Hepatocyte nuclear factors 1α and 4α control expression of proline oxidase in adult liver

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Abstract Adult liver functions are regulated by several hepatocyte nuclear factors (HNFs). HNF4α and HNF1α are involved in metabolic functions in the liver. The expression of proline oxidase (PO) and proline dehydrogenase was downregulated in the HNF4α liver-specific null mice. In addition, the expression of PO was also diminished in the liver derived from HNF1α-null mice. The −160 bp proximal promoter region of the PO gene has two HNF4α- and HNF1α-binding consensus sites. Transactivation, electrophoretic mobility shift and chromatin immunoprecipitation studies revealed that these regions are important for PO promoter activity. These results suggested that HNF4α and HNF1α regulate proline metabolism in adult liver.

Keywords: Amino acid metabolism; Liver-enriched transcription factor; Gene expression; Proline oxidase

1. Introduction

Adult liver is the central organ for intermediary metabolism, serum protein synthesis, lipogenesis, detoxification of xenobiotics, heme catabolism, amino acid catabolism and urea synthesis. During organ development, cell differentiation takes place which is a result of changes in gene expression that are mainly regulated at the level of transcription. Previous studies have suggested that hepatocyte nuclear factors (HNFs) 1α, 1β, 3α, 3β, 3γ, 4α and 6 as well as the CCAAT/enhancer-binding protein (C/EBP) family (α, β and δ) are involved in both liver development and the expression of mature liver functions.

HNF4α is a member of the nuclear hormone receptor superfamily and several studies revealed that it is important for both liver development and adult liver functions [1]. Indeed, adult liver-specific disruption of HNF4α results in several defects in liver functions such as the urea, lipid and bile acid metabolism [2–4]. HNF1α, a homeodomain-containing transcription factor preferentially expressed in the liver, regulates many genes including CYP2E1, serum albumin, phosphoenolpyruvate carboxykinase and phenylalanine hydroxylase (PAH), all of which have a functional HNF1α-binding sequence in their upstream regulatory regions [5–8]. A role for HNF1α in controlling mammalian development and metabolism was suggested by analysis of HNF1α-null mice [9,10]. HNF1α-null mice are characterized by a phenotype resembling non-insulin-dependent diabetes mellitus. In addition, the expression of PAH, the enzyme responsible for catabolism of phenylalanine, was markedly diminished in HNF1α-null mice, indicating that HNF1α regulates phenylalanine metabolism.

In addition to PAH, several enzymes regulating amino acid metabolism are expressed in the adult liver. All tissues have some capacity for synthesis of the non-essential amino acids, amino acid remodeling and conversion of non-amino acid carbon skeletons into amino acids and other derivatives that contain nitrogen. However, the liver is the major site of amino acid metabolism in the body. The amino acids including glutamine, proline, arginine, and histidine are ultimately converted to glutamate, which is deaminated to α-ketoglutarate. The first step of proline metabolism is the conversion of proline to L-1-proline-5-carboxylate, followed by conversion to ornithine, metabolism in the urea cycle or to glutamate and finally introduction into the TCA cycle [11]. Proline oxidase (proline dehydrogenase, PDH) is an important enzyme in the first step of proline metabolism and two mouse genes having the PDH domain were cloned as kidney and liver proline oxidase 1 (PO) and brain and kidney proline oxidase 2 (PDH) [12]. PDH has also been identified as p53-induced gene in a colorectal cell line [13]. However, the molecular mechanism regulating these two genes in adult liver remains unknown.

In this study, the liver specific transcriptional control of proline metabolic enzymes by HNF1α and HNF4α was analyzed. Disruption of HNF4α caused the downregulation of expression of PO and PDH. In addition, expression of PO was also decreased in the liver derived from HNF1α-null mice. The upstream regulatory region of the PO gene has both HNF4α and HNF1α binding sites and these sites were required for expression of PO induced by HNF4α and HNF1α. These data indicate that HNF4α and HNF1α regulate proline metabolism through expression of PO and PDH.

