The Winged Helix Transcriptional Activator HFH-3 Is Expressed in the Distal Tubules of Embryonic and Adult Mouse Kidney*

David G. Overdier‡, Honggang Ye, Richard S. Peterson, Derek E. Clevidence, and Robert H. Costa§

From the Department of Biochemistry, University of Illinois, Chicago, Illinois 60612-7334

The hepatocyte nuclear factor-3 (HNF-3)/fork head homolog (HFH) proteins are an extensive family of transcription factors, which share homology in the winged helix DNA binding domain. Members of the HFH/winged helix family have been implicated in cell fate determination during pattern formation, in organogenesis, and in cell-type-specific gene expression. In this study we isolated a full-length HFH-3 cDNA clone from a human kidney library which encoded a 351-amino acid protein containing a centrally located winged helix DNA binding domain. We demonstrate that HFH-3 is a potent transcriptional activator requiring 138 C-terminal residues for activity. We used in situ hybridization to demonstrate that HFH-3 expression is restricted to the epithelium of the renal distal convoluted tubules. We determined the HFH-3 DNA binding consensus sequence by in vitro DNA binding site selection using recombinant HFH-3 protein and used this consensus sequence to identify putative HFH-3 target genes expressed there. These putative HFH-3 target genes include the Na/K-ATPase, Na/K and anion exchangers, E-cadherin, and mineralocorticoid receptor genes as well as genes for the transcription factors HNF-1, vHNF-1, and HNF-4.

Deciphering the mechanisms that lead to cell-specific gene transcription is critical for understanding cellular differentiation during mammalian embryogenesis. Differential expression of protein encoding genes occurs at the point of transcriptional initiation (1) and involves the assembly of several well characterized basal factors with TATA-binding protein, TATA-binding protein-associated factors, and RNA polymerase II at the initiation site of the promoter region (2). Promoter and enhancer regions are also composed of multiple DNA sites that interact with sequence-specific transcription factors, which are believed to enhance the recruitment of basal factors to the initiation complex. Cell-restricted gene expression thus relies upon the combinatorial recognition of multiple cis-acting DNA sequences bound by families of cell-specific nuclear factors, which potentiate or repress transcriptional initiation (3). Because transcription factors play a central role in regulating cellular differentiation, the analysis of their molecular structure and expression patterns has facilitated elucidation of regulatory pathways involved in establishing tissue-specific gene transcription. In combination with other cell-specific transcription factors, the hepatocyte nuclear factor-3a (HNF-3a) and -3b proteins regulate cell-specific transcription in hepatocytes (4) and in respiratory (5–8) and intestinal epithelium (9). The HNF-3/fork head homolog (HFH) proteins are an extensive family of transcription factors that share homology in the winged helix DNA binding domain (10).

Members of the HFH/winged helix family have been implicated in cell fate determination during embryonic pattern formation, in organogenesis, and in cell-type-specific gene expression. Although the HNF-3a and HNF-3b genes are important for cell-type-specific gene regulation, their expression initiates during the primitive streak stage of embryogenesis (11–13). Disruption of the HNF-3b gene in homzygous knock-out mice results in an embryonic lethal phenotype, which exhibits defects in the formation of notochord, neurotube, somites, and gut endoderm (14, 15). Furthermore, targeted disruption of the winged helix of nude mouse (uhn) gene results in the phenotype of the nude mouse mutation (16). Aberrant expression of altered winged helix proteins has also been associated with neoplastic transformations (17–19). Taken together, these studies indicate that the winged helix protein family plays critical roles in cellular differentiation during embryonic development.

