Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9838–9842, December 1990
Biochemistry

Molecular cloning, functional expression, and chromosomal localization of mouse hepatocyte nuclear factor 1

(transcription factor/endoderm/human and mouse gene mapping)

CALVIN J. KUO, PAMELA B. CONLEY, CHIH-LIN HSIEH, UTA FRANCKE*, AND GERALD R. CRABTREE†

Howard Hughes Medical Institute, Unit in Molecular and Genetic Medicine, and Departments of *Genetics, *Pediatrics, and †Pathology, Stanford University School of Medicine, Stanford, CA 94305-5428

Communicated by Irving L. Weissman, August 30, 1990 (received for review June 1, 1990)

ABSTRACT **The homeodomain-containing transcription factor hepatocyte nuclear factor 1 (HNF-1) most likely plays an essential role during liver organogenesis by transactivating a family of >15 predominantly hepatic genes. We have isolated cDNA clones encoding mouse HNF-1 and expressed them in monkey COS cells and in the human T-cell line Jurkat, producing HNF-1 DNA-binding activity as well as transactivation of reporter constructs containing multimerized HNF-1 binding sites. In addition, the HNF-1 gene was assigned by somatic cell hybrids and recombinant inbred strain mapping to mouse chromosome 5 near *Bcd-1* and to human chromosome 12 region q22–qter, revealing a homologous chromosome region in these two species. The presence of HNF-1 mRNA in multiple endodermal tissues (liver, stomach, intestine) suggests that HNF-1 may constitute an early marker for endodermal, rather than hepatocyte, differentiation. Further, that HNF-1 DNA-binding and transcriptional activity can be conferred by transfecting the HNF-1 cDNA into several cell lines indicates that it is sufficient to activate transcription in the context of ubiquitously expressed factors.**

Molecular and genetic studies in a wide variety of organisms have suggested that transcription factors endowed with characteristic temporal and spatial expression patterns actively specify cellular phenotype through the coordinate activation of gene families. In this manner, the homeodomain-containing transcription factor hepatocyte nuclear factor 1 (HNF-1; also called LF-B1, APF) is believed to play an essential role during liver organogenesis by virtue of its selective expression in liver and its ability to transactivate a family of >15 hepatic genes (1–7) via a highly conserved, imperfectly palindromic, 13-base-pair (bp) HNF-1 binding site (2, 8, 9). Despite this apparent correlation with the liver phenotype, HNF-1 mRNA exists in several other endodermal and mesodermal tissues (10), suggesting that HNF-1 expression specifies cellular identity at a level more fundamental than hepatic determination. As HNF-1 can form homodimers through an N-terminal myosin-like domain (11), it has been speculated that HNF-1 may participate in the differentiation of several organs by heterodimerizing with different cofactors in different tissues with resultant alterations in DNA-binding specificity and target-gene spectrum.

In this work, we isolated cDNA clones encoding mouse HNF-1 (mHNF-1)‡ in efforts to take advantage of the broader genetic and developmental background of the mouse as opposed to the rat. We functionally expressed the mHNF-1 cDNA in a variety of cell types, establishing that mHNF-1 can transactivate an HNF-1-dependent transcription unit in cotransfection assays. In addition, mapping of the HNF-1 gene revealed a region of homology between human chromosome 12 and mouse chromosome 5.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Isolation and Analysis of cDNA Clones. Using rat HNF-1 cDNA (10) as probe, two phage bearing 1.1-kilobase (kb) (λ32) and 0.8-kb (λ24) inserts were isolated from 320,000 plaques of a mouse liver λgt10 library (Clontech) under low-stringency conditions. The λ32 insert was used to probe 1.8×10^6 plaques from a mouse liver λZAP cDNA library (Stratagene), from which 22 positives were recovered. Inserts extending farthest 5′ (λ4b) and 3′ (λ4a) were subcloned into pBS KS(−) (Stratagene) for DNA sequencing (12).

RNase Protection Assays. A 277-bp *Pst* I–*Eco*RI fragment of the λ24 insert was subcloned into pBS KS(−), linearized with *Pst* I, and transcribed with T3 polymerase, producing an antisense RNA probe in the mHNF-1 3′ untranslated region. RNase protection analysis of hybrids was carried out (13) against 15 μg of total RNA.

