Hepatocyte nuclear factor-4α regulates the human organic anion transporter 1 gene in the kidney

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Ogasawara K, Terada T, Asaka J, Katsura T, Inui K. Hepatocyte nuclear factor-4α regulates the human organic anion transporter 1 gene in the kidney. Am J Physiol Renal Physiol 292: F1819–F1826, 2007. First published March 6, 2007; doi:10.1152/ajprenal.00017.2007.—Human organic anion transporter 1 (OAT1, SLC22A6), which is localized to the basolateral membranes of renal tubular epithelial cells, plays a critical role in the excretion of anionic compounds. OAT1 is regulated by various pathophysiological conditions, but little is known about the molecular mechanisms regulating the expression of OAT1. In the present study, we investigated the transcriptional regulation of OAT1 and found that hepatocytic nuclear factor (HNF)-4α markedly transactivated the OAT1 promoter. A deletion analysis of the OAT1 promoter suggested that the regions spanning −1191 to −700 base pairs (bp) and −140 to −79 bp were essential for the transactivation by HNF-4α. These regions contained a direct repeat separated by two nucleotides (IR-2), which is one of the consensus sequences binding to HNF-4α, and an inverted repeat separated by eight nucleotides (IR-8), which was recently identified as a novel element for HNF-4α, respectively. An electrophoretic mobility shift assay showed that HNF-4α bound to DR-2 and IR-8 under the conditions of HNF-4α overexpression. Furthermore, under normal conditions, HNF-4α bound to IR-8, and a mutation in IR-8 markedly reduced the OAT1 promoter activity, indicating that HNF-4α regulates the basal transcription of OAT1 via IR-8. This paper reports the first characterization of the human OAT1 promoter and the first gene in the kidney whose promoter activity is regulated by HNF-4α.

SLC22A6; proximal tubule; promoter; inverted repeat-8

THE ORGANIC ANION TRANSPORTER (OAT) family plays an important role in the renal excretion of endogenous and exogenous organic anions, including drugs, toxins, and hormones (8, 18, 33, 41). Among the OAT family, OAT1 (SLC22A6) and OAT3 (SLC22A8) are localized to the basolateral membranes of the renal proximal tubular epithelial cells (26), and mRNA levels of both transporters were higher than those of other members of organic ion transporter (SLC22A) family in the human kidney cortex (42). So far, the molecular natures of OAT1 and OAT3 have been well characterized with regard to transport characteristics, structure-function relationships, and regulation (1, 8, 42, 47). As for regulation, various pathophysiological conditions, including hyperuricemia (14), renal failure (25, 36), bilateral ureteral obstruction (43), and acute biliary obstruction (6), affected the expression of OAT1 and/or OAT3. Although the elucidation of their regulatory mechanisms is quite important, studies that address the point are limited.

Recently, Kikuchi et al. (23) and we (28) reported the transcriptional regulation of the OAT3 gene. In contrast, as for the OAT1 promoter, a computational analysis but not a functional analysis has been conducted (2). In the present study, therefore, we attempted to characterize the transcriptional regulation of OAT1 by focusing on two kinds of transcription factors: hepatocytic nuclear factor (HNF)-1α/β and HNF-4α. The former is reported to stimulate the promoter activity of OAT3 (23), while the latter transactivates the OAT2 and OCT1 genes, which are other members of the SLC22A family mainly expressed in the liver (31, 34).

HNF-1α/β are homeodomain-containing transcription factors that are expressed in the liver, kidney, intestine, stomach, and pancreas (3, 10). In the kidney, HNF-1α is reported to control the expression of transporters such as sodium/glucose cotransporter 2 and sodium/phosphate cotransporter 1 besides OAT3 (9, 30). Furthermore, Oat1 had markedly lower renal mRNA levels in Hnf-1α-null mice than in wild-type mice (24). On the other hand, HNF-4α is an orphan member of the nuclear receptor superfamily that is expressed in the liver, kidney, intestine, stomach, and pancreas (27, 40). Although few reports are available on the physiological role of HNF-4α in the kidney, HNF-4α protein has been found only in proximal tubular epithelial cells in the kidney (22), where OAT1 protein is located (26). Accordingly, it is possible that the OAT1 gene is regulated by HNF-1α/β or HNF-4α. In the present study, we cloned the human OAT1 promoter region and examined whether the OAT1 gene is regulated by these transcription factors.

