Liver-Enriched Transcription Factors in Liver Function and Development. Part I: The Hepatocyte Nuclear Factor Network and Liver-Specific Gene Expression

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This article is dedicated to Jemima Ann Schrem.
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**Abstract**—Numerous studies have established the pivotal role of liver-enriched transcription factors in organ development and cellular function, and there is conclusive evidence for transcription factors to act in concert in liver-specific gene expression. During organ development and in progenitor cells the timely expression of certain transcription factors is necessary for cellular differentiation, and there is overwhelming evidence for hierarchical and cooperative principles in a networked environment of transcription factors. The search for molecular switches that control stem cell imprinting and liver-specific functions has lead to the discovery of many interactions between such different molecules as transcription factors, coactivators, corepressors, enzymes, DNA, and RNA. Many of these interactions either repress or activate liver-specific gene expression. It thus can be demonstrated that specific mutational changes in liver-enriched transcription factors lead to altered intermolecular interactions with the consequence of human disease. This review provides an overview of our current knowledge about liver-enriched transcription factors and their role in liver function and development. We review the basic principles of gene transcription, the role of liver-enriched transcription factors in liver gene regulation, and the classification of transcription factors by their DNA-binding domains.

**I. Transcription Factors and Gene Regulation**

**A. Principal Mechanisms**

Transcription factors are trans-acting DNA-binding proteins that bind to a specific cis-acting DNA sequence within the regulatory element of a gene. Usually, control regions can be found upstream of the start site of transcription, although in some cases binding occurs within the coding region. Transcription factors bound to their cognate cis-acting DNA sequence interact with the transcriptional machinery and enable selective gene expression and regulation. Frequently, this process is governed by the binding of many different proteins to cognate DNA-binding sites, which enables combinatorial control of gene expression. An additional level of complexity is provided by protein-protein interactions between transcription factors and coactivators or corepressors. Together with the transcriptional machinery, these proteins form a multiprotein complex that enables regulated mRNA synthesis (for review see Pabo and Sauer, 1992; Giordano and Avantaggiati, 1999; Klug, 1999; Wolberger, 1999; Goodman and Smolik, 2000).

Efficient gene transcription requires a permissive chromatin environment for successful interaction between the trans-acting transcription factors of the multiprotein complex and the respective cis-acting target DNA template of the nucleosome core particle. Therefore, the modulation of chromatin structure with its effects on gene transcription represents a key mechanism for transcriptional repression, derepression, and transcriptional activation. In the following sections we briefly summarize some of the fundamental mechanisms in the formation of the multiprotein complex to provide newfound knowledge on gene transcription and liver-
enriched transcription factors, and we deliberately exclude aspects of DNA repair and DNA miscoding, which have been reviewed elsewhere (Krokan et al., 2000; Thompson and Schild, 2001).

**B. Chromatin Higher Order Structure and Transcription Factor Function**

Chromatin is composed of a histone octamer, the DNA of the nucleosome core particle, and the linker DNA. The nucleosome core particle is formed by about 160 bp\(^{1}\) of DNA wrapped around an octamer composed of two copies of each of the four histones H2A, H2B, H3, and H4. Within the nucleosome core particle an \((H3)_2(H4)_2\) tetramer, as well as an H2A-H2B dimer, could be distinguished. These histone oligomers could be recombined with DNA in vitro to generate the characteristic X-ray diffraction pattern of chromatin (Kornberg and Thomas, 1974; Kornberg and Lorch, 1999). Figure 1 shows the basic entities that form chromatin, and Fig. 2 shows a schematic transection of the nucleosome core particle based on X-ray findings by Luger et al. (1997). X-Ray and electron crystallography revealed the coiling of DNA in left-handed superhelical turns around the histones (Finch et al., 1977). Crystallographic analysis of the nucleosome showed that the histones form a left-handed protein superhelix matching that of the DNA in the nucleosome core particle (Klug et al., 1980; Arents and Moudrianakis, 1995; Kornberg and Lorch, 1999).

The human genome consists of 2.91 billion base pairs, which would be theoretically about 1.8 m long if stretched out as one long chain (Venter et al., 2001). DNA is organized into chromatin to achieve the required high level of compaction to pack this DNA into a nucleus with a diameter less than 6 nm (Lewin, 1994). The orderly packaging of DNA in the nucleus plays an important role in the functional aspects of gene regulation. Only a small percentage of chromatin is made available to transcription factors and the transcriptional machinery, whereas the remainder of the genome is in a state that is essentially inaccessible to the RNA polymerases. ATP-dependent chromatin remodeling as well as chromatin modifications by acetylation of lysines, DNA methylation, phosphorylation of serines and threonines, and ubiquitination of lysines play key roles in altering chromatin higher order structure and function (Bradbury, 1992; Shilatifard, 1998; Giordano and Avantaggiati, 1999; Spencer and Davie, 1999; Stein et al., 1999). Acetylations and phosphorylations markedly affect the charge densities of well defined, very basic N- and C-terminal domains of histones (for details see also Fig. 2), whereas ubiquitination adds a bulky globular protein, ubiquitin, to lysines in the C-terminal tails of H2A and H2B (for review see Bradbury, 1992; Bird and Wolffe, 1999; Kornberg and Lorch, 1999).