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Abbreviations: HNF, hepatocyte nuclear factor; PO, proline oxidase; C/EBP, CCAAT/enhancer-binding protein; PAH, phenylalanine hydroxylase; PDH, proline dehydrogenase; DMEM, Dulbecco's Modified Eagle's Medium; FBS, Fetal bovine serum; RACE, rapid amplification of the cDNA ends methods; TO, tryptophan oxygenase; FLOX, HNF4α-floxed-allele; OTC, ornithine transcarbamylase; CPS, carbamoylphosphate synthetase-1; PPARα, peroxisome proliferator-activated receptor α

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2. Materials and methods

2.1. Materials

Liver-specific HNF4α-null mice were generated by Cre-loxP mediated deletion (in which the Cre gene is under the control of the albumin promoter) [2]. HNF1α-null mice were produced as described previously [10]. Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Gibco-BRL (Rockville, MD). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT). Goat polyclonal anti-human HNF4α antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). The expression vectors of HNF4α and HNF1α were previously described [3,14].

2.2. Northern blot analysis

Cellular and tissue total RNA samples were extracted with Trizol reagent (Invitrogen, Carlsbad, CA). Ten μg of total RNA from each sample was separated by electrophoresis on a 1.5% agarose gel containing 2% formaldehyde. RNA was transferred to GeneScreen Plus membranes (Dupont, Wilmington, DE) and the blots were hybridized with ^{32}P-labeled cDNA probes generated by random-prime labeling reactions. The membrane and probe were incubated at 65 °C in PerfectHyb Plus hybridization buffer (Sigma, St. Louis, MO) and following washing, the blot was exposed to a phosphorimager screen cassette and the signals visualized using a Molecular Dynamic Storm 860 Phosphorimager system (Sunnyvale, CA). All probe cDNAs were amplified from a mouse liver cDNA library by using gene-specific primers and cloned into pCR TOPOII (Invitrogen). The identity of the probes was confirmed by nucleotide sequencing.

2.3. Determination of the transcription start site

The transcription start site of the mouse PO gene was determined using 5′ rapid amplification of the cDNA ends methods (RACE) with the GeneRacer Kit (Invitrogen). After first-strand cDNA synthesis, PCR was performed with a gene-specific antisense primer (5′-AGAGCCCCCTTCCTGCCCACTCCATCCT-3′) and 5′ GeneRacer primer. To generate a gene-specific RACE PCR product, nested PCR was performed with a gene-specific antisense nested primer (5′-TGCTCACCGCTAGTCCATGAGTGA-3′) and 5′ GeneRacer nested primer. These primers were specific to the sequences in the PO exon 1. The transcription start site was determined by sequencing the resultant cloned PCR products. Since 19 out of 24 cDNAs had the exact sequence, this site was designated as the major transcription start site.

2.4. Construction of the mouse PO-luciferase promoter plasmids

The −775, −250, −160, −85 and −40/+24 bp fragments from the transcription start site of the mouse PO promoter were amplified by PCR and cloned into the luciferase reporter vector, pGL3basic (Promega, Madison, WI). Mutations were introduced into the HNF4α and HNF1α binding sites in the PO gene promoter using PCR-based site-directed mutagenesis. All plasmids were confirmed by nucleotide sequencing.

2.5. Transient transfection luciferase assay

HepG2 cells and CV-1 cells were cultured in DMEM containing 10% FBS, 100 units/ml penicillin/streptomycin (Invitrogen). The cells were seeded in 24-well tissue culture plates, grown to 90–95% confluency and transfected with pGL3basic reporter plasmid and pSG5-ratHNF4α or pCMV-ratHNF1α expression vectors using LipofectAMINE 2000 (Invitrogen). As an internal control, the plasmid pRL-TK containing the Renilla luciferase gene was co-transfected. Cells were cultured in medium for 48 h and then lysed with passive lysis buffer (Promega). Luciferase activity was measured according to the technical manual for the Dual-Luciferase Reporter Assay System (Promega).

