HNF4α REGULATES ORNITHINE TRANSCARBAMYLASE IN VIVO*

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Hepatocyte nuclear factor 4α (HNF4α) regulates the expression of many genes preferentially expressed in liver. HNF4α-null mice die during embryogenesis, precluding the analysis of its function in the adult. To circumvent this problem, liver-specific HNF4α-null mice were produced. Mice lacking hepatic HNF4α expression exhibited increased serum ammonia and reduced serum urea. This disruption in ureagenesis may be explained by a marked decrease in expression and activity of hepatic ornithine transcarbamylase (OTC). To determine the molecular mechanisms involved in transcriptional regulation of the mouse OTC gene, the OTC promoter region was analyzed. Sequence analysis revealed the presence of two putative HNF4α-binding sites in the mouse OTC promoter region. By using transient transfection analysis, it was established that high levels of promoter activity were dependent on both HNF4α-binding sites and the expression of HNF4α. Furthermore, the proximal HNF4α-binding site was found to be more important than the distal one for transactivating OTC promoter. These data demonstrate that HNF4α is critical for urea homeostasis by direct regulation of the OTC gene in vivo.

The liver plays an important role in the metabolism of carbohydrates, proteins, lipids, vitamins, and hormones, the synthesis and excretion of bile acids, the production of blood coagulation factors and plasma proteins, and the detoxification of various xenobiotics. Most of these functions are achieved by hepatocytes that account for over 60% of the liver mass. The expression of genes preferentially expressed in liver is controlled by liver-enriched transcription factors (LETF)1 in the HNF1, HNF3, HNF4, and C/EBP families (1). All of the LETFs described to date are expressed not only in the liver but also to some degree in other organs (2), indicating that the expression of liver-specific genes may be regulated by a combination of LETFs. Among these transcription factors, only the expression of HNF1α and HNF4α is strictly correlated with the differentiated state of hepatoma-derived cells (3, 4). Re-expression of HNF4 in de-differentiated hepatoma cells is sufficient to stimulate the re-expression of many liver-specific genes (5–7). Because HNF4α is a positive regulator of HNF1α (3), HNF4α may be a central factor among the liver-enriched transcription factors.

Hepatocyte nuclear factor 4α (HNF4α; NR2A1) is an orphan member of the nuclear receptor superfamily. Although fatty acyl-CoA derivatives such as (C14:0)-CoA and (C16:0)-CoA were identified as positive regulators of HNF4α (8), they are not good candidates for classical ligands for HNF4α as assessed by structural modeling and experimental analysis (9). Thus, it remains uncertain whether specific ligands exist for HNF4α. Both tissue distribution and amino acid sequence of HNF4α have been highly conserved throughout evolution (2, 10, 11), suggesting a critical role for this factor from embryonic development through the adult. Indeed, targeted disruption of the HNF4α gene was found to be embryonic lethal (12). HNF4α is an essential transcription factor that regulates several serum proteins such as apolipoproteins, blood coagulation factors, P450s, hepatitis B virus, and enzymes involved in glucose, lipids, steroid, and fatty acid metabolism (11, 13). However, most information on HNF4α target genes is derived from in vitro studies using cell lines and transfection and by overexpression of HNF4α. Although the majority of such in vitro studies could be confirmed in vivo, some genes that had been shown to be positively regulated by HNF4α in vitro are unaffected (apolipoproteins A-I and E) or even up-regulated (medium chain acyl-CoA dehydrogenase) in animals lacking hepatic HNF4α (14–17). Thus, it is important to determine whether HNF4α is actually an essential factor that regulates expression of liver-specific genes in an intact animal model.

Ammonia, derived from deamination reaction of α-amino groups of amino acids, is toxic to animals. A portion of the free ammonia is excreted in the urine, but most is used in the synthesis of urea. The urea cycle is an essential pathway to eliminate ammonia through its conversion to urea (Fig. 1). Because the enzymes involved in this pathway are highly expressed in the liver, this organ has an essential role in ammonia elimination. Ornithine transcarbamylase (OTC), the second enzyme in the urea cycle, catalyzes the condensation of ornithine and carbamoyl phosphate to form citrulline (18). OTC deficiency, the most common and severe inborn error in the urea cycle, causes hyperammonemia, mental retardation, and finally death because of hyperammonemic coma (19).

