HNF-1 α and HNF-1 β (vHNF-1) share dimerization and homeo domains, but not activation domains, and form heterodimers in vitro

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HNF-1 α (previously referred to as HNF-1, LPB1, and APF) is a vertebrate transcription factor that contains a divergent homeo domain and plays a prominent role in regulating genes that have the common characteristic of being expressed in hepatocytes and a complex group of endodermally and mesodermally derived tissues. HNF-1 α is unique among the vertebrate homeo domain-containing proteins in that it dimerizes in the absence of its DNA recognition sequence, suggesting the possibility that the function of HNF-1 α may be diversified by forming heterodimers with other related proteins. We report the initial characterization of HNF-1 β , which is closely related to HNF-1 α and is able to form heterodimers with HNF-1 α in vitro. Although HNF-1 α , but not HNF-1 β , is expressed in the liver, HNF-1 α and HNF-1 β are coexpressed in the murine Hepa1A cell line and in the mammalian kidney where a subset of hepatocyte genes are expressed. In contrast, exclusive expression of HNF-1 β is associated with repression of a subset of hepatocyte-specific genes in the dedifferentiated hepatocyte cell line C2, differentiated F9 cells, in somatic hybrids between hepatocytes and fibroblasts, and in the lung. The extent of heterodimerization may be regulated in a tissue-specific way because freely exchangeable heterodimers are formed in Jurkat T cells transfected with HNF-1 α and HNF-1 β , whereas in liver cells stable homodimers are present. These studies define a pair of homeo domain proteins that have the potential to interact to produce an embryologically complex pattern of gene expression.

[**Key Words:** Vertebrate transcription factor, HNF-1 α ; HNF-1 β ; homeo domain; heterodimer formation]

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A partial solution to the problem of controlling a very large number of genes with a limited number of transcription factors appears to have been reached by dimerization between members of classes of transcription factors. The earliest example to be discovered in vertebrates was the leucine zipper family (Landschulz et al. 1988b) including C/EBP, DBP, Fos, Jun, and others (Chiu et al. 1988; Landschulz et al. 1988a; Rauscher et al. 1988; Turner and Tjian et al. 1989; Mueller et al. 1990). Somewhat later the helix-loop-helix group including E12, E47, myoD, and Id were found to form weak but functionally important heterodimers (Tapscott et al. 1988; Murre et al. 1989; Benezra et al. 1990). These reports followed studies in prokaryotes where dimerization is a common characteristic of DNA-binding proteins (for review, see Johnson et al. 1981 and references therein). An obvious advantage of the ability of a transcription factor to form homodimers and/or heterodimers is that it provides an opportunity to diversify the number of regulatory mechanisms that can be associated with a single regulatory DNA-binding site. Another is the possibility of translating subtle alterations in the relative levels of

expression of members of a dimerizing pair into a substantial quantitative effect on transcription.

Despite the potential advantages offered by dimerization only two homeo domain proteins, Pit-1/GHF-1 and the products of the yeast *MAT* locus (for review, see Herskowitz 1989), have been shown to dimerize. In the life cycle of yeast, the extent of heterodimerization among the homeo domain-containing transcription factors MAT α 1 and α 2 (Keleher et al. 1988) is an important determinant of the diploid state. In diploid cells, α 2 represses α -specific genes, whereas α 1- α 2 heterodimers acquire a new sequence specificity and repress haploid-specific genes (Goutte and Johnson 1988). The extent of heterodimerization thus maintains gene expression in the diploid state. Among the mammalian homeo domain proteins, Pit-1 does not form dimers in solution but binds to its sequence as a homodimer (Ingraham et al. 1990). To date, a protein that pairs with Pit-1/GHF-1 has not been discovered.

Hepatocyte nuclear factor-1 (HNF-1) (Courtois et al. 1987), also called APF (Cereghini et al. 1988), LFB1 (Hardon et al. 1988), HP (Schorpp et al. 1988), and A box

factor (Maire et al. 1989), is a homeo domain-containing protein that acts as a transcriptional activator in vivo and in vitro to regulate the expression of a large group of genes that have the common characteristic of being expressed in hepatocytes (Courtois et al. 1988). Consistent with the presence of a homeo domain and sequences related to the POU-specific region (Frain et al. 1989; Baumhueter et al. 1990), HNF-1 has been suggested to play a role in establishing the hepatocyte phenotype.

Perhaps the strongest indication that HNF-1 plays a role in establishing and/or maintaining a cell-type-specific pattern of gene expression comes from recent work with mutants and revertants of the hepatic phenotype. Deschatrette and Weiss (1974) used morphologic criteria and single cell manipulation to select individual clones of dedifferentiated cells from the well-differentiated Fao hepatocyte cell line. Although the parental Fao cell line produces large numbers of genes expressed in the liver, the morphologically dedifferentiated cells selected by Deschatrette and Weiss (C2 cells) lack expression of nearly all liver-specific proteins, with the exceptions of tyrosine and alanine aminotransferases and PEPCK (Deschatrette and Weiss 1974). These cells probably represent mutants as their phenotype has been stable in culture for 13 years and they arise at a frequency of $\sim 10^{-7}$. The lack of expression of most of the gluconeogenic enzymes in the C2 cells allowed the selection of revertants by growth in glucose-free media (Deschatrette et al. 1980). The revertants (Rev7 cells), which occur at a frequency of 10^{-9} , express nearly all of the liver-specific proteins and are morphologically similar to the original differentiated cells (Deschatrette et al. 1980), perhaps because cells need several hepatocyte-specific proteins to perform gluconeogenesis and grow in media without glucose. Although the original differentiated Fao cells expressed the 88-kD form of HNF-1 found in liver extracts, the C2 cells expressed a 68-kD variant form of HNF-1 (vHNF-1) that exhibited DNA sequence specificity identical to the original HNF-1 protein (Baumhueter et al. 1988; Cereghini et al. 1988). The Rev7 revertant cells expressed the 88-kD form of HNF-1 as well as the entire group of HNF-1-dependent genes. Interestingly, the vHNF-1 protein was found in two other systems associated with dedifferentiation and/or repression of a subset of hepatocyte-specific genes: in the normal lung and in hybrid cell lines generated by fusing hepatocytes and fibroblasts (Baumhueter et al. 1988). Interestingly, the lung expresses some of the HNF-1-dependent genes that are selectively expressed in the liver, including γ -fibrinogen and α_1 -antitrypsin (Baumhueter et al. 1988).

HNF-1 is unique among mammalian homeo domain-containing proteins in that it contains a 21-amino-acid "loop" between helices 2 and 3 of the homeo domain (Baumhueter et al. 1990; Finney 1990) and it forms homodimers in solution (Chouard et al. 1990; Nicosia et al. 1990). The ability of HNF-1 to dimerize in solution, combined with the observation that a subset of the HNF-1-dependent genes are expressed in tissues and at various times in development, which appear inconsistent with the known expression pattern of HNF-1 (Baumhueter et

al. 1990), have raised the possibility that the function of the HNF-1-binding site could be diversified somehow developmentally either by the binding of other proteins or by developmental modifications of HNF-1. One likely candidate for a second protein that might be able to interact with the HNF-1-binding site to alter its function would be vHNF-1. Alternatively, a protein with a dimerization motif compatible with that of HNF-1 could also alter the activity of HNF-1.

We have cloned cDNA encoding the vHNF-1 protein and find that it contains a dimerization domain and a divergent homeo domain, including the 21-amino-acid loop, both of which are closely related to those of HNF-1 (Frain et al. 1989; Baumhueter et al. 1990). Consistent with this observation, HNF-1 and vHNF-1 expressed in Jurkat human T cells are able to form heterodimers. The fact that some tissues and cell lines express variable levels of vHNF-1 and HNF-1 suggests that heterodimerization may play a role in determining the pattern of gene expression in the large number of tissues that contain these proteins. Furthermore, we find that heterodimerization is regulated in a tissue-specific manner, as freely exchangeable heterodimers are formed in transfected Jurkat human T cells but stable nonexchangeable homodimers are present in liver extracts. For simplicity we will use the terms HNF-1 α for the original HNF-1 protein (Courtois et al. 1987) and HNF-1 β for the newly cloned vHNF-1 (Baumhueter et al. 1988; Cereghini et al. 1988).

Results

HNF-1 β is closely related to HNF-1 α

Several lines of indirect evidence suggested that there might be a protein closely related to HNF-1 α . We therefore screened a mouse liver cDNA library with a cDNA fragment containing the open reading frame of mouse HNF-1 α under low-stringency conditions. A screen of 600,000 plaques produced 22 positive clones. Sixteen of these clones remained positive when the filters were washed under highly stringent conditions. The later clones were partially sequenced and found to be clones for mouse HNF-1 α . The remaining six clones contained inserts ranging in size from ~ 500 bp to ~ 3 kb, with the two largest inserts both being ~ 3 kb. The two largest inserts were sequenced and found to be duplicates of the same clone, 28-1, which contained a single open reading frame encoding the 532 amino acids of HNF-1 β . The nucleotide sequence of this clone and the predicted amino acid sequence of HNF-1 β are shown in Figure 1.

