A murine transgenic model for transcriptional regulation of the Na/Pi-IIa major renal phosphate cotransporter

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Rosenberg T, Shachaf C, Tzukerman M, Skorecki K. A murine transgenic model for transcriptional regulation of the Na/Pi-IIa major renal phosphate cotransporter. Am J Physiol Renal Physiol 292: F1617–F1625, 2007. First published February 6, 2007; doi:10.1152/ajprenal.00412.2006.—Levels of the type IIa Na/Pi cotransporter, which serves as the principal mediator of phosphate reabsorption in the kidney, can be modulated through posttranscriptional or posttranslational mechanisms by dietary, hormonal, and pharmacological influences. Previous studies have not demonstrated clear-cut evidence for modulation of Na/Pi-IIa cotransporter levels through transcriptional mechanisms. We have previously demonstrated that a 4.7-kb rat genomic fragment upstream of the rodent Npt2 gene encoding the Na/Pi-IIa cotransporter, is sufficient to mediate its transcriptional activity in vitro (Shachaf C, Skorecki KL, Tzukerman M. Am J Physiol Renal Physiol 278: F406–F416, 2000). Accordingly, we have established an in vivo experimental model in which this Npt2 genomic fragment fused upstream of a Lac Z reporter gene was expressed as a transgene in mice. The nine independent transgenic founder lines exhibited transgene expression specifically in the renal cortex. This renal cortical-specific expression driven by the Npt2 promoter was confirmed at the mRNA and protein levels using RT-PCR, histochemistry, and Lac Z enzymatic activity. Furthermore, the expression of the transgene correlated with expression of the endogenous Npt2 gene during embryonic and early postnatal development. Thus we have generated a transgenic mouse model which will enable in vivo investigation of the contribution of transcriptional mechanisms to the overall regulation of Na/Pi-IIa expression under physiological and pathophysiological conditions.

Npt2; Lac Z

INORGANIC PHOSPHATE (Pi) is of critical importance to cellular function, as well to development of the skeletal system, especially during periods of growth (21). The kidneys regulate the excretion of Pi in response to varying intake, by adjusting the filtered load of phosphate in the normal mature kidney. Several renal phosphate-wasting disorders have been shown to involve perturbations in the expression or function of the Na/Pi-IIa cotransporter (28). Among others, these include the hereditary disorders X-linked hypophosphatemia and autosomal dominant hypophosphatemic rickets, as well as the acquired disorder oncogenic hypophosphatemia. These three disorders all involve reduced abundance of the Na/Pi-IIa cotransporter as a result of increased levels or action of the regulatory hormone FGF23. While experimental knockout of the homologous murine gene (Npt2) results in severe hypophosphaturia and hypophosphatemia, as yet no equivalent loss-of-function mutation for SLC34A1, encoding Na/Pi-IIa, has been described as a cause of hypophosphatemic rickets in humans (11). In contrast, loss-of-function mutations in the SLC34A3 gene, encoding Na/Pi-Iic, have recently been identified in patients with phosphaturia and renal stone formation (3, 12). A number of dietary

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and hormonal influences as well as pharmaceutical agents have been shown to modulate Na/Pi-IIa cotransporter levels, mostly through posttranscriptional or posttranslational mechanisms. No clear-cut evidence has been obtained to date of modulation of Na/Pi-IIa cotransporter levels through transcriptional mechanisms, during ontogeny or in response to physiological or pharmacological influences (29).

We have previously isolated and demonstrated that a 4.7-kb rat genomic fragment upstream of the rodent Npt2-coding region is sufficient to mediate its transcriptional activity (22). In particular, the isolated fragment induced transcription of a reporter gene in vitro in opossum kidney (OK) cells which express the endogenous Npt2 gene, but not in other cell lines which do not express an endogenous Npt2 gene. In these studies, we also identified a number of cis-acting promoter-regulatory sites responsible for cell-specific expression. To date, information about the Npt2 promoter in various species, has been derived exclusively from studies in tissue culture systems. Accordingly, we have established an in vivo experimental model in which the previously characterized 4.7-kb rat Npt2 gene promoter fragment fused upstream of the bacterial β-galactosidase (Lac Z) was expressed as a transgene in mice.