New findings on ATP-dependent chromatin remodeling as well as chromatin modifications by covalent acetylation, phosphorylation, ubiquitination, and DNA methylation demonstrate the importance of these alterations for the regulation of many genes, although their precise role in liver gene expression remains largely unknown. The following sections provide an overview of

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**Abbreviations:** bp, base pair; PEV, position-effect variegation; SWI/SNF, switch/sucrose nonfermenting; TBP, TATA-binding protein; CDK, cyclin-dependent kinase; GR, glucocorticoid receptor; HNF, hepatocyte nuclear factor; IFN, interferon; IL, interleukin; NuRD, nucleosome-remodeling histone; HDAC, histone deacetylase; HAT, histone acetyltransferase; RNA pol II, RNA polymerase II; TFII, transcription factor II; bZIP, basic region leucine zipper; BEF, bZIP-enhancing factor; C/EBP, CCAAT/enhancer-binding protein; DBP, D-binding protein; PPAR, peroxisome proliferator-activated receptor; SHP, short heterodimer partner; CBP, cAMP response element-binding protein; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; GH, growth hormone; STAT5, signal transducer and activator of transcription 5; pCAF, p300/CBP-associated factor; NCoA, nuclear receptor coactivator.
ATP-Utilizing Chromatin Remodeling Complexes: Switch/Sucrose Nonfermenting and Relatives. The activation of a gene requires accessibility for transcription factors, activators, coactivators, and transcription machinery to the various regulatory regions. Several ATP-consuming chromatin remodeling complexes have been identified that enable gene activation by altering the stable structure of nucleosomes (for review see Devine et al., 1999; Muchardt and Yaniv, 1999; Wade and Wolffe, 1999; Tyler and Kadonga, 1999; Sudarsanam and Winston, 2000).

The SWI/SNF complex (SWI = switch, SNF = sucrose nonfermenting) was initially discovered in Saccharomyces cerevisiae and represents the prototype of ATP-dependent chromatin remodeling complexes (Laurent et al., 1991; Peterson and Herskowitz, 1992). The altered nucleosome structure can be distinguished from normal nucleosomes by their slow electrophoretic mobility in nondenaturing gels, whereas the protein content in normal and altered nucleosomes remains unchanged (Schnitzler et al., 1998). It could be demonstrated that the SWI/SNF complex binds directly to nucleosome cores and uses the energy of ATP hydrolysis to disrupt DNA/histone interactions and to create an altered nucleosome core conformation that is also stable in the absence of SWI/SNF (Côté et al., 1998). It has been postulated that the alteration of the nucleosome structure by chromatin remodeling complexes affects the preferred bending of the DNA as it coils around the histone octamer, leading to facilitated binding of transcription factors to their DNA template. The reverse transition from altered to normal nucleosomes is also catalyzed by the same SWI/SNF complex using ATP hydrolysis (Côté et al., 1998; Schnitzler et al., 1998).

It could be demonstrated that the SWI/SNF complex contacts the DNA strand at two points creating a loop and that only nucleosomes within this loop are being altered (Bazett-Jones et al., 1999). In yeast only about 6% (329 of 5460) of the genes tested were affected 2-fold or more by the inactivation of SWI2/SNF2. Of these 329 genes, 203 genes were elevated 2-fold or more in the absence of SWI2/SNF2, indicating that chromatin remodeling can favor activation as well as repression of transcriptional activity (Holstege et al., 1998). These results prompted the hypothesis that the limited pool of SWI/SNF complexes is recruited to a small number of specific promoters, which in turn will bind either transcriptional activators or repressors (Holstege et al., 1998; Muchardt and Yaniv, 1999).