The HFH-3 winged helix DNA binding domain was previously isolated from rat kidney cDNA using a PCR protocol with degenerate primers synthesized to conserved sequences within this DNA binding domain (20). Northern blot analysis demonstrates that HFH-3 expression is restricted to the kidney. Subsequent to these studies, the winged helix domain of the human kidney-specific HFH-3 homolog, fork head-related activator-6 (Frea-6), was isolated from a human genomic library (21). Here, we report on the complete human HFH-3 cDNA sequence and its deduced amino acid sequence. We show that HFH-3 is a potent transcriptional activator and that expression is restricted to the epithelium of the renal distal tubules. We determined the HFH-3 DNA binding consensus sequence and used this consensus to identify putative target genes expressed there.

EXPERIMENTAL PROCEDURES

In Situ Hybridization of Mouse Embryos and Adult Kidney—In situ hybridization of paraffin embedded mouse embryos or adult kidney was performed with 33P-labeled antisense RNA probe generated from an

* This work was supported in part by Public Health Service Grant R01 GM42441-07 from the NIHMS, National Institutes of Health and grants from the Council for Tobacco Research and the American Heart Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Current address: Panduit Corp., 17301 Ridgeland Ave., Tinley Park, IL 60477.

‡ Established Investigator of the American Heart Association/Bristol-Myers Squibb. To whom correspondence should be addressed: Dept. of Biochemistry (M/C 536), College of Medicine, University of Illinois, 1819 W. Polk St., Chicago, IL 60612-7334. Tel.: 312-996-0474; Fax: 312-413-0364; E-mail: robcosta@uic.edu.

1 The abbreviations used are: HNF, hepatocyte nuclear factor; HFH, fork head homolog; PCR, polymerase chain reaction; GST, glutathione S-transferase; EMSA, electrophoretic mobility shift assay; CAT, chloramphenical acetyltransferase; CMV, cytomegalovirus.
EcoRI-linearized rat HFH-3 cDNA (nucleotides 208–517) was template using SP6 RNA polymerase and [33P]UTP (Amersham) as described (22). Antisense 33P-labeled HFH-3 RNA probes were hybridized to sectioned dewaxed mouse embryos or adult rat kidneys and rinsed at high stringency followed by autoradiography as described (22). A dark field condenser was used to enhance the visualization of the silver grains corresponding to specific HFH-3 hybridization.

In Vitro DNA Binding Site Selection and Electrophoretic Mobility Shift Assays—HFH-3 winged helix DNA binding domain (amino acids 87–208) was fused to the GST protein, and GST-HFH-3 fusion protein was isolated from bacterial cultures and purified to homogeneity via glutathione affinity chromatography (23). The affinity-purified GST-HFH-3 fusion protein was used to isolate high affinity HFH-3 binding sites from a pool of partially degenerate oligonucleotides containing 14

EcoRI-linearized rat HFH-3 cDNA (nucleotides 208–517) and EcoRI-linearized rat HFH-3 cDNA (nucleotides 208–517) pGEM-1 template using SP6 RNA polymerase and [33P]UTP (Amersham) as described (22). Antisense 33P-labeled HFH-3 RNA probes were hybridized to sectioned dewaxed mouse embryos or adult rat kidneys and rinsed at high stringency followed by autoradiography as described (22). A dark field condenser was used to enhance the visualization of the silver grains corresponding to specific HFH-3 hybridization.

In Vitro DNA Binding Site Selection and Electrophoretic Mobility Shift Assays—HFH-3 winged helix DNA binding domain (amino acids 87–208) was fused to the GST protein, and GST-HFH-3 fusion protein was isolated from bacterial cultures and purified to homogeneity via glutathione affinity chromatography (23). The affinity-purified GST-HFH-3 fusion protein was used to isolate high affinity HFH-3 binding sites from a pool of partially degenerate oligonucleotides containing 14