Nine founder lines were generated expressing the transgene, and three of these were subject to further breeding and confirmation as transgenic mouse founder lines. This analysis showed that the Lac Z reporter gene is expressed specifically in the proximal tubules of the renal cortex, and not in other organs. Furthermore, to confirm the validity of this transgenic model for the study of transcriptional regulation, we sought and found a correlation between expression of the transgene and the endogenous Npt2 gene during embryonic and early postnatal development.

MATERIALS AND METHODS

Establishment of Npt2 transgenic mice. A 4.7-kb genomic DNA fragment containing 4 kb of a 5′-flanking sequence (22) was fused upstream to the Lac Z reporter gene in the pPD4.21 vector (kindly provided by M. Shani) and was used to generate transgenic mice. The Lac Z reporter gene contained an SV40 nuclear localization signal (5).

Transgenic mice were maintained under a cycle of 12 h of light and 12 h of darkness. Transgenic mice were generated as described previously (9, 23). In brief, fertilized FVB/N eggs were microinjected with the Npt2 promoter-Lac Z reporter gene DNA cassette and reimplanted into the oviducts of pseudopregnant CD1 females. Transgenic founders were identified by Southern blot analysis of tail-reimplanted DNA. Five micrograms of genomic DNA from tail tips of the progeny were digested with EcoRV and NcoI restriction endonucleases (New England Biolabs, Ipswich, MA), fractionated on agarose gels, and transferred to nylon membranes. Southern blot analysis was performed using a labeled Npt2 gene fragment and Lac Z gene fragment as probes. To determine the copy number of the transgene inserted into the genome, the DNA blots were reprobed with the mouse C-Kappa gene fragment that exists as a single copy in the mouse genome. The intensity of the lac Z gene signal vs. the intensity of the C Kappa gene signal was quantified from the autoradiograms.

β-Galactosidase whole-organ staining. Adult mice were killed, and whole tissues were removed and fixed in 4% paraformaldehyde solution for 5 h. The tissues were washed three to four times in PBS and stained in PBS containing 2 mM MgCl2, 0.01% sodium deoxycholate, 0.02% NP-40, 5 mM K4Fe(CN)6, 5 mM K3Fe(CN)6, and 0.1% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; Roche, Indianapolis, IN) dissolved in N,N-dimethylformamide (CH3N2O) at 37°C under continuous agitation in the dark for several hours.

β-Galactosidase histochemical staining. Adult mice were anesthetized and perfused with fixative solution containing 3% paraformaldehyde and 0.05% picric acid in a 6:4 mixture of 0.1 M cacodylate buffer (pH 7.4 adjusted to 300 mosmol/kgH2O with sucrose) and 10% hydroxyethyl starch in saline, according to Lotscher et al. (13). The kidneys were removed, embedded in Tragant (Reidel de-Haen, Seelze, Germany) and snap-frozen in 2-methylbutane (Acros Organics, Geel, Belgium) cooled in liquid nitrogen. Ten-micrometer frozen sections were mounted on Super FrostPlus microscope slides (Menzel-Glaser, Braunschweig, Germany) and stained with X-gal solution as described.

RT-PCR. Whole organ tissues were snap-frozen in liquid nitrogen. Total RNA was extracted from homogenized tissues using 10 ml TRI Reagent (Molecular Research Center, Cincinnati, OH). RNA (10 µg) was treated with RNase-free DNase (10 U) at 37°C for 30 min and subsequently reverse transcribed using 1 µg oligo(dT)12-18 primer, 40 U RNAsin, and 200 U Moloney murine leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY) in a total reaction volume of 25 µl at 42°C. The cDNA product was used for PCR amplification. For Lac Z gene amplification, the following primers were used: Lac Z 524, 5′-ATG TGA GCG AGT AAC AAC 3′ and Lac Z 183, 5′-GAT CGA TCT CGT TTG ACA ACG TCG TGA-3′ (364-bp fragment). The PCR reaction was carried out for 36 cycles as follows: denaturing at 95°C for 1 min, annealing at 56°C for 1 min, and polymerization at 72°C for 1 min. The PCR products were then fractionated by electrophoresis on a 2% agarose gel. For amplification of the endogenous Npt2 gene transcript, the following primers were used: NaPi 1544, 5′-ATG TGA GCG AGT AAC AAC CCG TCG GAT TCT-3′ and NaPi 186, 5′-GAT CGA TCT CGT TTG ACA ACG TCG TGA-3′ (364-bp fragment). For β-actin gene amplification, the following primers were used: β-actin forward, 5′-CCG CGG CTC TGT GCA CTC C-3′ and β-actin reverse, 5′-CCG GCC AGC CAA GTC CAC GAC GG-3′ (508-bp fragment).