Two models have been proposed to explain why some promoters are SWI/SNF-dependent, whereas others are not. The first model describes the SWI/SNF complex as primarily regulating the DNA binding of transcriptional modulators. In this model SWI/SNF-dependent promoters are thought to have weak activator-binding sites covered by nucleosomes, whereas SWI/SNF-independent promoters either have high-affinity activator-binding sites or are located in a nucleosome-free region (Muchardt and Yaniv, 1999). In this context it is interesting to note that nucleosome-free linker DNA can be bound to linker histones (H1, H1*, H5, etc.) and that there is evidence for the involvement of linker histones in transcriptional regulation. A scenario has been proposed in which the reversible and controllable binding/displacement of linker histones to the nucleosomal entry/exit point determine the accessibility of nucleosomal DNA to
the transcriptional machinery (Zlatanova et al., 2000). The second proposed model on SWI/SNF-dependent and SWI/SNF-independent promoters suggests that the SWI/SNF complex exerts its major effect in transcriptional activation at a step subsequent to transcriptional activator-promoter recognition. The recruitment of the SWI/SNF complex by the DNA-binding protein may then allow the binding of secondary transcriptional regulators that in turn either facilitate or prevent the recruitment of the TATA-binding protein (TBP) (Ryan et al., 1998).

In some cases it could be found that the SWI/SNF complex is associated with the polymerase II holoenzyme, which lead to the hypothesis that the SWI/SNF complex is involved in the assembly of the preinitiation complex (Wilson et al., 1996). Furthermore, the SWI/SNF complex was shown to be able to facilitate the binding of TBP on nucleosomes in vitro (Imbalzano et al., 1994).

Drosophila and human cells contain complexes related to yeast SWI/SNF. These complexes contain about 10 subunits, and each contains a homolog of the yeast SWI2/SNF2 helicase-like subunit as well as one or two homologs of yeast SNF5 (SNF5 = sucrose nonfermenting 5), SWI3 (switch 3), and SWP73 (an associated protein) (Muchardt and Yaniv, 1999).

The composition of the mammalian SWI/SNF complex appears to be highly variable in contrast to the respective complex in yeast and drosophila. In human and mouse cells at least two homologs of the SWI2/SNF2 ATPase subunit exist, known as brm (also called Brahma or SNF2a) and brahma-related protein-1 (also known as BRG-1 or SNF2b). The brm and the BRG-1 protein are 75% identical. Purification experiments revealed that different mixtures of brm and BRG-1-associated complexes can be found in mammalian cells (Muchardt and Yaniv, 1999).

The second proposed model on SWI/SNF-dependent and SWI/SNF-independent complexes is dependent on the phase of the cell cycle, the stage of development, and the specific tissue, and it is likely that each form has a specific function (Muchardt and Yaniv, 1999). The phosphorylation of brm and BRG-1 during mitosis prevents the SWI/SNF complex from remodeling chromatin in vitro (Sif et al., 1998). Furthermore, it was proposed that mini-cycles of phosphorylation and dephosphorylation of the brm and BRG-1 proteins regulate the attachment of these proteins to nuclear structures during interphase (Muchardt and Yaniv, 1999).

It could be demonstrated that the retinoblastoma protein and BRG-1 form a complex and cooperate to induce cell cycle arrest (Dunaief et al., 1994). In the yeast two-hybrid system an interaction between BRG-1 and brm protein family members and retinoblastoma protein family members including pRB, p107, and p130 was observed. These interactions influence cellular proliferation because both BRG-1 and brm, but not mutants of these proteins, which are unable to bind pRB family members, inhibit the formation of drug-resistant colonies when transfected into the SW13 human adenocarcinoma cell line, which lacks endogenous BRG-1 or brm (Strober et al., 1996). Mouse brm null mutants (−/−) showed increased hepatocyte proliferation in the adult. In addition, embryonic fibroblasts isolated from the brm −/− mice showed defects in G1-checkpoint controls. In culture these cells fail to arrest at confluency. This lack of contact inhibition can be correlated with a lack of induction of the CDK inhibitor p27 at confluency (Reyes et al., 1998). One consequence of the interaction between brm/BRG-1 and the p105Rb retinoblastoma tumor suppressor could be demonstrated in transient transfection experiments as a synergistic repression of transcription factor E2F1, a protein known to regulate cell cycle progression (Trouche et al., 1997).