A comparison of the predicted amino acid sequences of murine HNF-1 α (Kuo et al. 1990) and HNF-1 β (Fig. 2) indicates that these two proteins are closely related, having an overall identity of 58% through the shorter HNF-1 β sequence. The similarity between these two proteins is greater in the amino half of the molecule, which is where the dimerization domain, the sequences related to the POU-specific box, and the divergent homeo domain are located. In these three functional re-

1	GAATTCCTACAGCCCGGGTGGGTGAGTCTGTTACGGGTGGGATGTGGGAACCTCAGGCTACTGGGAAATAATGGGGAAGGCGCTCCCGGTAAATGCCCTCGTCTTCTCCAGGAACAC	120
121	ACAGATGATGAGCCGATCCACCTTGTCCTTGCTGCTCCAGCCATCCCGCAGCGAAGCCAGCACTACTTCTGCAGCCTCTCATGGGGTAGCCAAACACGCCTGTGGAGATGCACGG	240
241	GAAAGCCACTGAGCGCAGCCGGTGTCCAGCAGCAGGTCCAGGCTGCTCAAGTAGCAGCTCGCGAGCTCGGCCCTGGCTGGCAGTGGGTGGCCACGGCGATGGGCCCCACCGTGTG	360
361	GATGACATACTTGGCTGGCAGCCGATAGCCGCAAGTGATCTTGGCTTTCGCCGTCTCGCAGTTCTGTAGGCTGCGGCATTCTGCTCGTGAGCAGGGATCCCGCGGCCGATGAATGCAGCC	480
481	GTCCACCCCTCCGCTTCAAGCAGGGAGCTGTTGGCAGCGTTGACAATGGCATCCACCTCCAGCTTGGTGAAGTCCAATCCCGGCCAAGTCTCTGCTCCGTTGGACTGTCCCTTAGGT	600
601	TCTCTTTTCCCCCTCCCAACCACTTTCATCTTCCGGAAGCTGAGCCCTTCCCATCTATTGCATATCTTATATGCGCTAATGGTGGAGATCATGGCAAGTTAGAAGTTTCTGA	720
721	CTCTCTCGGAGAGTCTCCGGACCCAGAGAGTAACAGGTGTCTGAAAGCTAAAGGGTGGAGGGTTCAGGACTTGGAGTTCTCTGTAAATCTCCCTCCACCTCTCTCTCACA	840
841	CCCACCCATCCCTCAACCCCTCTCTTTTCCGTCCTTGGAAATGGTGTCCAGCTCAGCTCGCTCCAGCAAGAACTCTGAGTGCCTGTGAGCTCCGGAGTCCCAAGGAAGTGTG	960
	M V S K L T S L Q Q E L L S A L L S S G V T K E V L	26
961	ATCCAGGCTTGGAGGAGTTACTGCGCTCCCGAATTTCCGGGTGAAGCTGGAGACTGCCCTGTCCCGGGAGCGGGCGGATCTGCACCAAGCCGGTTTTCATCTCTCACC	1080
	I Q A L E E L L P S P N F G V K L E T L P L S P G S G A D L D T K P V F H T L T	66
1081	AATGGCCACGCCAAGGGCCGCTTGTCTGGGACGAGGGCTCAGAGGACGGCGACACTATGACACTCTCCCATCTCTCAAAGAGCTCCAGGCGCTCAACACCGAGGAGGCGCGGAGCAG	1200
	N G H A K G R L S G D E G S E D G D D Y D T P P I L K E L Q A L N T E E A A E Q	106
1201	CGGCGGAGGTGGACGGATGCTCAGCGAGGACCGTGGAGGGCTGCCAAATGATCAAGGATACATGCAACAGCACAATATCCCCAGAGGGAGGTGGTGCATGTCACAGGCTGAAC	1320
	R A E V D R M L S E D P W R A A K M I K G Y M Q Q H N I P Q R E V V D V T G L N	146
1321	CAATCCCACTCTCTCAACACCTCAACAGGGCACCCCATGAAGACCCAGAAGAGAGTGCCTGTACACTTGGTACGTGAGAAAGCAACGGGAGATCTCCGACAGTTCAGTCAACAG	1440
	Q S H L S Q H L N K G T P M K T Q K R A A L Y T W Y V R K Q R E I L R Q F S Q Q	186
1441	AACAGGGGCTGGGAGTGGAGGACCTGCTCCGAGCCACCAACAGAAGATGCGCGCAACCGGTTTAAATGGGGCCCGCATCCCAAGCAATTTTGTACAGGCTACGACCGG	1560
	N Q G P G Q S E D T C S E P T N K K M R R N R F K W G P A S Q Q I L Y A S Q Q D R	226
1561	CAAAAGATCCAGCAAGGAGGAGGAGGCTTGTGGAGGAGTGAACAGGCAAGATCTTTCGCAAGGAGGCTCCCTTCCAAAGCCACGGCTAGGCTCCAATCTGGTACG	1680
	Q K N P S K E E R E A L V E E A L G E C L Y R V S P S K A H G L G S N L V T	266
1681	GAGTCCGTGTCTCAACTGGTTCGCAACCGCGGAAGAGGCGTTCAGGCAGAGCTGGCCATGGATGCCTATAGCTCCAACACAGCAGCACAACCTGAACCCCTGCTCACCAT	1800
	E V R V Y N W F A N R R K E E A F R Q K L A M D A Y S S N Q T H N L N P L L T H	306
1801	GGTCCCTCACCATCAGCCAAGCTCTCTCCACCAACAAGATGTGAGAGTGCCTACACAGCCGGGAAACAATGAGGTCACTTCTCTCGACAATCAGTCACCATGGTAACAGT	1920
	G S P H H Q P S S S P P N K M S G V R Y N Q P G N N E V T S S S T I S H H G N S	346
1921	GCCATGGTGACAGCAGTCCGGTTTACAGCAAGTCTCCCAAGCAGCCTGGACCCAGGCCAGCTCTCTCTACCTGACAGTAAATGAGATCAGTGTGCGGAGGAGGACTGCCT	2040
	A M V T S Q S V L Q Q V S P A S L D P G H S L L S P D S K M Q I T V S G G G L P	386
2041	CCCGTCAGCACCTTGACGAATATCCACAGCTCTCCACCAACAATCCCAAGCAATCTCAGAACCTCATCATGACCCCTGTCTGGAGTCAATGCCATGCACAGAGCTCAACACCTCC	2160
	P V S T L T N I H S L S H H N P Q Q S Q N L I M T P L S G V M A I A Q S L N T S	426
2161	CAAGCCAGGGTGTCCCGGTCATTACAGTGTGGCTAGCAGCCTGGCAGCCCTACAGCCCTCAGTTCTCTCAACAGCTGCACAGCCCTCACCAGCAGCCCTCATGACAGCAGGCCA	2280
	Q A Q G V P V I N S V A S S L A A L Q P V Q F S Q Q L H S P H Q Q P L M Q Q S P	466
2281	GGCAGTCACATGGCCAGCAGCCTTCATGGCCGGTGACTCAGTCAGCAACTCCACATGTATGCACATAAGCAGGAACCCCCAGTATTCCCAACCTCCCGGTTCCCATCTGCA	2400
	G S H M A Q Q P F M A A V T Q L Q N S H M Y A H K Q E P P Q Y S H T S R F P S A	506
2401	ATGGTGGTCACAGATACAGTAGCATCAACACTCTCACCAGCATGTCTTCCAGTAAACAGTGTCCACTGCAAGCCTGGTGATCTACATAAACCCTACTCTGTGCACAGCAACAAGGA	2520
	M V V T S S I N T L T S M S S L Q C P L Q A W *	532
2521	CCCCATTTCCACACCATCAGTCTCTGGGAAGTCCAGCAACCAGACAGGCACCTGGGAGCCAGGAGAGGGCCCTGCTCACCTGACATCTGCCAGGCACCTCAGATAAGCGTTC	2640
2641	AGAGCTGACAGCCGAAGCCAGCTTCCCTTCTTTGAGTATTGTGATGATGCTCTCCCAAGATGCCAAGTGTCTTGTGTCCCCAGAGCGGGCTGCTGCGAATGGTGAACGAGA	2760
2761	AGGGAGAAACCGTGAGTCTCCCTGCAACCCATCATCGAACAACATGATGCAAAAACCTGAATCTGTTACTGAAATAAGATGAGGACCAAGGAGGGTGTGTGCTAATGAACAGCCA	2880
2881	GACACTGTAATACTGACAGACCCCCCTCTCCCATCCAGATGATCTCAAGATTCTTCTAAAGAGTAAATTTGTCATGGGTGTAACCTATAAATACTGTAATTAAGTGAATTTT	3000
3001	CCTTCCAGTTTATCTACCCCTGCCCTGTATATAATACTAAAGTGTCTATCACTTTTCTTGTAAAGGTCAGAGTTGAAATTCACAAAAA	3099

Figure 1. Nucleotide sequence of the 28-1 cDNA and predicted amino acid sequence of HNF-1 β .

gions the identity between HNF-1 α and HNF-1 β is 72%, 66%, and 92%, respectively. Moreover, in the dimerization domain the hydrophobic and charged residues that would be expected to be important in maintaining the coiled-coil structure of this sequence (Nicosia et al. 1990) are conserved. Although the identity in the two motifs similar to the POU proteins is only 66%, the charged residues in the corresponding sequences are largely conserved and in the basic region, which has been implicated in sequence-specific binding of Pit-1 (Ingraham et al. 1990), the identity is 90%.

In the carboxyl half of the molecules there is less overall identity between the two proteins. In particular, the first 100 amino acids beyond the homeo domain are poorly conserved, and HNF-1 α contains a 20-amino-acid stretch (amino acids 414–434 of HNF-1 α) that is not

present in HNF-1 β . HNF-1 α also contains an additional 96 amino acids at its carboxyl terminus. Neither of the transcriptional activation domains (AD) described for HNF-1 α , a proline-rich region just beyond the carboxyl terminus of the homeo domain (AD II), and the carboxy-terminal 84 amino acids of HNF-1 α (AD I) (Nicosia et al. 1990), are present in HNF-1 β .

Mammalian tissues contain varying ratios HNF-1 α and HNF-1 β

Although an earlier study suggested that HNF-1 α message is restricted to the liver (Frain et al. 1989), we have subsequently shown that the intestine, stomach, and kidney also contain message for HNF-1 α at levels comparable to that found in the liver (Baumhueter et al.