2.6. Electrophoretic mobility shift assay

Nuclear extracts were prepared from wild-type and null mouse livers using NE-PER nuclear and cytoplasmic extraction reagents (Pierce, Rockford, IL). Three μg of protein extract was preincubated at 4 °C for 15 min in 10 mM Tris–HCl (pH 7.5), 60 mM KCl, 0.1% NP-40, 6% glycerol, 1 μg poly(dI-dC) and 1 mM dithiothreitol. For competition experiments, a 50-fold excess of unlabeled oligonucleotide was added to the reaction mixture. ^{32}P-end-labeled double stranded oligonucleotide was then added and the reaction mixture was incubated at 4 °C for 20 min. For gel mobility super-shift assays, and antibodies against HNF4α and HNF1α (kindly provided by Dr. Jorge Ferrer, Hospital Clinic Universitari, Spain) were incubated with the probe-nuclear extract mixtures for 30 min at 4 °C. DNA/protein complexes were resolved on a 5% polyacrylamide (19:1, acrylamide:bisacrylamide) gel in 0.5× Tris–borate–EDTA at 150 V for 2 h at 4 °C. The following oligonucleotides were used; the PO promoter/HNF4α-B1, 5′-TTCTGACCTCTGTCCCTAA-3′ and 3′-AAGACTGGAGACAGGGATT-5′; HNF4α-B1 mutant oligonucleotides, 5′-TTCTGACCTCTGTCAGTAA-3′ and 3′-AAGACTGGAGACAGTCATT-3′; HNF4α-B2, 5′-AGCTGGGCAAGGGGCATTT-3′ and 3′-TCGAC- CCGTTCCCCGTAAA-5′; HNF4α-B2 mutant oligonucleotides, 5′-AGCTTCGCAAGGGGCATTT-3′ and 3′-TCGAAGCGTTCCC- CGTAAA-5′; HNF1α-B1, 5′-CATTAATTATTAACCCT-3′ and 3′-GTAATTAATAATTGGGA-5′; HNF1α-B1 mutant oligonucleotides, 5′-CATCGGCTATTAACCCT-3′ and 3′-GTAGCCGATAATTGGGA-5′; HNF1α-B2, 5′-AGTTAATCAGTAACTGC-3′ and 3′-TCAATTAGTCATTGACG-5′; HNF1α-B2 mutant oligonucleotides, 5′-AGTCGGCCAGTAACTGC-3′ and 3′-TCAGCCGGTCATTGACG-3′ (mutations are underlined).

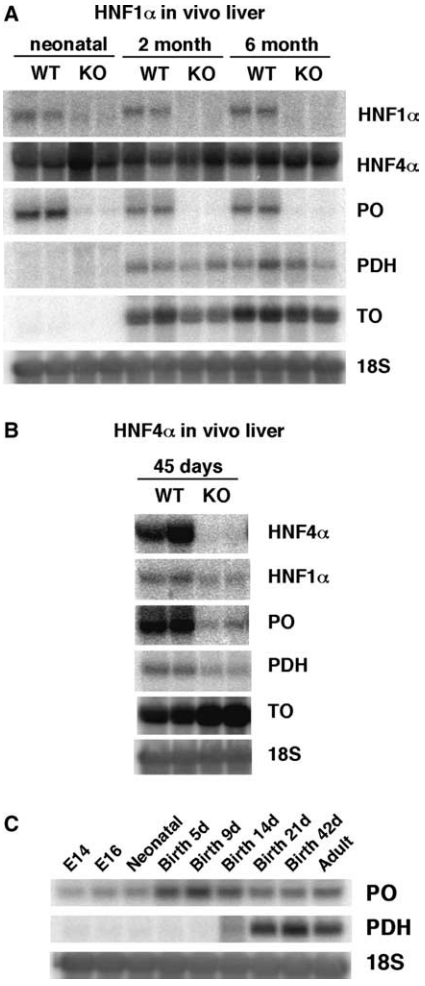

Fig. 1. Expression of amino acid metabolic enzymes regulated by HNF4α and HNF1α. (A) The neonatal, 2 and 6 month-old mice were dissected. Total RNAs were purified from the liver and subjected to Northern blot analysis. Expression of PDH and TO was barely changed by the HNF1α gene inactivation. (B) Expression of PO and PDH in the livers of control and HNF4α liver-null mice. The 45-days livers derived from HNF4α FLOX and null mice were used for Northern blot analysis. (C) Developmental changes of expression of PO and PDH in the liver. Total RNAs isolated from E14, E16, neonatal, 5-, 9-, 14-, 21-, 42-day-old, and adult livers were used for Northern blot analysis.

2.7. Chromatin immunoprecipitation assays

A total of 0.1 mg adult liver tissues derived from HNF4α FLOX and null mice were used for assays. Chromatin immunoprecipitation experiments were performed according to the technical manual for the Chromatin Immunoprecipitation Assay Kit (Upstate, Lake Placid, NY). Sonication was performed 8 times on ice for 10 s at 20-s intervals. A volume of sonicated DNA was then immunoprecipitated by using the anti-human HNF4α mouse monoclonal antibody (Clone No. K9218, Perseus Proteomics Inc.) [15]. All immunoprecipitations were subjected to a round of preclearing with an excess of protein A-Agarose to ensure the specificity of the reaction. Precipitated and non-precipitated (input) genomic DNA was then purified and resuspended and one tenth of the DNA preparations were PCR-amplified for 45 cycles (30 s at 95 °C, 30 s at 50 °C, and 30 s at 72 °C). The following primers were used; PO −250/+24, 5′ primer: 5′-TGCTTATTCAGCAATGCAAGCTG-3′ and 3′ primer: 5′-CAAGTGCCTGGTTACCACCACACTAG-3′; PO +1034/+1249, 5′ primer: 5′-TCAGTTGCTGAGCATGGTCGTG-3′ and 3′ primer: 5′-GGAGAGACGGCTGCATTTTCTG-3′.