β-Galactosidase solution assay. This was conducted using for the assay a β-galactosidase fluorescent activity detection kit (Sigma-Aldrich, Rechovot, Israel). Fresh transgenic and nontransgenic mice were homogenized in 50 mM HEPES buffer, pH 7.4, containing 5 mM CHAPS. Samples were frozen and thawed three times and centrifuged to remove cell debris. Protein concentrations were determined using a Bio-Rad protein assay (Bio-Rad, Hercules, CA). Two milligrams of protein were used to perform the β-galactosidase solution assay in 0.1 M sodium phosphate buffer, pH 7.3, 1 mM MgCl2, 50 mM β-mercaptoethanol, and 0.85 mM 4-methylumbelliferyl β-D-galactosidase as a substrate at 37°C for 90 min. Fluorescence was measured using a Fluorimeter plate reader (FLUOstar...
Real time RT-PCR. Forty nanograms of total RNA extracted from embryonic and newborn kidneys were subjected to one-step real-time RT-PCR using a Quantitect SYBR Green RT-PCR kit (Qiagen, Santa Clarita, CA) and Rotor-Gene RG-3000A apparatus (Corbett Research, Sydney, Australia). Each reaction mix contained the following: 40 ng RNA, 100 ng of each primer, 12.5 μl 2× PCR buffer, 0.5 μl RT mix, and DEPC-H2O to 25 μl. The following conditions were used: for reverse transcription, 30 min at 50°C; for the PCR initial activation step, 15 min at 95°C; and for amplification, 15 s at 94°C, 30 s at 56°C, and 30 s at 72°C for 40 cycles. Melting curve analysis of the RT-PCR products was performed to verify specificity and identity of the PCR products. The specificity of the RT-PCR products was examined by agarose gel electrophoresis. For each sample, real-time RT-PCR analysis was performed and compared with the β-actin gene, which served as an internal control. Primers used for gene amplification are listed above.

RESULTS

Generation of II Npt2-Lac Z transgenic founder mice. We have previously shown that a 4.7-kb fragment of the rat Npt2 genomic region from −4,035 to +706 bp concurrent to the promoter regulatory region was sufficient to drive cell-specific gene regulation in OK cells (22). To study the activity of this rat Npt2 promoter in vivo, this fragment was fused upstream to the coding sequence of an Escherichia coli-Lac Z reporter gene and used to produce transgenic mice (Fig. 1A). The chimeric cassette was constructed in the pPD46.21 vector and digested with Not I (3′)- and Ase I (5′)-flanking restriction enzymes to remove vector sequences. The cassette was injected into the pronucleus of the one-cell mouse embryo of FVB/N mice and then introduced to a pseudopregnant (CD1) female mouse that carried the eggs to term (as previously described in Refs. 9 and 23; see schematic presentation in Fig. 1B). Pups were screened for the presence of the transgene by Southern blot analyses of genomic DNA from tail clips to identify those harboring the transgene DNA. Nine of the 24 offspring carried the transgene construct in the genome (marked with an asterisk in Fig. 2A). Positively reacting fragments of 3.9 and 1.7 kb from the rat Npt2 gene and 1.7-kb fragment from the Lac Z gene were identified (Fig. 2A). To produce transgenic lines, each founder transgenic mouse was crossed with a nontransgenic mouse. Analysis of the F1 offspring showed that the transgene cassette was transmitted to ~50% of the litter as expected for Mendelian inheritance (Fig. 2B). Heterozygous male and female mice were crossed to produce F2 mice (Fig. 2C). As expected, ~75% of the F2 offspring expressed the transgene, in either heterozygotes or homozygotes. To identify the homozygous F2 offspring, candidate F2 transgenic mice were bred with nontransgenic mice, and F2 homozygosity was confirmed when 100% of the F3 offspring of such pair were transgenic (Fig. 2D).