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|--- Dimerization Domain ---|
HNF-1 $\alpha$  1  MVSKLSQLQTELLAALLESGLSKEALIQAIGE..PGPYLMVGEGPLDKGESCG...GSRGDLTELPNGLGETRGSEDDTDDDGEDF.APPIIKEPENLS
HNF-1 $\beta$  1  -----TS--Q---S---S--VT--V-----E-LL-S-NFG-KLET-PLSPGS-ADLDTKPVFHT-T--HAKG-L-G-EGSE--D-YDT-----LQA-NT

|----- POU-
94  EEAHQKAVVESLLQEDPWRVAKMVKSYLQQHNPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVRKQREVAQQFTHAGQGGLIEEPTGDEL
101 -----E-R-E-DRM-S-----A---I-G-M-----V-----I-L-R--SQQN--PGQS-D-CS-P

Motif -----|----- Homeodomain -----|
|--- 21-AA Loop ---|--- Third Helix ---|--- Activa-
194  PTKKGRNRNRFKWGPASQQILFQAYERQKNPSKEERETLVEECNRAECIQRGVSPSQAQGLGSLNLTVEVRVYNWFANRRKEEAFRHKLAMDYNGPPPGPG
201  TN--M-----Y--D-----A-----L-----K-H-----Q-----A-SSNQ..TH

tion Domain II -----|
294  PGPALPAHSSPGLPTTTLSPSKVHGVRVYQGSATSEAAEVPSSS...GGPLVTVSAALHQVSPPTGLEPS.SLLSTEAKL.VSATGGPLPPVSTLTALHSLE
299  NLNP-LT-G---HHQFSSSP-N-MS-----N-PGNN-VTSSSTI-HHGNSAM--SQSV-Q---AS-D-GH---PDS-MQITVS--G-----NI---S

389  QTSPGLNQQLIMASLPQVMTIGPGEASLGPTFTNTGASTLVIGLASTQAQSVPVINSMGSSLTTLQPVQFSQPLHPSYQQPLM.PPVQSHVAQSPF
399  HHN...--S-----TP-S---A-AQ.....S-NTS---G-----VA--AA-----Q--SPH-----QQSPG--M--Q--

|----- Activation
488  MATMAQLQSPHALYSHKPEVAQYTHSTLLPQTMLITDT.NLSTLASLTPTKQVFTSDTEASSEPLHEPPSPATTIHIPSQDPSNIQHLQPAHRLSTSP
476  --AVT---NS-.M-A--Q-PP--S---RF-SA-VV---SSIN--T-MSSS---.CPLQ-W* 532

Domain I -----|
587  VSSSSLVLYQSSDSNGSHLLPSNHSVIETFIQTMASSSQ* 628

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Figure 2. HNF-1 β is closely related to HNF-1 α . The homeo domain and dimerization domain, but not the activation domain, are conserved between HNF-1 α and HNF-1 β . The predicted amino acid sequences of murine HNF-1 α (Kuo et al. 1990) and HNF-1 β are compared according to the BestFit program of the Sequence Analysis Software Package of the Genetics Computer Group (University of Wisconsin). Amino acids in the HNF-1 β sequence that are identical to those in the HNF-1 α sequence are indicated by dashes; gaps in either sequence are indicated by dots. The dimerization domain, the POU-specific region, the homeo domain, and the two transcriptional activation domains are indicated as they appear in the HNF-1 α sequence (see text).

1990; Kuo et al. 1990), and that the kidney expresses substantial amounts of HNF-1-binding activity (Baumhueter et al. 1990). To determine whether HNF-1 α and HNF-1 β might be expressed in the same tissues we first used the RNase protection assay to investigate the tissue distribution of HNF-1 β message. The results are shown in Figure 3. Liver, intestine, stomach, and kidney, the four tissues that contain the highest levels of HNF-1 α message (Baumhueter et al. 1990; Kuo et al. 1990), also contain message for HNF-1 β (Fig. 3A). The message for HNF-1 β was also detected at comparable levels in the lung and ovaries, two tissues that contain low (Baumhueter et al. 1990) or undetectable (Kuo et al. 1990) amounts of the message for HNF-1 α . A direct comparison of the relative abundance of the messages for HNF-1 α and HNF-1 β in liver and kidney, the two tissues that have been most carefully investigated for HNF-1-binding activity, indicated that the liver contains similar amounts of the message for the two forms of HNF-1, whereas in the kidney the message for HNF-1 β is approximately three times as abundant as the message for HNF-1 α (Fig. 3B), suggesting that HNF-1 β might be the more abundant form of HNF-1 in the kidney.

Kuo et al. (1991) have shown that the expression of

HNF-1 α in F9 cells is either translationally regulated or highly unstable such that the message for HNF-1 α can be present at levels comparable to those in the liver, but the protein is either not expressed or is present at very low levels. As a means of investigating whether the two forms of HNF-1 might be differentially regulated in different tissues we used the gel mobility shift assay to test for the presence of the two forms of HNF-1 in nuclear extracts prepared from liver, kidney, and lung, three tissues that contain different relative amounts of message for HNF-1 α and HNF-1 β .

Because HNF-1 α and HNF-1 β have identical or near-identical sequence specificity (videa infra), we developed polyclonal antisera that distinguish HNF-1 α from HNF-1 β to detect the presence of each form of HNF-1 in a gel-shift assay. The ability of the antisera to discriminate between HNF-1 α and HNF-1 β was evaluated based on their ability to supershift the retarded complex generated by incubating the β 28 synthetic oligonucleotide probe with nuclear extracts of Jurkat human T cells transfected with an expression vector containing the cDNA encoding HNF-1 α or HNF-1 β . As is shown in Figure 4A, the anti- α antisera produces a supershift when added to a reaction containing nuclear extract from Jur-

Figure 3. Tissue distribution of the message for HNF-1 β . (A) Ten micrograms of total RNA from the indicated tissues of adult mice, or 10 μ g of tRNA as a negative control, was examined for the presence of HNF-1 β mRNA in an RNase protection assay as described in Materials and methods. The presence of the message for HNF-1 β was indicated by a 210-nucleotide radioactive fragment. Molecular mass markers are included at left. (B) Ten micrograms of total RNA from the liver and kidney was assayed for relative content of message for HNF-1 α (α) and HNF-1 β (β). The presence of the message for HNF-1 α is indicated by a protected fragment of 277 nucleotides (Kuo et al. 1990). After separation on a denaturing gel, the amount of radioactivity in each band was determined by direct quantitation using an AMBIS radioactivity scanner. In this experiment the bands corresponding to the protected regions of HNF-1 α and HNF-1 β message from the liver contained 1378 and 1279 counts, respectively. The bands corresponding to the message for HNF-1 α and HNF-1 β from the kidney contained 2663 and 8017 counts, respectively.

kat cells expressing HNF-1 α , but not when added to a reaction containing extract from cells expressing HNF-1 β . Similarly, the anti- β antisera only produces a supershift when added to a reaction containing nuclear

extract from cells transfected with HNF-1 β . Untransfected Jurkat cells do not have either HNF-1-binding activity (data not shown).

A similar analysis of nuclear extracts from liver, kid-

Figure 4. Tissue distribution of HNF-1 α and HNF-1 β protein as detected by specific antisera. (A) The ability of the anti- α and - β antisera to differentiate between HNF-1 α and HNF-1 β was tested by using nuclear extracts of Jurkat human T cells that had been transfected with either the HNF-1 α or HNF-1 β expression vector. Five micrograms of nuclear extract was incubated with 1 μ l of anti- α antiserum (α), 1 μ l of anti- β antiserum (β), or without the addition of antiserum (-) in a gel retardation assay reaction mixture containing the β 28 probe. The specificity of the antisera was demonstrated by the presence of a supershift of the retarded complex only in extracts of cells transfected with 10 μ g of the appropriate expression vector. (B) The anti- α (α) and anti- β (β) antisera were used to determine the relative contribution of HNF-1 α and HNF-1 β to the HNF-1-binding activity in liver, kidney, and lung nuclear extracts. Binding reactions were performed with 10 μ g of nuclear protein from each of the indicated tissues, the β 28 oligonucleotide, and 1 μ l of antiserum as described above. The presence of HNF-1 α or HNF-1 β in the nuclear extract was indicated by the presence of a slower migrating complex than the one observed in the absence of antisera. The HNF-1 complexes in kidney nuclear extracts migrate more rapidly than those of liver and lung due to proteolytic digestion of the protein during the preparation of extracts. We feel that the more rapidly migrating complex is not due to a smaller protein as the mRNA for HNF-1 α and HNF-1 β in the kidney seems to be identical to that cloned from the liver.

ney, and lung is shown in Figure 4B. The HNF-1 DNA-binding activity in liver nuclear extracts appears to be due primarily, if not completely, to HNF-1 α although the messages for the two forms of HNF-1 are present at comparable levels. This result is consistent with the observation that HNF-1 purified from rat liver does not contain the β form of HNF-1 based on sequence analysis of the purified protein (D. Mendel, unpubl.). The kidney appears to express both α and β forms of HNF-1, with HNF-1 β constituting the more predominant form in agreement with the relative abundance of the messages for HNF-1 α and HNF-1 β . HNF-1 α and HNF-1 β may be expressed in different cell types as the kidney is highly heterogeneous in cellular makeup. The lung appears to express exclusively the HNF-1 β protein in agreement with the apparent lack of HNF-1 α message in this tissue. The discrepancy between the relative levels of HNF-1 α and HNF-1 β message and protein in the liver indicates that HNF-1 β , like HNF-1 α , is subject to translational regulation or rapid degradation.

