HNF-1 shares three sequence motifs with the POU domain proteins and is identical to LF-B1 and APF

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The coordinate expression of genes during development and differentiation is thought to be accomplished by common transcription factors operating on the promoters of families of coexpressed genes. HNF-1 is a transcriptional factor involved in the expression of genes in the liver and was originally defined as playing a major role in coordinating the expression of the linked fibrinogen genes. We have isolated cDNA clones for HNF-1 using oligonucleotides prepared to the sequence of the purified protein. The sequence of HNF-1 shares the homeo domain, as well as short acidic and basic sequences with the POU family of transcriptional activators. Peptides from the protein interacting with the albumin proximal element, or B box (APF), and the factor interacting with the α_1 -antitrypsin promoter (LF-B1) are found in the predicted sequence of HNF-1. HNF-1 mRNA is not present in the dedifferentiated hepatoma variant, C2, but reappears upon selection for gluconeogenesis coincident with the re-expression of liver-specific genes. Finally, the mRNA is not present in somatic cell hybrids in which liver-specific gene expression is extinguished. In contrast to earlier published results, we find that in addition to being present in the liver, HNF is expressed in the kidney, intestine, and spleen, but not in other tissues. This pattern of expression mirrors the complex pattern of expression of many genes, such as α -fetoprotein, α_1 -antitrypsin, and fibrinogen, whose promoters contain HNF-1 sites. These data indicate that HNF-1 is a more broadly acting transcription factor than has been indicated by previous work.

[Key Words: HNF-1; transcription factors; POU domain proteins]

Received November 1, 1989; revised version accepted December 14, 1989.

Several classes of regulatory proteins have been identified that function by coordinating the activity of large families of genes to achieve a particular cellular fate or phenotype. Most notable are the homeotic genes of insects, mutation of which leads to specific alterations in cell fate. More recently, genes with related functions have been identified in mammals. For example, MyoD (Davis et al. 1987; Tapscott et al. 1988), myogenin (Wright et al. 1989), and Myd (Pinney et al. 1988) appear to be sufficient to impart the muscle phenotype to fibroblasts and other cell types. Despite their role in determining cell phenotype, these genes do not have a homeo domain. A third group of genes thought to have a role in determining cell phenotype was described recently. These genes contain an extensive region of sequence similarity including a homeo domain, and members of the group, referred to as the POU domain proteins (Herr et al. 1988), include the transcription factors Pit-1 (Ingraham et al. 1988) or growth hormone factor (GHF)

(Bodner et al. 1988), Oct-1 (Singh et al. 1986; Sturm et al. 1988), Oct-2 (Landolfi et al. 1986; Clerc et al. 1988; Ko et al. 1988; Muller et al. 1988; Scheidereit et al. 1988), and Unc-86 (Finney et al. 1988). The functions of this extensive region of sequence similarity are not clear, but one known function is DNA binding (Sturm and Herr 1988).

We have searched for transcription factors that might coordinate the expression of genes during liver differentiation and development by analyzing the promoters for the three fibrinogen genes (Courtois et al. 1987). These genes encode polypeptide chains that are incorporated into the mature fibrinogen molecule and are coordinately expressed (Crabtree and Kant 1982). These studies led to the recognition of a transcription factor, HNF-1, that interacts with sequences in the promoters of the α and β chains of fibrinogen essential for their transcriptional activation (Courtois et al. 1987, 1988). The tissue distribution, sequence specificity, and electrophoretic migration of DNA-protein complexes formed with the α - and β -chain sequences were shown to be identical to DNA-protein complexes formed with the promoter of the α_1 -antitrypsin gene, indicating that the same or sim-

