Identification of a new gene product (diphor-1) regulated by dietary phosphate

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Abstract

Chronic restriction of dietary Pi elicits an increased reabsorption of Pi in the kidney proximal tubules, which involves a stimulation of apical Na-Pi cotransport. This adaptation is in part a direct cellular response of which the mechanism(s) are poorly understood. In this study, the impact of dietary Pi restriction on the differential expression of rat kidney cortex mRNAs was visualized to identify gene products regulated by the Pi status. When kidney cortex mRNAs of rats fed a low- or a high-Pi diet were compared by differential display-polymerase chain reaction (DD-PCR), thirty modulated cDNA bands were observed, of which four were confirmed as being regulated. We focused on one of the upregulated bands, dietary Pi-regulated RNA-1 (diphor-1). A cDNA containing an open reading frame encoding a 52-kDa protein was cloned by library screening. Diphor-1 exhibits a high degree of identity to the Na/H exchanger regulatory factor and to a tyrosine kinase activating protein. Highest expression of diphor-1 mRNA was detected in the kidney (proximal tubules) and in small intestine. Expression experiments showed that diphor-1 specifically increases Na-Pi cotransport in oocytes of Xenopus laevis coinjected with renal type II Na-Pi contranporter cRNA. Further characterizations of diphor-1 will show whether diphor-1 is primarily or secondarily involved in the response to dietary Pi.
Identification of a new gene product (diphor-1) regulated by dietary phosphate

MARÍA CUSTER, BENJAMIN SPINDLER, FRANÇOIS VERREY, HEINI MURER, AND JÜRG BIBER
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Custer, MaríA, Benjamin Spindler, François Verrey, Heini Murer, and Jürg Biber. Identification of a new gene product (diphor-1) regulated by dietary phosphate. Am. J. Physiol. 273 (Renal Physiol. 42): F801–F806, 1997.—Chronic restriction of dietary P:\textsubscript{i} elicits an increased reabsorption of P:\textsubscript{i} in the kidney proximal tubules, which involves a stimulation of apical Na-P:\textsubscript{i} cotransport. This adaptation is in part a direct cellular response of which the mechanism(s) are poorly understood. In this study, the impact of dietary P:\textsubscript{i} restriction on the differential expression of rat kidney cortex mRNAs was visualized to identify gene products regulated by the P:\textsubscript{i} status. When kidney cortex mRNAs of rats fed a low- or a high-P:\textsubscript{i} diet were compared by differential display-polymerase chain reaction (DD-PCR), thirty modulated cDNA bands were observed, of which four were confirmed as being regulated. We focused on one of the upregulated bands, dietary P:\textsubscript{i}-regulated RNA-1 (diphor-1). A cDNA containing an open reading frame encoding a 52-kDa protein was cloned by library screening. Diphor-1 exhibits a high degree of identity to the Na/H exchanger regulatory factor and to a tyrosine kinase activating protein. Highest expression of diphor-1 mRNA was detected in the kidney (proximal tubules) and in small intestine. Expression experiments showed that diphor-1 specifically increases Na-P:\textsubscript{i} cotransport in oocytes of Xenopus laevis coexpressed with renal type II Na-P:\textsubscript{i} cotransporter cRNA. Further characterizations of diphor-1 will show whether diphor-1 is primarily or secondarily involved in the response to dietary P:\textsubscript{i}.

renal transport; phosphate; regulation; adaptation

REGULATION OF THE PLASMA level of P:\textsubscript{i} is crucial for the metabolism of P:\textsubscript{i}. Bone, kidney, and small intestine, regulated through interacting hormones, are the major organs involved in maintaining extracellular P:\textsubscript{i} concentrations constant. Regulated reabsorption of filtered P:\textsubscript{i} in the renal proximal tubules represents a key process in overall P:\textsubscript{i} homeostasis (2, 6).

