Dietary phosphate (P_i) is a most important regulator for renal P_i reabsorption. The type II sodium-diphosphate (Na/P_i) cotransporters (NPT2) are located at the apical membranes of renal proximal tubular cells and major functional transporters associated with renal P_i reabsorption. The consumption of a low-P_i diet induces the synthesis of NPT2, whereas a high P_i diet decreases it. The molecular mechanisms of regulation by dietary P_i are not yet known.

In this report, in weaning mice fed a low-P_i diet for 4 days, the NPT2 mRNA level was increased 1.8-fold compared with mice fed a normal P_i diet. This increase was due to an elevation of the transcriptional activity. In the NPT2 gene promoter, the DNA footprint analysis showed that six regions were masked by the binding protein, but at the position −1010 to −985 upstream of the transcription start site, the binding clearly corresponded to the levels of dietary P_i. The phosphate response element (PRE) of the NPT2 gene was found to consist of the motif related to the E box, 5′-CACGTG-3′. A yeast one-hybrid system was used to clone a transcription factor that binds to the PRE sequences in the proximal promoter of the NPT2 gene. Two cDNA clones that encoded protein of the mouse transcription factor E3 (TFE3) were isolated. This is a DNA-binding protein that activates transcription through the E3 site of the immunoglobulin heavy chain enhancer. TFE3 antibody completely inhibited the binding to the PRE. The coexpression of TFE3 in COS-7 cells transfected with the NPT2 gene promoter markedly stimulated the transcriptional activity. The feeding of a low P_i diet significantly increased the amount of TFE3 mRNA in the kidney. These findings suggest that TFE3 may participate in the transcriptional regulation of the NPT2 gene by dietary P_i.

The regulation of inorganic phosphate (P_i) in the human body is controlled mainly by reabsorption in the proximal tubules of the kidneys (1–3). Apical Na^+-dependent phosphate (NaP_i) cotransporter is central to the renal proximal tubular reabsorption of P_i (2, 3). Studies of isolated kidney tubules and of brush-border membranes have demonstrated that the physiological regulation of proximal tubule P_i transport involves complex hormonal and metabolic factors that affect the activity or expression of the transporter molecules (1–3). A major regulator of the NaP_i cotransporter is dietary P_i (4–7). Dietary P_i restriction is associated with an adaptive increase of the overall proximal tubular capacity to reabsorb P_i (4–7). Alterations of the dietary intake of P_i lead to an adaptation of renal P_i transport activity independent of extrarenal factors such as parathyroid hormone, growth hormone, and vitamin D (1–3, 9, 10). The molecular mechanisms of this adaptation are unknown.

Three types of Na^+-dependent P_i cotransporter have been isolated from a kidney library (11, 12). Recent studies suggest that the type II transporters (NPT2) may play an important role in P_i homeostasis in the kidney, that they are controlled by parathyroid hormone, and by the dietary intake of P_i (11–13). In a previous study, we investigated the cellular mechanism of the up-regulation of the NaP_i cotransporter in mice induced by the intake of a low P_i diet and found that the administration of a low P_i diet to the mice clearly stimulated the elevation of NPT2 mRNA and protein (7). We have been studying the NPT2 gene expression using dietary P_i feeding in mice. The NPT2 genes respond at the transcriptional and post-transcriptional level to an increased P_i concentration in the diet (6, 7). The DNA sequences responsible for the P_i response in the mouse kidney, which we have designated the phosphate response elements (PRE), have been mapped. The PRE of the NPT2 gene promoter shares a region with 9 of 10 bp identity to yeast phosphate-responsive transcription factor Pho4 binding element. At the center of this region is a CACGTG motif, the core recognition site for the helix-loop-helix of transcription factors. This segment contains a 5′-CACGTG-3′ motif that is sufficient to confer the transactivation by dietary P_i deprivation. We also isolated a transcription factor (a helix-loop-helix protein), which has structural features very similar to those of yeast Pho 4.