Transfections and Analysis of DNA-Binding Proteins. Ten micrograms of chloramphenicol acetyltransferase (CAT) reporter construct [either $(\beta 28)_3$-CAT, containing three copies of the β-fibrinogen HNF-1 binding site in front of the γ-fibrinogen basal promoter (1), or βSH-CAT, containing 5.2 kb of rat β-fibrinogen 5′ flanking sequences (J. Morgan and G.R.C., unpublished work)] was cotransfected with 10 μg of pBJ5mHNF or pBJ5 by the calcium phosphate method (1) for 12–16 hr. The pBSmHNF plasmid, containing the full-length mHNF-1 cDNA in pBS KS(−) was constructed by ligating the λ4b and λ4a clones at a unique *Bcl* I site (bp 1759). The 3176-bp pBSmHNF insert was then excised with *Eco*RI and inserted into the COS cell expression vector pBJ5 (34) to create pBJ5mHNF. Cell extracts were then prepared and CAT enzyme activity was assayed (14). Jurkat cells were transfected by electroporation with a Bio-Rad Gene Pulser at 960 mF, 250 V. CAT assays were quantitated on an AMBIS radioactivity scanner. Gel mobility-shift assays (1) used 5–20 μg of protein from whole cell extracts (15).

Somatic Cell Hybrids and DNA from Mouse Strains. A panel of 11 Chinese hamster–human hybrid clones containing various human chromosome subsets was used for primary chromosomal assignment of the human HNF-1 gene (*HNF1*). Two additional Chinese hamster–human hybrids, one containing human chromosome 12 region pter–q22 and the other containing the long arm of chromosome 12, were used for regional mapping. Localization of the mHNF-1 gene (*Hnf-1*) was determined by analyzing a panel of 12 Chinese hamster–mouse and one rat–mouse hybrid clones or subclones. Hybrid clones in these mapping panels were derived from several independent fusion experiments between Chinese hamster and human and between other rodent and mouse cell lines, respectively (16). DNA from inbred and recombinant

Abbreviations: HNF-1, hepatocyte nuclear factor 1; mHNF-1, mouse HNF-1; CAT, chloramphenicol acetyltransferase; RI, recombinant inbred.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M57966).

inbred (RI) mouse strains were purchased from The Jackson Laboratory for linkage study in mice.

Probes and Southern Blot Hybridization. A 1.3-kb rat HNF-1 cDNA probe (10) and a 1.8-kb mHNF-1 cDNA fragment (λ4b) were labeled with [α-^{32}P]dCTP. Genomic DNA was digested with restriction enzymes *Eco*RI and *Bgl* II for mapping of human and mouse genes, respectively. Six enzymes, *Bam*HI, *Bgl* II, *Eco*RI, *Hin*dIII, *Hin*cII, and *Pst* I, were used for polymorphism studies in inbred mouse strains. For linkage studies, DNA from the B×D set (progenitor strains are C57BL/6J and DBA/2J) of RI strains was digested with *Bam*HI. Restriction enzyme digestion, filter hybridization, and film exposure were carried out as described (17).

RESULTS

Molecular Cloning of mHNF-1 cDNA. To isolate mHNF-1 cDNA clones, we employed the previously isolated rat HNF-1 gene (10) to screen various mouse liver λgt10 libraries at low stringency. Sequence analysis of the λ4a and λ4b clones defined a 628-amino acid open reading frame exhibiting 99% amino acid identity with rat HNF-1 (9) (Fig. 1). The mouse and rat genes displayed an extraordinary nucleotide conservation, 96%, 95%, and 82% in the 5′ untranslated, coding, and 3′ untranslated regions, respectively. A mouse–mouse dot plot revealed no obvious internal repeats (data not shown). The consensus polyadenylylation signal AATAAA or a poly(A) tail was not identified, indicating that we had not isolated the 3′ end of the mHNF-1 transcription unit.

The rat HNF-1 homeodomain diverges strongly from all other known homeodomains in its possession of an additional 21-amino acid loop interposed between helix 2 and helix 3, the two α-helices functionally equivalent to the "helix–turn–helix" motif of bacteriophage λ repressor (10, 11, 18). Significantly, mHNF-1 displays exact amino acid conservation of this 21-amino acid loop, demonstrating that it is not unique to rat HNF-1 and suggesting that it may have functional importance, such as facilitating protein–protein interactions (11, 18). In addition, two short amino acid motifs that rat HNF-1 shares with the POU-specific domain of Pit-1, Oct-1/2, and unc-86 (9, 10) are also exactly conserved in the appropriate region of mHNF-1 (Fig. 1).