HFH-3 Expression in Epithelium of Renal Distal Tubules

Figure 1. Translation of the winged helix transcription factor HFH-3 cDNA. Panel a, nucleotide and amino acid sequence of HFH-3 cDNA. The HFH-3 cDNA was isolated from a human kidney cDNA library using a PCR-derived winged helix DNA binding domain probe. Translation of the 2.1-kilobase pair HFH-3 cDNA shows that the HFH-3 gene encodes a 351-amino acid polypeptide with a centrally located winged helix DNA binding domain (highlighted in bold). The original HFH-3 winged helix DNA binding domain sequence (the complete HFH-3 cDNA sequence has been appended to GenBank accession number L13203) has been modified to include the entire cDNA sequence. Panel b, amino acid alignment of the DNA binding domains of winged helix genes expressed in the kidney. The amino acid sequence of the HNF-3α winged helix DNA binding domain was used as comparison with those from the mesenchyme fork head 1 (MFH-1) (40), brain factor-2 (BF-2) (43), Fkh-6 (41), and HFH-11A and HFH-11B (42).

In Vitro DNA Binding Site Selection and Electrophoretic Mobility Shift Assays—HFH-3 winged helix DNA binding domain (amino acids 87–208) was fused to the GST protein, and GST-HFH-3 fusion protein was isolated from bacterial cultures and purified to homogeneity via glutathione affinity chromatography (23). The affinity-purified GST-HFH-3 fusion protein was used to isolate high affinity HFH-3 binding sites from a pool of partially degenerate oligonucleotides containing 14
HFH-3 protein-selected sites were cloned in pGEM1, and the DNA insert was labeled during PCR amplification using 5′-labeled T7 and SP6 primers and tested for HFH-3 protein complex formation by electrophoretic mobility shift assays (EMSA) with 60 ng of affinity-purified recombinant GST-HFH-3 protein using methods described previously (23). We chose 31 high affinity HFH-3 binding sites from a total of 55 selected DNA sites to determine the HFH-3 consensus sequence. The frequency of occurrence for each nucleotide was used to compile a 13-nucleotide HFH-3 binding consensus sequence (Table I; the 14th nucleotide position was degenerate). Double-stranded oligonucleotides were made to previously described winged helix DNA binding sites (23), which formed to the HFH-3 consensus sequence (e.g. HFH-1 site 25). Radioactively labeled oligonucleotides containing HFH-3 binding sites were used for EMSA with 60 ng of affinity-purified GST-HFH-3 fusion protein and 4 μg of poly(dI-dC·dI-dC) in a 20-μl binding reaction as described previously (23). We used the HFH-3 DNA binding consensus sequence to search 12 putative regulatory regions of genes expressed in distal convoluted tubules of the kidney (extracted from GenBank™). Eight of these promoters contained putative HFH-3 binding sites.

**Construction of HFH-3 cDNA Deletions and Cotransfection Assays**—To determine the HFH-3 transcriptional activity, cotransfection assays were performed in human hepatoma HepG2 cells, a cell line that does not express endogenous HFH-3 protein. This cotransfection assay consisted of a reporter plasmid containing four copies of the high affinity HFH-3 binding site (HFH-1 site 25) cloned upstream of a TATA box-driven chloramphenicol acetyltransferase (CAT) gene, an expression vector that used the CMV promoter to express the HFH-3 cDNA sequences and a CMV promoter-driven β-galactosidase control plasmid to normalize for differences in transfection efficiency (24). The full-length HFH-3 cDNA was cloned as an EcoRI in the CMV expression vector (24). HFH-3 cDNA deletions were created by a PCR-mediated strategy using Vent DNA polymerase (New England Biolabs) and primers containing XbaI (5′) or BamHI (5′) restriction sites as described previously (24). PCR-generated C-terminal or 5′ HFH-3 cDNA deletions terminated with an XbaI site, and the resulting EcoRI-XbaI fragment (5′ to 3′) was cloned into the CMV expression vector. PCR-generated N-terminal or 5′ HFH-3 cDNA deletions terminated with a BamHI site, and the resulting BamHI-XbaI fragment (5′ to 3′) was cloned in frame with a translational initiation sequence into the CMV expression vector. Internal deletions were made by cloning PCR-generated C-terminal HFH-3 XbaI fragments (247–351, 213–351) in frame with HFH-3 C-terminal deletion sequences (1–208, 1–246). The boundaries of the HFH-3 deletion constructs were confirmed by DNA sequencing.

