *et al.* 1958). Second, *Hyp*/Y and *Hyp*/+ offspring of *Hyp*/+ × +/Y matings (C57Bl/6J background) have very similar age-specific mean serum phosphorus values (Eicher *et al.* 1976). Third, obligate heterozygous (*Hyp*/+) offspring of *Hyp*/*Hyp* × +/Y matings have age-specific phosphorus values not significantly different from those of the homozygous (*Hyp*/*Hyp*) females (Fig. 4*a*) (Kronick J., C. R. Scriver, H. S. Tenenhouse, unpublished data). Fourth, *Gy*/+ and *Gy*/Y mice have similar serum phosphorus values after normalizing them to the corresponding control values (Lyon *et al.* 1986); (normalization was necessary because diet, age and other factors were different for the two sexes when serum phosphorus was measured).

(ii) *Renal phosphate transport*

Direct measures of Na^+-phosphate cotransport in brush border membrane vesicles prepared from +/+, *Hyp*/+, *Hyp*/*Hyp* female mice show that *Hyp*/+ and *Hyp*/*Hyp* females had similar values which were about half those of +/+ (Fig. 4*b*) (Kronick, J., C. R. Scriver, H. S. Tenenhouse, unpublished data). The similarity between the mutant female phenotypes and the mutant male phenotype (half normal Na^+-phosphate cotransport (see above)) provides evidence for a deviant gene dose effect.

(iii) *Possible mechanisms of the deviant gene dose effect in kidney*

Of several possible mechanisms, none has yet been investigated. They include: (i) Negative allelic complementation when the gene encodes a subunit in a homopolymer (Scriver, 1989). This corresponds to the dominant negative mutation hypothesis (Herskowitz, 1987). In clonal (Lyonized) tissue, the gene product would be a diffusible substance (see section 6). (ii) When there is random inactivation of X chromosomes followed by selection of the mutant clone. X-autosome translocations (Broadhead *et al.* 1986; Kapur *et al.* 1987) with this effect might explain a single case but not a population. A biochemical explanation for selection (Migeon *et al.* 1981) could explain a more universal phenotype among cases. (iii) Selective imprinting of an X-linked locus. This mechanism is equivalent to 'non-random' X chromosome inactivation (Nisen & Waber, 1989). Imprinting is influenced by parental origin of gamete, embryonic origin of the tissue, and DNA segment (Monk, 1988; Sapienza *et al.* 1988) and it is thought to explain the variable