Because HNF4α has been shown to control the expression of

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∥ The abbreviations used are: LETF, liver-enriched transcription factors; HNF4α, hepatocyte nuclear factor 4α; OTC, ornithine transcarbamylase; CPS I, carbamoyl-phosphate synthase I; ASS, argininosuccinate synthase; ASL, argininosuccinate lyase; WT, wild type.

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the rat OTC gene in vitro (20, 21), liver-specific HNF4α-null mice were examined for defects in ureagenesis. These mice exhibited high levels of serum ammonia and decreased serum urea concentrations. Furthermore, it was confirmed by using transient transfection studies that the mouse OTC is directly regulated by HNF4α. Expression levels of several key genes implicated in ureagenesis were altered in these mice. However, this phenomenon was only recapitulated at the protein levels for OTC. Thus, our data not only establish the in vivo significant of HNF4α in the control of ureagenesis but also suggest that post-transcriptional mechanisms may act at other parts of this key pathway.

EXPERIMENTAL PROCEDURES

Animals—Liver-specific HNF4α-null mice were generated by Cre-loxP-mediated deletion (in which theCre gene is under the control of the albumin promoter) to remove exons 4 and 5 of the HNF4α gene (17). All experiments were performed with 45-day-old HNF4αfl/−/fl mice and HNF4αfloxed/− (FLOX) mice. Mice were housed in a pathogen-free animal facility under standard 12-h light/12-h dark cycle with ad libitum water and chow.

Northern Blot Analysis—Total liver or intestine RNA (10 μg) extracted with Trizol reagent (Invitrogen, Carlsbad, CA) from frozen liver or intestine was reverse-transcribed with an oligo-dT primer by reverse transcriptase through a formaldehyde gel and then transferred to GeneScreen Plus membranes (Du Pont). Blots were hybridized at 42 °C in Ultraspin (Ambion, Austin, TX) with random primer-labeled cDNA probes and exposed to a PhosphorImager screen cassette following visualization using a Molecular Dynamic Storm 860 PhosphorImager system (Sunnyvale, CA). All probes were amplified from a mouse cDNA library using gene-specific primers and cloned into pCR TOPO II (Invitrogen). Sequences were verified using ABI Prism Big Dye Terminator kit (Applied Biosystems, Inc., Foster City, CA).

Western Blot Analysis—Frozen livers were crushed on dry ice using a mortar and pestle, washed with cold phosphate-buffered saline, homogenized in a lysis buffer (9 mM urea, 2% Triton X-100, 70 mM dithiothreitol, 1 μM aprotinin, 1 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride), and allowed to swell on ice for 30 min. The nuclear extracts were precleared at room temperature for 30 min after the addition of a 5 μg of anti-HNF4α antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and grown to 90% confluency. For transfections, 800 ng/well of pGL3 basic or the wild-type or mutated OTC promoters and 400 ng/well of pHVT7K (Promega) were used with the total amount of DNA adjusted to 1000 ng as a carrier DNA. For co-transfections, 400 ng/well of the rat HNF4α expression plasmid pSG5/HNF4α was used. Transfections were performed using LipofectAMINE 2000 reagent (Invitrogen) according to the manufacturer’s instructions. After 48 h, the cells were washed with phosphate-buffered saline and assayed for dual luciferase activity using commercial kits according to the manufacturer’s (Promega) instructions.

RESULTS

Liver-specific HNF4α-null Mice Have High Serum Ammonia and Low Serum Urea Concentrations—The urea cycle is an essential liver-specific pathway for the elimination of toxic ammonia derived from amino acid catabolism through production of non-toxic urea (Fig. 1). Because HNF4α is involved in many liver-specific functions (11, 13), the urea cycle was analyzed in liver-specific HNF4α-null (HNF4α-floxed/floxed, albumin-Cre; H4LivKO) mice. At first, ammonia, a primary product of the urea cycle, and urea, the terminal metabolite, were measured. Serum ammonia levels in H4LivKO mice were approximately twice as high as in control (HNF4α-floxed/floxed, no albumin-Cre; H4Flox) mice (Fig. 2A), and serum urea levels in H4LivKO mice were approximately one-half compared with H4Flox mice (Fig. 2B). From a time course study of serum ammonia levels, ammonia in H4LivKO mice increased from 36 days after birth, and its level in 54 day-old mice was 3-fold higher as compared with the control H4Flox mice (Fig. 2C).