As a first response, dietary P:\textsubscript{i} restriction provokes an adaptive increase in the capacity of the renal proximal tubules to reabsorb P:\textsubscript{i}. Part of this phenomenon has been described to be independent of extrarenal factors, such as parathyroid hormone, 1,25-dihydroxyvitamin D\textsubscript{\textgamma}, plasma calcium, or growth hormone, and therefore likely represents an intrinsic cellular response of the proximal tubular cell (2, 16). In response to chronic phosphate deprivation the activity of proximal tubular Na-dependent P:\textsubscript{i} cotransport mediated by the type II Na-P:\textsubscript{i} cotransporter (NaPi-2) is increased. Previous studies indicated that adaptation to chronic P:\textsubscript{i} restriction involves a small increase of the NaPi-2 mRNA and a larger increase of type II Na-P:\textsubscript{i} cotransporters in the apical membrane (11, 12, 20). The mechanisms leading to the apparent additional increase in Na-P:\textsubscript{i} cotransporter abundance compared with the relatively small increase in mRNA content are not known but may be explained by an involvement of other (regulatory) proteins also being regulated by chronic P:\textsubscript{i} deprivation.

In addition, dietary P:\textsubscript{i} restriction leads to an increase of the rat kidney parathyroid hormone receptor mRNA (9) and to an increase of serum 1,25-dihydroxyvitamin D\textsubscript{\textgamma}. The increase of vitamin D\textsubscript{\textgamma} can be explained, at least in part, by an increase of the activity of the renal 25-hydroxyvitamin D-1a-hydroxylase and by a decrease of proteins involved in the intracellular catabolism of 1,25-dihydroxyvitamin D\textsubscript{\textgamma} (18, 21). An increase of serum 1,25-dihydroxyvitamin D\textsubscript{\textgamma} largely explains the increase of small intestinal Na-P:\textsubscript{i} cotransporter (4), which occurs secondary to the renal response to low-P:\textsubscript{i} diet.

The signaling mechanisms that link dietary P:\textsubscript{i} deprivation to proximal tubular cell adaptation, such as altered Na-P:\textsubscript{i} cotransport, are still unknown. In this report, differential display-polymerase chain reaction (DD-PCR) was used to identify differences in the mRNA populations of rat kidney cortex of animals fed chronically a low- (0.1%) or a control (high, 1.2%) P:\textsubscript{i} diet. A newly identified mRNA, which is regulated by dietary P:\textsubscript{i} (diphor-1), is described.

MATERIALS AND METHODS

Experimental animals. Experiments were performed with male Sprague-Dawley rats weighing 180–200 g. For experimental purposes, the animals were fed chronically for 3 days a low- (0.1%) or a control (high, 1.2%) phosphate chow, which was obtained from Kiliba Mühlen (Kaiseraugst, Switzerland).

Differential display. Total RNA was isolated from the kidney cortex using the cesium trifluoroacetate/guanidinium thiocyanate (CSTFA) centrifugation method according to the protocol given by the manufacturer (Pharmacia). After isolation, 50 µg of total RNA were incubated for 30 min at 37°C with 10 U deoxyribonuclease I (Pharmacia) in 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 8.5, 10 mM MgCl\textsubscript{\textgamma}, 100 mM KCl, 1 mM dithiothreitol (DTT), and 10 U RNAsin (Promega) and used as template for reverse transcription in a volume of 40 µl using 2.4 µM oligo(dT)\textsubscript{\textgamma} primer, 50 µM dNTPs, 5 mM MgCl\textsubscript{\textgamma}, and ethanol precipitated. Differential display was performed using a modification (S. Gullans, personal communication) of the protocol originally described by Liang and Pardee (13). The reverse transcriptase reaction was performed using oligo(dT)\textsubscript{\textgamma} primers, and, for PCR, pairs of the following arbitrary 13-mers were used in all possible combinations: TGGATTTGGTCTCC, CTTTCTACCCGAC, GATCATGGTGCAC, TCCATACTGAGGC, GATCATGAAGTCG, and TACAAGCGGCCG. Two micrograms of total RNA were denatured at 65°C for 10 min in the presence of 50 U RNAsin (Promega) and 2 mM DTT and used as template for reverse transcription in a volume of 40 µl using buffer II (Perkin-Elmer), 2.4 µM oligo(dT)\textsubscript{\textgamma} primer, 50 µM dNTPs, 5 mM MgCl\textsubscript{\textgamma}, and 400 U Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL) for 1 h at 37°C. After heat inactivation (5

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min at 95°C), 2 µl of the cDNA sample were used as a template for the PCR reaction in a total volume of 20 µl composed of buffer II, 500 nM of two 13-mer primers, 1.25 mM MgCl2, 30 mM (α-32P)dCTP (3,000 Ci/mmol, NEN), and 1.25 U AmpliTaq DNA polymerase (Perkin Elmer). The PCR reaction was performed using a Bio-Rad thermocycler with the following setting: an initial denaturation step at 94°C for 1 min, followed by 40 cycles for 30 s at 94°C, 2 min at 55°C, and 30 s at 72°C. The reaction was terminated for 5 min at 72°C.