3. Results

3.1. Expression of proline oxidase enzymes regulated by HNF1α and HNF4α

Proline oxidation (dehydrogenation) is the first step of proline metabolism. Two mouse genes, PO and PDH, have a proline dehydrogenase consensus domain. However, there is little information on the mechanism of regulation of these genes. Recently, mouse oligonucleotide microarrays using HNF1α-null mice revealed that several known genes and EST clones displayed a greater than 50% decrease in expression in HNF1α-null mouse liver [16]. One of these genes was PO and consistent with the microarray result, the expression of this gene was significantly downregulated in HNF1α-null liver as assessed by Northern blots (Fig. 1A). In contrast, the expression of other amino acid metabolic enzymes, PDH and tryptophan oxygenase (TO), was not changed in adult liver derived from HNF1α-null mice. The expression of genes involved in proline metabolism was analyzed in HNF4α-null livers (Fig. 1B). Expression of both PO and PDH was significantly decreased by liver-specific deletion of the HNF4α gene, whereas expression of TO was not downregulated by targeted inactivation of HNF4α in the liver. These results suggest that expression of genes encoding proline oxidase enzymes was regulated by HNF4α and HNF1α either directly or indirectly. During liver development, the expression of PDH was not detected in the fetal and perinatal livers, whereas PO was expressed during both the fetal and adult stages (Fig. 1C).

3.2. The PO proximal promoter region has HNF4α and HNF1α putative binding consensus sequences

The inactivation of HNF4α resulted in a 50% less lower HNF1α expression (Fig. 1B). In contrast, the expression of HNF4α was not changed in HNF1α-null livers (Fig. 1A). To

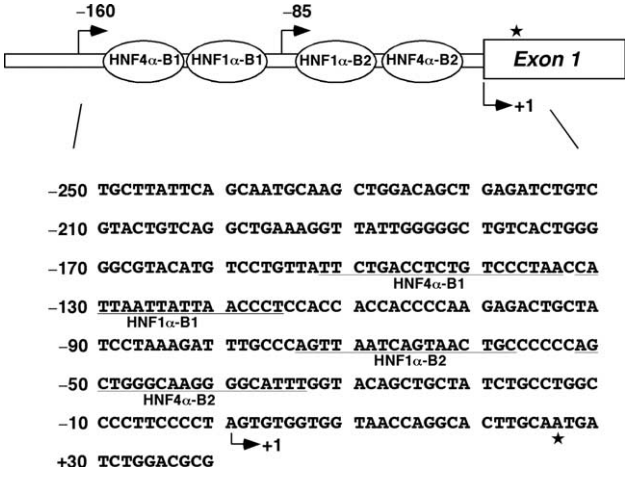

Fig. 2. Nucleotide sequence of the −250 bp fragment of the PO proximal promoter. Numbering of nucleotides is relative to the transcription start site (nucleotide +1, arrow). The asterisk marks the translation start site. The two putative HNF4α binding sites (HNF4α-B1 and HNF4α-B2) and two putative HNF1α binding sites (HNF1α-B1 and HNF1α-B2) are found in the proximal promoter region of the PO gene.