Cyclin E, another cell cycle protein, was found to associate with both BRG-1 and BAF155, a human homolog of SWI3. The interaction with cyclin E, which is independent of p105Rb, leads to phosphorylation of the SWI/SNF subunits by cyclin E-associated kinase activity, and

b. Switch/Sucrose Nonfermenting Complex and Cell Cycle Control: Impact on Liver Regeneration? Several observations suggest that the SWI/SNF complex is also involved in cell cycle control (Muchardt and Yaniv, 1999; Sudarsanam and Winston, 2000). It has been observed that growth arrest or differentiation leads to increased accumulation of brm protein, whereas rapidly dividing cells contain mainly BRG-1 (Muchardt et al., 1998). The levels of brm and BRG-1 are also regulated during the cell cycle. At the G1/M transition the two proteins are phosphorylated. This phosphorylation leads to proteolytic degradation of the brm protein, whereas BRG-1 remains stable through mitosis (Muchardt et al., 1996; Sif et al., 1998; Muchardt and Yaniv, 1999). From these observations it is likely that the ratio between brm and BRG-1-associated complexes is dependent on the phase of the cell cycle, the stage of development, and the specific tissue, and it is likely that each form has a specific function (Muchardt and Yaniv, 1999). The phosphorylation of brm and BRG-1 during mitosis prevents the SWI/SNF complex from remodeling chromatin in vitro (Sif et al., 1998). Furthermore, it was proposed that mini-cycles of phosphorylation and dephosphorylation of the brm and BRG-1 proteins regulate the attachment of these proteins to nuclear structures during interphase (Muchardt and Yaniv, 1999).
cyclin E and cyclin D1 can partially rescue BRG-1-induced growth arrest (Shanahan et al., 1999).

After liver resection mammalian liver regeneration leads to the controlled induction of a proliferative response in hepatocytes that terminates as soon as the hepatic mass has been restored. Among other proteins the retinoblastoma protein family members as well as the cyclins E and D1 have been associated with different roles in hepatocyte cell cycle control after partial hepatectomy in mice and rats (Trautwein et al., 1999). The role of brm and BRG-1 in mammalian liver regeneration remains to be determined. Since several lines of evidence suggest that brm and BRG-1 play important roles in cell cycle control, and since brm and BRG-1 are known to influence the transcription of several genes through chromatin remodeling, it seems likely that these mammalian SWI/SNF subunits play an important role in liver regeneration. It would be valuable to investigate the role of brm and BRG-1 during liver regeneration. Furthermore, studies on protein-protein interactions of brm and BRG-1 with the retinoblastoma protein family and cyclin E would be interesting, because it is likely that liver regeneration may influence the phosphorylation status of brm and BRG-1 and thus hepatocyte proliferation and/or senescence.

c. Components of the Switch/Sucrose Nonfermenting Complex as Cofactors for Nuclear Receptors. Components of the SWI/SNF complex can function as coactivators for several nuclear receptors including the glucocorticoid receptor, the retinoic acid receptor, and the estrogen receptor (Muchardt and Yaniv, 1993; Chiba et al., 1994). A ligand-dependent interaction of the estrogen receptor, the glucocorticoid receptor (GR) or the progesterone receptor with the BRG-1 protein has been demonstrated (Ichinose et al., 1997; Fryer and Archer, 1998). Prebinding of GR to a nucleosomal template in vitro facilitates nucleosome disruption by the SWI/SNF complex (Ostlund-Farrants et al., 1997). On the other hand, it could be shown that GR-induced chromatin remodeling requires the SWI/SNF complex (Fryer and Archer, 1998).

GR as well as the liver-enriched hepatocyte nuclear factor-4 (HNF-4) belong both to the superfamily of nuclear receptors that share several structural similarities (Hadzopoulou-Cladaras et al., 1997). Whether components of the SWI/SNF complex also interact with HNF-4 as coactivators or corepressors would be an interesting field of research in view of the known impact of HNF-4 on the regulation of liver function.

d. Further Multiprotein Complexes with Homology to the Switch/Sucrose Nonfermenting ATPase. In the last few years several multiprotein complexes with homology to the SWI/SNF ATPase subunit have been identified [e.g., NURF (nucleosome remodeling factor), CHRAC (chromatin-accessibility complex), ACF (ATP-utilizing chromatin assembly and remodeling factor), RSF (remodeling and spacing factor), NuRD (nucleosome remodeling histone deacetylase complex), and RSC (remodel the structure of chromatin)] (Zhang et al., 1998, 1999; Muchardt and Yaniv, 1999; Stein et al., 1999; Ahringer, 2000). This diversity suggests that chromatin remodeling complexes are numerous and may each be involved in specific cellular pathways.

Molecular analysis of the NuRD subunits revealed that this ATP-utilizing chromatin remodeling complex contains the human dermatomyositis-specific autoantigen Mi-2 and a histone deacetylase core complex. Furthermore, the NuRD complex has been involved in DNA methylation (Zhang et al., 1998, 1999). Therefore, the NuRD complex represents an example of a protein complex that is able to influence transcriptional activity by several different mechanisms: ATP-dependent chromatin remodeling, chromatin deacetylation, and DNA methylation (Wade and Wolfe, 1999; Ahringer, 2000; Guschin et al., 2000). The NuRD complex (also known as the Mi-2 complex) has been associated with transcriptional silencing (Wade and Wolfe, 1999). The histone deacetylases HDAC1 and HDAC2, as well as the two histone-binding proteins RbAp46 and RbAp48, belong to this complex and to the SIN3 complex. The NuRD and the SIN3 complex represent the two major HDAC complexes that have specific functions in development rather than being required for general cellular processes (Ahringer, 2000).