All aliquots of the PCR reaction (3 µl) were loaded on denaturing 6% polyacrylamide gels. After drying on a Whatman 3-M paper and autoradiography, the cDNA bands of interest were cut out of the gel, rehydrated in 200 µl water overnight, and boiled for 10 min, and the DNA was precipitated using glycogen (0.5 mg/ml) as a carrier. The DNA was reamplified using the corresponding primer set, and the same PCR conditions as above, except that no tracer was used.

Cloning of amplified cDNA fragments and sequencing. Reamplified cDNA fragments were first used as probes for Northern blots (see below) and then cloned using the PCR-Script Amp SK (+) Cloning Kit (Stratagene). Sequencing of the cloned cDNAs was performed with the T7 Sequencing Kit (Pharmacia) using standard T7 and T3 primers. Sequence similarities to known DNA sequences were searched with the basic local alignment search tool [BLAST; Altschul et al. (1)].

Northern blot analysis. Total cellular RNA of rat kidney cortex was isolated using the CsTFA method as above. Polyadenylated RNA was prepared from total RNA using oligo(dT) cellulose (Pharmacia). Samples containing 5 µg of poly(A)+ RNA were run on 1.5% agarose-formaldehyde gels, transferred to Pall transfer membranes (Biodyne), and immobilized with ultraviolet light, according to standard protocols. [α-32P]dCTP-labeled probes were generated using a random-priming oligolabeling Kit (Pharmacia). Hybridization and washes were performed according to standard protocols. The blots were exposed and scanned using a Phosphorlmager and analyzed using the ImageQuant software (Molecular Dynamics).

Library screening and characterization of diphor-1 cDNA. A rat kidney cortex cDNA library constructed in psport1 (14) was screened first by PCR with diphor-1-specific primers. Positive sublibraries were further tested by colony hybridization with the original diphor-1 cDNA fragment. The longest of the four cDNAs obtained was sequenced on both strands as above. In vitro translation of diphor-1 was performed with a rabbit reticulocyte lysate system in the absence and in the presence of microsomes (TNT Kit, Promega).

Xenopus laevis oocytes and transport assays. All techniques and methods concerning the handling of oocytes as well as the assay for transport were performed as described (19). Oocytes were injected with 50 nl of water containing 100 ng/µl of the indicated cRNA(s). After 2–3 days, uptake of the substrates H32PO4, 35SO4, or α-[methyl-3H]glucopyranoside (NEC Radiochemicals) was measured either in the presence or absence of sodium as described. For in vitro transcription, plasmid DNAs were isolated by standard procedures, linearized by Not I, and transcribed with T7 polymerase (Promega).

Reverse transcription-PCR with microdissected tubules. Microdissection of nephron segments and reverse transcription (RT)-PCR was performed as described (5). For RT, oligo(dT) primers were used, and the PCR reaction was carried out using the following diphor-1-specific primers: sense (position 1207–1230), CGACACGTACGGCTTTCCACCTGAA; and antisense (position 1511–1533), GCTGAGTGTGCTAGTCTCTCC. The expected size of the PCR product obtained with these primers is 326 bp.

RESULTS

DD-PCR was used to analyze changes in mRNA expression in rat kidney cortex after chronic dietary Pi deprivation. Rat kidney cortex RNAs of animals fed a low- (0.1%) or a control (high, 1.2%) Pi diet for 3 days were compared using all combinations of the primers described in MATERIALS AND METHODS. This series of differential display reactions yielded a total of ~1,000 cDNA bands, 30 of which showed reproducible differences in intensity. These bands were excised, and the cDNA fragments reamplified by PCR using the corresponding primer combinations. Such reamplified PCR products were used for Northern blots using poly(A)+ RNA isolated from kidney cortex of rats fed a low- or a control (high) Pi diet. Ten of such eluted bands produced a detectable and regulated signal and were subsequently subcloned and tested again by Northern blotting using the same poly(A)+ RNAs. Four of these subcloned DD-PCR products produced on Northern blots the expected regulated signals. One product was found to be downregulated, and three were found to be upregulated. In this study, the analysis of one of the Pi-upregulated candidates obtained by DD-PCR is described. This upregulated product was called diphor-1 (for dietary Pi-regulated RNA-1) and was studied in more detail.