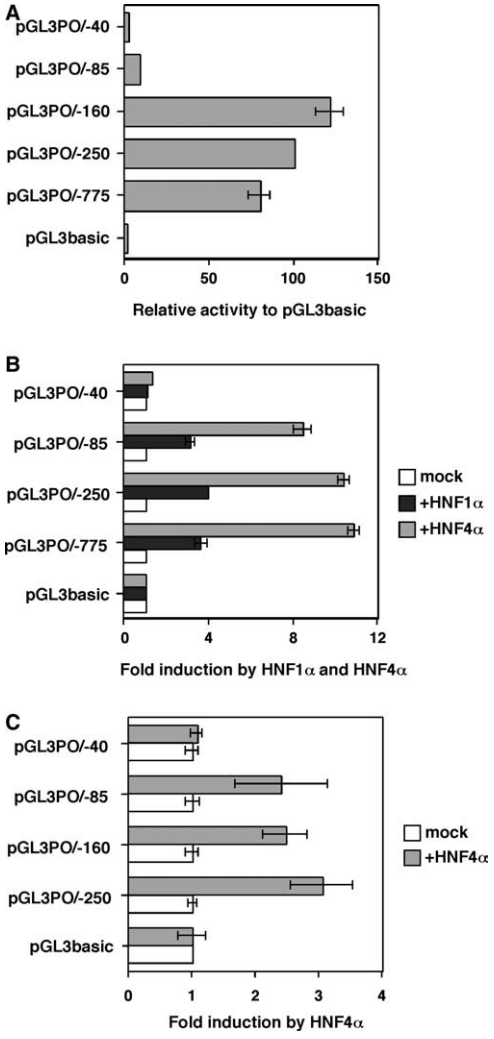

Fig. 3. Promoter analyses of the mouse PO gene. (A) Various luciferase reporter plasmids containing mouse PO promoter (−775, −250, −160, −85 and −40/+24 bp from the transcription start site) were transfected into HepG2 cells. (B) CV-1 cells were cotransfected with the HNF4α or HNF1α expression vector as indicated. The relative activity to pGL3basic of the most active promoter (pGL3PO/−775 + HNF4α) was 19.2. (C) HepG2 cells were cotransfected with the HNF4α expression vector as indicated. "Mock" shows that the cells transfected with PUC19 instead of the expression vectors. The relative activity to pGL3basic of the most active promoter (pGL3PO/−250 + HNF4α) was 170. The fold induction was calculated. Each point is the mean and SD of triplicate assays.

determine whether downregulation of PO expression in HNF4α-null livers is due to HNF1α, a DNA fragment containing the 5′ flanking region of the mouse PO gene was isolated and the precise transcription start site was determined by 5′ RACE using the sense and antisense nested primers specific to the sequences within the coding region of the PO gene. After sequencing the PCR products, the major transcription start site was determined to be −26 bp upstream of the translation start site (Fig. 2). Interestingly, the −160 bp upstream fragment of the PO gene has two putative tandem HNF4α and HNF1α binding sites.

Several PO promoter-luciferase reporter plasmids were constructed and analyzed by transient transfections. When HepG2 cells were used for transient transfections, the promoter activities of the −160 bp and longer fragments were significantly higher than that obtained with the −85 and −40 bp fragments (Fig. 3A). This indicated that the elements important for hepatic transcription are within 160 bp of the PO gene transcription start site that has HNF4α and HNF1α binding sequences. Next, to determine the effects of HNF4α and HNF1α on the PO promoter, non-hepatic CV-1 cells were used. Basal activities of the −85 and −40 bp fragments were lower than the activities of the −250 and −775 bp fragments (data not shown). However, the activity of the −85 bp fragment, in addition to the −250 and −775 bp fragments, was also increased by co-expression of HNF4α and HNF1α (Fig. 3B). This result is consistent with the −85 bp upstream region of the PO promoter having HNF4α and HNF1α binding sites (Fig. 2). In contrast, the −40 bp upstream fragment, which has no HNF4α or HNF1α binding site, was not activated by co-expression of HNF4α and HNF1α. Similarly to CV-1 cells, HNF4α induced the activity of the −85 bp upstream region of the PO promoter but not the −40 bp upstream fragment in HepG2 cells (Fig. 3C). In contrast, the overexpression of HNF1α induced the activity of pGL3basic almost ten times in HepG2 cells. It is possible that some associating protein in HepG2 cells affects the vector sequence of pGL3basic in co-operation with HNF1α (data not shown).

3.3. Identification of HNF4α and HNF1α binding sites in the PO proximal promoter

The −160 bp fragment derived from the PO promoter was found to have two putative HNF4α and HNF1α binding sites. To prove that these sites directly bind to HNF4α, electrophoretic mobility shift assays (EMSAs) were performed using crude liver nuclear extracts from HNF4α liver null and control (HNF4α-floxed allele, FLOX) mice (Fig. 4A). Liver nuclear extracts from HNF4α FLOX mice contained proteins that bound to both HNF4α binding site oligonucleotides (WT) (Fig. 4A, lanes 1 and 8). In addition, super-shifted fragments were detected using the HNF4α-specific antibody (Fig. 4A, lanes 3 and 10). In contrast, the super-shifted fragments were not found in samples derived from HNF4α null liver nuclear extracts (Fig. 4A, lanes 5 and 12). To confirm the specificity of HNF4α binding to these sites, mutations were introduced to change 2 nucleotides (CC to AG of HNF4α-B1 and GG to TC of HNF4α-B2). The mutated oligonucleotides were unable to bind to HNF4α (Fig. 4A, lanes 7 and 14).