**EXPERIMENTAL PROCEDURES**

*Animals and Diets—Male ICR mice (3 weeks after birth) were purchased from SLC (Shizuoka, Japan). They were housed in plastic cages and the animals were fed standard mouse chow (Oriental, Osaka, Japan) ad libitum. For the first week, they were fed the diet. After the period, they received a diet containing 1.2% calcium, 0.6% phosphorus and 4.4 IU vitamin D_3/g for 5 days. Thereafter, they were fed a diet with sequence; TFE3, mouse transcription factor μE3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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a normal Pi level (0.6%) for 7 days, given between 11:00 a.m. and 1:00 p.m. (6, 7). On the 8th day, the following three groups of six mice each were established: the normal Pi group, mice that were chronically fed a diet containing 0.6% Pi; the low Pi group, mice that received a diet containing a low percentage (0.02%) of Pi; and the high Pi group, in which mice received a high percentage (1.2%) of Pi diet. After a 4-day period, each of the given diet, all of the mice were anesthetized with intraperitoneal pentobarbital, and the kidneys were rapidly removed. One-half of each kidney was used for RNA isolation and the other half was used for the isolation of brush-border membrane vesicles (7).

Northern Blot Analysis—Total RNA was isolated from the kidney by extraction with acid guanidine thiocyanate/phenol/chloroform using the method of Chomczynski et al. (13). A NaPi-7 cDNA probe (2.4 kilobase pairs) was obtained from a mouse kidney cDNA library (5). The internal standard was GAPDH cDNA.

Cloning of DNA-binding Proteins Using the Yeast One-hybrid System—A reporter gene, NPT2 PRE-TC1-HIS3, for yeast one-hybrid study (14) was constructed as follows. The Saccharomyces cerevisiae HIS3 coding region connected downstream of the UAS (upstream activation sequence)-less S. cerevisiae CYC1 promoter was constructed on a pUC19-based plasmid containing the S. cerevisiae ADE2 gene fragment. This plasmid was designated as pWHNaPi0. The five tandem copies of 36-bp double-stranded oligonucleotide, which originates from the sequences corresponding to the nucleotide positions from −9100 to −9800 of the NPT2 promoter, were inserted into the upstream of the CYC1 promoter on pWHNaPi0 as its UAS sequences. The resulting plasmid, pWHNaPi5, was integrated into an ade2 locus on chromosome of S. cerevisiae strain HPH100 (MATA ura3–52 leu2–3,112 trp1–1 his3Δ ade2–101 lys2–801; our stock strain), and this integrant strain was designated as YBH5. The other yeast strain YBH0 was constructed by the similar integration using pWHNaPi0 instead of pWHNaPi5.

More than 2 × 10⁶ colonies of YBH5 transformants by mouse kidney cDNA library (mouse kidney MATCHMAKER cDNA, CLONTECH Inc., Palo Alto, CA) were screened by their growth on the histidine omission media. Among the candidates, colonies were obtained for identification and analysis of protein and DNA components of gel-shift assays. Approximately 20 μg of nuclear protein was used in a preparative gel shift. After transfer onto DE81 paper, the protein–DNA complexes were identified by autoradiography. Proteins were eluted once in acetone and twice in cold 100% ethanol, vacuum-dried, resuspended, denaturated in sample buffer, and subjected to SDS/10% polyacrylamide gel electrophoresis and Western blotting (21).

Nuclei were prepared from renal proximal tubules of mice fed a low Pi diet for 4 days, or from mice fed a low Pi diet for 4 days and then treated with a high Pi diet for 4 days. The levels of NPT2 mRNA were determined using Northern blot analysis (B). The density of the signals was measured using an imaging analyzer (Fuji-BAS2000). The relative intensity was based on the comparison using GAPDH (GAPDH) as an internal control. The data are mean ± S.E. of six mice per group, D, in vitro transcription in isolated nuclei of renal cells from mice fed the low Pi diet. Nuclei were isolated from the renal cortex of mice fed a normal Pi diet (LP), or a high Pi diet (HP), and were assayed for transcription in vivo. 32P-Labeled transcripts were hybridized to NPT1, NPT2, and GAPDH cDNA. Lane CP, the normal Pi diet; lane LP, the low Pi diet. (SureTec Footprinting Kit, Amersham Pharmacia Biotech, Upssala, Sweden.) Shifting of Western Blotting—Shifting of Western blotting was performed according to the method of Demczuk et al. (21). This method was developed for identification and analysis of protein and DNA components of gel-shift assays. Approximately 20 μg of nuclear protein was used in a preparative gel shift. After transfer onto DE81 paper, the protein–DNA complexes were identified by autoradiography. Proteins were eluted once in acetone and twice in cold 100% ethanol, vacuum-dried, resuspended, denaturated in sample buffer, and subjected to SDS/10% polyacrylamide gel electrophoresis and Western blotting (21).