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ilar proteins may bind to all three sequences (Courtois et al. 1987). In subsequent studies, HNF-1 was purified and found to be 88 kD (Baumhueter et al. 1988; Courtois et al. 1988), and the purified protein was shown to bind to functional sequences within the promoters of albumin at a site that had previously been termed the albumin B box (Lichtsteiner et al. 1987) or the albumin proximal region (Cereghini et al. 1987). In addition, purified HNF-1 was found to bind to the α_1 -antitrypsin, α fetoprotein, transthyretin, and the pre-S1 promoter of the hepatitis B virus (Courtois et al. 1988) at sites that had been implicated in the tissue-specific expression of these genes (Scott et al. 1984; Costa et al. 1986, 1989; Godbout et al. 1986; Shen et al. 1987; Burk et al. 1988; Hardon et al. 1988; Li et al. 1988; Monaci et al. 1988). Furthermore, most of the tissue specificity of the albumin promoter could be attributed to a site for the C/EBP protein (Johnson et al. 1987; Landschulz et al. 1988) and to a region termed the albumin B box or the albumin proximal sequence (Gorski et al. 1986; Cereghini et al. 1988; Lichtsteiner and Schibler 1989). The latter interacted with a protein estimated to be 90 kD by UV cross-linking and was found to have a sequence specificity similar to the protein binding to the fibrinogen sequence (Cereghini et al. 1988; Lichtsteiner and Schibler 1989). Subsequent characterization of the protein that interacted with the promoter for α_1 -antitrypsin (LF-B1) indicated that the sequences protected with nuclear extracts of liver cells differed slightly from that originally thought to be due to HNF-1 (Hardon et al. 1988; Monaci

et al. 1988). Furthermore, the protein purified with the α_1 -antitrypsin sequence was 45 kD, as opposed to 88 kD (Frain et al. 1989). These results indicated that a different protein might interact with the α_1 -antitrypsin gene.

We have used the HNF-1 site in the β chain of fibrinogen to purify HNF-1. The determined amino acid sequence of several fragments of this protein was used to isolate cDNA clones. The sequence of the cDNA clone is identical to that of LF-B1, which was recently cloned using oligonucleotides corresponding to the protein purified by affinity chromatography with the α_1 -antitrypsin promoter (Frain et al. 1989). The clone for LF-B1 has been shown to produce a protein that binds the LF-B1 site and is functional in promoting in vitro transcription at the α_1 -antitrypsin promoter (Frain et al. 1989). In addition, peptides derived from the protein purified with the putative HNF-1 site in the albumin promoter are present in the cDNA sequence of HNF-1 (M. Yaniv, pers. comm.). These data establish HNF-1 as a broadly acting transcription factor dictating the pattern of expression of a group of proteins in the liver and selected other tissues.

Results

Isolation of HNF-1 cDNA clones

We have purified HNF-1 from rat liver nuclear extracts by DNA sequence affinity chromatography (Herrick 1980; Kadonaga and Tjian 1986). The purified material

Figure 1. (a) Purified HNF-1, analyzed by SDS-polyacrylamide gel electrophoresis and visualized by silver staining, migrates as a single broad band with an apparent molecular mass of 88 kD. (Right) The positions of molecular weight markers. (b) The amino acid sequence of two peptides derived from purified HNF-1. Below is the nucleotide sequence of the degenerate oligonucleotides that were used as primers in the PCR to amplify a partial HNF-1 cDNA. Choices in the degenerate oligonucleotides are indicated by asterisks (*). The amino acids that were not included in the synthetic oligomers are also shown.

migrated as a single, relatively broad band in an SDS-polyacrylamide gel with an apparent molecular mass of 88 kD (Fig. 1a). This protein was further purified by reverse-phase high-performance liquid chromatography (HPLC), and the single peak renatured. This renatured protein exhibited DNA-binding activity identical to that of HNF-1. Peptides obtained from cyanogen bromide or endoproteinase ArgC were sequenced after HPLC separation. On the basis of the amino acid sequence of two of the peptides, degenerate oligonucleotides were designed (Fig. 1b) and used as primers in a polymerase chain reaction (PCR) in which first-strand cDNA synthesized from poly(A)-selected mRNA of the cell line Rev7 (Deschattre et al. 1980) served as the template. The PCR product of 144 bp was sequenced and found to encode the 10 additional amino acids that had not been included in the two oligonucleotides. This short HNF-1 cDNA was then used to screen two λ gt10 cDNA libraries, constructed from Rev7 and rat liver poly(A)⁺ mRNA. Twenty clones were isolated in a screen of 3×10^6 primary recombinants; the largest cDNA insert was 3.6 kb. The nucleotide sequence of one of the partial cDNA clones is shown in Figure 2. Three of the peptides derived from purified HNF-1 appeared in the predicted amino acid sequence (Fig. 2), providing evidence that the cDNA clone encodes HNF-1.