HNF-1 β is vHNF-1, which has been associated with repression of a subset of hepatocyte-specific genes

A 68-kD variant form of HNF-1, known as vHNF-1, has been identified previously in the C2 dedifferentiated hepatocyte cell line, somatic hybrids, and the lung (Baum-

hueter et al. 1988). Our demonstration that the HNF-1-binding activity in lung nuclear extracts is HNF-1 β , combined with the observation that the predicted molecular mass of HNF-1 β , ~60 kD, is similar to the molecular mass of vHNF-1 (Baumhueter et al. 1988), suggested that HNF-1 β might be vHNF. We therefore used the anti- α and anti- β antisera to test whether this is the case. As is shown in Figure 5A, the HNF-1 binding activity in nuclear extracts of the Fao parent cell line and the spontaneous revertant Rev7 cell line is supershifted only when anti- α antisera is added to the reaction mixture. This result is consistent with earlier reports that the HNF-1-binding activity in these cells is associated with an 88-kD protein and that these cells contain the message for HNF-1 α (Baumhueter et al. 1988). In contrast, the HNF-1-binding activity in the C2 nuclear extract is supershifted only with the anti- β antisera, indicating that HNF-1 β is vHNF. The anti- β antisera, but not the anti- α antisera, is also able to supershift the HNF-1-binding activity in FF3-3 and FF5-1 somatic hybrids, generated by fusing Fao hepatocytes with mouse fibroblasts (Killary and Fournier 1984), which have been shown to express vHNF but not HNF-1 α (Baumhueter et al. 1988) (Fig. 5B). This evidence indicates that vHNF-1 or an immunologically and biochemically very similar protein is encoded by HNF-1 β cDNA clone. As with vHNF-1, HNF-1 β has been shown to have identical se-

Figure 5. HNF-1 β is the HNF-1-binding activity expressed in hepatocyte/fibroblast somatic hybrids and in the C2 dedifferentiated hepatocyte cell line. Nuclear extracts from a variety of cell lines were investigated for the presence of HNF-1 α and HNF-1 β by using the specific anti- α and anti- β antisera as described in the text and the legend to Fig. 4. The cell lines tested were the differentiated Fao cell line, the C2 dedifferentiated variant of the Fao cell line, and the Rev7 spontaneous revertant isolated from the C2 cell line (A); the FF3-3 and FF5-1 somatic hybrids generated by fusing the rat hepatocyte Fao cell line with mouse fibroblasts (B); and the differentiated Hepa1A murine hepatocyte cell line (C). The presence of HNF-1 α or HNF-1 β was indicated by the appearance of a new complex, which migrated more slowly than the original complex, after the addition of the specific antiserum.

quence specificity as HNF-1 α (Baumhueter et al. 1988; Cereghini et al. 1988).

Of the cell lines tested, only the mouse hepatocyte cell line Hepa1A contains detectable amounts of both HNF-1 α and HNF-1 β protein (Fig. 5C).

HNF-1 β is able to form heterodimers with HNF-1 α in vitro

Previous studies have shown that HNF-1 α , as originally postulated (Courtois et al. 1988), binds to its recognition sequence as a dimer (Frain et al. 1989; Chouard et al. 1990; Nicosia et al. 1990) and that this dimerization, which occurs in the absence of DNA, is dependent on the 30 amino acids at the extreme amino terminus of HNF-1 α (Chouard et al. 1990; Nicosia et al. 1990). The observation that the dimerization domain of HNF-1 α is conserved in HNF-1 β , and that the dimerization domain is required for HNF-1 β to bind DNA (Fig. 6A), suggested that HNF-1 α and HNF-1 β could form heterodimers. This was directly tested using the approach of Hope and Struhl (1987) after expressing full-length HNF-1 α and a truncated version of HNF-1 β in Jurkat cells. As shown in Figure 6B, nuclear extracts of Jurkat cells cotransfected with HNF-1 α and truncated HNF-1 β contained α - α homodimers, β - β homodimers, and α - β heterodimers. Similar results were obtained when nuclear extracts of Jurkat cells singly transfected with either full length HNF-1 α or truncated HNF-1 β were mixed in the presence of the β 28 probe (Fig. 6B), indicating that dimers formed in the Jurkat cells are freely exchangeable. Identical results are obtained when HNF-1 α and HNF-1 β are produced by in vitro translation in a rabbit reticulocyte lysate system (data not shown) and are similar to what has been reported for HeLa cells expressing HNF-1 α dimers (Nicosia et al. 1990).

The instability of the HNF-1 dimer is surprising because it is in apparent contradiction to what we had come to regard as a stable dimer for the native protein in liver nuclear extracts (D.B. Mendel and G.R. Crabtree, unpubl.). We therefore tested whether the truncated

HNF-1 β produced in Jurkat cells could exchange with the subunits of the HNF-1 complex in liver nuclear extracts. The results shown on the right of Figure 6B demonstrate that the HNF-1 complex present in liver nuclear extracts, which is most likely a homodimer of HNF-1 α , is stable and cannot be induced to exchange subunits when challenged by adding an excess of the truncated HNF-1 β subunit. We have also tested the stability of the β - β dimers from the lung and find that they, too, are not subject to exchange when challenged with a high concentration of the recombinant truncated β chain (data not shown). These results indicate that the stability of the HNF-1 dimer extends to both α - α and β - β homodimers produced in vivo. Thus, we sought to examine more carefully the stability of the native dimers.

To address this question we used gel filtration chromatography and density gradient centrifugation to determine the molecular mass of the native HNF-1 complex in liver nuclear extracts. As shown in Figure 7, native HNF-1 elutes from an S-300 gel filtration column as a 7.1-nm complex, both in the absence and in the presence of the β 28 synthetic oligonucleotide, and sediments through a density gradient as a 4.5S or a 5.0S complex in the absence or presence of probe, respectively. As a control, we have also assayed these samples for Oct-1, which is ubiquitously expressed, and found that it behaves as a 6.1-nm, 3.6S complex in agreement with previous measurements (O'Neill and Kelly 1988; Poellinger and Roeder 1989).

On the basis of these values we calculated that the apparent molecular mass of the native HNF-1 complex in liver extracts is $\sim$ 140 kD in the absence of the β 28 probe and $\sim$ 160 kD once bound to the probe. The 20-kD increase in the molecular mass calculated for the HNF-1 complex in the presence of the probe is most likely due to the presence of the 28-bp probe itself, which has a calculated molecular mass of 18.5 kD, and probably does not reflect the addition of subunits upon DNA binding.

These results agree with earlier studies that have reported that HNF-1 is a dimer in the presence or absence of DNA (Frain et al. 1989; Chouard et al. 1990; Nicosia

Figure 6. The stability of the HNF-1 dimer is tissue dependent and requires the dimerization domain. (A) The ability of HNF-1 β to bind DNA depends on the presence of the dimerization domain. Nuclear extracts of Jurkat cells transfected with expression vectors encoding HNF-1 β (β), HNF-1 β missing amino acids 2–30, which constitute the dimerization domain (dimerless β), or a truncated form of HNF-1 β (trunc β), which only expresses the first 350 amino acids of the protein, were tested for DNA-binding activity in a gel-mobility-shift reaction by using the β 28 probe. (Left) A comparison of the first 32 amino acids of HNF-1 α and HNF-1 β , which constitute the dimerization domain. Amino acids included in both sequences are enclosed in shaded boxes. The asterisks (*) mark the position of regularly occurring hydrophobic residues. (B) HNF-1 α and HNF-1 β form heterodimers in vitro. (Left) Nuclear extracts of Jurkat cells transfected with the expression vector encoding HNF-1 α (α), HNF-1 β (β), or the first 350 amino acids of HNF-1 β (trunc β), were tested for HNF-1 binding in a gel mobility shift assay by using the β 28 probe. The migration position of the full-length α - α or β - β homodimer is indicated by a solid arrowhead. The migration position of the truncated β homodimer is indicated by an open arrowhead. To test for heterodimerization, Jurkat cells were cotransfected with the expression vector for HNF-1 α or HNF-1 β in combination with the expression vector for truncated HNF-1 β at the indicated molar ratios, with the expression vector for the full-length protein always being more abundant. The nuclear extracts of the cotransfected cells were analyzed for the presence of a complex that migrated between the two homodimeric complexes. A Western blot of the extract shown in lane 3 (1 : 10) revealed very similar amounts of the α and β proteins. (Right) Decreasing amounts of nuclear extract (3 μ g–5 ng) from Jurkat cells transfected with the expression vector encoding the truncated form of HNF-1 β were mixed with 3 μ g of nuclear extract from Jurkat cells transfected with the expression vector encoding HNF-1 α or with 3 μ g of liver nuclear extract. The mixed extracts were left at room temperature for 1 hr in the presence of β 28 probe and then analyzed by gel mobility shift for the appearance of a new complex that migrated between the full-length homodimer (solid arrowhead) and the homodimer of the truncated HNF-1 β (open arrowhead).

et al. 1990). The ability of the native HNF-1 complex to remain intact during these lengthy biochemical analyses, however, supports our conclusion that, unlike the unstable HNF-1 dimers formed in vitro, HNF-1 dimers present in liver nuclear extracts are extremely stable. Our observation that the apparent molecular mass of the native HNF-1 complex is twice the predicted molecular mass of the HNF-1 α monomer (~70 kD) suggests that HNF-1 is not stabilized by association with large, non-HNF-1 subunits as part of a larger complex in liver nuclear extracts. We do not, however, exclude the possibil-

ity that the native HNF-1 complex in liver extracts contains small tissue-specific factors that could act to stabilize the dimer, or that the liver modifies HNF-1 α in such a way that the dimer becomes stable.

HNF-1 β does not block the transcriptional activity of HNF-1 α

Kuo et al. (1990) have previously demonstrated that expression of HNF-1 α in COS cells cotransfected with a reporter construct driven by a synthetic promoter con-

Figure 6. (See facing page for legend.)