Human hepatoma HepG2 cells were maintained in monolayer cultures and transfected (25) using Lipofectin reagent (Life Technologies, Inc.) according to manufacturer's protocol (35 mm plates, 400 ng of CMV-HFH-3 expression vector, 1600 ng of 4× HFH-1 site 25 CAT reporter, 100 ng of CMV-β-galactosidase construct, and 10 μl of Lipofectin). Cellular protein extracts were prepared from transfected cells 48 h after transfection and analyzed for CAT, and β-galactosidase enzyme activity was determined as described previously (25). To determine the expression of HFH-3 deletion mutants during cotransfection experiments, nuclear extracts were prepared from HepG2 cells transfected with the HFH-3 cDNA constructs and analyzed by EMSA as described previously (24).

**RESULTS**

**Isolation of HFH-3 cDNA from Human Kidney cDNA Library**—The rat HFH-3 winged helix DNA binding domain was used as a probe to screen a human cDNA library propagated in Agt11 phage. The HFH-3 cDNA clone consists of 2089 nucleotides and contains an open reading frame between nucleotides 79 and 1131 encoding a 351-amino acid polypeptide (the complete HFH-3 cDNA sequence has been appended to GenBank accession number L13203; Fig. 1a). Allowing for a 21-nucleotide poly(A) tail, the HFH-3 cDNA sequence represents a full-length cDNA clone because the human HFH-3/Freac-6 mRNA size is 2.3 kilobase pairs as evidenced by Northern blot analysis (21). The HFH-3 winged helix DNA binding domain exhibits 56% amino acid identity compared with the first identified winged helix family member HNF-3α (26, 27). Included in the amino acid alignment figure are other winged helix proteins, which are expressed in the developing and/or adult kidney.

However, the HFH-3 protein does not exhibit sequence similarity outside of the winged helix DNA binding motif with other transcriptional activation domains shared with members of the winged helix family (5, 24, 27, 28).

**HFH-3 Is Expressed in the Distal Tubule Epithelium of the Adult Kidney**—To determine the HFH-3 cellular expression patterns, we performed in situ hybridization of paraffin-embedded sections of a 16-day post coitum mouse embryos or adult kidney with a 32P-labeled antisense HFH-3 RNA probe (Fig. 2). After hybridization, stringent washes, and autoradiography, dark field microscopy was used to visualize HFH-3-expressing cells in the tissues. Shown is a paraffin section of the 16-day post coitum embryonic kidney, demonstrating that the HFH-3 gene was expressed in the epithelium of the convoluted tubules (Fig. 2a). HFH-3 expression continued in the tubule epithelium...
Consensus binding sites with recombinant HFH-3 protein in EMSA (Fig. 3, panel a). DNA sequences that adhered to the HFH-3 consensus formed complexes with recombinant HFH-3 protein as described previously (23). Shown is the percent nucleotide occurrence for each nucleotide position in the HFH-3 binding site.

| Position | 1 2 3 4 5 6 7 8 9 10 11 12 13 |
|----------|---------------------------------|
| G        | 45 39 27 58 13 13 13 16         |
| A        | 20 12 34 42 48 26 7 5 55         |
| T        | 32 23 27 100 100 100 100 77 58 77 23 |
| C        | 3 26 12 23 3 3 6                 |

Consensus:

|        | G | G | G | T | G | T | G | T | T | A | A | C | T | A |
|--------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Position| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10| 11| 12| 13|

The HFH-3 DNA binding consensus sequences was compiled from 31 high affinity binding sites isolated by in vitro binding site selection using recombinant HFH-3 protein as described previously (23). Shown is the percent nucleotide occurrence for each nucleotide position in the HFH-3 binding site.