HNF-1 is a homeo domain protein

Sequence analysis of previously identified DNA-binding proteins has shown that only a small number of conserved motifs are used to form a DNA-binding domain. The most commonly found are zinc fingers (Miller et al. 1985) and the homeo box, which is present in *Drosophila* homeotic gene products, some yeast proteins, the mammalian *hox* proteins (Scott et al. 1989), and a recently established subgroup called POU domain proteins (Herr et al. 1988). Figure 3a shows that HNF-1 displays 20% sequence identity with the 60-amino-acid region that constitutes the homeo domain of the *Drosophila* paired (*prd*) protein and the mammalian transcription factor Oct-2. The highest degree of sequence identity is centered around the WFXNRR motif in the third helix, which is the most conserved among the known homeo domain proteins. HNF-1 exhibits little sequence identity with other homeo domain proteins throughout the other two helices. Comparison of the amino acid sequence of HNF-1 with that of LF-B1, purified by affinity chromatography using the human α_1 -antitrypsin gene (Frain et al. 1989), indicates that the two sequences are completely identical, with the exception of two additional cytosines in the 3'-noncoding sequence not present in our sequence indicated in Figure 2. These additional cytosines most likely represent sequencing errors because there is only a single gene for HNF-1 detected by high-stringency Southern blots (Frain et al. 1989).

Interestingly, two short sequence motifs located upstream of the homeo domain in HNF-1 are similar to regions within and slightly upstream of the A box of the

POU-specific box (Fig. 3b). A region corresponding to the B box of the POU-specific domain is not present in HNF-1. The POU domain has been found in a limited