Figure 7. The molecular mass of native HNF-1 is 140 kD. Liver nuclear extracts (●), or liver nuclear extracts that had been preincubated with the $\beta 28$ probe (○), were separated by gel filtration on a Sephacryl S-300 column (A) or by density gradient centrifugation on 7.5–30% glycerol gradients (C). After separation, the position of the HNF-1/DNA complex was assayed by counting aliquots of the eluted fractions for the presence of ^{32}P (○). In the absence of the $\beta 28$ probe, the presence of HNF-1 was identified by quantitation of the amount of $\beta 28$ probe retarded in a gel retardation assay. Quantitation of the retarded complex was performed by optical scanning of the autoradiogram, and the results are therefore given in arbitrary O.D. units. The Stokes radius (R_s) (B) and sedimentation coefficient ($S_{20,w}$) (D) of HNF-1 in the absence (●) or presence (○) of the $\beta 28$ probe was determined by comparing the elution profile of the complex obtained in A or C with the elution profiles of standard proteins (x). The Stokes radii and sedimentation coefficients of the standard proteins are given in Materials and methods. In A, V_o and V_i indicate the void volume and internal volume, respectively, of the S-300 column as determined by the elution profiles of Blue dextran 2000 and [^3H]leucine.

taining three HNF-1-binding sites can activate transcription. Because HNF-1 β is also able to bind to HNF-1-binding sites, we used this same reporter construct to test whether HNF-1 β acts as a transcriptional activator in COS cells.

The results from three transfections are presented in Figure 8. Although HNF-1 α is able to activate transcription of a cotransfected HNF-1-directed target gene, in agreement with earlier results, HNF-1 β cannot. The inability of HNF-1 β to activate transcription cannot be attributed to a lack of HNF-1 β protein being produced in the COS system, as comparable amounts of HNF-

1-binding activity was detected in nuclear extracts of COS cells transfected with either the HNF-1 α or HNF-1 β expression vector (data not shown).

The inability of HNF-1 β to activate transcription by this promoter is not altogether surprising as HNF-1 β lacks both transcription activation domains, which have been reported for HNF-1 α (Nicosia et al. 1990 and data shown above), although the serine/threonine-rich region at the carboxyl terminus of HNF-1 β might be expected to substitute for the serine/threonine-rich activation domain (AD I) of HNF-1 α .

One potential consequence of the apparent ability of

Figure 8. HNF-1 β does not activate or repress transcription directed by the β -fibrinogen HNF-1-binding site. Cellular extracts of COS cells cotransfected with 5 μ g of RSV-luciferase, 5 μ g of the (28)3-CAT reporter, and the indicated amounts of the expression vector encoding HNF-1 α (alpha) or HNF-1 β (beta) were assayed for chloramphenicol acetyltransferase (CAT) activity, which was indicated by the percent of chloramphenicol converted to the mono and diacetylated form. All values were normalized to the amount of luciferase activity present in duplicate extracts. The height of the boxes indicates the average value of three independent experiments. The individual values are indicated by the solid ovals.

HNF-1 β to heterodimerize with HNF-1 α but its lack of transcriptional activity is that HNF-1 β might act as a negative regulator of HNF-1 α transcriptional activity. Such a result would be expected if HNF-1 β were a dominant repressor of HNF-1 activity, as had been suggested based on the presence of vHNF-1 in several systems that do not express the majority of hepatocyte-specific gene products [Baumhueter et al. 1988]. We therefore tested whether cotransfection of HNF-1 β with HNF-1 α would cause a decrease in the ability of HNF-1 α to activate transcription of the reporter construct. However, this does not appear to be the case. Transfection studies in which various ratios of the HNF-1 α and HNF-1 β expression vectors were cotransfected with the reporter plasmid resulted in transcriptional activity that was proportional to the amount of expression vector included in the transfection. This result indicates that even when HNF-1 β is present in vast excess, HNF-1 α is able to activate transcription of this reporter construct.

Discussion

Heterodimerization of HNF-1 α and HNF-1 β is regulated in a cell type-selective manner

In this discussion we report the initial characterization of HNF-1 β , a protein that is closely related to HNF-1 α and is able to heterodimerize with HNF-1 α when the two proteins are expressed in vitro.

In a complex biological system, heterodimerization as a means of diversifying the function of component proteins would be most effective if the extent of het-

erodimerization could be controlled by factors other than the relative abundance of the two subunits. Our results suggest that HNF-1 dimerization may be regulated in such a fashion in tissues that normally express HNF-1 α or HNF-1 β . When the proteins are simply coexpressed in the Jurkat human T-lymphoid cell line, heterodimers and homodimers are formed at stoichiometric ratios that reflect the relative abundance of each of the two monomers. These dimers are unstable, with the subunits of the dimers being freely exchangeable at room temperature. When HNF-1 from liver nuclear extract is challenged with either the free peptide representing the dimerization domain [D.B. Mendel and G.R. Crabtree, unpubl.] or the truncated form of HNF-1 β produced in Jurkat cells, the dimers produced in vivo in the liver do not exchange with the second protein. Thus, in tissues that naturally express HNF-1, a modification or second molecule appears to stabilize or select for a specific pairing of the two chains of the protein. A similar mechanism has recently been proposed for a cell-specific inhibitor of c-Jun transcriptional activity, which is believed to act by interfering with the transcriptional activity of the c-Jun/c-Jun homodimer [Baichwal and Tjian 1990]. The full implications and biologic occurrence of heterodimerization of HNF-1 α and HNF-1 β must await the definition of this second process.

Our demonstration that HNF-1 in liver nuclear extracts exists as a ~140-kD complex, which is approximately twice the predicted size of the HNF-1 α monomer, indicates that whatever is responsible for stabilizing the native dimer cannot be a large protein. However, we have recently purified a small protein that binds to the homodimeric form of HNF-1 in liver, and that we believe to be a candidate for a cell type-specific regulator of HNF-1 dimerization [D.B. Mendel and G.R. Crabtree, in prep.].

Translational or post-translational control is a major regulator of both HNF-1 α and HNF-1 β

In this study we report that liver nuclear extracts contain undetectable amounts of HNF-1 β although the liver contains the message for HNF-1 β at essentially the same abundance as the message for HNF-1 α , indicating that in the liver HNF-1 β , but not HNF-1 α , is subject to negative translational regulation or that this protein is selectively unstable. Translational regulation has been reported for several transcription factors including GCN-4 [Roussou et al. 1988], LAP [Descombes et al. 1990], and Pit-1/GHF-1 [Dollé et al. 1990], and on the basis of these results, translation regulation rather than very rapid turnover of the protein seems the more likely explanation of the discordance between protein and RNA levels. In the kidney, the relative abundance of the HNF-1 α and HNF-1 β protein appears to be reasonably well correlated to the relative abundance of their respective messages, suggesting that neither form of HNF-1 is subject to translational control in this tissue.

In a separate study, Kuo et al. (1991) have investigated whether HNF-1-binding activity is induced in F9 embry-

onal carcinoma cells treated with retinoic acid. F9 cells induced to differentiate with retinoic acid go through changes similar to those of the preimplantation blastocyst (Strickland et al. 1980; Strickland 1981) and during this process, certain, but not all, target genes for HNF-1 are induced. HNF-1 β appears 16–24 hr after treatment, whereas HNF-1 α does not appear until 3–5 days after differentiation is induced. Surprisingly, the F9 cells contain similar levels of HNF-1 α mRNA during the entire time course of retinoic acid-induced differentiation. These results indicate that the mRNA for HNF-1 α must be translated at a low level until 3–5 days after retinoic acid treatment, or that the HNF-1 α protein is exceptionally unstable until day 3 to 5. The latter possibility, however, seems unlikely as mixing studies show no evidence that HNF-1 α protein becomes unstable when added to nuclear extracts of retinoic acid-stimulated F9 cells (C. Kuo, unpubl.).

Taken together, these observations point out that HNF-1 α and HNF-1 β are both subject to extensive translational regulation and that the magnitude and selectivity of the translational regulation is tissue/system-dependent. These observations also point out the necessity to study the potential interaction of these two factors on the basis of direct assays of the individual proteins rather than on the more convenient measurements of their respective message levels.

HNF-1 α and HNF-1 β expression in cell lines is reciprocally regulated by a locus that exerts a pleiotropic influence

We have found that formation of somatic cell hybrids generated by fusing liver cells, which express only HNF-1 α , and fibroblasts, which express neither HNF-1 α nor HNF-1 β , inactivates HNF-1 α expression, activates HNF-1 β expression, and leads to a loss of liver-specific gene expression. This reciprocal regulation of HNF-1 α and HNF-1 β expression also occurs upon selection of morphologically and biochemically dedifferentiated hepatocytes. Here, differentiated cells that express only HNF-1 α give rise to dedifferentiated cells that express only HNF-1 β . Selection of revertants for differentiation gives cell lines that express HNF-1 α but not HNF-1 β . This reciprocal regulation is accompanied by patterns of HNF-1 target gene expression that indicate that HNF-1 β expression is associated with repression of a subset of target genes and that HNF-1 α expression is associated with activation of a subset of target genes. The frequency with which revertants are selected (10^{-9}) (Deschatrette and Weiss 1974) strongly suggests that a single genetic locus controls the activation of HNF-1 α and the inactivation of HNF-1 β . Furthermore, this locus appears to exert pleiotropic effects as only part of the change in gene expression associated with revertants can be attributed to the HNF-1-binding sequence. The tissue-specific extinguishing locus, *tse-2*, on chromosome 1, which has been mapped in somatic cell hybrids that extinguish albumin, fibrinogen, and other liver-specific gene expression (Gourdeau et al. 1989), could direct the activation of

HNF-1 β and the inactivation of HNF-1 α . This hypothesis is consistent with the observation that genes controlled by *tse-2*, but not *tse-1*, are HNF-1 dependent (Courtois et al. 1988).

HNF-1 β may function indirectly

The functional role of HNF-1 β is still unclear. Our demonstration that HNF-1 β is vHNF-1, the previously identified variant form of HNF-1, and that it is HNF-1 β that is initially induced in F9 cells upon retinoic acid stimulation (Kuo et al., 1991) enables us to use information gained from these two systems in considering the function of HNF-1 β . From these studies we know that HNF-1 α and HNF-1 β exhibit similar DNA sequence specificity when assayed in gel-shift reactions, that they utilize the same contact Gs in the β -fibrinogen (Baumhueter et al. 1988) and albumin (Cereghini et al. 1988) binding sites, and that, with the possible exception that HNF-1 β may produce a slightly shortened footprint, there is no detectable difference in footprint pattern.