In addition, the binding activities of HNF1α binding consensus sequences were studied. Shifted bands were detected using liver nuclear extracts from wild-type mice (Fig. 4B, lanes 1 and 7), but not from HNF1α-null mice (lanes 3 and 9). To confirm the specificity of HNF1α binding to these sites, mutated oligonucleotides were synthesized within 4 nucleotides changes (TAAT to CGGC) of the HNF1α binding sites. HNF1α did not bind to these mutated oligo-

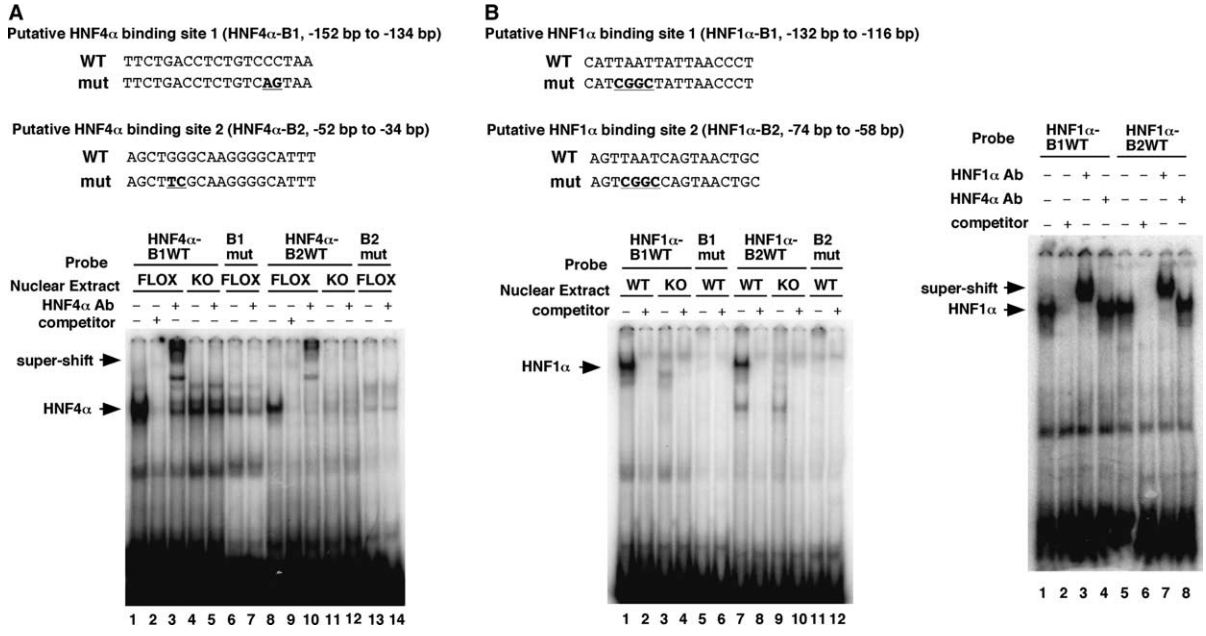

Fig. 4. Electrophoretic mobility shift assays for HNF4α and HNF1 binding sites in the PO promoter. (A) Nuclear extract proteins (3 μg) from HNF4α FLOX or null livers were incubated with labeled wild-type (B1WT and B2WT) and mutant (B1mut and B2mut) PO HNF4α binding site oligonucleotides. For super-shift assays, 1 μg of an HNF4α antibody was added to probe-extract complexes. For competition experiments, a 50-fold excess of unlabeled oligonucleotides was added to the reaction mixture. (B) Nuclear extract proteins from HNF1α wild-type (WT) and null livers were incubated with labeled wild-type (B1WT and B2WT) and mutant (B1mut and B2mut) PO HNF1α binding site oligonucleotides. The right panel shows the effect of HNF1α and HNF4α antibodies on the HNF1α-B1WT and HNF1αB2WT oligonucleotide-bound complexes.

nucleotides (Fig. 4B, lanes 5 and 11). Antibodies against HNF1α produced super-shifted the complexes with both HNF1α-B1WT and HNF1α-B2WT (Fig. 4B, right panel). No super-shifted fragments were detected with either oligonucleotide with antibody against HNF4α. These results suggest that the HNF4α and HNF1α putative binding sites in the PO proximal promoter are capable of binding to HNF4α and HNF1α.