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5' CGGTACGTCGCGAAGCAGCGAGAGTGGCTCAGCAATTACCCACGCGGGCAGGGCGGA
  R Y V R K Q R E V A Q Q F T H A G Q G G
  CTGATTGAAGAGCCACAGGTGATGAGCTGCCAACCAAAAGGGCGGAGGAACCGGTTTC
  L I E E P T G D E L P T K K G R R N R F
  AAGTGGGGCCCGCATCCAGCAGATCCTGTTCCAGGCTTACGAGAGGAGCAAGAACCC
  K W G P A S Q Q I L F Q A Y E R Q K N P
  AGCAAGGAAGAGCGAGAGACCTTGGTGGAGGAGTGCAATAGGGCGGAGTGATCCAGAGA
  S K E E R E T L V E E C N R A E C I Q R
  GGGGTGTACCATCGCAGGCGCCAGGGCTAGGCTCCAACTTGTACCGAGGTGCGTGTG
  G V S P S Q A Q G L G S N L V T E V R V
  TACAACCTGGTTTGCACCGCGCAAGAGAGACCTTTCGGCATAAGCTGGCCATGGAC
  Y N W F A N R R R K E E A G F R H K L A M Q
  ACGTATAACGGGCTCCACCGGGCCAGGCGCGGCGCTGGCTACCTGCCACAGTTCC
  T Y N G P P P G P G P G P A L P A H S S
  CCGGGCTGCCACAACCCCTCTCTCCAGTAAGTCCAGGTGTGGGTATGGACAG
  P G L P T T T L S P S K V H G V R Y A G Q
  TCTGCAACAGCGAGGCGAGTGGTGCCTCCAGCGGAGGTGCTTGTGTACAGTGTG
  S A T S E A A E V P S S S G G P L V T V
  TCTGGCGCTTACACCAAGTGTCCCGCCAGAGGCTGGAGCCAGCAGCTGTGAGCACC
  S A A L H Q V S P T G L E P S S L L S T
  GAGGCAAGCTGGTCTCAGCCACGGGGGTCCCTGCTCCCGTCAGCACCTGACAGCA
  E A K L V S A T G G L P P V S T L T A
  CTGCACAGCTTGGAGCAGCTCTCCAGGTCTCAACAGCAGCGCAGAACCTTATCATG
  L H S L E Q T S P G L N Q Q P Q N L I M
  GCCTGCTGCTGGGTGATGACCATCGGCCAGGGAGCGCGCTCCCTGGGTCCACAG
  A S L P G V M T I G P G E P A S L G P T
  TTCCTAACACGGGTGCTCTACCTGGTTATTGGTCTGGCTCCACAGGACAGAGC
  F T N T G A S T L G L A S T Q A Q S
  GTGCAGTCATCAACAGCATGGGGAGCAGCTGACCCCTGCAGCGGTCCAGTTTTC
  V P V I N S M G S S L T T L Q P V Q F S
  CAGCCACTGCACCTTCTCTCAGCAGCTCTCATGCCCTGTACAGAGCCAGTGGCC
  Q P L H P S Y Q Q P L M P P V Q S H V A
  CAGAGTCCTTTCAGCAACCATGGCCAGCTGACAGAGCCCGCCCTGTACAGCCAC
  Q S P F M A T M A Q L Q S P H A L Y S H
  AAGCTGAGGTGGCCAGTACACGCATACAAGCTGCTTCCGAGACCATGCTGATCACA
  K P E V A Q Y T H T S L L P Q T M L I T
  GACACCACTCAGCACCTTGCCAGCTCAGCCGCCAACAGAGGTCTTCACTCAGAC
  D T N L S T L A S L T P T K Q V F T S D
  ACAGAGCCTCCAGTGAGCCTGGGCTTCAAGCCGCTGCTCCAGCCACACCACTTAC
  T E A S S E P G L H E P S S P A T T I H
  ATCCCCAGCCAGGACCGCTCAACATCAGCACCTGAGCCTGCTCACCGGCTCAGCACC
  I P S Q D P S N I Q H L Q P A H R L S T
  AGTCCACAGTGTCTCCAGCAGCTGGTGTGTACAGAGTCTGACTCCACGGGCAC
  S P T V S S S S L V L Y Q S S D S N G H
  AGCCACTCTGCTCCATCAACCCAGGTGTATCAGAGACTTTTATCTCCACCCAGATGGCC
  S H L L P S N H G V I D T F I S T Q M A
  TCCTCTCCAGTAACCATGGTGAAGTCTCCAGGAGCTGGGTCCAGAGCTGCACA
  S S S Q *
  GGGGAGAGGAGGGCCACAGGCATGCTGCTGGAGGTGTGGAGCTGCCACCTGCCAC
  AGGTGCTGGCTTCCAGAACTCTATGCTTCTGCTGAGCTGCTCTCCATCATCAG
  AAAGGATGGCTCTGAGGTGTCTCTCAGCTGAGGGGCAAGCTCAAGGACCGGAGGA
  CAGCCCAATCTGGACCGCCATCCCTGCTGGTTAGAAATAGAAACTTAATGCTTGAACAG
  AAGGGGAAGCCTGTATTGCTGGCACCTCCAGTCAGAGCTTGACGGCCCTGAAGATCT
  CTGCTGAGCTC

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Figure 2. Nucleotide sequence and predicted amino acid sequence of an HNF-1 cDNA clone. The three peptides obtained from purified HNF-1 that occur in the sequence are underlined. Two additional cytosines present in the sequence of Frain et al. (1989) are indicated (*).