Cell Culture and Transient Transfection—Cos-7 cells (Riken Cell Bank, Tokyo) were cultured at 37 °C and under 5% CO₂ in Dulbecco’s modified Eagle’s medium (Sanko-junyaku, Tokyo) with 10% fetal bovine serum (Sigma). OK cells (ATCC:CRL1840) were maintained in F-12/ethylene glycol (8,000), 1.5% saline/sodium phosphate/EDTA. Aliquots of RNA from treated and untreated samples were counted in a scintillation counter, and an equal number of counts from each condition (1–2 × 10⁶ cpm) were hybridized to linearized cDNAs (5 μg) for NPT1, NPT2, GAPDH, pBluescript II DNA, which were immobilized to Hybond filters using a slot blot apparatus.

Stastical Analysis—Data are expressed as the mean ± S.E. Differences between experimental groups were determined by analysis of variance, and p values < 0.05 were accepted as indicating a significant difference.

RESULTS

Effects of Low Pi Diet on the Expression of the Type II Transporter Gene—The brush-border membrane vesicles isolated from renal proximal tubules of mice fed a diet low in Pi, for 4 days, were prepared and used for the assay of Pi transport activity. The analysis of the Na⁺-dependent Pi uptake at 1 min revealed an approximately 1.7-fold increase in these mice compared with mice that received a control diet (P < 0.01) (Fig. 1A). As shown in Fig. 1B, the NPT2 mRNA levels were significantly increased (by about 1.8-fold) in the mice fed the low Pi diet for 4 days. In addition, the amounts of the NPT2 protein...
(the 80–90-kDa bands) were significantly increased (by about 2.5-fold for the 90-kDa band) compared with those in mice fed the control diet (Fig. 1C). In contrast, the high Pi diet significantly suppressed these three parameters (transport activity, mRNA, and protein).

We studied the in vitro transcription in isolated nuclei of renal cortex cells from mice fed the low Pi diet. As shown in Fig. 1D, the transcriptional activity in the NPT2 is significantly increased in the mice fed the low Pi diet compared with those in the mice fed the control Pi. The transcriptional activity of the type I NaPi cotransporter NPT1 gene was not significantly different between the normal Pi and low Pi groups. Thus, the elevation (1.8-fold) of the NPT2 mRNA levels in the mice fed the low Pi diet was, at least in part, due to the increase in the transcription of the NPT2 gene.

A Phosphate Response Factor in the NPT2 Gene Promoter—To clarify the protein binding region in the NPT-2 gene promoter induced by the feeding of a low Pi diet, we incubated DNAse I and 20 μg of kidney extracts isolated from mice fed a low Pi diet. Nucleotide sequence of a portion of NPT2 and exon 1. Footprints are underlined by a black bar (FP-1 to FP-6). The major transcription start site is marked by an arrow.
diet, we performed DNase footprint assays using nuclear extracts from the renal cortex isolated from mice fed a low Pi diet (Fig. 2). The footprints in the nuclear extract isolated from mouse renal proximal tubular cells had a series of hypersensitive sites in common and a protected region extending from mouse kidneys (Fig. 2). There was also additional protection within the regions of -779 to -757 nt (FP-4), -635 to -612 nt (FP-5) and -321 to -298 nt (FP-6), indicating that an additional factor binds the DNA in mice kidney (data not shown). In the comparison of these elements between the mice fed a low Pi, normal Pi, or a high Pi diet. As shown in Fig. 6, the binding activity in the PRE-WT oligonucleotide decreased in parallel with the elevation of plasma Pi levels. There was a good correlation with both parameters (PRE binding activity and plasma Pi concentration).

Cloning of PRE-binding Protein by the Yeast One-hybrid System—To begin to identify some of the proteins that bind this segment, we used this DNA (nucleotide positions from -1010 to -985) as a UAS of the reporter gene's promoter in the yeast one-hybrid system and screened a mouse kidney cDNA library. The yeast strain YBH5 is histidine auxotroph and 3-amino-1,2,4-triazole-sensitive. The yeast strain YBH5 is histidine auxotroph and 3-amino-1,2,4-triazole-sensitive. We isolated a cDNA which produced fusion proteins from mice fed a low P_i diet, the increased DNA/protein complex was observed in EMSA (Fig. 4), but the increase in the DNA/protein complex was not observed with FP-1, FP-2, FP-4, FP-5, and FP-6 probes (data not shown). The protein-DNA complex was completely inhibited unlabeled PRE oligonucleotide.