2. Chromatin Modification: Reversible Acetylation of Histone Lysines. Expressed genes are located in highly acetylated chromatin. The acetylation status of nucleosomes is regulated by a group of enzymes, histone acetyltransferases (HATs) and HDACs. Examples of acetylation sites of histones H3 and H4 are shown in Fig. 2. Both groups of enzymes contain numerous family members, most of which have been highly conserved during evolution. The noncatalytic components of these complexes can either target the catalytic unit to specific sites of the genome or regulate its enzymatic specificity. DNA methylation and histone acetylation have also been linked together, whereby methylation is used to direct gene repression through a histone deacetylase complex (Gray et al., 1999) (see also Fig. 3).

Recent studies have suggested a strong link between histone acetylation, chromatin remodeling, and gene regulation (reviewed in Grunstein, 1997; Wade and Wolfe, 1997; Workman and Kingston, 1998). In particular, a number of transcriptional regulatory proteins, including GCN5, PCAF, p300/CPB, TFIID250, and the nuclear hormone receptor coactivators ACTR and SRC-1, have been found to possess intrinsic HAT activity (Kuo et al., 1998; Wang et al., 1998a,b; Chen et al., 1999). Mutational analyses of yeast GCN5 indicated a direct role for the HAT activity in histone acetylation and transcriptional activation of target genes in vivo (Kuo et al., 1998, Wang et al., 1998a,b). These findings suggest a mechanism whereby the activators recruit HAT complexes to the promoters of target genes, allow-
ing for acetylation of histones to increase the accessibility of transcription factors. In addition, it has recently been shown that p160 coactivators such as the acetylase SRC-3 can be acetylated by p300/CBP and that such acetylation disrupts hormone receptor-coactivator interaction. These findings show the possible role of histone acetylation in gene activation and the possible role of acetylase protein acetylation in transcriptional attenuation (Chen et al., 1999). However, the precise role of histone acetylation and nonhistone protein acetylation in the process of transcriptional activation in vivo remains largely unclear. The role of reversible acetylations of histone lysines and transcription factors for the regulation of liver-specific genes is becoming increasingly evident, as could be shown for several coactivators of the liver-enriched transcription factors HNF-1 and HNF-4. In the second part of this review (see section “Molecular Regulation of Liver Function”), the HNF-1 and HNF-4 coactivators will be discussed in some detail.

3. Chromatin Modification: Reversible Phosphorylation of Histone Serines and Threonines. Histone H1 and H3 phosphorylations correlate with the process of chromosome condensation. The subunits of histone H1 kinase have now been shown to be cyclins and the p34CDC2 kinase product of the cell cycle control gene CDC2. It is probable that all of the processes that control chromosome structure and function relationships are also involved in the control of the cell cycle (Bradbury, 1992; Spencer and Davie, 1999).

In addition to phosphorylating specific transcription factors, MAP kinases and their downstream kinases are implicated in eliciting rapidly targeted alterations in the chromatin environment of specific genes by modulating the phosphorylation and/or acetylation of nucleosomal and chromatin proteins (Thomson et al., 1999).

4. Chromatin Modification: Reversible Ubiquitination of Histone Lysines. Ubiquitin-dependent proteolytic pathways are largely responsible for selective protein turnover in the cytosol of eukaryotes. Although ubiquitinated histones are present in substantial levels in vertebrate cells, the roles they play in specific biological processes and the cellular factors that regulate this modification are not well characterized. Ubiquitinated H2B (uH2B) has been identified in the yeast S. cerevisiae, and mutation of the conserved ubiquitination site could confer defects in mitotic cell growth and meiosis. uH2B was not detected in rad6 mutants, which are defective for the ubiquitin-conjugating enzyme Ubc2, thus identifying Rad6 as the major cellular activity that ubiquitinates H2B in yeast (Robzyk et al., 2000).