MATERIALS AND METHODS

Materials. [γ-32P]ATP was obtained from GE Healthcare (Little Chalfont, Buckinghamshire, UK). Restriction enzymes were from New England Biolabs (Beverly, MA). An antibody against HNF-4α (H-171) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The human HNF-1α and HNF-1β expression vectors were kindly supplied by Dr. Marco Pontoglio (Institute Pasteur, Paris, France). The human HNF-4α (transcript variant 2) expression vector was from OriGene Technologies (Rockville, MD).

5′-Rapid amplification of cDNA ends. To identify the transcription start site of human OAT1, 5′-rapid amplification of cDNA ends (5′-RACE) was carried out using Human Kidney Marathon-Ready cDNA (Clontech, Mountain View, CA) according to the manufacturer’s instructions. The primers for 5′-RACE were as follows: a gene-specific primer for OAT1 (accession number NM_004790), 5′-GGTCCCCACT-CAGTCACGATGGTAGATGG-3′ (683 to 656); and a nested genespecific primer for OAT1, 5′-CGACACCCCCACCTGCTGCAG-GAGG-3′ (347 to 322). The PCR products were subcloned into the pGEM-T Easy Vector (Promega, Madison, WI) and sequenced using a multipipillary DNA sequencer RISA384 system (Shimadzu, Kyoto, Japan).

Cloning of the 5′-regulatory region of the OAT1 gene. Based on the human genomic sequence (accession number NT_033903), the 2,747-

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Table 1. Oligonucleotide sequence of primers

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<td>+88 to +64</td>
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<td><strong>Primers for the site-directed mutagenesis</strong></td>
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<tr>
<td>OAT1/mutIR-8-B-F</td>
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<td>−129 to −90</td>
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<tr>
<td>OAT1/mutIR-8-R-B</td>
<td>CATCTAGTGCACCTGGTCCGATCTCGATGG</td>
<td>−90 to −129</td>
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**Oligonucleotides for EMSA**

| (−882) to −853-F | CTAGATTTCCGACAATGGAGAGGCCTTCCGAG          | −882 to −853 |
| (−852) to −833-R | CGCCGTTTGGCAGACCGAGAGGCCTTCCGAG          | −853 to −882 |
| (−127) to −100-F | ATTTGCTACCGTCCGAGACCGAGAGGCCTTCCGAG       | −127 to −100 |
| (−127) to −100-R | ATTTGCTACCGTCCGAGACCGAGAGGCCTTCCGAG       | −127 to −100 |
| mutDR-2-A-F       | CTAGATTTCCGACAATGGAGAGGCCTTCCGAG          | −882 to −853 |
| mutDR-2-A-R       | CGCCGTTTGGCAGACCGAGAGGCCTTCCGAG          | −853 to −882 |
| mutDR-2-B-F       | CTAGATTTCCGACAATGGAGAGGCCTTCCGAG          | −882 to −853 |
| mutDR-2-B-R       | CGCCGTTTGGCAGACCGAGAGGCCTTCCGAG          | −853 to −882 |
| mutDR-2-AB-F      | CTAGATTTCCGACAATGGAGAGGCCTTCCGAG          | −882 to −853 |
| mutDR-2-AB-R      | CGCCGTTTGGCAGACCGAGAGGCCTTCCGAG          | −853 to −882 |
| mutIR-8-A-F       | ATTTGCTACCGTCCGAGACCGAGAGGCCTTCCGAG       | −127 to −100 |
| mutIR-8-A-R       | ATTTGCTACCGTCCGAGACCGAGAGGCCTTCCGAG       | −127 to −100 |
| mutIR-8-B-F       | ATTTGCTACCGTCCGAGACCGAGAGGCCTTCCGAG       | −127 to −100 |
| mutIR-8-B-R       | ATTTGCTACCGTCCGAGACCGAGAGGCCTTCCGAG       | −127 to −100 |

OAT1, organic anion transporter 1; F, forward; R, reverse; mut, mutant; DR, direct repeat; IR, inverted repeat. *Hin*1 and *Xho*1 sites are underlined. Mutations introduced into the oligonucleotides are shown in bold.

base pair (bp) flanking region upstream of the transcription start site was cloned by PCR using the primers listed in Table 1 and human genomic DNA (Promega). The PCR product was isolated by electrophoresis and subcloned into the firefly luciferase reporter vector pGL3-Basic (Promega) at the *Hin*1 and *Xho*1 sites. This full-length reporter plasmid is hereafter referred to as −2,747/+88.