Predominantly Endodermal Tissue Distribution of mHNF-1 mRNA. The mHNF-1 cDNA was used in RNase protection assays to examine the tissue distribution of HNF-1 transcripts in the mouse. An antisense RNA probe derived from the HNF-1 3′ untranslated region detected HNF-1 mRNA in the endodermal organs liver, stomach, and intestine and in kidney, a mesodermal derivative, but not in ovary, brain, heart, or lung (Fig. 2). In addition, HNF-1 mRNA was

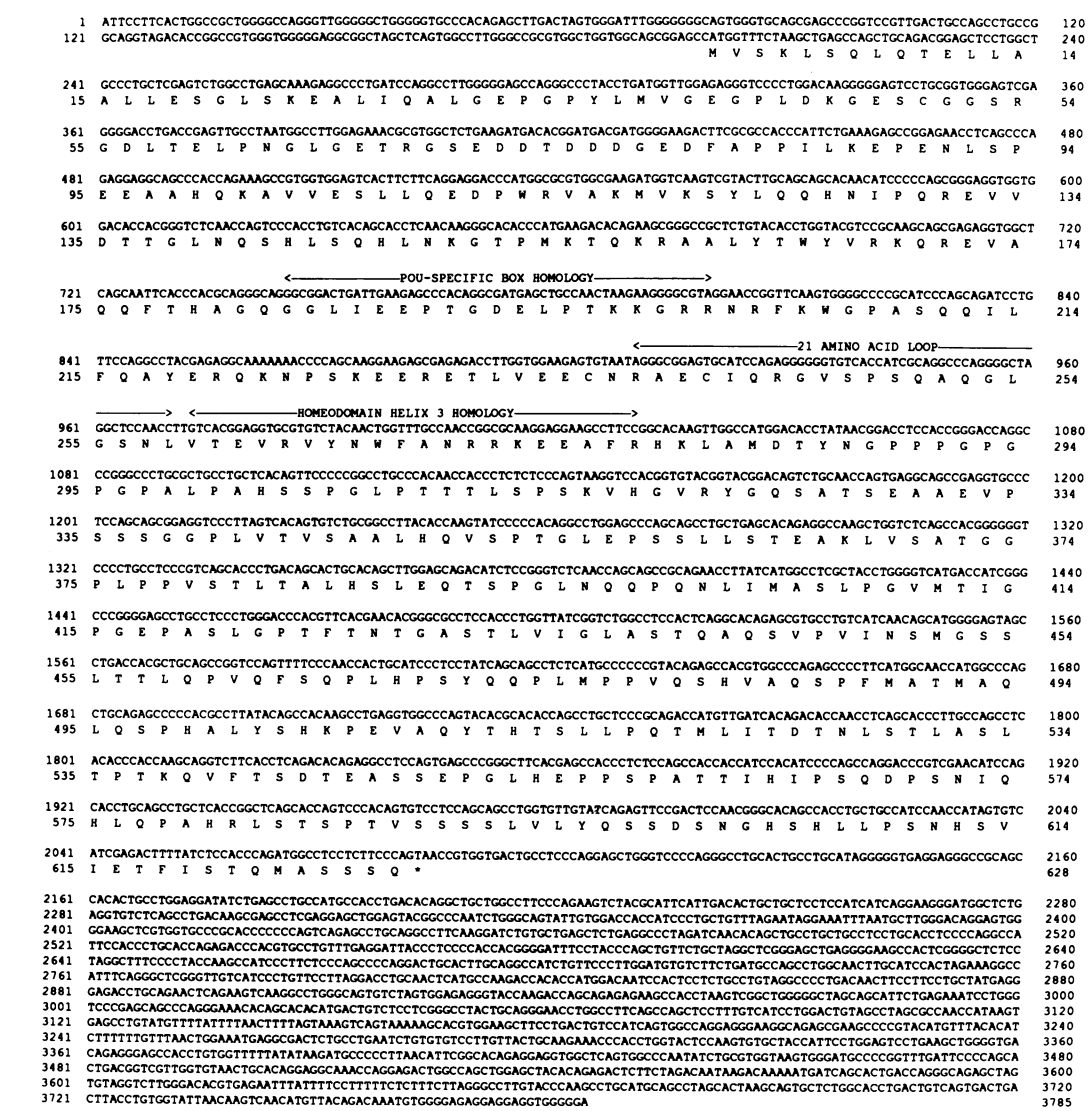

FIG. 1. mHNF-1 cDNA and predicted amino acid sequence. Homologies with homeobox and POU gene families are indicated.

detected at low levels in undifferentiated F9 teratocarcinoma cells, a mouse cell line believed to mimic certain aspects of the primitive endoderm (hypoblast) of the preimplantation embryo (Fig. 2).