Diphor-1 probes hybridized with two transcripts of ~2.8 and 2.3 kb on Northern blots (Fig. 1). Both transcripts were upregulated approximately twofold after 3 days of Pi deprivation (Fig. 1). The mRNA corresponding to the rat renal Na+-dependent Pi co-transporter of type II (NaPi-2), which has been demonstrated to be upregulated approximately twofold under...
dietary P_i deprivation conditions (12, 20), was used as a control for adaptation, and β-actin was used as a negative control.

With the diphor-1 fragment as a probe, an ~2-kb long cDNA was isolated from a rat kidney cortex cDNA library. The clone identified in which the nucleotide sequence of the corresponding subclone was confirmed contained an open reading frame of 1,422 bp (coding for a protein of 52 kDa), preceded by 46-bp 5'-untranslated region (5'-UTR) and followed by 546-bp 3'-UTR. A database search with the amino acid sequence deduced from the diphor-1 open reading frame revealed that diphor-1 is 33% identical (57% similar) to the Na/H exchanger regulatory factor (NHE-RF) of rabbit and mouse (Ref. 18 and unpublished data) and 28% identical (49% similar) to a tyrosine kinase activator protein 1 (TKA-1) (K. Seedorf and A. Ullrich, unpublished observations). As illustrated by the hydrophobicity plot (Fig. 2B), the protein diphor-1 likely represents a hydrophilic protein, since no obvious transmembrane region is evident.

In vitro translation experiments using the full-length clone resulted in a major product of ~55 kDa (Fig. 2C), in agreement with the size of the protein deduced from the open reading frame. When in vitro translation was performed in the absence of diphor-1 cDNA, no product

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**Fig. 2.** A: comparison of amino acid sequence deduced from diphor-1 with related sequences Na/H exchanger regulatory factor (NHE-RF) and tyrosine kinase activator protein 1 (TKA-1). Diphor-1 exhibits a similarity of 33% and 28% to NHE-RF and TKA-1, respectively. Complete nucleotide sequence of diphor-1 has been deposited in the GenBank; accession no. AF013145. B: hydrophobicity profile of diphor-1 deduced amino acid sequence obtained using algorithm of Kyte and Doolittle (10) with a window of 20 amino acids. C: cell-free translation of diphor-1 cDNA in a reticulocyte lysate system in the presence and absence of microsomes. Under both conditions, major product appeared as a protein with an apparent mol mass of 55 kDa.
of 55 kDa was obtained indicating the specificity of the translated protein (not shown). The apparent molecular weight of the in vitro translated products was identical in the absence or in the presence of pancreatic microsomes (Fig. 2C), despite the presence of two consensus sites for N-glycosylation (asparagine residues 190 and 346) (Fig. 2A).

Expression of diphor-1 mRNA in different rat tissues was tested by Northern blot analysis using poly(A)+ RNA of small intestine, liver, and skeletal muscle. As illustrated in Fig. 3, besides in the kidney, diphor-1 mRNA is significantly expressed in the small intestine, slightly in the liver, and could not be detected in skeletal muscle. Interestingly, this analysis revealed that diphor-1 mRNA is upregulated by dietary Pi deprivation not only in the kidney cortex (2-fold, see Figs. 1 and 3) but also in the small intestine (3- to 4-fold, Fig. 3). Diphor-1 localization along the rat nephron was studied in single microdissected nephron segments by RT-PCR. RT-PCR reaction was performed with 21 proximal tubules, 14 papillary collecting ducts, and 5 glomeruli, of which 20, 10, and 2, respectively, were positive. As illustrated in Fig. 4, single PCR products of the expected size of 326 bp were obtained in proximal tubules and collecting ducts. RT-PCR reaction was strictly dependent on the presence of mRNA, since no diphor-1-related product was detected in the absence of reverse transcriptase (Fig. 4). In approximately half of the experiments, a diphor-1 signal was also observed in microdissected glomeruli, which is explained by a variable amount of proximal tubular cells attached to the glomerulus.