5. Chromatin Modification: Reversible DNA Methylation. Cytosine residues in the sequence 5′CpG (cytosine-guanine) are often postsynthetically methylated in animal genomes. The methyl-CpG-binding proteins MeCP1 and MeCP2 interact specifically with methylated DNA and mediate transcriptional repression.
MeCP2 is an abundant nuclear protein that is essential for mouse embryogenesis (Nan et al., 1998). MeCP2 binds tightly to chromosomes in a methylation-dependent manner. It contains a transcriptional-repression domain that can function at a distance in vitro and in vivo. A region of MeCP2 that localizes with the transcriptional-repression domain associates with a corepressor complex containing the transcriptional repressor mSin3A and histone deacetylases (see Fig. 3). Transcriptional repression in vivo is relieved by the deacetylase inhibitor trichostatin A, indicating that deacetylation of histones (and/or of other proteins) is an essential component of this repression mechanism. Two global mechanisms of gene regulation, DNA methylation and histone deacetylation, can be linked by MeCP2 (Nan et al., 1998).

The strong effect of 5-methylcytosine (5 mC) in mammalian promoter regions suggests that DNA methylation inhibits transcription by interfering with transcription initiation. DNA methylation has been shown to reduce the binding affinity of sequence-specific transcription factors like Sp1 and c-Myc (Prendergast and Ziff, 1991; Clark et al., 1997). In addition, methylation-dependent, sequence-specific DNA-binding proteins such as MDBP may act as transcriptional repressors (Asiedu et al., 1994).

There are several situations in which 5’CpG islands in the promoter region of genes become de novo methylated in normal development, thereby silencing the expression of the associated gene (Feil and Khosla, 1999; Jones and Laird, 1999). Examples of genes silenced by 5’CpG island methylation include genes that are transcriptionally repressed by parental-specific imprinting and genes on the inactive X chromosome in female mammals (Issa et al., 1994, 1996; Jaenisch, 1997). During aging, CpG islands associated with nonimprinted autosomal genes can show gradual increases in methylation (Issa et al., 1994 and 1996). DNA methylation may also contribute to immobilization of mammalian transposons, suppression of transcriptional noise, and the control of tissue-specific gene expression, but decisive evidence on these points is lacking (Bird and Wolffe, 1999). The methylation of tumor suppressor gene promoters (e.g., RB1, VHL, CDKN2, CDKN2B, MLH1, and APC) is regarded as one potential hit paving the way to carcinogenesis together with loss of heterozygosity or mutational inactivation in such tumors as retinoblastoma, renal cell carcinoma, melanoma, and colorectal cancer (Jones and Laird, 1999). CpG methylation is involved in the repression of viral genomes, while the methylation of exogenous DNA introduced into cells compromises efforts at gene therapy (Garrick et al., 1998). A striking and widespread de novo methylation of CpG islands occurs as a consequence of in vitro cell culture of immortal cell lines (Jones and Laird, 1999).

Figure 4 shows a model proposed by Bird and Wolffe (1999) on the effect of DNA methylation on the range of transcriptional activity.

![Figure 4](image-url)
transcriptional regulation beyond that which could be achieved by chromatin modification alone. It is assumed that DNA methylation is able to contribute a significant additional level of gene repression.

**C. Epigenetics**

The inheritance of information during cell replication on the basis of gene expression levels is known as epigenetics, as opposed to genetics, which refers to information inherited on the basis of gene sequence. Enzymatic methylation of the C-5 position of cytosine residues can effect epigenetic inheritance by altering the expression of genes and by transmission of DNA methylation patterns through cell division (Bird and Wolffe, 1999; Jones and Laird, 1999; Wolffe and Matzke, 1999). Epigenetic control of gene expression can be considered from the standpoint of normal development, which requires stable repression of genes not required in specific cell types (Wolffe and Matzke, 1999). Interactions between repeated DNA sequences can trigger the formation and the transmission of inactive genetic states and DNA modifications. Methylation induced by DNA repeats can template chromatin modifications and transcriptional repression by MeCP2 binding to methylated CpG with subsequent recruitment of histone deacetylase (Nan et al., 1998; Jones and Laird, 1999) (see also Fig. 3).

**D. Position-Effect Variegation**

The chromosomes of most higher eukaryotes consist of distinct regions that are cytologically distinguishable owing to differences in condensation. In a typical chromosome, heterochromatin differs from euchromatin in sequence composition, function, and cytological appearance and is predominantly located in the pericentric region. The DNA of heterochromatin consists almost entirely of repetitive sequences and encodes relatively few genes. In Drosophila, genes juxtaposed to heterochromatin are frequently inactivated, a phenomenon known as PEV. Inactivation is believed to result from the spreading of the heterochromatin state along the chromosome (Dorer and Henikoff 1994, 1997). The extent of PEV spreading may vary from cell to cell, producing mosaic expression of nearby genes. In contrast with the growing understanding of transacting factors, little is known of cis-acting requirements for heterochromatin formation and PEV. Experiments with Drosophila using a mini-white reporter gene, a commonly used eye color marker in Drosophila P transposons, and PEV to explore the requirements for heterochromatin formation revealed that variegated expression of mini-white occurs when it is present in repeat arrays. Variegation was particularly strong for repeated transposons at a euchromatic site near heterochromatin, but also resulted from repeats at a site distant from heterochromatin (Dorer and Henikoff, 1994). Inactivation strengthened with increasing copy number, a phenomenon that can also be observed for the transgene in numerous transgenic animals and plants (Dorer and Henikoff, 1997; Garrick et al., 1998). Experiments using the lox/Cre system of site-specific recombination to generate transgenic mouse lines showed that the reduction in copy number results in a methylation at the transgene locus (Garrick et al., 1998).