Expression of mHNF-1 DNA-Binding Activity *in Vivo* and *in Vitro*. Whole cell extracts prepared from COS cells transfected with the expression vector pBJ5mHNF and the parental vector pBJ5 were analyzed for HNF-1 binding activity in gel shift assays. Incubation of extract from pBJ5mHNF-transfected COS cells with an oligonucleotide containing the β-fibrinogen HNF-1 binding site (β28; ref. 1) (Fig. 3*a*, lanes 1–3) produced a retarded band (arrow 1) at the same mobility as HNF-1 in crude liver nuclear extract (lane 7). Formation of this labeled complex was specifically inhibited with a 1000-fold excess of unlabeled β28 oligomer (lane 2) but not with an oligomer specific for the NF-AT (nuclear factor of activated T cells) transcription factor (19) (lane 3). In extract from pBJ5-transfected COS cells, no specific retardation of the HNF-1 oligomer was observed (lanes 4–6). Similarly, transfection of COS cells with a C/EBP expression vector (20) did not induce HNF-1 binding activity (data not shown). Thus, COS cells transfected with the HNF-1 expression vector encode a binding activity identical, by these criteria, to HNF-1 in liver nuclear extract. Interestingly, the COS cell-expresed HNF-1 migrated as two distinct species, in contrast to HNF-1 in crude extract, possibly resulting from differential cell-type- or species-specific posttranslational modifications in COS versus liver cells.

Similar experiments using a transfected human T-cell line, Jurkat, also produced a retarded complex with the same mobility as native HNF-1 (Fig. 3*b*, lane 1). Less HNF-1 protein was produced in Jurkat cells than in COS cells, probably due to the ability of the pBJ5 vector to undergo T-antigen-stimulated replication in COS cells but not in Jurkat cells. However, the retarded complex in Jurkat transfectants (arrow) appears to represent specific HNF-1 binding, since its formation was prevented with an excess of unlabeled β28 oligomer (lane 2), whereas formation of the other retarded complexes was not affected. Nontransfected Jurkat cells did not exhibit endogenous HNF-1 DNA-binding activity in gel shift assays (data not shown).

Transactivation of Reporter Genes by the mHNF-1 cDNA. HNF-1 has not been reported to transactivate cotransfected reporter plasmids. To examine this possibility, we cotransfected the pBJ5mHNF expression vector with CAT reporter

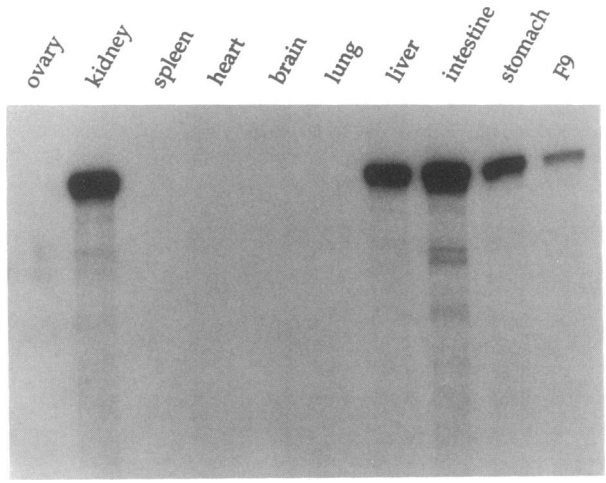

FIG. 2. Tissue distribution of mHNF-1 transcripts. Total RNA (15 μg) from the indicated mouse tissues or from F9 teratocarcinoma cells was hybridized to a mHNF-1 antisense RNA probe and digested with RNases AIII and T1. The predominant signal corresponds to full-length protection of 277 nucleotides.

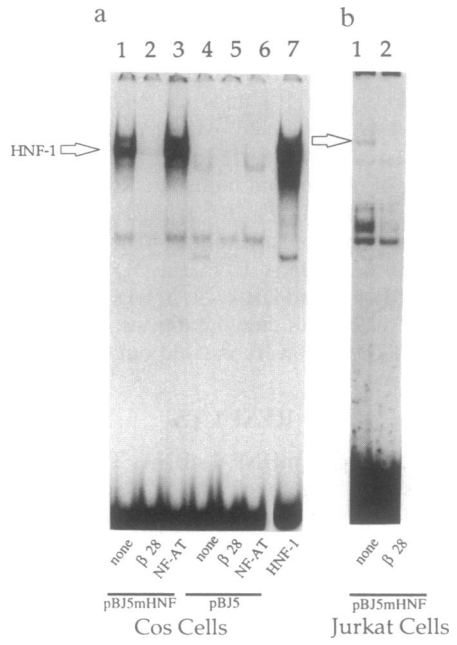

FIG. 3. Transfection of pBJ5mHNF induces HNF-1 DNA-binding in COS cells and T lymphocytes. COS cells (*a*) and the Jurkat T-lymphocyte line (*b*) were transfected with either pBJ5mHNF or pBJ5, and whole cell extracts were analyzed in gel shift assays with β28 probe. Arrows indicate the mobility of the retarded complex formed by HNF-1 from crude liver nuclear extract. Competitor oligomer is denoted below each lane except lane 7, where HNF-1 indicates the use of protein from crude liver nuclear extract.

plasmids bearing either an artificial promoter [$(\beta 28)_3$-CAT] or the natural β-fibrinogen promoter (βSH-CAT) into both COS and Jurkat cells (Table 1). The pBJ5mHNF vector transactivated the β28-CAT reporter construct 27-fold and 12-fold in COS and Jurkat cells, respectively, while transactivating the βSH-CAT construct 16-fold and 3-fold in COS and Jurkat, respectively. mHNF-1 expressed from pBJ5mHNF activated both reporter genes in COS and Jurkat cells, indicating that there is no tissue-specific modification (unique to liver cells) required for HNF-1 to function as a transcriptional activator.