Preparation of deletion reporter constructs. The 5′-deleted constructs (−1851/+88, −1191/+88) were generated by digestion of the −2747/+88 construct with *MluI* and either *Nde*I or *Pst*I. The ends were blunted with T4 DNA polymerase (Takara Bio, Otsu, Japan) and then self-ligated. The other 5′-deleted constructs were generated by PCR with primers containing a *Hin*1 site and *Xho*1 site (Table 1). The site-directed mutation in direct repeat (DR)-2 was introduced into the −2747/+88 construct and the mutation in inverted repeat (IR)-8 was introduced into the −2747/+88 construct and −140/+88 construct with a QuikChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA) using the primers listed in Table 1. The nucleotide sequences of these deleted or mutated constructs were verified.

Cell culture, transfection, and luciferase assay. Opossum kidney (OK) cells were cultured in medium 199 (Invitrogen, Carlsbad, CA) containing 10% FBS (Invitrogen) without antibiotics, in an atmosphere of 5% CO2-95% air at 37°C, and subcultured every 7 days using 0.02% EDTA and 0.05% trypsin. OK cells were plated into 24-well plates (4 × 10⁵ cells/well) and transfected the following day with the reporter constructs and expression vector, and 25 ng of the *Renilla reniformis* vector pRL-TK (Promega), using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s recommendation.

![Fig. 1. Functional analysis of the human organic anion transporter 1 (OAT1) promoter in kidney-derived cell lines [opossum kidney (OK) and HEK293] and a human intestinal cell line (Caco-2). The longest OAT1 construct (−2747/+88 (500 ng) was transfected into these cells for luciferase assays. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Data are reported as the relative fold-increase compared with pGL3-Basic and represent the means ± SD of 3 replicates.](http://ajprenal.physiology.org/ by 10.22103/3.6 on October 29, 2017)
The firefly and Renilla activities were determined 48 h after the transfection using a dual luciferase assay kit (Promega) and a LB940 luminometer (Berthold, Bad Wildbad, Germany). The firefly activity was normalized to Renilla activity.

**EMSA.** Nuclear extract was prepared from OK cells transiently transfected with HNF-4α or not, according to the method of Shimakura et al. (37). The double-stranded oligonucleotides used in the EMSA are listed in Table 1. The OAT1 probes (−882/−853) and (−127/−100) were end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (Takara Bio) and purified through a Sephadex G-25 column (GE Healthcare). EMSA was performed according to Prieur et al. (32) but with some modifications. The OK nuclear extract (10 μg) was incubated in binding buffer [40 mM KCl, 10 mM Tris HCl (pH 8.0), 1 mM DTT, 6% glycerol, and 0.05% Nonidet P-40] for 10 min at 4°C. Thereafter, one of the labeled probes was added and the mixture was incubated for a further 10 min at 4°C. For competition experiments and supershift assays, excess (50-fold) unlabeled oligonucleotides and antibody (2 μg) were added 10 min and 1 h before the addition of the labeled probe, respectively. The volume of the binding mixture was 20 μl throughout the experiment. The DNA–protein complex was then separated on a 4% polyacrylamide gel for 1.5 h at 200 V and room temperature in 0.5× Tris borate-EDTA buffer. Gels were dried and exposed to X-ray film for autoradiography.