To study whether diphor-1 might be involved in the regulation of proximal tubular Na⁺-dependent Pi cotransport, diphor-1 and NaPi-2 were coexpressed in X. laevis oocytes. As shown in Fig. 5A and Table 1, coinjection of diphor-1 with NaPi-2 cRNA stimulated Na-dependent Pi uptake −1.7 times compared with oocytes injected with NaPi-2 cRNA alone. This effect was observed using different oocytes from different frogs and also different cRNA preparations (Table 1). The stimulation of Pi uptake was sodium dependent because no stimulation was observed when the uptake was performed in the presence of choline instead of sodium (Fig. 5A). To study whether this effect of diphor-1 was specific for NaPi-2 or if it could also

![Fig. 3. Tissue distribution of diphor-1 studied by Northern blot analysis. From tissues indicated, poly(A)+ RNA was isolated from rats chronically fed a low- or a high-Pi diet. β-Actin was used as a control for equal loading. In the case of liver poly(A)+ RNA, equal loading was confirmed by probing with glyceraldehyde-3-phosphate dehydrogenase (GAPDH).](image)

![Fig. 4. Localization of diphor-1 in microdissected nephron segments and glomeruli by reverse transcription-polymerase chain reaction (RT-PCR). PCR reaction was performed with samples treated with or without reverse transcriptase (RT).](image)

![Fig. 5. Effect of diphor-1 cRNA on expression of NaPi-2-associated Na-Pi cotransport (A), NaSi-1-associated Na-sulfate cotransport (B), and intrinsic Na-Pi cotransport (C). Oocytes of Xenopus laevis were injected with 5 ng of each cRNA, and, after 3 days, uptake of phosphate (at 0.5 mM) or sulfate (at 1 mM) was determined. Solute transport was determined either in the presence of sodium (solid bars) or choline (open bars). Results are means ± SD of 10 oocytes.](image)
Table 1. Effect of diphor-1 cRNA on NaPi-2- and NaSi-1-associated Na-dependent transports of phosphate and sulfate

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Oocytes Injected With</th>
<th>Stimulation, %</th>
<th>Oocytes Injected With</th>
<th>Stimulation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaPi-2</td>
<td>NaPi-2 + diphor-1</td>
<td>NaSi-1</td>
<td>NaSi-1 + diphor-1</td>
</tr>
<tr>
<td>1</td>
<td>9.7 ± 2.8</td>
<td>16.9 ± 2.7</td>
<td>74</td>
<td>46.9 ± 6.6</td>
</tr>
<tr>
<td>2</td>
<td>11.9 ± 3.5</td>
<td>16.3 ± 5.8</td>
<td>40</td>
<td>44.1 ± 5.0</td>
</tr>
<tr>
<td>3</td>
<td>12.5 ± 3.2</td>
<td>20.2 ± 2.7</td>
<td>60</td>
<td>-6</td>
</tr>
<tr>
<td>4</td>
<td>4.9 ± 1.3</td>
<td>12.0 ± 4.5</td>
<td>140</td>
<td>72.5 ± 18.1</td>
</tr>
<tr>
<td>5</td>
<td>6.9 ± 3.3</td>
<td>12.4 ± 4.8</td>
<td>70</td>
<td>73.8 ± 10.5</td>
</tr>
<tr>
<td>6</td>
<td>16.2 ± 3.9</td>
<td>20.4 ± 6.3</td>
<td>26</td>
<td>103.7 ± 21.5</td>
</tr>
</tbody>
</table>

Values are means ± SD of 10 oocytes. Units are pmol·oocyte⁻¹·min⁻¹ of net sodium-dependent uptake. Oocytes of X. laevis were injected with 5 ng cRNAs, and, after 3 days, transport of phosphate or sulfate was measured in the presence of sodium or choline. NaPi-2, Na-dependent type II Pi cotransporter; NaSi-1, Na-dependent type I sulfate cotransporter.

stimulate other Na-dependent cotransporters, experiments were performed in parallel with the renal Na-dependent sulfate transporter NaSi-1 (15). As demonstrated in Fig. 5B, diphor-1 did not affect the transport characteristics of NaSi-1. Furthermore, injection of diphor-1 cRNA alone did not affect the intrinsic Na-dependent transport of the oocytes (Fig. 5C).