3.4. HNF4α and HNF1α activate the PO promoter through the HNF4α and HNF1α binding sites in the −160 bp upstream region

To determine the functional relevance of these HNF4α and HNF1α binding sites, CV-1 cells were transiently transfected with the PO promoter plasmid containing mutated binding sites shown in Fig. 4. The basal activities of −250 bp fragment of PO were not significantly changed by introducing mutations in the HNF4α binding sites (data not shown). In the cells transfected with the wild-type and HNF4α-binding site mutant promoter plasmids, the fold induction of the luciferase activities by the overexpression of HNF1α was similarly detected (Fig. 5A, black bars). In contrast, mutation of each HNF4α binding site caused significant decrease of the induction by the co-expression of HNFα and the minimum induction was detected using the promoter having both mutated HNF4α binding sites (Fig. 5A, gray bars). In addition, mutations were introduced into the HNF1α-binding sites in the PO promoter. Mutation of HNF1α binding sites decreased basal luciferase activities of the PO promoter (data not shown). When both HNF1α binding sites were mutated, the PO promoter activity was not significantly induced when co-transfected with the HNF1α expression plasmid (Fig. 5B, black bars), but not HNF4α (Fig. 5B, gray bars). These results suggested that the HNF4α and HNF1α binding sites were important for the activity of the PO promoter.

3.5. HNF4α occupies the HNF4α binding sites in the −160 bp upstream fragment of the PO gene in vivo

Occupancy of the PO promoter by HNF4α in intact hepatocytes was studied by using chromatin immunoprecipitation assays performed on DNA from HNF4α FLOX and null adult livers. Chromatin DNA was precipitated using anti-HNF4α antibody and the sequence covering the HNF4α binding sites was amplified using specific primers. As a negative control, when the same DNA samples were amplified with primers covering a distal region of the PO gene, no signal was observed (Fig. 6, middle panel). The PO −250/+24 fragment was significantly amplified when samples were precipitated using anti-HNF4α antibody but not without antibodies (Fig. 6, lower panel). These results suggested the binding of HNF4α to this DNA region in the in vivo adult liver.

4. Discussion

This study revealed that the expression of PO and PDH was regulated by HNF1α and HNF4α. In the HNF4α liver-specific null mice, expression of PO and PDH was significantly decreased. In addition, deletion of the HNF1α gene suppressed expression of PO but not PDH, suggesting that both factors were involved in the control of this gene. Indeed, two HNF4α and HNF1α binding sites were found in the proximal pro-