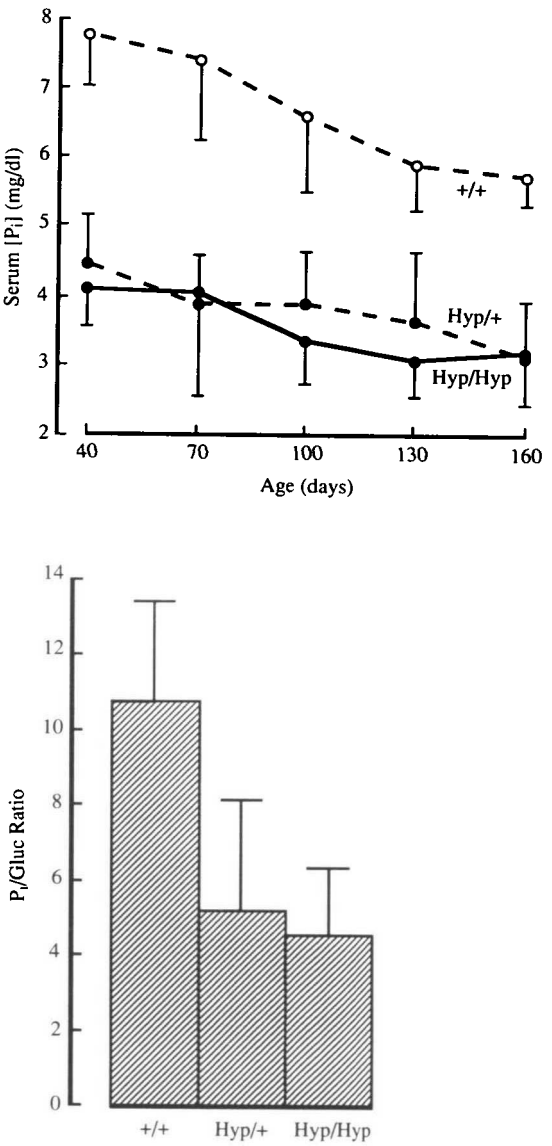

Fig. 4. A. Age-related serum phosphorus values (mean ± S.D.). B. Na^+-phosphate cotransport (mean ± S.D.) normalized to the simultaneous rate of Na^+-glucose cotransport in renal brush border membrane vesicles. The mice were C57Bl/6J normal (+/+) females, homozygous mutant females (*Hyp*/*Hyp*) and obligate heterozgotes (*Hyp*/+); the latter were produced by mating +/Y males with *Hyp*/*Hyp* females. Brush border membrane vesicles were prepared as described by Tenenhouse & Scriver (1978). Serum phosphorus values (panel A) and transport rates (panel B) were not significantly different in *Hyp*/+ and *Hyp*/*Hyp* animals; values in mutants are significantly decreased from controls ($P < 0{\cdot}01$) (J. Kronick, C. R. Scriver and H. S. Tenenhouse, unpublished data).

penetrance of some alleles (Searle *et al.* 1989). The transmitting parent was female in the mouse studies (Eicher *et al.* 1976; Lyon *et al.* 1986; Kronick *et al.* unpublished data and Fig. 4). The effect of the transmitting parent on serum phosphorus values in affected patients has not been measured, to our knowledge. (iv) Allelic restriction (Coleclough *et al.* 1981) is an unlikely mechanism here but it would be compatible with the renal phenotype.

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(iv) *Mineralized tissues in XLH*

Winters *et al.* (1958) and others (Burnett *et al.* 1964; McNair & Stickler, 1969) observed that severity of bone disease and shortness of stature in affected females did not correspond consistently to the severity of hypophosphatemia. The phenomenon led to the idea (Frost, 1958; Frame *et al.* 1965; Steendijk *et al.* 1965) that bone lesions might be the consequence of two phenomena: low phosphorus concentration in extracellular fluid; and expression of the mutant gene in mineralized tissues. Support for the hypothesis was found, for example, in: (i) Expression of the *Gy* gene in both the kidney and the inner ear (Lyon *et al.* 1986). (ii) Periosteocytic lesions in XLH compact bone (Marie & Glorieux, 1983) and in cultured or transplanted *Hyp* calvarial bone (Glorieux & Ecarot-Charrier, 1987; Ecarot-Charrier *et al.* 1988) that were unrelated to environmental phosphate concentration. (iii) Lower bone mineral content in *Hyp*/Y mice relative to *Hyp*/+ mice although serum phosphorus values were similar in the sexes (Kay *et al.* 1985). (iv) Milder bone disease (Scriver *et al.* 1977) and tooth abnormalities (Schwartz *et al.* 1988) in patients with autosomal dominant hypophosphatemia (McKusick 14635) than in patients with XLH, even though serum phosphorus values are similar in the two diseases. New observations on tooth development in XLH patients now provide evidence for extrarenal expression of the HPDR gene; it is based on evidence for a normal gene dose effect on teeth (Shields *et al.* 1990). By normal we mean that the metrical value in heterozygotes lies midway between the values for mutant hemizygotes and wild type controls.