Despite the fact that HNF-1 α and HNF-1 β bind to the same sites in vitro, HNF-1 β does not appear to activate transcription by the β -fibrinogen-binding site. The lack of transcriptional activity is consistent with the observation that HNF-1 β is present as the sole HNF-1-binding activity in C2 dedifferentiated hepatocyte cells and in somatic hybrids, neither of which express the majority of hepatocyte-specific genes. However, the observation that HNF-1 β is present as the sole HNF-1-binding activity in the lung, which does express some of the HNF-1-dependent hepatocyte-specific genes, and as the predominant form in kidney, which also exhibits a subset of HNF-1-dependent hepatocyte genes, would argue that HNF-1 β may be transcriptionally active on certain promoters in vivo. This suggests that these two factors may regulate a different set of genes in vivo, although they do not distinguish between the DNA-binding sites in vitro. Alternatively, HNF-1 β may be similar to Oct-1 in that Oct-1 lacks an activation domain (Tanaka and Herr 1990), rendering it unable to activate transcription in transfection studies, but is able to induce *trans*-activation in the presence of specific coactivators ((Gerster and Roeder 1988; Stern et al. 1989); K. Ullman et al., in prep.).

Previous studies have used nuclear run-on transcription assays to show that the appearance and disappearance of HNF-1 β in the dedifferentiated variants/mutants is associated with only a moderate effect on the transcription of genes characteristic of the terminal stage of differentiation (Clayton et al. 1985; Friedman et al. 1987; Baumhueter et al. 1988). These studies led to the unanticipated conclusion that post-transcriptional control is an important element of the control associated with induction and reversal of vHNF-1 (HNF-1 β) expression in the dedifferentiated cells and the revertants (Baumhueter et al. 1988). These results suggest that HNF-1 β may function indirectly to regulate other transcription or processing factors.

Developmental implications of HNF-1 heterodimerization

The finding that HNF-1 α and HNF-1 β can form stable dimers suggests that this mechanism could be used during early development to generate tissue-selective patterns of gene expression from overlapping patterns of HNF-1 α and HNF-1 β expression. Kuo et al. (1991) have demonstrated that the two chains are subject to different regulatory influences during F9 differentiation, a result that is consistent with our finding that the two chains are expressed at widely varying levels in different tissues. Although several transcription factors have been described that are selectively expressed in the liver, including C/EBP (Johnson et al. 1987; Landschulz et al. 1988a), DBP (Mueller et al. 1990), LAP (Descombes et al. 1990), HNF-3 (Lai et al. 1990), and HNF-4 (Sladek et al. 1991), none are entirely liver specific and none have the potential, such as MyoD or myogenin (Lassar et al. 1986), to convert another cell type into a hepatocyte. Furthermore, most of these factors are expressed at the time of birth, too late in development to play a primary role in organogenesis.

The pattern of expression of HNF-1 α and HNF-1 β in adult tissues is unusual in that both mesodermal and endodermal structures express the proteins. This could reflect an embryonic segmental pattern in that foregut and hindgut endodermally derived structures, such as the salivary glands and uterus, lack these mRNAs, whereas mesoderm-derived structures, such as the kidney, contain them as well as all of the midgut endodermally derived structures. Segmental patterns of expression of some of the mammalian homeo domain proteins have been demonstrated (Wilkinson et al. 1989) and may be a common feature of these proteins. Murine HNF-1 α and HNF-1 β appear ~8–9 days postconception, coincident with embryonic inductive influences required for organogenesis (Le Douarin 1968; Darnell et al. 1986). The inductive influence exerted by the lateral plate mesoderm on the midgut endoderm could be controlled either by HNF-1 proteins (as they are potentially expressed in both germ layers) or it could be that the inductive influence operates by regulating HNF-1 and other proteins required for organogenesis. The ability of lateral plate mesoderm to induce hepatocytes and structures characteristic of liver, from foregut endoderm in cell culture (Le Douarin 1964, 1975), should allow direct investigation of the developmental meaning of the regulated dimerization of HNF-1 α and HNF-1 β .

Materials and methods

Cloning and sequencing of HNF-1 β

A ~2.1-kb *EcoRI*–*EcoRV* fragment of the murine HNF-1 α cDNA corresponding to the entire open reading frame plus ~250 bp and ~50 bp of the 5' and 3' UTR, respectively, was labeled with [α^{32} P]dCTP and used to screen a λ ZAP mouse liver cDNA library (Stratagene). Filters were washed under low stringency conditions (1 $\times$ SSC, 0.1% SDS at 37°C). Twenty-two positives were isolated from an initial screen of 600,000 plaques.

Sixteen of these positives could not be washed off under high-stringency conditions (0.1 $\times$ SSC, 0.1% SDS at 65°C) and were found to be clones for murine HNF-1 α . Of the remaining six positives, the two with the longest inserts (~3 kb each) were subcloned into Bluescript KS+ (Stratagene) and subjected to dideoxyl sequencing using Sequenase version 2.0 (U.S. Biochemicals) according to the manufacturer's instructions.

RNase protection assays

The riboprobe used to detect the murine HNF-1 α message has been described previously (Kuo et al. 1990). To detect the HNF-1 β message, an ~1.1-kb *Apal*–*Apal* fragment of the 28-1 clone was subcloned into Bluescript KS+, linearized with *Stu*I, and an antisense riboprobe transcribed in the presence of [α^{32} P]UTP using T7 polymerase. Full-length riboprobes were purified under denaturing conditions on 6% acrylamide/7 M urea gels before use. RNase protection assays were performed as described previously (Baumhueter et al. 1990) using 10 μ g of total RNA, or tRNA, and 2×10^5 to 5×10^5 cpm of riboprobe. The protected fragments for HNF-1 α and HNF-1 β are 277 and 210 nucleotides, respectively. The protected fragment of the HNF-1 β message corresponds to nucleotides 1314–1524 of the 28-1 cDNA insert.

Nuclear extractions

Nuclear extracts were prepared from mouse and rat tissues with minor modification of published methods (Courtois et al. 1987). Briefly, tissues were excised immediately after the animals were sacrificed and homogenized in ice-cold buffer A [10 mM HEPES (pH 7.8 at 4°C), 15 mM KCl, 2 mM MgCl₂, 1 mM DTT, 0.1 mM EDTA, 1 mM PMSF, 10 μ g/ml leupeptin, and 1 μ g/ml antipain]. The nuclei were pelleted by centrifugation, washed twice in buffer A, and resuspended in buffer A. Ammonium sulfate was added to a final concentration of 300 mM from a 3 M stock, and the mixture was rotated at 4°C for 30 min. Chromatin was precipitated by centrifugation, and solid ammonium sulfate was added to the supernatant to a final concentration of 0.3 g/ml. After 60 min at 4°C the precipitated nuclear proteins were pelleted by centrifugation and the pellet was resuspended in a minimal volume of buffer A. The extract was then dialyzed for 2 hr against at least 100 volumes of buffer C [50 mM HEPES (pH 7.8) at 4°C, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 1 mM PMSF, 10% glycerol], the dialysate changed, and the extract dialyzed for another 2 hr. The protein content of the extract was determined by Bradford assay (Bradford 1976) using bovine serum albumin as a standard. Nuclear extracts were stored frozen at –20°C or –70°C until use.

Nuclear extracts from tissue culture cells were prepared by the same procedure with the exception that cells were lysed in buffer A containing 0.2% NP-40.

Gel retardation assays

Gel retardation assays were performed as described previously (Fried and Crothers 1981; Courtois et al. 1987) using 5–20 μ g of nuclear extract protein. Extracts were incubated with 5,000–20,000 cpm of the kinased, blunt-ended β 28 probe (Baumhueter et al. 1988) in a reaction mixture containing 10 mM Tris (pH 7.8), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 5% glycerol, and poly[d(I-C)], generally 1–2 μ g, was added as competitor DNA. The sequence of the 28-bp β 28 probe is based on the HNF-1-binding site identified in the promoter for β -fibrinogen (Courtois et al. 1987). The reactions were left at room temperature for 30–60 min before being run on a 4% nondenaturing acrylamide

gel in $1 \times$ TBE buffer at 10 V/cm. After fixing in 20% methanol, 10% acetic acid, gels were dried and exposed to film at -70°C in the presence of an intensifying screen.

When indicated, 1 μl of antisera was added to the gel-shift reactions along with the other components. Reactions containing antisera were treated the same as those without serum.

Generation of antisera against HNF-1 α and HNF-1 β

Antiserum directed against HNF-1 β was generated by immunizing rabbits with a synthetic peptide, corresponding to amino acids 66–94 of HNF-1 β , coupled to keyhole limpet hemocyanin (KLH). Antiserum directed against HNF-1 α was generated by immunizing rabbits with a fusion protein corresponding to amino acids 211–524 of HNF-1 α , generated using the pET3a vector (a gift of Dr. Studier, Brookhaven National Laboratories) overexpressed in *Escherichia coli* containing the T7 expression vector (Ramadori et al. 1985). The fusion protein was isolated from *E. coli* lysates as part of an inclusion body preparation (Maniatis et al. 1982) and further purified on SDS-PAGE gels. The fusion protein was then electroeluted from the gel, dialyzed against PBS containing 0.01% SDS, and used without further modification as an antigen. After initial immunization, the animals were boosted at 6-week intervals and bled 2 weeks after each boost. TDM emulsion (Ribi Immunochem) was used as adjuvant.

Calculation of apparent molecular mass

Apparent molecular mass was calculated from the Stokes radii (R_s) and sedimentation coefficients, as described by Siegel and Monty (1966), using a standard value of 0.74 ml/g for the partial specific volume.