Interestingly, the sequence of PRE was very similar to those of the P_i responsible element for the promoter of the P_i transporter gene PHO4 and acid phosphatase gene PHO5 in yeast (23). This element is known to be a Pho4 binding site. Pho4 is a helix-loop-helix transcription factor for the genes associated with yeast P_i metabolism and binds the E box sequence 5'-CACGTG-3'. To confirm that the E box in the target sequence of the putative binding protein, we performed EMSAs with oligonucleotides containing specific mutations of this sequence (Fig. 3B). The PRE-MT1 oligonucleotide, in which GG in the 5' half-site of the PRE sequence was changed to TT, showed binding activity similar to that of the PRE-WT (Figs. 3B and 4B). The mutation of TG in the E-box to AA (PRE-MT2) abolished the ability to interact with PRE-WT (Fig. 3B). Mutation of the second and third nucleotides (PRE-MT3) of the E-box also abolished the binding activity (Fig. 4B), suggesting that the putative binding protein recognized the sequence 5'-CACGTG-3' in the promoter of the NPT2 gene.

Functional Role of PRE in the NPT2 Promoter—We investigated the role of the PRE in the basic promoter activity of the NPT2 gene. The vector (p3P1170) was constructed with the NPT2 gene promoter (-1289 to +54 nt), which contains the PRE (-110/-985 nt), linked to the luciferase reporter gene in OK cells. Transfection of p3P1170 into OK cells showed a 6-fold increase in the activity of control vector-transfected OK cells. Mutation of the E box in the PRE (p3P1170MT) markedly decreased the basic promoter activity, suggesting that the PRE is important for the expression of the NPT2 gene in OK cells (Fig. 5).

Relationship between Plasma P_i Levels and PRE Binding Activity—To further analyze whether the binding activity in the PRE is regulated by plasma P_i concentration, we measured plasma P_i levels and PRE binding activity in the nuclei isolated from mice fed a low P_i, normal P_i, or a high P_i diet. As shown in Fig. 6, the binding activity in the PRE-WT oligonucleotide decreased in parallel with the elevation of plasma P_i levels. There was a good correlation with both parameters (PRE binding activity and plasma P_i concentration).

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Mutation Analysis of the PRE Sequence of the NPT2 Gene Promoter—Next, we used the oligonucleotide of PRE (-1010/-985 nt) and performed EMSA. In nuclear extract isolated from mice fed a low P_i diet, the increased DNA/protein complex was observed in EMSA (Fig. 4), but the increase in the DNA/protein complex was not observed with FP-1, FP-2, FP-4, FP-5, and FP-6 probes (data not shown). The protein-DNA complex was completely inhibited unlabeled PRE oligonucleotide.

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One cDNA contained an almost full-length coding sequence for TFE3 and the other was a shorter partial cDNA for TFE3. TFE3 is a DNA-binding protein that activates transcription through the \( mE3 \) site of the immunoglobulin heavy chain enhancer (17, 18). To isolate the functional TFE3 full-length cDNA clone, we screened the mouse kidney cDNA library (from mice fed a low Pi diet). We isolated TFE3L and TFE3S cDNA clones. The full-length TFE3 was termed TFE3L, and TFE3S is the isoform for TFE3L and is truncated at the N-terminal region (transactivation domain) of TFE3L (32) (Fig. 7A).

**Functional Analysis of the Effects of TFE3L and TFE3S on the Transactivation of the NPT2 Gene Promoter**—To determine whether TFE3 can activate the transcription of the \( NPT2 \) gene, the TFE3L cDNA was cloned in a mammalian expression vector, and the vector was cotransfected with the \( NPT2 \) gene promoter (\([-1289 \rightarrow -54] \)) linked to the luciferase reporter gene (p3P1170WT) in COS-7 cells. TFE3L stimulated the luciferase activity 6-fold compared with that of the control vector (Fig. 7B). TFE3S also stimulated the transcription (2.8-fold). TFE3L induced the luciferase activity in the COS-7 cells transfected with the \( NPT2 \) gene promoter with the mutation of the PRE (p3P1170MT), but the induction was lower compared with that in the p3P1170WT clone (Fig. 7B).