**RESULTS**

**Determination of the transcription start site of OAT1.** To identify the transcription start site of human OAT1, 5′-RACE was performed. Sequencing of the longest RACE product showed that the terminal position of OAT1 cDNA was located 265 nucleotides above the start codon, which was 43 bp downstream of the 5′-end of OAT1 cDNA registered in the National Center for Biotechnology Information (NCBI) database (accession number NM_004790). No unreported intron was identified in the 5′-flanking region of OAT1, so the 5′-end of OAT1 cDNA registered in the NCBI database was numbered with +1 as the transcription start site in this study.

**OAT1 promoter is transactivated by HNF-4α.** To perform the functional analysis of the human OAT1 promoter, the 2,747-bp flanking region upstream of the transcription start site of OAT1 was subcloned into the pGL3-Basic vector. At first, the OAT1 promoter construct −2747/+88 was transiently transfected into a renal epithelial cell line derived from OK, a cell line derived from human embryonic kidney (HEK293), and a human intestinal cell line (Caco-2), and luciferase activity was measured. As shown in Fig. 1, the −2747/+88 construct showed a significant increase in luciferase activity compared with pGL3-Basic in OK cells, which have many characteristics of renal proximal tubule cells, including an organic anion transport system (17). However, this construct had little promoter activity in HEK293 and Caco-2 cells, which lack an organic anion transport system. Therefore, OK cells were used in the subsequent experiments.

Next, to investigate whether the OAT1 promoter is regulated by HNF-1α or HNF-4α, the −2747/+88 construct was transiently transfected into OK cells simultaneously with the HNF-1α, HNF-1β, or HNF-4α expression plasmid. Only HNF-4α could transactivate the OAT1 promoter activity markedly; HNF-1α and HNF-1β could not (Fig. 2). These results suggested that the OAT1 promoter is regulated by HNF-4α.

**Identification of HNF-4α response elements.** To determine the elements required for the transactivation of OAT1 promoter activity by HNF-4α, a series of deletion constructs of OAT1 were transfected into OK cells simultaneously with HNF-4α.

**Fig. 2. Effects of hepatocyte nuclear factor (HNF)-1α, HNF-1β, and HNF-4α overexpression on human OAT1 promoter activity. OK cells were transiently transfected with 500 ng of a −2747/+88 construct and 500 ng of the expression vector for HNF-1α, HNF-1β, HNF-4α, or empty vector. Firefly luciferase activity was normalized to Renilla luciferase activity. Data are reported as the relative fold-increase compared with pGL3-Basic and represent the means ± SD of 3 replicates.**

**Fig. 3. Identification of the HNF-4α-responsive region in the human OAT1 promoter. A series of deleted promoter constructs (equimolar amounts of the −2747/+88 construct (500 ng/l) and 500 ng of the HNF-4α expression vector or empty vector were transiently transfected into OK cells for luciferase assays. Firefly luciferase activity was normalized to Renilla luciferase activity. Data are reported as the relative fold-increase compared with pGL3-Basic (A) or as the ratio of HNF-4α expression vector to empty vector (B) and represent the means ± SD of 3 replicates.**
and luciferase activity was measured (Fig. 3). With the $-2747/ +88$ to $-1191/ +88$ constructs, the promoter activity in the presence of HNF-4$\alpha$ increased seven- to ninefold compared with that in the absence of HNF-4$\alpha$. Deletion of the fragment spanning $-1191$ to $-700$ bp reduced the activation of the OAT1 promoter by HNF-4$\alpha$, and the $-536/ +88$ to $-140/ +88$ constructs showed the same level of activation by HNF-4$\alpha$ as the $-700/ +88$ construct. Further deletion of the fragment spanning $-140$ to $-79$ bp almost abolished the responsiveness. These results suggested that the regions spanning $-1191$ to $-700$ and $-700$ to $-140$ contain the elements important for the transactivation of OAT1 promoter activity by HNF-4$\alpha$.