**E. Formation of the Multiprotein Complex**

The expression of any gene is accomplished primarily through the interaction of protein transcription factors with characteristic nucleotide sequences located in the control regions of the gene, which are most commonly located near to, or upstream from, the actual coding region. The binding of a set of such factors, or regulatory proteins, acts as a molecular switch for the activation of the RNA polymerase II (RNA pol II) and other components of the transcriptional machinery, which are common to all genes. The supply of a particular combination of such transcription factors ensures that a gene is switched on in the right cell or tissue and at the right time (Duncan et al., 1998; Klug, 1999).

Transcription initiation by RNA pol II requires interaction between cis-acting promoter elements and trans-acting factors. The eukaryotic promoter consists of core elements, which include the TATA and CAAT box and other DNA sequences that define transcription start sites, and regulatory elements, which either enhance or repress transcription in a gene-specific manner. The core promoter is the site for assembly of the transcription preinitiation complex, which includes RNA pol II and the general transcription factors TBP, transcription factor IIB (TFIIB), TFIIE, TFIIF, and TFIIH (for review see Roeder, 1996; Hampsey, 1998; Shilatifard, 1998).

Regulatory elements bind gene-specific factors, which affect the rate of transcription by interacting, either directly or indirectly, with components of the general transcriptional machinery. A third class of transcription factors, termed coactivators, is not required for basal transcription in vitro but often mediates activation by a broad spectrum of activators. Accordingly, coactivators are neither gene-specific nor general transcription factors, although gene-specific coactivators have been described in metazoan systems including humans. Transcriptional repressors include both gene-specific and general factors. Similar to coactivators, general transcriptional repressors affect the expression of a broad spectrum of genes yet do not repress all genes. General repressors either act through the core transcriptional machinery or are histone-related and presumably affect chromatin function, thus preventing RNA transcription (Chang and Jaehning, 1997; Hampsey, 1998; Yamaguchi et al., 1998).

Figure 5 depicts a schematic model on the formation of the multiprotein complex within the promoter region of a gene. In this model acetylated chromatin is made accessible for transcription factors (DNA-binding trans-
activators) in the control region (promoter regions and enhancer-binding sites) of the respective gene by ATP-dependent chromatin remodeling complexes. After transcription factor binding, the RNA polymerase II, general initiation factors, and mediators bind at the promoter region. Then RNA polymerase II elongation factors bind additionally to the multiprotein complex to enable mRNA transcription. In this process extensive protein-protein interactions occur that enable the fine tuning of an orchestrated regulation of gene transcription (for review see Roeder, 1996; Hampsey, 1998; Shilatifard, 1998; Wolberger, 1999).

II. Classification of Liver-Enriched Transcription Factors

Transcription factors achieve recognition of the DNA-binding site through protein-DNA and protein-protein interactions via discrete substructures or protein domains that serve binding to DNA. The DNA-binding motifs of transcription factors contain characteristic amino acid sequences and form characteristic threedimensional structures that allow the classification of different types of transcription factors. The three-dimensional structure of these DNA-binding motifs leads to DNA sequence-specific DNA binding through the formation of hydrogen bonds and Van der Waals contacts (Pabo and Sauer, 1992; Klug, 1999).

A. DNA-Binding Domain of Hepatocyte Nuclear Factor-1

The first DNA-binding motif identified in X-ray crystallographic studies is the helix-turn-helix (HTH) motif (Wintjens and Rooman, 1996). POU domain transcription factors have two separate helix-turn-helix DNA-binding subdomains, the POU homeodomain (POUhd) and the POU-specific domain (POUs). Each subdomain recognizes a specific subsite of 4 or 5 bp in the octamer recognition sequence (Van Leeuwen et al., 1997). The POU domain family of transcription factors was defined after the observation that the products of three mammalian genes, Pit-1, Oct-1, and Oct-2, and the protein encoded by the Caenorhabditis elegans gene unc-86, shared a region of homology, known as the POU domain (Schonemann et al., 1998).