**Binding of TFE3 to the PRE**—To further confirm the specific binding to TFE3L and TFE3S in the promoter of the \( NPT2 \) gene, EMSAs were performed using a \( ^32P \)-labeled \([-1010 \rightarrow -985] \) oligonucleotide as a probe (PRE-WT) and several competitor DNA oligonucleotides (PRE-MT1, PRE-MT2, and PRE-MT3) corresponding to the E box (Fig. 8). In this experiment, the mouse TFE3L expression vector was transfected into COS-7 cells, and the nuclear extract expressing mouse TFE3L was used for EMSA. The results of competition experiments are shown in the lanes with the triangles, with the competing oligonucleotide indicated above the triangle. The competitors were added 25-(\( \times 25 \)), 50-fold (\( \times 50 \)), and 100-fold (\( \times 100 \)) in the EMSA. The data presented are representative of three independent experiments.
protein bound to the −1010 to −985 oligonucleotide.

To further confirm that the DNA-binding protein is TFE3, we performed the shift-Western analysis. The nuclear extract isolated from mice fed a low Pi diet was incubated with 32P-labeled PRE oligonucleotide and then performed the shift-Western analysis (Fig. 8B, lanes 1 and 2). This method was developed for identification and analysis of protein and DNA components of gel-shift assays. The protein-DNA complexes, separated in polyacrylamide gels, were transferred onto stacked nitrocellulose and anion-exchange membranes. The proteins bound to nitrocellulose were identified by immunoblotting by TFE3-specific antibody (lane 2), while the DNA, which bound only to the anion-exchange membrane, was detected by autoradiography (lane 1). Nuclear proteins were prepared from the kidneys of mice fed a low Pi diet. C, effect of TFE3 antibody on nuclear protein binding to PRE. The nuclear extract from kidney cortex of mice fed a low Pi diet incubated with 32P-labeled PRE-WT oligonucleotide. The TFE3 antibody was added to EMSA reaction medium. Lane 1, DNA protein complex in EMSA; lane 2, added TFE3 antibody.
mice fed a low Pi diet. The intensity of each hybridization was normalized to the GAPDH mRNA. Data represent means ± S.E. of five animals.

**DISCUSSION**

Chronic dietary Pi restriction leads to an increased NaPi cotransport rate, along with increased NPT2 protein and mRNA (3–8). In the present study, the transcription rate was significantly increased in nuclei isolated from the kidney cortex of mice fed a low Pi diet. Recent reports suggest that the up-regulation is due to the elevation of the type II transporter synthesis by Pi deprivation and is the elevation of the stability for the type II transporter mRNA, but not transcription (24, 25). However, in the present study, we concluded that the elevation of the NPT2 mRNA level is due, at least in part, to an increase in the transcription rate. The difference in the findings of run-on assay may be based on the feeding schedule or animal age, because our feeding schedule included meal feeding. In this schedule, the animals can feed only during one period of 2 h in a day. This schedule is useful to investigate the effect of dietary Pi on the regulation of NPT2 synthesis (6). In the low Pi group, the plasma Pi levels were suddenly decreased compared to those in the normal Pi group. When the animals were fed a low Pi diet ad libitum, the plasma Pi levels were gradually decreased. The differences of the feeding schedule might have affected the regulation of NPT2 in the kidney.

In a nuclear run-on assay, we analyzed the four marker genes used: neutral basic amino acid transporter (NBAT), peptide transporters (PepT1), type I NaPi cotransporter (Npt1), and GAPDH (data not shown). In these conditions, we clearly found that all cDNA did not respond to dietary Pi in a run-on assay. We also observed the increase in the stability of the NPT2 transcripts in vitro assay (26). This step may also be an important regulatory point as proposed by the Murer and Ghishan studies (24, 25).

The present DNA footprinting analysis showed that six regions of the NPT2 gene promoter were masked by the nuclear protein isolated from the mice fed a low Pi diet. In addition, the gel-shift mobility assay demonstrated that the binding for the element was markedly increased in the nuclei isolated from the kidney cortex of the mice fed the low Pi diet. This binding protein recognized the consensus sequence 5′-CACGTG-3′ known as the E box. The PRE of the NPT2 gene was further investigated by EMSA with various oligonucleotides as probes and competitors.