Chromosomal Mapping of the mHNF-1 Gene. *Hnf-1* was assigned with a panel of 13 mouse–rodent somatic cell hybrids that retained overlapping subsets of mouse chromosomes. After hybridization of the ^{32}P-labeled 1.8-kb mouse cDNA probe to *Bgl* II-digested genomic DNA, three fragments (5.4, 2.6, and 2.3 kb) were detected in mouse control DNA (Fig. 4*a*, lane 1) that were separable from Chinese hamster control DNA (lane 2), and rat control DNA (lane 8). In hybrids that contained mouse chromosome 5, all three mouse signals were present as well as the Chinese hamster fragments (Fig. 4*a*, lanes 3–5). No mouse signal was detected in hybrids not containing mouse chromosome 5 (lanes 6 and 7). The presence of mouse *Hnf-1* bands therefore exhibited

Table 1. Cotransfection of pBJ5mHNF transactivates β-fibrinogen constructs

Cotransfected plasmid	COS cells CAT* −	COS cells CAT* +	COS cells Fold induction	Jurkat cells CAT* −	Jurkat cells CAT* +	Jurkat cells Fold induction
$p(\beta 28)_3$-CAT	1.15	31	27	0.9	10.6	11.8
pβSH-CAT	3.4	5.6	16	0.9	3	3.3

*CAT activity, as percent conversion of [^{14}C]chloramphenicol to mono- and diacetylated forms. Each number is an average of at least two independent determinations. − or + indicates cells were transfected with pBJ5 or pBJ5mHNF, respectively.

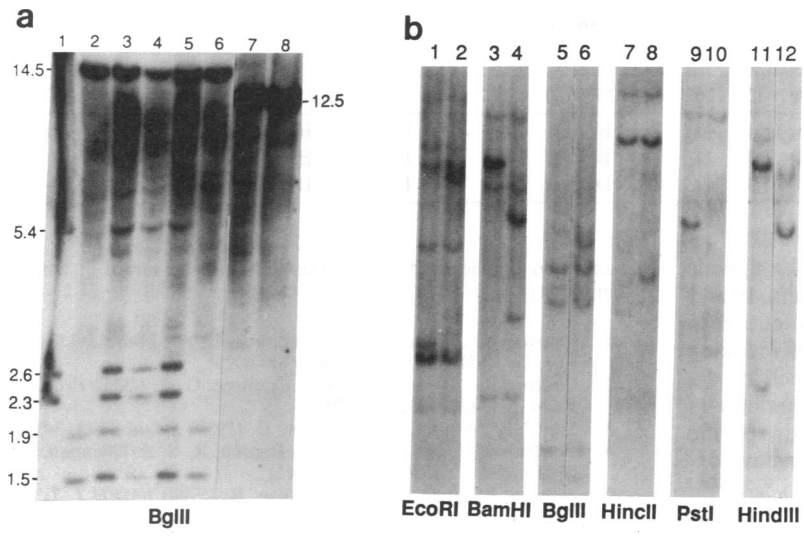

FIG. 4. (*a*) Mapping of the mHNF-1 gene. Lane 1, mouse 3T3 cells; lane 2, Chinese hamster (CH) cells V79/380-6; lanes 3–6, CH–mouse hybrid cell lines; lane 7, rat–mouse hybrid cell line; lane 8, rat hepatoma cells. Lanes 6 and 7 contained only CH or rat HNF-1 sequences and were scored as negative. Lanes 3–5 contained both mouse and CH HNF-1 sequences and were scored as positive. Sizes are indicated in kilobases. (*b*) Polymorphism in mice. Lanes 1, 3, 5, 7, 9, and 11 are the C57BL/6J digestion patterns of six restriction enzymes; strains C3H/HeJ and C57L/J have the same patterns as C57BL/6J. Lanes 2, 4, 6, 8, 10, and 12 are the restriction enzyme digestion patterns of strain DBA/2J; strain AKR/J has the same patterns.

perfect concordance with the presence of mouse chromosome 5 in hybrid cell lines, and all other mouse chromosomes were excluded by 3–10 discordant hybrids.