HNF-4$\alpha$ binds as a homodimer to a DNA sequence consisting of a direct repeat of AGGTCA-like hexamers separated by one or two nucleotides, which were designated as DR-1 and DR-2, respectively (12, 21). We investigated whether the regions mentioned above had DR-1 and DR-2 sites using NUBIScan, an in silico tool to identify nuclear receptor response elements (29). As a result, a DR-2-like element was identified between $-875$ and $-862$ bp in the $-1191/-700$

![Fig. 4. EMSA using nuclear extract from OK cells transiently transfected with HNF-4$\alpha$ (OK-HNF-4$\alpha$ NE) and two human OAT1 probes, $(-882/-853)$ and $(-127/-100)$. A: sequence of the oligonucleotides used in EMSA. Mutations introduced into the oligonucleotides are shown in bold. B: OK-HNF-4$\alpha$ NE was incubated with the $^{32}$P-labeled OAT1 oligonucleotide probe alone (lanes 2 and 9), or in the presence of excess unlabeled wild-type oligonucleotides (lanes 3 and 10), excess mutated oligonucleotides (lanes 4–6 and 11–13), or antibody against HNF-4$\alpha$ (lanes 7 and 14). In lanes 1 and 8, nuclear extract was not added.](http://ajprenal.physiology.org/)

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**A**

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**B**

<table>
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**Fig. 4. EMSA using nuclear extract from OK cells transiently transfected with HNF-4$\alpha$ (OK-HNF-4$\alpha$ NE) and two human OAT1 probes, $(-882/-853)$ and $(-127/-100)$. A: sequence of the oligonucleotides used in EMSA. Mutations introduced into the oligonucleotides are shown in bold. B: OK-HNF-4$\alpha$ NE was incubated with the $^{32}$P-labeled OAT1 oligonucleotide probe alone (lanes 2 and 9), or in the presence of excess unlabeled wild-type oligonucleotides (lanes 3 and 10), excess mutated oligonucleotides (lanes 4–6 and 11–13), or antibody against HNF-4$\alpha$ (lanes 7 and 14). In lanes 1 and 8, nuclear extract was not added.**

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HNF-4α REGULATES HUMAN OAT1 GENE IN THE KIDNEY

Fragment, while neither a DR-1 nor DR-2 site was found in the −140/−79 fragment. Between −123 and −104, however, the −140/−79 fragment contained an inverted repeat of hexamers separated by eight nucleotides (IR-8), which was recently identified as a HNF-4α response element in the human apolipoprotein AV promoter (32).

HNF-4α binds to DR-2 and IR-8 elements. To confirm that HNF-4α is able to bind to the DR-2 and IR-8 elements within the OAT1 promoter, EMSA was performed using an OAT1 probe (−882/−853) containing the DR-2 element or OAT1 probe (−127/−100) containing the IR-8 element and nuclear extract from OK cells transiently transfected with HNF-4α (OK-HNF-4α NE). Both OAT1 probes formed a DNA-protein complex (Fig. 4B, lanes 2 and 9), and the formation of the complex was completely impaired by the addition of an excess amount of the unlabeled wild-type oligonucleotides (Fig. 4B, lanes 3 and 10). Mutations in DR-2 (Fig. 4A, mutDR-2-B, mutDR-2-AB) and in IR-8 (Fig. 4A, mutIR-8-A, mutIR-8-B, mutIR-8-AB) abolished the ability to compete with the formation of the complex, while mutDR-2-A weakly competed with the formation of the complex (Fig. 4B, lanes 4–6, 11–13). Furthermore, the DNA-protein complex was supershifted on the addition of the HNF-4α antibody (Fig. 4B, lanes 7 and 14). These results indicate that HNF-4α binds to DR-2 and IR-8 elements within the OAT1 promoter.

Mutagenesis of DR-2 and IR-8 elements. To examine the contribution of the DR-2 and IR-8 elements to the responsiveness to HNF-4α, mutations at these sites were introduced into the −2747/+88 construct, and OK cells were transfected. Mutations in DR-2-B and IR-8-B reduced the transactivation of OAT1 promoter activity by HNF-4α more than mutations in DR-2-A and IR-8-A, respectively (data not shown); hereafter, mutations in DR-2-B and IR-8-B are regarded as mutations in DR-2 and IR-8, respectively. As shown in Fig. 5, mutations in DR-2 and IR-8 reduced the responsiveness to HNF-4α to two-thirds and one-half of the wild-type level, respectively. Furthermore, mutations in both DR-2 and IR-8 reduced the responsiveness to one-third of the wild-type. These results suggest that the DR-2 and IR-8 elements in the OAT1 promoter play an important role in the responsiveness to HNF-4α and that IR-8 contributed to the activation by HNF-4α more than did DR-2.