To identify putative target genes expressed in distal tubules or in the loops of Henle (data not shown). Because the morphology of the distal straight and convoluted tubules is indistinguishable, we are not able to rule out the possibility that HFH-3 is expressed in the distal ascending straight tubules. HFH-3 expression was not detected in the medullary ray, which is devoid of glomeruli and contains the collecting ducts (Fig. 2b). HFH-3 hybridization signals were not detected in the descending straight tubules, which are the continuation of the proximal convoluted tubules or in the loops of Henle (data not shown).

The HFH-3 Transcriptional Activation Domain Resides in the C-terminal Sequences—To determine the HFH-3 transcriptional activation domain(s), we used cotransfection assays (24, 29) to compare the activation levels between wild-type and truncated HFH-3 proteins (see “Experimental Procedures”). To avoid complications with endogenous HFH-3 protein, we chose the human hepatoma HepG2 cell line that does not express HFH-3 to perform the cotransfection assays and monitored HFH-3 protein expression via EMSA with nuclear extracts (data not shown). None of the deletion constructs disrupted the winged helix DNA binding domain, which is sufficient to direct nuclear localization (29).

Cotransfection assays with the full-length HFH-3 cDNA expression plasmid provided an approximately 40–60-fold increase in reporter gene transcription compared with the CMV control plasmid (Fig. 4, constructs 1 and 2). This activation was dependent on retention of the HFH-3 recognition sequence in the reporter construct (data not shown). Removal of the first 86 N-terminal residues from the HFH-3 protein did not reduce transcriptional activity (Fig. 4, construct 3). Further C-terminal deletions eliminated detectable HFH-3 transcriptional activation (Fig. 4, constructs 5 and 6). Internal deletions were created using an XbaI site at the ends of the C-terminal deletions. A 50% reduction in transcriptional activity was observed when an XbaI linker was inserted between amino acid residues 246 and 247 of the HFH-3 protein (Fig. 4, construct 7). An internal deletion that removed amino acid residues 209–246 of the HFH-3 protein eliminated transcriptional activity in cotransfection assays and exhibited activity identical to the 1–208 C-terminal HFH-3 deletion construct (Fig. 4, compare constructs 8 and 9). However, a smaller internal deletion that removed amino acid residues between 209 and 212 was almost as active as the wild-type protein. These functional studies suggest that the minimal HFH-3 transcriptional activation domain resides in the amino acid 213–351 C-terminal sequences, which do not resemble those of other winged helix transcription factors.

a Uppercase letter indicates nucleotides which are highly represented in DNA sites. If there are only two or three prevalent nucleotides occurring in one position, a lower case letter indicates a nucleotide that occurs less frequently. The nucleotides of the HFH-3 DNA binding core sequence—DBDTRTTY—located in both cortex and medulla of the adult kidney (Fig. 2b). Magnification of the renal cortex demonstrates that HFH-3 was expressed in the distal convoluted tubules but not in the proximal convoluted tubules or in the glomeruli within the renal corpuscles (Fig. 2, c and d). Because the morphology of the distal straight and convoluted tubules is indistinguishable, we are not able to rule out the possibility that HFH-3 is expressed in the distal ascending straight tubules. HFH-3 expression was not detected in the medullary ray, which is devoid of glomeruli and contains the collecting ducts (Fig. 2b). HFH-3 hybridization signals were not detected in the descending straight tubules, which are the continuation of the proximal convoluted tubules or in the loops of Henle (data not shown).

The binding affinity is abbreviated as follows: +++, strong; ++, moderate; +, weak; −, none. The HFH-3 DNA binding consensus sequence was derived by DNA site selection with recombinant HFH-3 protein (Table I, where D is not C, B is not A, R is A or G, and Y is C or T).
Protein are expressed in each transfection as evidenced by EMSA (data not shown). This region does not share sequence similarity with transcription factors that share homology in the winged helix DNA binding domain. The HFH-3 activation domain also exhibits sequence similarity in three amino acid regions (246–257, 415–432, and 313–324). The HFH-3 activation motif is defined in the experiments described in this figure. Approximately equal amounts of HFH-3 protein deletions assayed in HepG2 cell cotransfection assays with the 4 x HFH-1 site 25 CAT reporter construct (Fig. 3). Data are presented as percent of wild-type activity, and error bars (white) represent standard deviation from three separate experiments. The stippled box represents the HFH-3 transcriptional activation motif defined in the experiments described in this figure. Approximately equal amounts of HFH-3 protein are expressed in each transfection as evidenced by EMSA (data not shown).