HNF-1 is a broadly expressed homeo domain protein

Figure 3. (a) Alignment of the homeo domain sequence of HNF-1 with several other homeo domains. Identical residues are indicated by boldface letters and dark shading; similar amino acids (K-R, E-D, P-G, L-V-I-A, Y-W-F-H, M-C, and T-S) are lightly shaded. (Right) Percent overall identity (60 amino acids), percent similarity, and percent identity in the third helix only (outlined above). (b) Alignment of HNF-1 with Oct-2, Pit-1, and *unc-86* in the region of the A box of the POU-specific domain.

number of mammalian transcription factors. These include Oct-1 (Singh et al. 1986; Sturm et al. 1988), which is ubiquitously expressed, and two tissue-specific nuclear proteins, Oct-2 (Landolfi et al. 1986; Clerc et al. 1988; Ko et al. 1988; Muller et al. 1988; Scheidreit et al. 1988) and Pit-1 (Ingraham et al. 1988; Bodner et al. 1988), whose expression is restricted to lymphocytes and the somatotroph cell type of the pituitary, respectively. In addition, He et al. (1989) have recently isolated a number of cDNAs encoding POU domain proteins that are expressed mainly in the developing nervous system (*brn1-3*) and testis (*tst1*) but whose functions are not yet known. Studies with in vitro-expressed Oct-1 indicate that the A box of the POU-specific domain is required for DNA binding, because deletion or alanine substitution mutation (Sturm and Herr 1988) in this region results in a loss of binding activity.

HNF-1 is not liver-specific

On the basis of earlier results, we have speculated that HNF-1 is involved in specifying the hepatocyte phenotype. We used the sensitive ribonuclease protection assay to analyze the tissue distribution of HNF-1 mRNA and test its expression in different cell types. Surprisingly, HNF-1 mRNA is present in similar amounts in liver and kidney (Fig. 4b, lanes 8 and 4, respectively) and at substantially lower levels in intestine, spleen, and thymus (4-fold, 10-fold, and 24-fold, respectively). These differences are not likely to be attributable to tissue-spe-

cific alternative splicing, because probes from the extreme 3' and 5' ends of the clone detect mRNA in the kidney, intestine, and spleen similar in size to those seen on Northern blots of liver RNA (Fig. 4a). HNF-1 transcripts were not detectable in skin, lung, heart, or brain. To establish whether kidney cells not only contain HNF-1 transcripts but also synthesize the protein, we prepared nuclear extracts and performed a DNase I footprint analysis. Figure 4c shows that kidney nuclear extracts protect the functional HNF-1-binding site in the β -fibrinogen promoter; this footprint is indistinguishable from that obtained with liver nuclear extracts (cf. lanes 3 and 5). Finally, to determine whether the HNF-1 is transcriptionally active in the kidney, we examined the expression of the three fibrinogen chains in the liver, lung, brain, and kidney. As shown in Figure 5, the α and β chains of fibrinogen are expressed in the liver and kidney, whereas the γ chain is expressed in the liver and lung. The γ chain does not have an HNF-1 binding site in its promoter and is under the control of adenovirus major late transcription factor (MLTF), Sp1, CBP2, and a NF- κ B-like protein (Chodosh et al. 1988a,b; Morgan et al. 1988).

Expression of HNF-1 in dedifferentiated cells and somatic hybrids

In earlier experiments we analyzed the dedifferentiated hepatoma cell line C2 and the revertant Rev7 for HNF-1-binding activity. The Rev7 cell line was selected

Figure 4. (a) Northern blot using 10 μ g of poly(A)-selected rat liver RNA. (b) RNase mapping of HNF-1 transcripts in adult rat tissues. (Lane 1) Spleen; (lane 2) skin; (lane 3) lung; (lane 4) kidney; (lane 5) intestine; (lane 6) heart; (lane 7) brain; (lane 8) liver; (lane 9) thymus; (lane 10) probe alone. (c) DNase I footprint analysis of the β -fibrinogen gene promoter with kidney and liver nuclear extracts. (Lane 1) 25 μ g of BSA; (lanes 2 and 3) 25 μ g or 50 μ g of kidney extract; (lane 4) 50 μ g of kidney extract in the presence of 100-fold excess of the synthetic oligonucleotide containing the HNF-1 binding site; (lane 5) 25 μ g of liver extract. (d) RNase mapping of HNF-1 transcripts in hepatoma cell lines and somatic hybrids. (Lane 1) Rat hepatoma cell line Fao; (lane 2) dedifferentiated variant C2; (lane 3) redifferentiated revertant Rev7; (lane 4) somatic rat hepatoma–mouse fibroblast hybrid; (lane 5) tRNA control; (lane 6) DNA molecular weight markers (MWM). The position of the full-size protected fragment is indicated by open arrowheads.