Stokes radii were determined using a $15 \times 1\text{-cm}$ column of Sephacryl S-300 equilibrated with buffer C. Liver nuclear extract (100–200 μg), in a total volume of 100 μl buffer C containing 15% glycerol, was loaded onto the column and allowed to fractionate on the column at a flow rate of 2 ml/cm²/hr. Fifty-drop fractions were collected and analyzed for the presence of HNF-1-binding activity in a gel retardation assay in a total reaction volume of 20 μl containing 15 μl of each fraction, 1 μg of poly[d(I-C)] as competitor DNA, and 5,000–20,000 cpm of ³²P-labeled $\beta 28$ probe. The amount of HNF-1-binding activity in each fraction was determined by densitometry scanning of the exposed autoradiogram to determine the amount of radioactive probe that migrated at the position of the HNF-1 complex.

Samples containing HNF-1-DNA complexes were incubated at room temperature with $\sim 50,000$ cpm of the $\beta 28$ probe in the presence of 5 μg of poly[d(I-C)] before being loaded onto the column. The elution profile of the HNF-1-DNA complex was determined by assaying the eluted fractions for radioactivity. Elution volumes (V_e) were determined and compared with the elution volume of Blue Dextran 2000 as an indicator of the void volume (V_o). Stokes radii were estimated from the V_e/V_o measured for the test samples based on a standard plot of V_e/V_o versus $\log R_s$ for standard proteins of known R_s . The standard proteins and their R_s are myoglobin (2.0 nm), carbonic anhydrase (2.3 nm), ovalbumin (2.9 nm), bovine serum albumin (3.6 nm), β -amylase (4.8 nm), catalase (5.2 nm), and ferritin (6.1 nm) (Holbrook et al. 1983).

Sedimentation coefficients ($S_{20,w}$) were determined using 11 ml linear gradients of 7.5–30% glycerol in buffer C. Approximately 150 μg of liver nuclear extract in a final volume of 150 μl of buffer, containing 5% glycerol, was layered onto the top of the gradients, and the samples were centrifuged at 40,000 rpm in a SW 45 rotor for 22 hr at 3°C . At the end of the centrifuga-

tion, 20-drop fractions were collected from the bottom of the tubes, and these fractions were assayed for the presence of HNF-1-binding activity as described for the fractions collected after gel filtration. Luciferase and catalase were included as internal standards and their presence was detected by the assays of de Wet et al. (1987) and Beers and Sizer (1952), respectively. Sedimentation coefficients were estimated from a standard plot of fraction number versus log sedimentation coefficient using standard proteins of known sedimentation coefficients. The standard proteins and their sedimentation coefficients are carbonic anhydrase (3.2S), bovine serum albumin (4.4S), β -amylase (9.4S), and catalase (11.3S) (Holbrook et al. 1983). A sedimentation value of 4.05S was determined for luciferase on the basis of several measurements relative to the standard proteins.

Expression of HNF-1 α and HNF-1 β in mammalian cells

The HNF-1 α expression vector containing the HNF-1 α cDNA in the SR α -based pBJ5 vector has been described previously (Kuo et al. 1990). The $\sim 3\text{-kb}$ cDNA for HNF-1 β was excised from Bluescript KS+ with *EcoRI* and inserted into the pBJ5 expression vector in the correct orientation at a unique *EcoRI* site. To prepare the HNF-1 β dimerless expression vector containing HNF-1 β lacking amino acids 2–30, the synthetic oligonucleotides 5'-CGCGGATCCAAAATGGAGGAGTTACTGCCG-3' and 5'-CTGGTATCTGTGACCAC-3' were used as coding and noncoding primers, respectively, in a PCR reaction in which the 28-1 insert was used as template. The $\sim 1.4\text{-kb}$ PCR product encoding a start methionine, in the context of the AAAATGG Kozak sequence (Kozak 1986) found in the message for HNF-1 β , followed by amino acids 31–512 of HNF-1 β , was cut with *BamHI* and *ApaI*, and the $\sim 1\text{-kb}$ DNA fragment corresponding to the 5' end of the PCR product was subcloned into Bluescript K5+. A $\sim 2\text{-kb}$ *SmaI*-*KpnI* fragment corresponding to the 3' end of the 28-1 insert extending 5' to amino acid 51 of HNF-1 β was used to replace the *SmaI*-*KpnI* fragment of the PCR product in Bluescript. This enabled us to recreate the full open reading frame of HNF-1 β , minus amino acids 2–30, without any of the 5'UTR. Only 75 bases of the final construct were generated by PCR, and the first 150 bases of the construct were sequenced to ensure that there had been no mutations due to PCR or subsequent cloning steps. The dimerless expression vector was generated by cutting the insert out of Bluescript KS+ using *NotI* and *EcoRI* and inserting this fragment into pBJ5 at the *NotI* and *EcoRI* sites in the polylinker. The same approach was used to generate an expression vector containing the full open reading frame of HNF-1 β without the 5' UTR, except that the coding primer used in the PCR reaction had the sequence 5'-CGCGGATCCTGGAAAATGGTGTCC-3'. This latter expression vector was then cut at *BstEII* and *EcoRI*, the sites were bluntended, and the plasmid was reclosed to create the expression vector lacking the last 182 amino acids of HNF-1 β (truncated β).

COS cells, cultured in DMEM plus 10% FCS, were plated at a density of 8×10^5 cells per dish and, 6–12 hr later, were cotransfected with 5 μg of the ($\beta 28$)₃-CAT reporter gene and 1–10 μg of either or both the HNF-1 α or HNF-1 β expression vectors, as described previously (Courtois et al. 1987; Kuo et al. 1990). Jurkat cells expressing large T antigen (J. Northrop, unpubl.), cultured in RPMI plus 10% FCS, were transfected by electroporation with a Bio-Rad Gene Pulser at 960 μF and 250 V. Cells were harvested and CAT activity was determined (Gorman et al. 1982) at 36–48 hr after transfection. CAT assays were quantitated on an AMBIS radioactivity scanner. Transfections of cells to be assayed for CAT activity contained 10 μg of an RSV-luciferase plasmid (Courtois et al. 1987) as an internal control for transfection efficiency. The CAT activity in the extracts

of the transfected cells was normalized according to the luciferase activity (de Wet et al. 1987) assayed in separate aliquots of the cells.

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Note added in proof

Sequence data described in this paper have been submitted to the EMBL/GenBank Data Libraries.

References

- Baichwal, V.R. and R. Tjian. 1990. Control of c-Jun activity by interaction of a cell-specific inhibitor with regulatory domain δ : Differences between v- and c-Jun. *Cell* **63**: 815–825.
- Baumhueter, S., G. Courtois, and G.R. Crabtree. (1988). A variant nuclear protein in dedifferentiated hepatoma cells binds to the same functional sequences in the β fibrinogen gene promoter as HNF-1. *EMBO J.* **7**: 2485–2493.
- Baumhueter, S., D.B. Mendel, P.B. Conley, C.J. Kuo, C. Turk, M.K. Graves, C.A. Edwards, G. Courtois, and G.R. Crabtree. 1990. HNF-1 shares three sequence motifs with the POU domain proteins and is identical to LF-B1 and APF. *Genes & Dev.* **4**: 372–379.
- Beers, R.F. Jr. and I.W. Sizer. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **195**: 133–140.
- Benezra, R., R.L. Davis, D. Lockshon, D.L. Turner, and H. Weintraub. 1990. The protein Id: A negative regulator of helix-loop-helix DNA binding proteins. *Cell* **61**: 49–59.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254.
- Cereghini, S., M. Blumenfeld, and M. Yaniv. 1988. A liver-specific factor essential for albumin transcription differs between differentiated and dedifferentiated rat hepatoma cells. *Genes & Dev.* **2**: 957–974.
- Chiu, R., W.J. Boyle, J. Meek, T. Smeal, T. Hunter, and M. Karin. 1988. The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* **54**: 541–552.
- Chouard, T., M. Blumenfeld, I. Bach, J. Vandekerckhove, S. Cereghini, and M. Yaniv. 1990. A distal dimerization domain is essential for DNA-binding by the atypical HNF1 homeo domain. *Nucleic Acids Res.* **18**: 5853–5863.
- Clayton, D.F., M. Weiss, and J.E. Parrell. 1985. Liver-specific RNA metabolism in hepatoma cells: Variations in transcription rates in mRNA levels. *Mol. Cell. Biol.* **5**: 2633–2641.
- Courtois, G., J.G. Morgan, L.A. Campbell, G. Fourel, and G.R. Crabtree. 1987. Interaction of a liver-specific nuclear factor with the fibrinogen and alpha 1-antitrypsin promoters. *Science* **238**: 688–692.
- Courtois, G., S. Baumhueter, and G.R. Crabtree. 1988. Purified hepatocyte nuclear factor 1 interacts with a family of hepatocyte-specific promoters. *Proc. Natl. Acad. Sci.* **85**: 7937–7941.
- Darnell, J.E., H. Lodish, and D. Baltimore. 1986. *Molecular cell biology*. Scientific American Books, New York.
- Deschatrette, J. and M.C. Weiss. 1974. Characterization of differentiated and dedifferentiated clones of a rat hepatoma. *Biochimie* **56**: 1603–1611.
- Deschatrette, J., E.E. Moore, M. Dubois, and M. Weiss. 1980. Dedifferentiated variants of a rat hepatoma: Reversion analysis. *Cell* **19**: 1043–1051.
- Descombes, P., M. Chojkier, S. Lichtsteiner, E. Falvey, and U. Schibler. 1990. LAP, a novel member of the C/EBP gene family, encodes a liver-enriched transcriptional activator protein. *Genes & Dev.* **4**: 1541–1551.
- Dollé, P., J.-L. Castrillo, L.E. Theill, T. Deerinck, M. Ellisman, and M. Karin. 1990. Expression of GHF-1 protein in mouse pituitaries correlates both temporally and spatially with the onset of growth hormone gene activity. *Cell* **60**: 809–820.
- Finney, M. 1990. The homeodomain of the transcription factor LF-B1 has a 21 amino acid loop between helix 2 and helix 3. *Cell* **60**: 5–6.
- Frain, M., G. Swart, P. Monaci, A. Nicosia, S. Stämpfli, R. Frank, and R. Cortese. 1989. The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. *Cell* **59**: 145–157.
- Fried, M.G. and D.M. Crothers. 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res.* **9**: 6505–6526.
- Friedman, J.M., L.E. Babiss, M. Weiss, and J.E. Darnell, Jr. 1987. Hepatoma variants (C2) are defective for transcriptional and post-transcriptional actions from both endogenous and viral genomes. *EMBO J.* **6**: 1727–1731.
- Gerster, T. and R.G. Roeder. 1988. A herpesvirus trans-activating protein interacts with transcription factor OTF-1 and other cellular proteins. *Proc. Natl. Acad. Sci.* **85**: 6347–6351.
- Gorman, C.M., L.F. Moffat, and B.H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2**: 1044–1050.
- Gourdeau, H., T.C. Peterson, and R.E.K. Fournier. 1989. Differential activity of a tissue-specific extinguisher locus in hepatic and nonhepatic cells. *Mol. Cell. Biol.* **9**: 1813–1822.
- Goutte, C. and A.D. Johnson. 1988. α 1 protein alters the DNA binding specificity of β 2 repressor. *Cell* **52**: 875–882.
- Hardon, E.M., M. Frain, G. Paonessa, and R. Cortese. 1988. Two distinct factors interact with the promoter regions of several liver-specific genes. *EMBO J.* **7**: 1711–1719.
- Herskowitz, I. 1989. A regulatory hierarchy for cell specialization in yeast. *Nature* **342**: 749–757.
- Holbrook, N.J., J.E. Bodwell, M. Jeffries, and A. Munck. 1983. Characterization of nonactivated and activated glucocorticoid-receptor complexes from intact rat thymus cells. *J. Biol. Chem.* **258**: 6477–6485.
- Hope, I.A. and K. Struhl. 1987. GCN4, a eukaryotic transcriptional activator protein, binds as a dimer to target DNA. *EMBO J.* **6**: 2781–2784.
- Ingraham, H.A., S.E. Glynn, J.W. Voss, V.R. Albert, M.S. Kapiloff, L. Wilson, and M.G. Rosenfeld. 1990. The POU-specific domain of Pit-1 is essential for sequence-specific, high affinity DNA binding and DNA-dependent Pit-1-Pit-1 interactions. *Cell* **61**: 1021–1033.
- Johnson, A.D., A.R. Poteete, G. Lauer, R.T. Sauer, G.K. Ackers, and M. Ptashne. 1981. γ repressor and *cro*-components of an