**DISCUSSION**

Dietary P_i deprivation produces, among other responses, a stimulation of the proximal tubular Na-dependent P_i cotransport via an increase of the type II Na-P_i cotransporter in the apical membrane (11). The aim of this study was to identify other gene products involved in this adaptive process by studying changes in the renal cortex mRNA expression due to dietary P_i restriction by DD-PCR.

DD-PCR has been mainly used to detect differences of gene expression between transformed and nontransformed cells and also to identify developmentally or hormonally regulated genes (e.g., Ref. 7). However, as reported, nutritional regulation of mRNA levels is not necessarily as pronounced as that of hormonal or developmental mRNA regulation (3). Therefore, to apply the DD-PCR technique to study genes regulated by dietary P_i, a generous selection criterion for the differentially expressed cDNAs had to be used, meaning that all cDNA bands arising from kidney RNA of animals fed a low P_i with different visual intensities relative to the control RNA (from animals fed a high-P_i diet) were selected. However, the major problem of such a selection criterion is the high probability of selecting false positives. This could be an explanation for the fact that only 4 of the 30 bands initially selected could be confirmed by Northern blot analysis of kidney cortex mRNA of low- and high-P_i-fed animals as being regulated.

In this work, one of the four P_i-regulated gene products obtained by DD-PCR, diphor-1, is further described. The deduced amino acid sequence of diphor-1 exhibits high homology to two previously described proteins, namely, to the rat Na/H exchanger regulatory factor (NHE-RF, Ref. 18) and to TKA-1 (Sedendorf et al., unpublished observations). NHE-RF has been suggested to be a mediator of protein kinase A action on the Na/H exchanger (18), but no functional evidence for the TKA-1 has been reported so far.

Northern blot analysis of diphor-1 with kidney cortex mRNA isolated from rats fed a low- or a high-P_i diet for 3 days indicated hybridization with two transcripts (2.3 and 2.8 kb), which were equally upregulated (~2-fold) after P_i deprivation. This magnitude of mRNA regulation is similar to that described for NaPi-2 mRNA induced by P_i deprivation (11, 12, 20). Therefore, to study whether diphor-1 could be implicated in the regulation of the Na-P_i cotransporter NaPi-2, functional studies were performed in X. laevis oocytes. Coinjection of diphor-1 and NaPi-2 cRNA into X. laevis oocytes followed by measurement of P_i uptake demonstrated that diphor-1 indeed influences the extent of NaPi-2-associated transport. This effect of diphor-1 was specific for NaPi-2 because no alteration of the transport of the NaSi-1 or the intestinal Na-dependent glucose transporter (not shown) was noticed when transport of the corresponding substrates was measured in X. laevis oocytes after coinjection of diphor-1 cRNA with the cRNAs corresponding to these transporters. Furthermore, injection of diphor-1 cRNA alone did not stimulate intrinsic P_i transport of X. laevis oocytes. Taken together, these results indicate that the described protein diphor-1 specifically exerts an effect on the expression of NaPi-2.

The mechanism by which diphor-1 stimulates NaPi-2-associated Na-P_i cotransport in oocytes has not been yet elucidated. Because transport studies performed at different concentrations of phosphate indicated an increase of Vmax without change of the Michaelis constant of Na-P_i cotransport in oocytes coinjected with NaPi-2 and diphor-1 (data not shown), the following two possibilities need to be considered: 1) diphor-1 leads to an increase of the number of transporters at the cell membrane, e.g., by influencing the turnover of NaPi-2 protein; or 2) the NaPi-2 transporters present in the membrane are more active in the presence of diphor-1.

Tissue distribution studies revealed that diphor-1 related mRNA is present in the kidney and in the small intestine. Interestingly, diphor-1 mRNA was found to be regulated by P_i deprivation in the small intestine as well. Because dietary P_i deprivation leads to an increased concentration of the active form of vitamin D (1,25-dihydroxyvitamin D_3) in serum, which stimulates the Na-dependent P_i transport in the small intestine (4), this suggests that diphor-1 could also be involved.
in the adaptive response of small intestinal Na-P\textsubscript{i} transport.

In summary, in this study, we describe a newly identified gene product that is differentially expressed in rat kidney cortex of animals fed a high-P\textsubscript{i} diet, compared with animals fed chronically a low-P\textsubscript{i} diet for 3 days. The identification of diphor-1 may provide an additional target molecule to help to understand the mechanisms involved in dietary P\textsubscript{i} adaptation.

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