from C2 for its ability to perform gluconeogenesis, a liver-specific trait. These studies demonstrated that C2 expresses a variant protein termed vHNF, which had binding characteristics very similar to HNF-1 (Baumhueter et al. 1988). Furthermore, somatic hybrids between the hepatoma cell line Fao and fibroblasts, which extinguish liver gene expression (Killary and Fournier 1984; Gourdeau et al. 1989), also contain a binding activity indistinguishable from vHNF (Baumhueter et al. 1988). Interestingly, we cannot detect HNF-1 mRNA in either the C2 cell line or the somatic hybrids, yet HNF-1

transcripts are present in the parent cell line Fao, as well as in the revertant Rev7 (Fig. 4d). This result was confirmed by ribonuclease protection using probes derived from two different parts of the HNF-1 cDNA clone, including one that contained the homeo domain. Thus, it seems that HNF-1 expression is repressed as a consequence of dedifferentiation and extinction of the hepatocyte phenotype in somatic cell hybrids that contain the full complement of fibroblast chromosomes. More extensive analysis of hybrids having only part of the fibroblast genome will be necessary before concluding that

Figure 5. Expression in the mRNA for the α and β chains of fibrinogen in the kidney and the γ chain in the lung. Five micrograms of total cellular RNA from each tissue was examined. The position of properly initiated transcripts corresponds to the dark band in lanes 1 (A–D). Properly initiated transcripts detected with the α , β , and γ fibrinogen cDNAs and the actin cDNA are shown in A, B, C, and D respectively. (Lanes 1) liver; (lanes 2) lung; (lanes 3) brain; (lanes 4) spleen; (lanes 5) kidney. A distortion in the sequencing gel makes the size of the α -fibrinogen fragment (A) in the kidney (lane 5) and in the liver (lane 1) appear different; they are actually the same size.

HNF-1 is directly repressed by the tissue-specific extinguisher and determining which extinguishing locus is involved.

Discussion

HNF-1 is expressed more widely than was initially suspected on the basis of studies of complexes formed at the recognition site of the β -fibrinogen gene (Courtois et al. 1987) or previous studies of the mRNA (Frain et al. 1989). The detection of mRNA and DNA binding in the kidney and intestine, however, is consistent with the pattern of expression of the promoters to which it binds. For example, the α_1 -antitrypsin promoter is expressed in the kidney and intestine and in macrophages such as those in the spleen (Koopman et al. 1989). α -Fetoprotein

is expressed in both the intestine and the liver (Tilghman and Belayew 1982; Krumlauf et al. 1985). Most of the genes thought to be liver specific have not been rigorously examined with sensitive techniques such as ribonuclease protection and could possibly be more broadly expressed than indicated from initial studies. For example, we have found that the α and β chains of fibrinogen are expressed at very low levels in the kidney (Fig. 5). Because the kidney is derived from mesoderm, the pattern of expression of HNF-1 does not follow traditional embryologic lines, and the logic of this pattern of expression remains to be elucidated.

We found two short sequence motifs within HNF-1 that are similar to regions in the POU family of transcriptional activators. Although the function of the basic motif in Oct-1 has been documented, there is yet no evidence that the acidic motif is essential. HNF-1 is distantly related to the homeo domain family (Scott et al. 1989), but it does not have sufficient similarity to the POU group to be included in this family.