Interestingly, the sequence of the PRE was very similar to those in the Pi-responsive element for the promoter of the Pi transporter gene PHO84 and acid phosphatase gene PHO5 (23, 27, 28) in the yeast S. cerevisiae. This element is known to be a Pho4 binding site. Pho4 is a helix-loop-helix transcription factor for the genes associated with yeast Pi metabolism (23, 27, 28). An EMSA demonstrated the formation of two complexes between oligonucleotides containing PRE and nuclear extract. The binding of the sequence with isolated nuclei was detected in the mice fed the low-Pi diet, but not in those fed the normal diet. The formation of the protein-DNA complex was inhibited in the presence of an oligonucleotide containing the Pho4 binding site of the yeast PHO84 gene promoter.

We isolated cDNAs for the protein TFE3L/S by the yeast one-hybrid system. The expression of TFE3 markedly stimulated the promoter activity in the NPT2 gene, but not in the NPT2 gene with the mutation sequence in the E box. This suggested the possibility that TFE3L/S might bind specifically to the -1010 to -985-bp segment of the human NPT2 gene in Pi deprivation. Indeed, the incubation with antibodies for mouse TFE3 completely inhibited DNA/nuclear protein binding, suggesting that protein-DNA complex in EMSA using the renal nuclear extract are TFE3.

In addition, we identified a similar PRE in the NPT2 gene of opossum kidney (29) cells. The similar sequences are located at position –2453 to –2441 relative to the transcription start site of the opossum NPT2 gene promoter. The sequence is 5′-CAC-NNTGC-3′, and TFE3 can bind this E box sequence. In addition, 25-hydroxyvitamin D$_3$ is a potent hormone that stimulates the transcription of the vitamin D biosynthesis (30). Dietary Pi restriction increases 1α-hydroxylase activity, while high Pi diet decreases it (31). In the mouse and human 1α-hydroxylase gene promoters, the similar sequence is located at position –660 to –655 relative to the transcription start site of the mouse 1α-hydroxylase gene (32, 33). It is possible that the PRE sequence in the 1α-hydroxylase gene promoter may also be important for dietary Pi regulation (data not shown). To further clarify the role of the PRE in the NPT2 gene promoter, we are cloning mouse and rat NPT2 gene promoter.

What is the nature of the Pi-responsive factor? Factors binding to the CACGTG motif have been shown to belong to the c-Myc family. While many members of this family have been identified, TFE3 is the predominant factor in renal extracts that binds to the PRE in vitro. A recent study demonstrated...
that the TFE3 gene was a candidate for papillary cell carcinoma (34), suggesting that the gene may function as the tumor repressor in renal cells. In addition, Hua et al. (35) reported that TFE3 is an important transcription factor in at least one TGF-β-activated signal transduction pathway. TGF-β is known to have widespread regulatory effects on extracellular matrix and has been implicated as a major cause of increased extracellular matrix synthesis and buildup of pathological matrix within glomeruli in experimental glomerulonephritis (36). A mechanism of the rapid therapeutic effect of a low protein diet on experimental glomerulonephritis is through suppression of TGF-β expression and prevention of the induction of extracellular matrix synthesis within the injured glomeruli (36). It is possible that TFE3 regulated the expression of TGF-β in this model. Furthermore, in uremic animals, a low Pi diet prevented hyperparathyroidism, while a high Pi diet produced hyperplasia of the parathyroid glands (37). These data suggest that the Pi response factor TFE3 may regulate cell proliferation and matrix synthesis.

Finally, we used the yeast one-hybrid system to clone a transcription factor (TFE3) that binds to a specific sequence in the promoter of the NPT2 gene. TFE3 is known to activate matrix synthesis within the injured glomeruli (36). It is known that TFE3 is an important transcription factor in at least one repressor in renal cells. In addition, Hua et al. (35) reported that the TGF-β-activated signal transduction pathway is through suppression of TGF-β expression and prevention of the induction of extracellular matrix synthesis within the injured glomeruli (36). It is possible that TFE3 regulated the expression of TGF-β in this model. Furthermore, in uremic animals, a low Pi diet prevented hyperparathyroidism, while a high Pi diet produced hyperplasia of the parathyroid glands (37). These data suggest that the Pi response factor TFE3 may regulate cell proliferation and matrix synthesis.

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