Lymphocytes of the H4LivKO mice (17). From these results, it was expected that enzymes involved in the catabolism of amino acids may be affected in the H4LivKO mice.

Ornithine Transcarbamylase Is Decreased in Liver-specific HNF4α-null Mice—Catabolism of amino acids is initiated by transamination, followed by oxidative deamination and,
nally, ammonia elimination via the urea cycle (Fig. 1). Northern blot analysis was performed for genes encoding enzymes involved in these steps to determine whether there is any change in gene expression in the liver-specific HNF4α-null mice (Fig. 3). a-Amino groups from almost all amino acids were transferred to α-ketoglutarate by transamination. Expression of mRNA encoding alanine aminotransferase and aspartate aminotransferase-1 and -2, among the most important aminotransferases, were not different between H4LivKO and H4Flox mice. The next step, oxidative deamination, is mainly catalyzed by glutamate dehydrogenase and glutaminase, and free ammonia is released by both reactions. In the reverse reaction catalyzed by glutaminase, ammonia is removed from glutamine by glutamine synthase. Expression of glutamate dehydrogenase mRNA was higher in H4LivKO mice compared with H4Flox mice. The expression of glutamine synthase was not different between H4LivKO and H4Flox mice. In the last step, the urea cycle is composed of five enzymes; carbamoyl-phosphate synthase I (CPS I), ornithine transcarbamylase (OTC), argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), and arginase I. Free ammonia is then catabolized into urea. Expression of mRNA encoding the second enzyme, OTC, was dramatically decreased in H4LivKO mice, and the levels were 20-fold lower than in H4Flox mice. The expression of the fourth enzyme, ASL, was also decreased by 60% in H4LivKO mice. On the other hand, mRNAs encoding the first (CPS I), third (ASS), and last enzyme (arginase I) were up-regulated approximately twice as high in H4LivKO mice as compared with H4Flox mice.

To determine whether mRNA levels reflected protein expression, Western blot analysis was performed. The expression of OTC protein in liver was not significantly detected in H4LivKO mice (Fig. 4). This result is consistent with the result of Northern blot analysis (Fig. 3). On the other hand, there was no significant difference in the expression of the other proteins (CPS I, ASS, ASL, and arginase I) between H4LivKO and H4Flox mice.

To determine further whether OTC activity in liver is also reduced in H4LivKO mice, activity was measured using a mitochondrial fraction. Liver OTC activity in the H4LivKO mice was markedly reduced to ~5.5% of controls (4.783 ± 1.075 versus 86.103 ± 9.604 μM of citrulline/mg of protein/h, respec-

![FIG. 1. Schematic representation of ureagenesis pathway. Amino acids were converted into ammonia (NH₃) via transamination by aminotransferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) followed by oxidative deamination by glutamate dehydrogenase and glutaminase. Ammonia is then converted into urea via the urea cycle (CPS I, OTC, ASS, ASL, and arginase I). The abbreviations used are as follows: ALT, alanine aminotransferase; GD, glutamate dehydrogenase; GS, glutamine synthase.](http://www.jbc.org/)

![FIG. 2. Serum ammonia and urea levels in liver-specific HNF4α-null and control mice. Serum ammonia (A) and urea (B) levels in 45-day-old mice. Data are mean ± S.E. (FLOX (n = 13), KO (n = 10)). C; time course study of serum ammonia at 36, 42, 48, and 54 days old of age. Data are mean ± S.E. (n = 5). Significant differences compared with FLOX mice: *, p < 0.01; **, p < 0.001.](http://www.jbc.org/)
Regulation of Ornithine Transcarbamylase Expression