The number of copies of the transgene cassette insertion was determined by comparison of the Lac Z gene radioactive signal to the C Kappa gene, which is a single copy 2.4 of kb. The number of integration sites varied among the founders from 3 to 38 copies, thus providing evidence of separate integration events, as shown in Table 1. Founder line F4, which carried 38 copies, showed the highest level of Lac Z gene transcription.

![Fig. 1. Schematic representation of generation of transgenic mice. Shown is a map of the kidney cortex-specific Npt2 promoter-Lac Z construct (A). To generate transgenic mice, a 4.7-kb fragment of genomic DNA from −4035 to +706 bp corresponding to the rat Npt2-regulatory regions that drive cell-specific gene regulation in OK cells was used. The arrow indicates the transcription initiation site of the Npt2 gene. Restriction enzymes were as follows: B, Bam HI at −3458; Hp, Hpa I at −1514; and A, Acc 65 I at −954. The cassette was constructed in the pPD46.21 vector and digested and used to produce transgenic mice as described in B.](http://ajprenal.physiology.org/)
Tissue- and cell-specific expression of Npt2-Lac Z transgene. To determine the specific expression of the Lac Z reporter gene, adult transgenic progeny of each founder line were examined. Whole organs were fixed in paraformaldehyde and stained with X-gal (Fig. 3). Blue staining for Lac Z activity was observed specifically in the cortex of the transgenic mouse kidney (Fig. 3A, left) but not in the brain, heart, lung, liver, spleen, and skeletal muscle (Fig. 3, B–G, respectively). Non-specific staining was observed in the stomach and intestine of both transgenic and nontransgenic mice (not shown). The kidney cortex-specific staining was observed in progeny of all nine transgenic founder lines but not in nontransgenic mice.

Histochemical staining of the kidney was also carried out in frozen sections. Staining of β-galactosidase was detected exclusively in the cortex in the proximal tubule and not in the medulla (Fig. 4, C–E). Proximal tubule cells were readily identified by their brush-border membrane.

In addition, whole mounts of neonatal transgenic mouse were examined for Lac Z gene expression (Fig. 5). Staining for β-galactosidase was carried out during the first week postpartum (5 days). Intense staining was observed in the renal cortex. Some staining was also observed in the choroid plexus, lacrimal gland, submandibular gland, reproductive organs, and neonatal bone in the whole mount, but its specificity could not be ascertained.

Detection of Lac Z transgene using RT-PCR. The expression of the Lac Z transgene was also verified at the level of transcription using RT-PCR analysis. Total RNA was extracted from the kidney, brain, lung, heart, liver, intestine, stomach, and muscle of transgenic mice. Samples were quantified using densitometry following separation on a 1.2% agarose gel. RNase-free DNase digestion was performed to remove residual genomic DNA from the samples. Each RNA sample was reverse transcribed and subjected to PCR amplification using Lac Z gene-specific primers. The amplification product (364 bp) corresponding to the Lac Z gene was detected specifically in the transgenic mouse kidney together with the product (420 bp) corresponding to the endogenous murine Npt2 (Fig. 6A). No Lac Z gene or Npt2 gene expression was detected in other tissues examined (Fig. 6A). β-Actin gene expression was monitored as an internal control in all of the samples.

Solution β-galactosidase assay for detection of Lac Z reporter gene. To compare β-galactosidase tissue-specific staining and activity, a solution enzymatic assay was performed. Total protein was extracted from whole organs of transgenic and nontransgenic mice, and β-galactosidase activity was measured as fluorescence intensity in a solution assay using a Fluorimeter plate reader. High-level β-galactosidase activity was observed exclusively in the kidneys of transgenic mice (Fig. 6B). β-Galactosidase activity was not observed in the other tissues examined (Fig. 6B).