- efficient molecular switch. *Nature* **294**: 217–233.
- Johnson, P.F., W.H. Landschulz, B.J. Graves, and S.L. McKnight. 1987. Identification of a rat liver nuclear protein that binds to the enhancer core element of three animal viruses. *Genes & Dev.* **1**: 133–146.
- Keleher, C.A., C. Goutte, and A.D. Johnson. 1988. The yeast cell-type-specific repressor $\alpha 2$ acts cooperatively with a non-cell-type-specific protein. *Cell* **53**: 927–936.
- Killary, A.M. and R.E. Fournier. 1984. A genetic analysis of extinction: *trans*-dominant loci regulate expression of liver-specific traits in hepatoma hybrid cells. *Cell* **38**: 523–534.
- Kozak, M. 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* **44**: 283–292.
- Kuo, C.J., P.B. Conley, C. Hsieh, U. Franke, and G.R. Crabtree. 1990. Molecular cloning, functional expression, and chromosomal localization of mouse HNF-1. *Proc. Natl. Acad. Sci.* **87**: 9838–9842.
- Kuo, C.J., D.B. Mendel, L.P. Hansen, and G.R. Crabtree. 1991. Independent regulation of HNF-1a and HNF-1b by retinoic acid in F9 teratocarcinoma cells. *EMBO J.* (in press).
- Lai, E., V.R. Prezioso, E. Smith, O. Litvin, R.H. Costa, and J.E. Darnell Jr. 1990. HNF-3A, a hepatocyte-enriched transcription factor of novel structure is regulated transcriptionally. *Genes & Dev.* **4**: 1427–1436.
- Landschulz, W.H., P.F. Johnson, E.Y. Adashi, B.J. Graves, and S.L. McKnight. 1988a. Isolation of a recombinant copy of the gene encoding C/EBP. *Genes & Dev.* **2**: 786–800.
- Landschulz, W.H., P.F. Johnson, and S.L. McKnight. 1988b. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. *Science* **240**: 1759–1764.
- Lassar, A.B., B.M. Paterson, and H. Weintraub. 1986. Transfection of a DNA locus that mediates the conversion of 10T1/2 fibroblasts to myoblasts. *Cell* **47**: 649–656.
- Le Douarin, N. 1964. Isolement experimental du mesenchyme propre du foie et role morphogene de la composante mso-dermique dans l'organogenese hpatique. *J. Embryol. Exp. Morphol.* **12**: 141–160.
- Le Douarin, N. 1968. Synthse du glycogne dans les hepatocytes en voie de differenciation: Role des mesenchymes homologues et heterologues. *Dev. Biol.* **17**: 101–114.
- Le Douarin, N. 1975. An experimental analysis of liver development. *Med. Biol.* **53**: 427–455.
- Maire, P., J. Wuarin, and U. Schibler. 1989. The role of *cis*-acting promoter elements in tissue-specific albumin gene expression. *Science* **244**: 343–346.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Mueller, C.R., P. Maire, and U. Schibler. 1990. DBP, a liver-enriched transcriptional activator, is expressed late in ontogeny and its tissue specificity is determined posttranscriptionally. *Cell* **61**: 279–291.
- Murre, C., P. Schonleber McCaw, and D. Baltimore. 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, MyoD, and *myc* proteins. *Cell* **56**: 777–783.
- Nicosia, A., P. Monaci, L. Tomei, R. De Francesco, M. Nuzzo, H. Stunnenberg, and R. Cortese. 1990. A myosin-like dimerization helix and an extra-large homeodomain are essential elements of the tripartite DNA binding structure of LFB1. *Cell* **61**: 1225–1236.
- O'Neill, E.A. and T.J. Kelly. 1988. Purification and characterization of nuclear factor III (origin recognition protein C), a sequence-specific DNA binding protein required for efficient initiation of adenovirus DNA replication. *J. Biol. Chem.* **263**: 931–937.
- Poellinger, L. and R.G. Roeder. 1989. Octamer transcription factors 1 and 2 each bind to two different functional elements in the immunoglobulin heavy-chain promoter. *Mol. Cell. Biol.* **9**: 747–756.
- Ramadori, G., J.D. Sipe, C.A. Dinarello, S.B. Mizel, and H.R. Colten. 1985. Pretranslational modulation of acute-phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. *J. Exp. Med.* **162**: 930–942.
- Rauscher, F.J. III, P.J. Voulalas, B.R. Franza, Jr., and T. Curran. 1988. Fos and Jun bind cooperatively to the AP-1 site: Reconstitution in vitro. *Genes & Dev.* **2**: 1687–1699.
- Roussou, I., G. Thireos, and B.M. Hauge. 1988. Transcriptional-translational regulatory circuit in *Saccharomyces cerevisiae* which involves the GCN4 transcriptional activator and the GCN2 protein kinase. *Mol. Cell. Biol.* **8**: 2132–2139.
- Schorpp, M., W. Kugler, U. Wagner, and G.U. Ryffel. 1988. Hepatocyte-specific promoter element HPI of the *Xenopus* albumin gene interacts with transcriptional factors of mammalian hepatocytes. *J. Mol. Biol.* **202**: 307–320.
- Siegel, L.M. and K.J. Monty. 1966. Determination of molecular weights and frictional ratios of proteins in impure systems by the use of gel filtration and density gradient centrifugation. Application to crude preparations of sulfite and hydroxylamine reductases. *Biochem. Biophys. Acta* **112**: 346–362.
- Stern, S., M. Tanaka, and W. Herr. 1989. The Oct-1 homeodomain directs formation of a multiprotein RNA complex with the HSV transactivator VP16. *Nature* **341**: 624–630.
- Strickland, S. 1981. Mouse teratocarcinoma cells: Prospects for the study of embryogenesis and neoplasia. *Cell* **24**: 277–278.
- Strickland, S., K.K. Smith, and K.R. Marotti. 1980. Hormonal induction of differentiation in teratocarcinoma stem cells: Generation of parietal endoderm by retinoic acid and dibutyryl cAMP. *Cell* **347**: 347–355.
- Tanaka, M. and W. Herr. 1990. Differential transcriptional activation by Oct-1 and Oct-2: Interdependent activation domains induce Oct-2 phosphorylation. *Cell* **60**: 375–386.
- Tapscott, S.J., R.L. Davis, M.J. Thayer, P.-F. Cheng, H. Weintraub, and A.B. Lassar. 1988. MyoD1: A nuclear phosphoprotein requiring a Myc homology region to convert fibroblasts to myoblasts. *Science* **242**: 405–411.
- Turner, R. and R. Tjian. 1989. Leucine repeats and an adjacent DNA binding domain mediate the formation of functional c-Fos–c-Jun heterodimers. *Science* **243**: 1689–1694.
- De Wet, J.R., K.V. Wood, M. DeLuca, D.R. Helinski, and S. Subramani. 1987. The firefly luciferase gene: Structure and expression in mammalian cells. *Mol. Cell. Biol.* **7**: 725–737.
- Wilkinson, D.G., S. Bhatt, M. Cook, E. Boncinelli, and R. Krumlauf. 1989. Segmental expression of Hox-2 homeobox-containing genes in the developing mouse hindbrain. *Nature* **341**: 405–409.

HNF-1 alpha and HNF-1 beta (vHNF-1) share dimerization and homeo domains, but not activation domains, and form heterodimers in vitro.

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