Fig. 3. Northern blot analysis of the genes involved in amino acid catabolism. Total liver RNA was isolated, and 10 μg was separated on a 1% agarose gel, transferred to a nylon membrane, and hybridized with the indicated 32P-labeled cDNA probes. The abbreviations used are as follows: ALT, alanine aminotransferase; GD, glutamate dehydrogenase; GS, glutamine synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Fig. 4. Western blot analysis of five urea cycle enzymes. Liver total protein (30 μg) was separated by SDS-PAGE (7%; CPS I, 10%; HNF4α, OTC, ASS, ASL, arginase I, and actin) and transferred to a polyvinylidene difluoride membrane. Goat or rabbit polyclonal antibodies (goat, HNF4α and actin; rabbit, CPS I, OTC, ASS, ASL, and arginase I) were used to assess protein expression, respectively. Thus, mRNA, protein, and activity for OTC are decreased in the livers of H4LivKO mice. These results are consistent with the observed alterations in serum ammonia and urea in these animals.

Because OTC is also expressed in intestine (21) as well as CPS I, OTC expression in the intestine of H4LivKO mice was investigated. As expected, the expression of HNF4α mRNA was unchanged in the intestine of H4LivKO and H4Floxed mice (Fig. 5). Likewise, the expression of OTC and CPS-I mRNAs was also unchanged in the intestine of H4LivKO and H4Floxed mice. Thus, OTC expressed in intestine may help to compensate for lack of OTC in liver in the elimination of serum ammonia in the liver-specific HNF4α-null mice.

HNF4α Activates the Mouse OTC Promoter—Sequence analysis of the mouse OTC promoter revealed two HNF4α-binding sites at −203 to −191 bp and −123 to −111 bp from the predicted translation start site based on the rat OTC promoter (20). To determine whether HNF4α also has the potential to activate the mouse OTC promoter, several OTC promoter-luciferase reporter plasmids were constructed (Fig. 6A). When the human hepatoblastoma-derived HepG2 cells, which express HNF4α (28), were used for transient transfections, the promoter activity of the −173-bp fragment containing a putative HNF4α-binding site was about 2-fold higher than that of the −104-bp fragment (Fig. 6B). The promoter activity of the −235-bp fragment, which is suspected to have two HNF4α-binding sites, was about 5.5-fold higher than that of the −173-bp fragment. The same results were obtained using longer fragments such as the −497 and −707-bp fragments. Their activities were similar to that obtained with the −235-bp fragment. To determine whether this effect was due to HNF4α, non-hepatic CV-1 cells, which do not express HNF4α (29), were used. Basal activity of the −104-bp fragment was higher than that of a promoterless vector (pGL3/basic) and those of longer fragments (Fig. 6C). The promoter activity of this fragment was unchanged by co-transfection of the HNF4α-expression vector, consistent with the absence of an HNF4α. However, the −173-bp fragment was up-regulated by HNF4α. The same results were obtained from experiments using the −235, −497, and −707-bp fragments. However, these promoter activities were much higher than that of the −173-bp fragment (5–11 versus 2.3-fold).

Identification of HNF4α-binding Sites in the Mouse OTC Promoter—To prove further that binding of HNF4α is necessary for expression of the mouse OTC gene, mutations were introduced into either or both of the two HNF4α-binding sites of the mouse OTC promoter. The HNF4α homodimer recognizes a response element with a 13-bp core sequence (direct repeat 1, DR1). To determine whether HNF4α can bind to the both DR1-like elements in the mouse OTC promoter, gel shift analysis was performed using crude liver nuclear extracts from H4LivKO and H4Floxed mice, together with 32P-labeled probes representing the distal and the proximal HNF4α-binding site and mutated sites (Fig. 7A). Liver nuclear extracts from H4Floxed mice contained proteins that bound to the distal (WT1) and the proximal (WT2) HNF4α-binding sites (the lower arrow in Fig. 7B, lanes 1 and 7). These bands were diminished by the addition of excess amounts of unlabeled probes, indicating that specific protein bound to both sites (Fig. 7B, lanes 2 and 8). Furthermore, these bands were supershifted by addition of anti-HNF4α antibody, indicating that the protein bound to both HNF4α-binding sites was indeed HNF4α (the upper arrow in Fig. 7B, lanes 3 and 9). On the other hand, specific proteins that bound to both HNF4α-binding sites were not detected using liver nuclear extracts from the H4LivKO mice, consistent with the fact that these mice do not express HNF4α in liver (Fig. 7C, lanes 1–3 and 7–9). To characterize further the binding of HNF4α to both HNF4α-binding sites, mutations were introduced into the first two nucleotides (GG to CT) of both HNF4α-binding sites (Fig. 7A). Neither mutated distal (M1) nor proximal (M2) HNF4α-binding sites could bind to HNF4α using liver nuclear extracts from H4Floxed mice (Fig. 7B, lanes 4–6 and 10–12). The same results were obtained using liver nuclear extracts from H4LivKO mice (Fig. 7C, lanes 4–6 and 10–12). These results indicate that both HNF4α-binding sites in the mouse OTC promoter are capable of binding HNF4α.