Genetic Linkage Analysis using B×D RI Strains. Two distinct restriction fragment patterns were observed in inbred mouse strains with all six enzymes used. Strains AKR/J and DBA/2J exhibited identical patterns (Fig. 4*b*, even-numbered lanes), while C3H/HeJ, C57/J, and C57BL/6J strains shared another pattern (odd-numbered lanes). The strain distribution pattern of 26 strains of B×D RI mice was determined (Fig. 5, Table 2). From comparison of the strain distribution pattern of *Hnf-1* with that of *Bcd-1* (butyryl-CoA dehydrogenase) and of *Fla* (*Flp*, F liver protein), close linkage was apparent (21). No recombinant between *Bcd-1* and *Hnf-1* and four recombinants between *Fla* and *Hnf-1* were found among 24 strains compared (Table 2). This result indicates that *Hnf-1* is also proximal to *Fla* and closely linked to the *Bcd-1* locus on mouse chromosome 5 (22).

Chromosomal Mapping of the Human HNF-1 Gene. After hybridization of the ^{32}P-labeled rat cDNA probe (10) to *Eco*RI-digested genomic DNA, three restriction fragments (13.5, 10.5, and 7.6 kb) were detected in human DNA (Fig. 6*a*, lane 2). Since the 7.6-kb fragment was not separable from a hamster fragment (lane 1), only the two major 13.5- and 10.5-kb human bands were scored. These two human bands segregated together and were present in hybrids containing human chromosome 12 (lanes 5 and 7) and absent from hybrids lacking it (lanes 3, 4, and 6). All other human chromosomes were excluded by at least two discordant hybrids. By studying two hybrids that contained regions of human chromosome 12 without an intact chromosome 12 present, we localized *HNF1* to chromosome 12q22–qter (Fig. 6*b*).

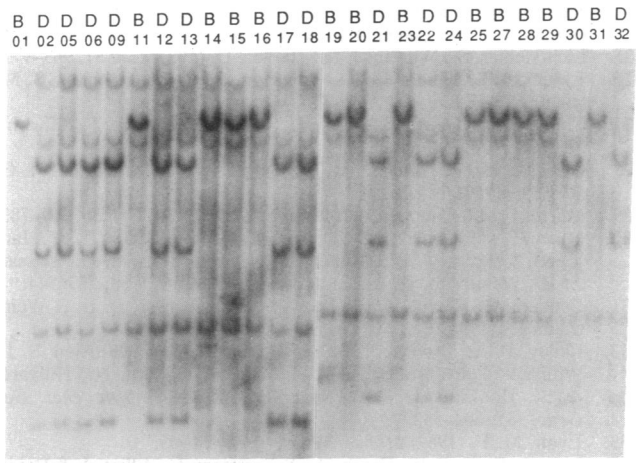

FIG. 5. Patterns of *Bam*HI-digested DNA from 26 B×D (progenitor strains are C57BL/6J and DBA/2J) RI mouse strains.

DISCUSSION

The mHNF-1 cDNA shared an extraordinary homology with rat HNF-1, displaying 99% amino acid and 95% nucleotide conservation in the 628-amino acid open reading frame, with 96% and 82% homology in the 5′ and 3′ untranslated regions, respectively. This similarity suggests stringent evolutionary constraints on the structure of both the HNF-1 protein and mRNA. Strikingly, other transcription factor genes such as Pit-1/GHF-1 (23) and C/EBP (24) demonstrate far less variation in amino acid sequence between species than do their target genes. Such high degree of conservation may reflect the tendency of transcription factors to assemble into multiprotein complexes, and the consequent need to maintain an exactly defined, permissive surface topology. HNF-1, indeed, binds in close proximity to other nuclear factors in various liver-specific promoters, as well as self-associating to form homodimers (5, 6, 9, 25).

Significantly, all non-liver tissues in which HNF-1 mRNA has been detected express, perhaps obligately, hepatic markers. For example, the kidney synthesizes α- and β-fibrinogen