HNF-4α is responsible for basal OAT1 promoter activity. Focusing on the promoter activity in the absence of HNF-4α (Fig. 5), we found that mutations in IR-8 also reduced OAT1 promoter activity, suggesting that IR-8 is essential for basal promoter activity of OAT1. To investigate the influence of IR-8 on basal activity, we carried out a progressive deletion analysis and a mutational analysis. Transfection of the −2747/+88 construct resulted in a fivefold increase in luciferase activity compared with that of pGL3-Basic, and serial 5′ deletions of the construct from −2747 to −88 significantly reduced the luciferase activity (Fig. 6A). However, deletion of the fragment spanning −140 to −79 bp significantly reduced the luciferase activity (Fig. 6A). These results suggested that the −140/−79 region containing IR-8 is essential for basal transcriptional activity.

Next, a mutation in IR-8 was introduced into the −140/+88 construct, and luciferase activity was measured. The mutation reduced luciferase activity to one-third of the wild-type level,
which was the same level of activity as the -79/+88 construct (Fig. 6B), suggesting that IR-8 is responsible for basal promoter activity of OAT1.

To examine whether endogenous HNF-4α binds to IR-8 without the overexpression of HNF-4α, EMSA was performed using an OAT1 probe (-127/-100) and nuclear extract from intact OK cells (OK-intact NE). Like OK-HNF-4α NE, OK-intact NE also formed a DNA-protein complex which was supershifted on the addition of the HNF-4α antibody (Fig. 7, lanes 2 and 7), suggesting that endogenous HNF-4α binds to IR-8 without the overexpression of HNF-4α. In competition experiments, mutIR-8-B and mutIR-8-AB did not impair the formation of the DNA-protein complex, while mutIR-8-A weakly competed with the formation of the complex (Fig. 7, lanes 4–6).

DISCUSSION

HNF-4α transactivates human OAT1 promoter activity, and this effect occurs mainly via DR-2 and IR-8 elements, as demonstrated by the deletion analysis, mutational analysis, and EMSA. Furthermore, it was found that HNF-4α is also responsible for basal promoter activity of OAT1 via DR-2 and IR-8 elements. IR-8 contributes to the activation by HNF-4α more than DR-2 (HNF-4α overexpression). Under normal conditions, IR-8 is responsible for the basal promoter activity of OAT1, and HNF-4α binds to IR-8 (constitutive expression).

Fig. 8. Schematic model of transcriptional regulation of the human OAT1 gene. In the abundance of HNF-4α, HNF-4α transactivates human OAT1 promoter activity via DR-2 and IR-8 elements. IR-8 contributes to the activation by HNF-4α more than DR-2 (HNF-4α overexpression). Under normal conditions, IR-8 is responsible for the basal promoter activity of OAT1, and HNF-4α binds to IR-8 (constitutive expression).

HNF-4α is an orphan member of the nuclear receptor superfamily that is expressed in the liver, kidney, intestine, stomach, and pancreas (27, 40). Liver-specific disruption of the HNF-4α gene indicated that HNF-4α is central to the maintenance of hepatocyte differentiation and lipid homeostasis (16). On the other hand, mutations in the gene encoding HNF-4α cause maturity-onset diabetes of the young (MODY), which is a genetically heterogeneous monogenic form of type 2 diabetes, associated with pancreatic β-cell dysfunction (46). In contrast to the roles of HNF-4α in the liver and pancreas, little has been reported on the physiological implications of HNF-4α in the kidney. In the present study, we clearly indicated that the renal OAT1 gene is transcriptionally regulated by HNF-4α. Because HNF-4α and OAT1 protein showed the restricted intrarenal localization, that is, in the renal proximal tubular epithelial cells (22, 26), it is strongly suggested that HNF-4α is a key factor regulating the intrarenal expression of OAT1 protein.