The Mouse OTC Promoter Is Activated through Both HNF4α-binding Sites—To determine whether disruption of HNF4α-binding sites has an effect on promoter activity, the mutations used in Fig. 7A were introduced into either or both of HNF4α-
binding sites in the mouse OTC-luciferase constructs. From the results in Fig. 6, the −235-bp fragment is sufficient to activate the OTC promoter so that the mutations were introduced into the −173- and −235-bp fragments (Fig. 8A). As shown in Fig. 8B, when HepG2 cells were used for transient transfections, promoter activity of the mutated −173-bp fragment (−173/M2; mutation in proximal HNF4α-binding site) was decreased to 25% compared with wild-type (−173/WT), and this value was almost same as the promoterless vector. When a longer fragment (−235/M2) was used, promoter activity was repressed to
10% compared with wild-type (WT). Next, when a distal HNF4α-binding site was disrupted (M1), promoter activity was also repressed, but this activity was 3-fold higher than that of the M2. When mutations were introduced into both HNF4α-binding sites (M1 + M2), promoter activity was decreased to the level of empty vector. These results indicate that both HNF4α-binding sites are important for activation of the OTC promoter, but the proximal site is more important than the distal one.

To confirm this finding, CV-1 cells were used for transient...
transfections. As shown in Fig. 8C, basal activities of all constructs were almost same as empty vector. When the HNF4α expression vector was co-transfected, the promoter activity of the −173/M2 was decreased to basal level of the promoterless vector. Similarly, the promoter activity of the −235/M2 was also repressed as compared with wild-type (−235/WT), but this construct was still activated by HNF4α. Surprisingly, the promoter activity of the −235/M1 was as high as wild type (−235/WT). Furthermore, mutants that disrupted both HNF4α-binding sites (−235/M1 + M2) resulted in no activation of the mouse OTC promoter in the presence of the HNF4α expression vector. These results indicate the importance of both HNF4α-
binding sites, and especially the proximal binding site as compared with the distal one in regulation of the mouse OTC gene.  

**DISCUSSION**

HNF4\(\alpha\) plays a central role in the regulation of the genes preferentially expressed in liver (1, 11, 13). Because most of these results with individual gene promoters were obtained using cell culture studies and/or HNF4\(\alpha\)-overexpression systems, it was important to determine whether they can be translated to the function of HNF4\(\alpha\) in vivo (17). Because the HNF4\(\alpha\)-null mouse is embryonic lethal (12), liver-specific HNF4\(\alpha\)-null mice were made using the Cre-loxP system to analyze the function of this factor in vivo. By using this model, HNF4\(\alpha\) was found to be essential for lipid homeostasis (17). Expression of apolipoprotein (apo) A-II, A-IV, C-II, C-III, and microsomal triglyceride transfer protein was markedly reduced in the livers of liver-specific HNF4\(\alpha\)-null mice. These results confirmed previous reports (30–34) using in vitro cell culture system. However, apoA-I and apoE were unaffected, and medium chain acyl-CoA dehydrogenase was up-regulated in liver of these mice, even though the promoters for these genes are regulated by HNF4\(\alpha\) in transfection studies (14–16). These results indicate that apoA-I and apoE may not be direct targets for HNF4\(\alpha\) in vivo, thus emphasizing the importance for studying gene expression in an intact animal model. Because HNF4\(\alpha\) is a central factor for the development and maintenance of liver function (35), it is expected that dramatic changes in gene expression patterns would occur in the livers of liver-specific HNF4\(\alpha\)-null mice. Thus, it is important to determine which genes are regulated directly or indirectly by HNF4\(\alpha\).