Comparison of expression between endogenous Npt2 gene and Lac Z transgene. The foregoing results prompted us to evaluate the potential usefulness of the Npt2 transgenic mice in
studying transcriptional regulation of the Npt2 gene. To this end, we compared RT-PCR signals of the murine Npt2 transcript driven by the endogenous promoter with the Lac Z transcript driven by the exogenous 4.7-kb transgenic promoter. During mouse kidney organogenesis, although kidney rudiments are observable at embryonic day 11 (E11), demarcation into outer cortical and inner medullary regions with proximal and distal tubule segments are evident only at 16 days gestation (dpc). Therefore, for this analysis, pregnant females of transgenic line 4 were killed at 16, 17, 18, and 19 dpc, and 10 fetal kidneys were harvested at each time point. In addition, neonatal kidneys for days 1, 4, 5, and 7 postpartum (pp) were harvested. For each such time point, total RNA was extracted, treated with RNase-free DNase, and subjected to semiquantitative RT-PCR analysis using primers for the endogenous mouse gene Npt2 and for the 4.7-kb promoter Lac Z transgene (Fig. 7). Expression of the Lac Z transgene increased progressively from day 17 dpc during prenatal fetal development and during postnatal growth pari passu with a corresponding rise in expression of the endogenous murine Npt2 gene (Fig. 7). These results validate the use of these transgenic mice as a model for studying the transcriptional regulation of the Npt2 gene under varying physiological and pathophysiological circumstances.

**DISCUSSION**

The use of mice harboring transgene constructs consisting of 5′-regulatory regions of a specific gene driving reporter gene expression plays an important role in studying cell-specific gene expression and transcriptional regulation in vivo (14, 26). In such studies, the promoter-reporter gene cassette is injected into the male pronuclei of fertilized eggs that are carried to term by pseudopregnant implanted females. The gene cassette is stably integrated, as tandem head-to-tail arrays of variable length, into the mouse genome at random integration sites.
where the regulatory sequences upstream of the reporter gene drive the expression of the reporter gene.

The kidney represents an ideal system with which to investigate mechanisms of gene regulation that are involved in cell differentiation. The nephron, composed of >18 different epithelial cell types, is organized into tubule segments with specific transport and metabolic functions. Normal nephron function depends on the appropriate architectural arrangement and integrated activity of these tubule segments. The specialized function of each tubule segment depends on the specific pattern of genes expressed in each of the component cell types. The SLC34A1 gene in humans that encodes the Na/Pi-IIa sodium-phosphate cotransporter is specifically expressed in the proximal convoluted tubule. Correspondingly, the rodent and murine Npt2 genes encode the homolog Na/Pi-IIa cotransporters in these species and therefore provide an important resource in elucidating the process of cell-specific gene expression during renal cell differentiation in normal and disease states.

We have previously isolated and characterized the 5′-flanking region of the rat Npt2 gene (22). Computer analysis of consensus sequences for transcription factor binding sites within the 5′-flanking region revealed putative binding sites for multiple transcription factors (among others, these included AP2 binding sites for the forkhead family of DNA binding protein that mediates gene activation in response to cAMP and phorbol esters, E-box enhancer element, members of the steroid receptor superfamily which bind receptors for vitamin D, peroxisome proliferator-activated receptor-γ, thyroid hormone, and retinoic acid). Transient transfection studies using OK cells that express the endogenous opposum Npt2 gene vs. HEK and NIH3T3 cells that do not confirmed cell specificity of promoter activity. However, to ensure that such cell-specific transcriptional regulation was also applicable in the kidney in vivo, we used the same 5′-promoter segment cloned upstream to a Lac Z reporter gene to generate nine transgenic mouse founders. All transgenic lines generated from these founders were investigated for the number of integration events of the transgene as well as the expression pattern of the reporter gene. Each of the founders was shown to harbor a different set of integration sites and to contain a different integration copy number as indicated by Southern blot analysis. Thus each of the founders could be used to produce homozygous transgenic lines, where progeny of each line were studied for different levels of expression of the transgene. The identical pattern of transgene reporter localization and developmental expression, for the different transgenic lines, ensures that the pattern observed reflects the regulatory effect of the transgenic promoter sequence, rather than of the surrounding genomic regions at the integration sites. Staining of whole tissues of the adult mouse showed specific staining in the kidney localized to
the cortex but not the medulla. To examine the structures where the expression occurs, histochemical studies of frozen adult kidney sections were carried out. Staining of frozen sections showed that the staining occurred only in the cortical proximal tubules, as evidenced by their brush-border membrane. Whole mount staining of neonatal transgenic mice confirmed results obtained by histochemistry and adult organs. Further confirmation was obtained using an assay for measurement of β-galactosidase activity in tissue protein extracts.

These studies confirmed that the β-galactosidase protein is expressed specifically in the kidney.