In the EMSA using OK-HNF-4α NE, the mutDR-2-A oligonucleotide containing an intact DR-2-B competed more with the formation of the DNA-protein complex than did the mutDR-2-B oligonucleotide containing an intact DR-2-A (Fig. 4B, lanes 4 and 5). Similarly, in the EMSA using OK-intact NE, HNF-4α bound to the mutIR-8-A oligonucleotide containing an intact IR-8-B with higher affinity than the mutIR-8-B oligonucleotide containing an intact IR-8-A (Fig. 7, lanes 4 and 5). These results indicate that DR-2-B and IR-8-B had greater activity to bind HNF-4α than did DR-2-A and IR-8-A, respectively, which are consistent with the finding that mutations in DR-2-B and IR-8-B reduced the responsiveness to...
HNF-4α more than mutations in DR-2-A and IR-8-A, respectively (data not shown). These findings suggest that DR-2-B and IR-8-B are important to the transactivation of the OAT1 promoter via HNF-4α compared with DR-2-A and IR-8-A. In the EMSA using OK-HNF-4α NE, both oligonucleotides mutIR-8-A and mutIR-8-B failed to impair the formation of the DNA-protein complex (Fig. 4B, lanes 11 and 12). It is possible that the abundance of HNF-4α caused the mutIR-8-A oligonucleotide to fail to completely compete with the formation of the complex.

It has been demonstrated that individual variation in the expression levels of drug transporters is responsible for the individual variation in pharmacokinetics by experiments using human tissue (15, 35, 36) and laboratory animals (11, 14, 20). Single nucleotide polymorphisms in the coding region (coding SNPs (cSNPs)) of drug transporter genes are also thought to be responsible for the variation in drug responses among individuals (19). Although there have been few reports on cSNPs of the human OAT1 gene, allele frequencies of these cSNPs were very low (5, 13, 45). In addition, Fujita et al. (13) reported that there was no significant decrease in the renal secretory clearance of adefovir in subjects with R454Q, which resulted in a complete loss of OAT1 function in vitro. These reports suggest that cSNPs of human OAT1 are unlikely to influence the interindividual variation in the drug responses and that OAT1 expression is an alternative candidate for the cause of the variation in pharmacokinetics among individuals. Recent studies suggest that SNPs in the promoter region [regulatory SNPs (rSNPs)] can alter the transcription of genes (7). In the OAT1 gene, Bhatnagar et al. (4) found one rSNP ~3 kb upstream of the transcription start site, but it is unclear whether this rSNP affects the mRNA level of OAT1. We previously reported that the OAT1 mRNA level varies and is significantly lower in the kidney of patients with renal diseases than in the normal kidney cortex (36). Accordingly, it is important to clarify the cause of the interindividual differences in OAT1 mRNA expression and the reduction in renal diseases. The present study suggests that HNF-4α regulates OAT1 expression at the mRNA level. Regulatory SNPs of OAT1, the HNF-4α expression level, or cSNPs in the HNF-4α gene that led to a loss of function may be the cause of the interindividual variation in OAT1 mRNA levels.

Tissue-specific gene expression is generally regulated by transcription factors (48). For example, we previously demonstrated that the intestine-specific transcription factor Cdx2 is responsible for the intestine-specific expression of H+/peptide cotransporter 1 (38). OAT1 mRNA is mainly expressed in the kidney (1), and we found that HNF-4α is responsible for the transcriptional regulation of the OAT1 gene. Interestingly, the major expression site of HNF-4α is the liver, but OAT1 mRNA is not expressed in the liver. Furthermore, the human OCT1 gene is also under the control of HNF-4α (34), but OCT1 mRNA is expressed in the liver but not in the kidney. Thus it is difficult to explain the renal-specific expression of OAT1 by HNF-4α itself. Other transcription factors that suppress the hepatic expression of OAT1 (or renal expression of OCT1) may contribute to tissue-specific expression. Alternatively, microRNA and DNA methylation of the promoter may be involved in the tissue-specific expression of genes (39, 44). Further studies are needed to elucidate the mechanism of tissue-specific expression of the SLC22A family.

In conclusion, the present study indicates that the human OAT1 gene is regulated by HNF-4α. This is the first report on the transcriptional regulation of OAT1. In addition, OAT1 is the first gene in the kidney whose promoter activity has been demonstrated to be regulated by HNF-4α.

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