Liver-specific HNF4\(\alpha\)-null mice lose weight coincident with a reduction in hepatic HNF4\(\alpha\) mRNA, and they usually exhibit a high mortality rate by 8 weeks after birth (17). Because the expression of various genes involved in lipid metabolism is drastically reduced in liver-specific HNF4\(\alpha\)-null mice, the possibility exists that the altered maintenance of lipid homeostasis may be one reason for their low viability. However, another reason might be high levels of serum ammonia in these mice due to reduction of hepatic OTC expression. High concentrations of ammonia are known to be a neurotoxin (27), and the levels of serum ammonia in liver-specific HNF4\(\alpha\)-null mice were 2–3-fold higher relative to those in controls. Because OTC is expressed in both liver and intestine (21) and its expression in intestine is unchanged in liver-specific HNF4\(\alpha\)-null mice, it is conceivable that intestinal OTC has an important role in preventing the accumulation of ammonia concentrations to higher levels. On the other hand, two OTC-deficient animal models, *spf* and *spf-ash* mice that exhibit missense mutation or abnormal RNA processing, have been characterized (36, 37). *spf* mice were shown to have 13% of hepatic OTC activity and plasma ammonia levels that were 2–3 times higher than normal (38). Interestingly, the levels of serum ammonia in liver-specific HNF4\(\alpha\)-null mice are almost the same as compared with *spf* mice.

With regard to other enzymes involved in the urea cycle, expression of the CPS-I, ASS, and arginase I mRNAs in liver of liver-specific HNF4\(\alpha\)-null mice were ~2-fold higher than controls, but no significant difference was detected in the protein levels with the exception of OTC. The increase of these mRNAs may reflect secondary effects caused by the reduction of OTC mRNA. Because the expression of mRNAs encoding urea cycle enzymes except OTC is reported to be increased by treatment with cAMP and/or glucocorticoids (39), these levels may increase in the livers of liver-specific HNF4\(\alpha\)-null mice. However, ASL mRNA was reduced in the null mice to 60% as compared with controls. Other transcription factors such as C/EBP\(\alpha\) and -\(\beta\) and Sp-1 are thought to regulate positively expression of these genes (40–42). However, the expression of Sp-1 was not different and C/EBP\(\beta\) and -\(\beta\) were conversely reduced in the livers of liver-specific HNF4\(\alpha\)-null mice as compared with controls (data not shown). Another reason for the elevated expression of CPS I, ASS, and arginase I mRNAs may be an increased DNA binding activity of these transcription factors mediated by cAMP and/or hormones. By using primary hepatocytes derived from C/EBP\(\beta\)-null mice, it was reported that C/EBP\(\beta\) is required for hormonal induction of the expression of CPS I and arginase I (43). Finally, HNF4\(\alpha\) may negatively regulate the expression of CPS I, ASS, and arginase I mRNAs. However, it has not been reported that there is a HNF4\(\alpha\)-binding site in promoter and enhancer regions of these genes. It was recently reported that activation of the peroxisome proliferator-activated receptor \(\alpha\) represses the expression of CPS I, OTC, ASS, and arginase I (44). The increased expression of these genes is therefore consistent with the down-regulation of peroxisome proliferator-activated receptor \(\alpha\) mRNA in livers of liver-specific HNF4\(\alpha\)-null mice (17).