In addition to measurement of the protein level by immunologic and enzymatic activity, Lac Z mRNA expression levels were ascertained using both RT-PCR and Northern blot (data not shown) analyses and also confirmed specific expression in the transgenic but not control nontransgenic kidneys. Taken together, these results indicate that the 5′-upstream regulatory sequence of the rodent Npt2 gene whose cell spec-

K-kidney, S-stomach, L-liver, SC-spinal cord, B-brain

Figs. 5 and 6 show the expression of Lac Z gene in neonatal transgenic mouse and adult tissue extracts. Lac Z expression was monitored by X-gal staining and RT-PCR analysis. The results confirm specific expression in the kidney of transgenic mice. No Lac Z expression was detected in any of the other tissues. β-Galactosidase activity of transgenic mouse tissues was monitored using a β-galactosidase solution assay. Total protein was extracted from whole organs of transgenic and nontransgenic mice, and β-galactosidase activity was measured as fluorescence intensity using a fluorimeter plate reader. Fluorescence values were normalized for protein content. The results shown represent the means of 1 of 4 representative experiments, each performed in quadruplicate.
ficity was shown in cell culture studies previously, also drives cell-specific expression in the renal proximal tubule in vivo. Thus it will be possible to conduct further in vivo studies using deletion and mutational constructs based on this promoter region to try to ascertain the cis-acting, and subsequently trans-acting, elements responsible for specifying proximal tubule expression and thereby gain insight with respect to differentiation of specialized nephron segments, during development, or in response to renal injury (such as in recovery from acute tubule necrosis). Although we and others have conducted such deletion and mutation analyses in cell culture (7, 10, 22, 27), the transgenic model provides a superior opportunity for such analysis in an in vivo system.

Experimental deletion of the endogenous Npt2 gene in knockout mice results in a 70% decrease in proximal tubule cortical membrane Na/Pi cotransport, with attendant severe massive phosphaturia and hypophosphatemia (8). The residual activity has been attributed to activity of the related transporter encoded by the murine equivalent of the SLC34A3 gene encoding the Na/Pi-IIc cotransporter. Of interest, maximum expression of this latter transporter has been shown to be localized in proximal tubules of superficial and midcortical nephrons of weanling rat kidneys. Furthermore, homozygous, loss-of-function mutations of SLC34A3 in humans have been identified in kindreds with autosomal recessive inheritance of hypercalciuric hypophosphatemic rickets (3, 12).

In contrast, no disease-causing or phenotype-altering mutations have been identified to date in the human SLC34A1 gene, encoding the Na/Pi-IIa cotransporter in humans. This may possibly point to incompatibility of its loss of function with normal fetal development, even though, as noted above, homozygous deletion in mice is not embryonically lethal.

The abundance and activity of the Na/Pi-IIa cotransporter is dynamically regulated under many physiological and pathophysiological conditions, including changing dietary phosphate intake, among others (2). Much of this modulation is mediated by circulating hormonal factors such as PTH and FGF23 among others (15, 20) and appears to occur, at least in part, at the posttranscriptional and posttranslational levels involving regulated internalization and lysosomal degradation. Cyclic nucleotide signaling cascades appear to be involved in this process (19).

To utilize the current model to determine to what extent transcriptional modulation also contributes to modulation of Na/Pi-IIa expression under physiological and pathophysiological conditions, it was necessary to show that the transgene mRNA expression mirrored mRNA expression of the endogenous Npt2 gene. This was indeed evident during the developmental modulation in renal ontogeny and early postnatal growth of transgenic mice. Thus it should indeed be possible to utilize this model to focus on the relative contribution of regulation at the transcriptional level to the modulation of Na/Pi-IIa expression in response to a variety of influences (1, 15–19, 27). In particular, experiments to date have not clarified definitively in a whole-organism model the potential promoter-mediated transcriptional modulation of Na/Pi-IIa expression in response to the influence of hormones acting through nuclear receptors such as vitamin D and thyroid...
hormone (4, 17, 18, 27). In preliminary studies using the current transgenic model, 1,24-Oh vitamin D3 administered according to previously published protocols (6, 27) was not observed to modulate transgene reporter levels, but confirmatory studies are needed to demonstrate a concomitant metabolic effect of administered vitamin D using the regimen employed before a firm conclusion can be reached. The same approach can be applied to other factors affecting whole-organism phosphate balance, and in addition to using the transgenic model to further investigate the molecular basis for cell-specific gene expression and differentiation.

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