Amino acid numbers of proline, serine, and threonine amino acid residues that exhibit an estimated pI of 4.65 as well as significant similarity in three amino acid regions (246–257, 415–432, and 313–324). The HFH-3 activation domain also exhibits sequence similarity in three amino acid regions (246–257, 415–432, and 513–528) with the transcriptional factors Oct-1 (GenBank accession number X70324) and of the herpesvirus 3 ICP4 (GenBank accession number X67648).

The winged helix/HFH proteins are a large family of transcription factors that share homology in the winged helix DNA binding domain and are involved in the differentiation of diverse cellular lineages (for review, see Refs. 4 and 30). In support of the important role of HFH-3 in regulating genes involved in the function of the renal distal tubules, we used the HFH-3 consensus binding sequence (Table I) to search promoters for genes that are expressed in the epithelium of the renal distal tubules.

### DISCUSSION

The winged helix/HFH proteins are a large family of transcription factors that share homology in the winged helix DNA binding domain and are involved in the differentiation of diverse cellular lineages (for review, see Refs. 4 and 30). In this study we report on the isolation of the kidney-specific HFH-3 cDNA clone and show that it is a potent transcriptional activator with activity residing in the C-terminal 138 amino acids. This region does not share sequence similarity with transcriptional activation motifs previously identified for related winged helix family members (5, 24, 27, 28). The HFH-3 activation domain possesses features found in other transcriptional activation motifs and includes an acidic amino acid composition that exhibits an estimated pI of 4.65 as well as significant numbers of proline, serine, and threonine amino acid residues (31, 32). The HFH-3 activation domain also exhibits sequence similarity in three amino acid regions (246–257, 415–432, and 513–528) with the transcriptional factors Oct-1 (GenBank accession number X70324) and of the herpesvirus 3 ICP4 (GenBank accession number X02132). These sequence comparisons suggest that the HFH-3 protein utilizes activation motifs in common with other eukaryotic transcription factors but not with the winged helix family.

We used in situ hybridization to demonstrate that HFH-3 is restricted to the distal tubule epithelium of embryonic and adult kidney. The distal tubules possess regulated ion channels, which are involved in the reabsorption of sodium and bicarbonate ions from the urine in exchange for the excretion of potassium and hydrogen ions, thus rendering the urine acidic (33, 34). This reabsorption process is regulated by the hormone aldosterone, a ligand for the mineralocorticoid receptor involved in activation of genes involved in Na/H exchange (35, 36). In support of the important role of HFH-3 in regulating genes involved in the function of the renal distal tubules, we used the HFH-3 DNA binding consensus sequence to identify several potential target genes involved in the function of the renal distal tubules (Table II). Potential HFH-3 binding sites were found in the genes encoding Na/H exchanger, anion exchanger, and two subunits of the Na/K-ATPase proteins (Table II). Furthermore, we found potential HFH-3 binding sites in the mineralocorticoid receptor gene, which encodes an aldosterone-dependent transcription expression that activates the expression of genes involved in renal distal tubule function. In the adult kidney, LFB1/HNF1-1 expression is restricted to the proximal and distal tubules, whereas LFB3/vHNF-1 expression is also detected in the collecting ducts (37) and HNF-4 expression is...
detected throughout the nephrons (22). We also found potential HFH-3 binding sites in the genes for tissue-specific transcription factors HNF-1, vHNF-1, and HNF-4 (Table II). Consistent with this prediction, one of the potential HFH-3 binding sites in the vHNF-1 promoter is DNase I-footprinted with kidney nuclear extracts (38). Although these transcription factors are expressed more broadly than the HFH-3 gene, HFH-3 may participate in regulating their expression in the distal tubule epithelium of the kidney.