The 5’-flanking region of mouse OTC genes contains sufficient elements to control OTC expression in a hepatoma cell line (45). The same result was obtained in the present study using a fragment of the mouse OTC promoter region. Furthermore, it was reported that a 1.3-kbp fragment of the rat OTC promoter region is active in a hepatoma cell line (46), and there are two HNF4\(\alpha\)-binding sites in this promoter region (20). Indeed, there are also two HNF4\(\alpha\)-binding sites in the mouse promoter. From the results using mutated constructs, the proximal HNF4\(\alpha\)-binding site was found to be more important than the distal site. Furthermore, mutation of the distal HNF4\(\alpha\)-binding site (~235/M1) yielded a higher activity in CV-1 cells than in HepG2 cells. These results indicate that an additional factor may be needed to activate the mouse OTC promoter in HepG2 cells, and the expression of such a factor may be lower in HepG2 cells as compared with CV-1 cells.

In humans, homozygous mutations in the HNF4\(\alpha\) gene have not been described, probably due to embryonic lethality. However, heterozygous mutations were identified in the coding regions (13, 47). These facts indicate that HNF4\(\alpha\) is essential for the development and maintenance of liver function in humans. Heterozygous mutations in HNF4\(\alpha\) are known to cause maturity of onset diabetes of young 1 (MODY-1), a form of non-insulin-dependent diabetes mellitus (48). Patients with MODY-1 inherit the disease in an autosomal dominant pattern and develop symptoms under the age of 25. MODY-1 is characterized by defective secretion of insulin by the pancreatic \(\beta\)-cells, but the function of liver and kidney is normal as is insulin sensitivity (13, 49). Recently, triglyceride and apoC-III levels were reported to be significantly reduced in these patients (50). This result is in agreement with the previous study (17) using liver-specific HNF4\(\alpha\)-null mice.

OTC deficiency in humans is the most common inherited disease in urea cycle enzymes (19). Many mutations were identified in the exons and exon/intron borders (51, 52), but no mutations have yet been reported in the regulatory elements. However, mutations in the HNF4\(\alpha\)-binding site were reported in other human genes, such as blood coagulation factor VII (53), IX (54–56), and HNF1\(\alpha\) (57). Because HNF4\(\alpha\)-binding sites in the promoter region are critical for regulation of OTC gene expression, any mutation in these sites could potentially cause human OTC deficiency.

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**REFERENCES**

1. Cereghini, S. (1996) *FASEB J.* 10, 267–282
2. Xanthopoulos, K. G., Prezioso, V. R., Chen, W. S., Sladek, F. M., Cortese, R.,
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27. Largillier, C. (1995) Proc. Natl. Acad. Sci. U. S. A. 88, 3807–3811
23. Kanazawa, M., Terada, K., Kato, S., and Mori, M. (1997) J. Biochem. 111, 2173–2178
29. Garnier, G., Circolo, A., and Colton, H. R. (1996) J. Biol. Chem. 271, 30205–30211
20. Kimura, A., Takiguchi, M., Tsukamoto, T., Hata, S., Osumi, T., Murachi, T., and Cohen, P. P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 952–957
12. Sladek, F. M. (1994) in \textit{Regulation of Ornithine Transcarbamylase Expression} (Burris, T. P., and McCabe, E., eds) pp. 309–313, McGraw-Hill Inc., New York
30. Ribeiro, A., Pastier, D., Kardassios, D., Chambaz, J., and Cardot, P. (1999) J. Biol. Chem. 274, 1216–1225
24. Yu, Y., Terada, K., Nagasaki, A., Takiguchi, M., and Mori, M. (1995) J. Biochem. 118, 706–7094
28. Inoue, Y., Miyaizaki, M., Tsuji, T., Sakaguchi, M., Fukaya, K., Huh, N.-H., and Namba, M. (2001) Int. J. Mol. Med. 8, 481–487
22. Mori, M., Miura, S., Tatibana, M., and Cohen, P. P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 952–957
15. Dang, Q., Walker, D., Taylor, S., Allan, C., Chin, P., Fan, J., and Taylor, J. (1995) J. Biol. Chem. 270, 22577–22585
14. Harnish, D. C., Malik, S., Kilbourne, E., Costa, R., and Karathanasis, S. K. (1996) Nuclear Receptors and Genetic Disease (Burris, T. P., and McCabe, E., eds) pp. 309–313, McGraw-Hill Inc., New York
26. Ktistaki, E., Lacorte, J.-M., Katrakili, N., Zannis, V. I., and Talianidis, I. (1996) J. Biol. Chem. 271, 1232–1236
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