Dental pulp profile area (PRATIO = pulp area/tooth area) and serum phosphorus values were measured in male and female XLH patients, uniformly and well-treated throughout their lifetime (Shields *et al.* 1990). The metabolic environment of tooth development was measured by using the serum phosphorus values obtained in the patients between 1 mo and 26 years of age. PRATIO values, calculated from standard Rinn radiographs, were used as outcome measures of tooth development. Treated XLH males and females had similar and near normal serum phosphorus (because of treatment) throughout the period of tooth development. Patients with teeth forming secondary dentin showed a gene dosage effect on PRATIO. The value was high in XLH male teeth ($0{\cdot}163 \pm 0{\cdot}046$, mean $\pm$ S.D.), intermediate in XLH female teeth ($0{\cdot}137 \pm 0{\cdot}039$) and low in control teeth ($0{\cdot}116 \pm 0{\cdot}023$); the differences were highly significant. (High PRATIO values indicate impaired development or mineralization of dentin). Alternative analysis by individual tooth class corroborated the gene dosage effect (Fig. 5). Since serum phosphorus values during the long period of tooth development were similar in the treated male and female XLH patients, this evidence of a gene dose effect on teeth implies that the HPDR gene was expressed in teeth during their development. The gene product and the developmental process are unknown.

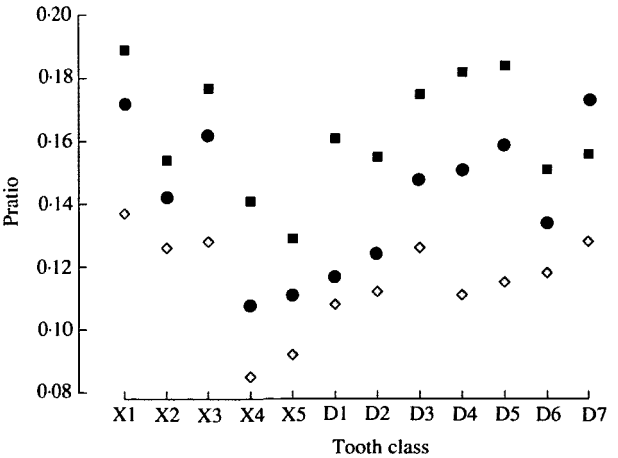

Fig. 5. A gene dose effect gives evidence that the HPDR gene is expressed in teeth. PRATIO (pulp area/tooth area) values are shown by tooth class and genotype (■ = XLH males, 65 teeth; ● = XLH females, 75 teeth; ◇ = controls, 209 teeth). Values for right and left teeth were pooled and averaged in each class (2–9 teeth/class); values for male and female controls were combined (15–21 teeth/class). Tooth-class nomenclature: X = maxilla; D = mandible; 1 = central incisors; 2 = lateral incisors; 3 = canines (cuspids); 4 = first premolars (bicuspids); 5 = second premolars; 6 = first molars; 7 = second molars (from Shields, E. D. *et al.* 1990, with permission).

The other mendelian hypophosphatemias

Autosomal loci as well as X-linked genes are involved in phosphate homeostasis (Table 1). In none of the former is the mechanism of hypophosphatemia yet well understood. The autosomal dominant trait called Hypophosphatemic bone disease (HBD) (McKusick 14625) alters renal phosphate transport in a manner readily distinguishable from that seen in XLH (Scriver *et al.* 1977), the circulating $1,25(OH)_2D$ level is higher in HBD than in XLH (Scriver *et al.* 1978); and while serum phosphorus values are similar in untreated HBD and XLH patients, their bone disease (Scriver *et al.* 1977) and tooth abnormalities (Schwartz *et al.* 1988) are not similar; they are less severe in the former. The autosomal recessive form of hypophosphatemic rickets with hypercalciuria (HHRH) (McKusick 24153) has severely impaired renal phosphate reabsorption in homozygotes (Tieder *et al.* 1985) and partial impairment in heterozygotes (Tieder *et al.* 1987); the serum $1,25(OH)_2D$ value is appropriately elevated. These metabolic peculiarities in autosomal and X-linked phenotypes suggest that the corresponding mutant gene products could be expressed in different segments of the nephron where there are important differences in the transport of phosphate and metabolism of vitamin D (Rasmussen & Tenenhouse, 1989).

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The mutant gene products in the hereditary hypophosphatemias may be components in the array of renal phosphate transporters identified by physiological analysis or they may be regulators of them. We would like to believe the former and with that hypothesis we began this work years ago. But the more we know about XLH, *Hyp* and gyro phenotypes, and about their autosomal counterparts, the less we seem to know.

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