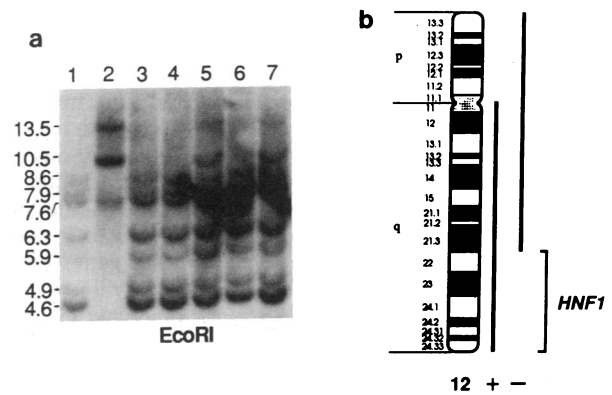

FIG. 6. (*a*) Mapping of the human HNF-1 gene. Lane 1, Chinese hamster (CH) cells V79/380-6; lane 2, human diploid lymphoblastoid cells; lanes 3–7, CH–human hybrid cell lines. Lanes 5 and 7 are positive for the 13.5- and 10.5-kb human *HNF1* fragments. Lanes 3, 4, and 6 contain only CH fragments and were scored as negative. (*b*) Ideogram of G-banding patterns of human chromosome 12 with vertical bars illustrating different portions of chromosome 12 present in somatic cell hybrids that were positive (+) or negative (−) for human HNF-1 sequence. Bracket shows the localization of *HNF1*.

Table 2. RI (B×D) strain distribution pattern of chromosome 5 loci in 26 strains

	Strain number																								
Locus	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Hnf-1	B	D	D	D	D	B	D	D	B	B	B	D	D	B	B	D	D	B	D	B	B	B	B	D	B	D
*Bcd-1**	B	D	D	D	D	B	D	D	B	B	B	D	D	B	B	D	D	B	D	B	B	B	B	D	—	—
*Flp**	B	D	D	B	D	B	D	D	D	B	B	D	D	D	B	D	D	B	D	D	B	B	B	D	—	—

*From ref. 21.

and α_1-antitrypsin, and α-fetoprotein is expressed throughout the developing gastrointestinal tract (reviewed in refs. 10 and 26). It is therefore apparent that HNF-1 does not function as a master hepatocyte regulatory gene, capable of inducing complete phenotypic conversion of expressing cell types. Rather, HNF-1 most likely acts in concert with other factors to subtly modify cellular phenotype, permitting liver-specific patterns of gene expression to coexist with other developmental hierarchies.

The ability of HNF-1 to form homodimers, unique among characterized homeoproteins (18), indicated that the active form of HNF-1 might be a heterodimeric complex, as described for Fos/Jun (27), the retinoic acid receptor (28), and members of the helix–loop–helix transcription factor family (MyoD1, E47/E12, Id) (29–31). As an initial step to examine this possibility, we transiently expressed the mHNF-1 cDNA in COS (kidney) and Jurkat (T-cell) lines, producing both HNF-1 DNA-binding activity and transactivation during cotransfection assays. The ability of the transfected HNF-1 cDNA to activate HNF-1-dependent transcription units in both cell types strongly suggests that HNF-1 DNA-binding and transcription factor activity can be specified by a single polypeptide in the context of ubiquitous proteins.

Stringent hybridization of genomic Southern blots of hybrid cell lines and parental control DNA provided evidence for single genes encoding HNF-1 in mouse and human. We mapped *HNF1* to human chromosome 12q22–qter and *Hnf-1* to mouse chromosome 5 by study of somatic cell hybrids. With RI mouse strains, we mapped *Hnf-1* close to *Bcd-1* on mouse chromosome 5, a region that also contains the genes for the liver-specific proteins albumin (*Alb*) and α-fetoprotein (*Afp*). The human *ALB/AFP* gene cluster is located on chromosome 4, not 12, however. On the other hand, genes on human chromosome (HSA) 12 have been mapped to mouse chromosomes (MMU) 4, 6, 10, and 15: the short arm, proximal long arm, and region q21–q24.1 of HSA12 are homologous to regions of MMU6, MMU15, and MMU10, respectively (32). Since the HNF-1 gene is not a member of these three conserved syntenic groups, we predict that *HNF1* is distal to band 12q24.1. The human homologs of genes on MMU5 have been localized on HSA4 and -7. The region where *Bcd-1* and *Hnf-1* are located corresponds to region F of MMU5 as determined by chromosome translocations (33). A new region of homology involving HSA12q24.1–qter and MMU5F may exist as suggested by the chromosome localizations of the HNF-1 gene in humans and mice. It should prove interesting to determine whether other genes homologous to the divergent HNF-1 homeobox will, with the HNF-1 locus, define a syntenic group/homeobox cluster.

We thank Mary Graves, Lei Chen, and Linda Hansen for invaluable assistance in the isolation and analysis of the mHNF-1 clones and Susanne Baumhueter for rat HNF-1 cDNA. We thank John Morgan for the pβSH-CAT vector, Mark Davis for the pBJ5 expression vector, and Steven McKnight for the C/EBP expression vector. Generous support was provided by the Howard Hughes Medical Institute and by National Institutes of Health Grants CA39612 and HL33942 (G.R.C.), HD07201 (P.B.C.), and GM26105 (U.F.). U.F. is an Investigator, G.R.C. an Associate Investigator, and C.-L.H. an Associate of the Howard Hughes Medical Institute. C.J.K. was supported by the Medical Scientist Training Program at Stanford University.

1. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G. & Crabtree, G. R. (1987) *Science* **238**, 688–692.
2. Courtois, G., Baumhueter, S. & Crabtree, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7937–7941.
3. Maire, P., Wuarin, J. & Schibler, U. (1989) *Science* **244**, 343–346.
4. Feuerman, M. H., Godbout, R., Ingram, R. S. & Tilghman, S. M. (1989) *Mol. Cell. Biol.* **9**, 4204–4212.
5. Monaci, P., Nicosia, A. & Cortese, R. (1988) *EMBO J.* **7**, 2075–2087.
6. Costa, R. H., Grayson, D. R. & Darnell, J. E., Jr. (1989) *Mol. Cell. Biol.* **9**, 1415–1425.
7. Tyner, A. L., Godbout, R., Compton, R. S. & Tilghman, S. M. (1990) *J. Cell Biol.* **110**, 915–927.
8. Cereghini, S., Blumenfeld, M. & Yaniv, M. (1988) *Genes Dev.* **8**, 957–974.
9. Frain, M., Swart, G., Monaci, P., Nicosia, A., Stämpfli, S., Frank, R. & Cortese, R. (1989) *Cell* **59**, 145–157.
10. Baumhueter, S., Mendel, D. B., Conley, P. B., Kuo, C. J., Turk, C., Graves, M. K., Edwards, C. A., Courtois, G. & Crabtree, G. R. (1990) *Genes Dev.* **4**, 372–379.
11. Nicosia, A., Monaci, P., Tomei, L., De Francesco, R., Nuzzo, M., Stunnenberg, H. & Cortese, R. (1990) *Cell* **61**, 1225–1236.
12. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
13. Baumhueter, S., Courtois, G. & Crabtree, G. R. (1988) *EMBO J.* **7**, 2485–2493.
14. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1050.
15. Fiering, S., Northrop, J. P., Nolan, G. P., Matilla, P. S., Crabtree, G. R. & Herzenberg, L. A. (1990) *Genes Dev.*, in press.
16. Yang-Feng, T. L., DeGennaro, L. J. & Francke, U. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8679–8683.
17. Hsieh, C.-L., Sturm, R., Herr, W. & Francke, U. (1990) *Genomics* **6**, 666–672.
18. Finney, M. (1990) *Cell* **60**, 5–6.
19. Shaw, J.-P., Utz, P. J., Durand, D. B., Toole, J. J., Emmel, E. A. & Crabtree, G. R. (1988) *Science* **241**, 202–205.
20. Friedman, A. D., Landschultz, W. H. & McKnight, S. L. (1989) *Genes Dev.* **3**, 1314–1322.
21. Winchester, G., Mitchison, N. A. & Taylor, B. A. (1987) *Immunogenetics* **26**, 356–358.
22. Davisson, M. T., Roderick, T. H., Hillyard, A. L. & Doolittle, D. P. (1989) *J. Cell Biol.* **84**, 15–23.
23. Bodner, M., Castrillo, J.-L., Theill, L. E., Deerinck, T., Ellisman, M. & Karin, M. (1988) *Cell* **55**, 505–518.
24. Xanthopoulos, K. G., Mirkovitch, J., Decker, T., Kuo, C. F. & Darnell, J. E., Jr. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4117–4121.
25. Lichtsteiner, S., Wuarin, J. & Schibler, U. (1987) *Cell* **51**, 963–973.
26. Tyner, A. L., Godbout, R., Compton, R. S. & Tilghman, S. M. (1990) *J. Cell Biol.* **110**, 915–927.
27. Chiu, R., Boyle, W. J., Meek, J., Smeal, T., Hunter, T. & Karin, M. (1988) *Cell* **54**, 541–552.
28. Glass, C. K., Lipkin, S. M., Devary, O. V. & Rosenfeld, M. G. (1989) *Cell* **59**, 697–708.
29. Murre, C., McCaw, P. S. & Baltimore, D. (1989) *Cell* **56**, 777–783.
30. Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. & Baltimore, D. (1989) *Cell* **58**, 537–544.
31. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990) *Cell* **61**, 49–59.
32. Lalley, P. A., Davisson, M. T., Graves, J. A. M., O'Brien, S. J., Womack, J. E., Roderick, T. H., Creau-Goldberg, N., Hillyard, A. L., Doolittle, D. P. & Rogers, J. A. (1989) *Cytogenet. Cell Genet.* **51**, 503–532.
33. Lyon, M. F. (1989) *Mouse Newslett.* **84**, 24–45.
34. Lin, A., Devaux, B., Green, A., Sagerstrom, C., Elliot, J. & Davis, M. (1990) *Science* **249**, 677–679.