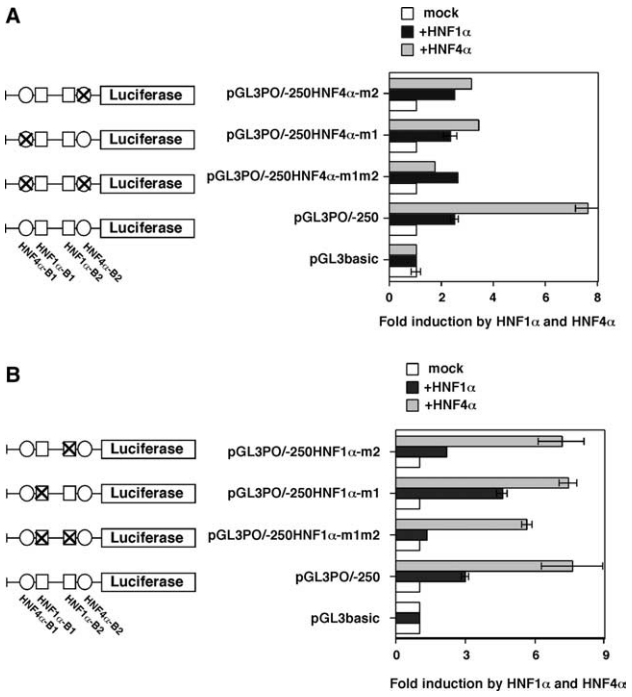

Fig. 5. Analysis of HNF4α and HNF1α binding sites in the PO promoter by transactivation assay. (A) Reporter plasmids and an expression vector (HNF4α or HNF1α) were co-transfected into CV-1 cells. The induction of PO promoter plasmids containing mutation in both HNF4α binding sites (−250 HNF4α-m1m2) was significantly decreased by the overexpression of HNF4α. The relative activity to pGL3basic of the most active promoter (pGL3PO/−250 + HNF4α) was 26.1. (B) Analysis of constructs containing mutations of both HNF1α binding sites in the PO promoter. The relative activity to pGL3basic of the most active promoter (pGL3PO/−250 + HNF4α) was 23.0. The fold induction was calculated. "Mock" shows that the cells transfected with PUC19 instead of the expression vectors. Each point is the mean and SD of triplicate assays.

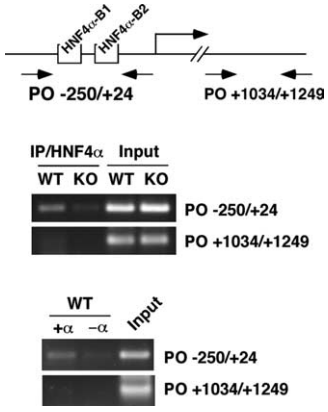

Fig. 6. HNF4α binds to the HNF4α binding sites in the PO promoter in the in vivo liver. Soluble chromatin was prepared from the HNF4α FLOX and KO adult livers, and immunoprecipitated with the anti-HNF4α antibody. Samples immunoprecipitated and incubated with protein A-Agarose (IP/HNF4α) or extracted DNA (Input) were amplified using pair of primers covering the HNF4α binding sites (PO −250/+24) or a distal region of the PO gene (PO +1034/+1249) as a negative control. The samples derived from HNF4α FLOX mice were also precipitated with the anti-HNF4α antibody (+α) or without antibodies (−α). Then, DNA was amplified.

moter region of the PO gene. HNF4α and HNF1α were found to directly bind to these regions and thus indicating that these binding sites were important for the activity of the PO promoter.

Human and mouse PDH was previously cloned as homolog of sluggish-A, a Drosophila melanogaster gene responsible for the behavioral phenotype of the slgA mutant [17,18]. Here, it was shown that expression of PDH was downregulated by HNF4α inactivation. However, the proximal PDH promoter was not activated by co-expression of HNF4α (data not shown). Expression of PDH was induced by p53 in tumor cell lines [13,19], yet, the molecular mechanism of regulation of PDH expression in intact animals remains unknown. The current study indicated that PO is expressed in both neonatal and adult stage livers. However, PDH mRNA was mainly expressed in the adult liver. It was previously found that plasma and brain proline levels were upregulated in mice containing a mutation in the PDH gene, suggesting that this gene is important for proline metabolism [18]. In contrast to adult mice, expression of PDH was not detected in the fetal and neonatal liver, suggesting that PO might be important for the first step of proline conversion in the fetal and neonatal stages. Expression of PO mRNA was regulated by HNF4α and HNF1α in the liver. It is noteworthy that HNF1α is directly regulated by HNF4α in the developing liver [20] and both HNFs are important for liver development. Previous results indicated that the expression of HNF4α was induced by hepatic maturation factor in fetal hepatic primary culture [21]. Thus, it is possible that these liver-enriched transcription factors regulate proline metabolism through the regulation of PO gene expression during embryonic and postnatal development.

In the HNF4α liver-specific null mice, the expression of two proline oxidase genes was significantly decreased in the liver. Proline is ultimately metabolized in the converged pathways of glutamate or arginine catabolism. The main arginine metabolic pathway is the urea cycle. Ammonia, derived from deamination reaction of α-amino groups of amino acids, is toxic to animals and thus the urea cycle is an essential pathway for elimination of ammonia through its conversion to urea. Since the enzymes involved in this pathway are highly expressed in the liver, this organ has an essential role in the urea cycle. Previous studies revealed that ornithine transcarbamylase (OTC), the second enzyme in the urea cycle, is directly regulated by HNF4α. In contrast, HNF4α may negatively regulate the expression of carbamoylphosphate synthetase-1 (CPS) and arginase [3]. The activation of peroxisome proliferator-activated receptor α (PPARα) repressed the expression of CPS and arginase [22]. Expression of PPARα was downregulated in the HNF4α null livers [2], indicating that this decrease caused the upregulation of these genes in the HNF4α liver-null mice. These results suggested that HNF4α is the key transcription factor in the amino acid and urea metabolic pathways.

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