Our data established that LF-B1 is identical to HNF-1. The sequences used to purify HNF-1 were derived from the β -fibrinogen promoter and share 11 of 17 nucleotides with the LF-B1 site in the α_1 -antitrypsin promoter used for the purification of LF-B1. Although initial studies suggested that these proteins were either very similar or identical (Courtois et al. 1987, 1988), later work indicated that the protein binding to the α_1 -antitrypsin promoter might be different from HNF-1 (Hardon et al. 1988; Monaci et al. 1988). Most notably, the protein purified with the LF-B1 site was 45 kD (Frain et al. 1989), whereas HNF-1 was found to be 88 kD by UV cross-linking and direct purification (Baumhueter et al. 1988; Courtois et al. 1988). However, the sequence of the cDNA clone isolated with oligonucleotides prepared from the determined amino acid sequence of LF-B1 predicts a protein of 70 kD. Recently, the protein binding to the albumin B box, or albumin proximal factor (APF), has been purified and the sequence of several peptides obtained. These peptides appear in the HNF-1 sequence (Fig. 2) (M. Yaniv, pers. comm.). Thus, the proteins binding to the albumin, α_1 -antitrypsin, and the fibrinogen β -chain promoters are identical. Other promoters that have functional interactions with purified HNF-1 in which binding has been documented have been compiled (Courtois et al. 1988). In most of these promoters, the HNF-1 site plays an important role in transcriptional activation, because elimination of the site reduces the activity of the promoter to only a few percent of the wild-type promoter. We therefore suggest that HNF-1 plays the role of a broadly acting transcriptional regulator involved in the expression of genes in both endodermal and mesodermally derived tissues.

Methods

HNF purification, cloning, and sequencing

HNF-1 was purified from rat liver nuclear extracts by DNA affinity chromatography (Herrick 1980; Kadonaga and Tjian 1986)

and subjected to digestion with cyanogen bromide, endoproteinase ArgC, or V8 endoproteinase. The products were separated by reverse-phase HPLC, and the purified peptides sequenced on an Applied Biosystems model 470A gas phase sequencer. From the determined amino acid sequence, primers were synthesized including all codon degeneracies except for the first four amino acids of peptide 1, where choices were made on the basis of codon preference. These primers were used for the PCR using 5 μ g of poly(A) RNA isolated from the Rev7 hepatoma cell line. A 144-bp product of the PCR was identified and its sequence determined by asymmetric PCRs. A cDNA library was constructed using random hexanucleotide primers and cloned into λ gt10 (Young and Davis 1983). This library was screened with the 144-bp α -³²P-labeled PCR product encoding HNF-1. A total of 20 clones were identified, ranging from 0.8 to 3.6 kb in length. Dideoxy sequencing was carried out with Sequenase according to the supplier's instructions.

Isolation of RNA and ribonuclease protection

RNA was isolated using the guanidine thiocyanate method (Chirgwin et al. 1979) from rat tissues and cell lines. For RNase mapping (Melton et al. 1984), three different probes were generated from the homeo domain and the 3' and 5' ends of the HNF-cDNA. Each gave similar results. The α -³²P-labeled antisense strand was generated using T3 RNA polymerase and [α -³²P]UTP. The probe was hybridized in solution to 10 mg of total cytoplasmic RNA from the indicated tissues and cell lines. After treatment with RNase A and T1, the protected fragments were analyzed on 6% polyacrylamide/7 M urea denaturing sequencing gels.

Cell culture

Cell lines, including C2, FaO, and Rev7 (courtesy of M. Weiss), as well as the somatic cell hybrid line (courtesy of K. Fornier), were grown in 10% CO₂ and in Dulbecco's modified Eagle media, supplemented with 10% fetal bovine serum.

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HNF-1 shares three sequence motifs with the POU domain proteins and is identical to LF-B1 and APF.

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Genes Dev. 1990, **4**:

Access the most recent version at doi:[10.1101/gad.4.3.372](https://doi.org/10.1101/gad.4.3.372)

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