In addition to the HFH-3 gene, other members of the winged helix transcription factors exhibit restricted cellular expression patterns in the kidney (Fig. 1b). The HFN-3a gene is expressed in the urothelium of the embryonic and adult renal pelvis and may regulate genes involved in reabsorption of water from urine (9). Epithelial expression of HFH-3 and HNF-3 in the kidney thus forms a continuum from the distal convoluted tubules to the collecting ducts. Another winged helix transcription factor, BF-2, is expressed in the metanephric mesenchyme, which gives rise to both the nephron epithelium and stromal cells of the mature kidney (39). Targeted disruption of the BF-2 gene in mice has inhibited the induction of renal mesenchyme into tubular epithelium and branching of the ureter and renal collecting system (39). Two other winged helix transcription factors are also expressed in the mesenchyme of the developing embryonic kidney, including the mesenchyme fork head 1 (MFH-1) (40) and fkh-6 gene (41). Furthermore, a new member of the winged helix family, HFH-11, is transiently expressed in the cortical epithelium and mesenchyme of the embryonic mouse kidney but its expression is extinguished in the adult kidney (42). Like the kidney, HFH-11 is also expressed in the proliferating cells of the embryonic intestine, lung, and liver but its expression is extinguished in non-replicating cells of these organs in the adult. We have shown that HFH-11 expression is reactivated in response to proliferative signals induced following organ injury. It is therefore likely that the winged helix HFH-11 gene is reactivated in the renal cortex in response to cellular injury. The winged helix family of transcription factors thus appears to play an important role in kidney morphogenesis and terminal differentiation of various cell types in the adult kidney.

In summary, we demonstrated that HFH-3 expression is restricted to the epithelium of the distal convoluted tubules in the developing and adult kidney. We determined an HFH-3 DNA binding consensus sequence and identified potential target genes in cells co-expressing HFH-3. Transfection studies demonstrate that HFH-3 is a potent transcriptional activator whose activation domain resides in sequences located at the C terminus and that these sequences possess features in common with other transcriptional activation domains, but not with other HFH family members.

Acknowledgments—We thank P. Raychaudhuri for critically reading the manuscript. The DNA sequence for the human HFH-3 cDNA was determined by the DNA Sequencing and Synthesis Facility at Iowa State University of Science and Technology.

REFERENCES
1. Derman, E., Krauter, K., Walling, L., Weinberger, C., Ray, M., and Darnell, J., Jr. (1981) Cell 25, 731–739
2. Orphanides, G., Lagrange, T., and Reinberg, D. (1996) Genes Dev. 10, 2657–2683
3. Cereghini, S. (1996) FASEB J. 10, 267–282
4. Costa, R. H. (1994) in Liver Gene Transcription (Tronche, F., and Yaniv, M., eds) pp. 183–206, R. G. Landes Co., Austin, TX
5. Cereghini, S., E., Overdier, D. G., Peterson, R. S., Porcella, A., Ye, H., and Lai, E., Jr. (1994) in HNF-3 Expression in Epithelium of Renal Distal Tubules
The Winged Helix Transcriptional Activator HFH-3 Is Expressed in the Distal Tubules of Embryonic and Adult Mouse Kidney
David G. Overdier, Honggang Ye, Richard S. Peterson, Derek E. Clevidence and Robert H. Costa

J. Biol. Chem. 1997, 272:13725-13730.
doi: 10.1074/jbc.272.21.13725

Access the most updated version of this article at http://www.jbc.org/content/272/21/13725

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 51 references, 31 of which can be accessed free at http://www.jbc.org/content/272/21/13725.full.html#ref-list-1