Molecular characterization of the genes whose sequence alterations cause impressive phenotypes in the fruit fly, Drosophila melanogaster, has led to the identification of the human homeobox genes, also referred to as the HOX genes and defined as “master genes” for their crucial role in embryogenesis (McGinnis and Krumlauf, 1992). They all share a homeobox region, known as a 180-bp highly conserved sequence encoding a 60-amino acid DNA-binding domain, also called the “homeodomain”, conferring to the resulting proteins the ability to act as transcription factors (Gehring et al., 1994; Chariot et al., 1999). The 39 human HOX genes are organized in four distinct clusters (loci A, B, C, and D) and can be aligned on the basis of homology within the homeobox to define paralogues (Acampora et al., 1989; Scott, 1992). Besides a critical involvement in cell phenotype determination along the anterior-posterior axis during embryonic development, the HOX genes also play a key role in differentiation and tumoral development (Chariot et al., 1999; Cillo et al., 1999; Morata and Sanchez-Herrero, 1999).
The liver-enriched transcription factor hepatocyte nuclear factor-1 (HNF-1) contains a variant homeodomain and shares homeodomain, as well as short acidic and basic sequences, with the POU family of transcriptional activators (Baumhueter et al., 1990). HNF-1 is composed of HNF-1α or HNF-1β homo- or heterodimers (Song et al., 1998).

**B. DNA-Binding Domain of Hepatocyte Nuclear Factor-3**

The hepatocyte nuclear factor-3 (HNF-3)/fork head (fkh) family contains a large number of transcription factors and folds into a winged helix motif. Despite having almost invariable amino acid sequences in their principal DNA-binding helices, HNF-3/fkh proteins show a wide diversity of sequence-specific binding. Previous studies of chimeric HNF-3/fkh proteins demonstrated that the binding specificity is primarily influenced by a region directly adjacent to the binding helix (Marsden et al., 1998; Jin et al., 1999). In NMR and X-ray crystallographic studies it is found that in comparison with HNF-3, the HNF-3/fork head (fkh) family member Genesis contains an extra small helix directly prior to the N terminus of the primary DNA contact helix. Due to the insertion of this helix, a shorter and slightly repositioned primary DNA contact helix is observed, which is believed to lead to the DNA-binding specificity differences among various family members (Marsden et al., 1998).

**C. DNA-Binding Domain of Hepatocyte Nuclear Factor-4**

The liver-enriched transcription factor hepatocyte nuclear factor-4 (HNF-4) belongs to the group of zinc finger proteins and is frequently seen as a member of the nuclear receptor superfamily with unknown ligand (Taraviras et al., 1994). Zinc-fingers are small DNA-binding peptide motifs. These motifs can be used as modular building blocks for the construction of larger protein domains that recognize and bind to specific DNA sequences (Klug, 1999). Steroids and thyroid hormones, as well as vitamin D, retinoids, and some nutrient metabolites (fatty acids, prostaglandins, farnesol metabolites) act through binding to members of the zinc-finger containing superfamilies of nuclear hormone receptors. These receptor proteins bind directly to specific DNA recognition sequences (hormone response elements) in the promoter region of target genes to facilitate transcription. The formation of several sets of heterodimers among family members as well as cross-talk with other signaling systems results in an intricate regulatory network with distinct particularities for each receptor type (Meier, 1997).

**D. DNA-Binding Domain of Hepatocyte Nuclear Factor-6**

HNF-6 is a liver-enriched transcription factor that contains a single-cut domain and a novel type of homeodomain. Comparative trees of mammalian, *Drosophila*, and *C. elegans* proteins showed that HNF-6 defines a new class of homeodomain proteins called one-cut class. It could be demonstrated that *C. elegans* proteins of this class bind to HNF-6 DNA targets. Thus, depending on their sequence, these targets determine for HNF-6 at least two modes of DNA binding, which hinge on the homeodomain and on the linker that separates it from the cut domain, and two modes of transcriptional stimulation, which hinge on the homeodomain (Lannoy et al., 1998).

**E. DNA-Binding Domain of CCAAT/Enhancer-Binding Proteins**

Many transcription factors bind DNA to form dimeric (2:1) protein-DNA complexes. Examples include basic region leucine zipper (bZIP) proteins and basic region helix-loop-helix zipper (bHLHZIP) proteins. These two families of transcription factors follow an assembly pathway in which two protein monomers bind DNA sequentially and form their dimerization interface while bound to DNA (Kohler et al., 1999). Dimerization of these transcription factors stabilizes the protein-DNA complexes and can lead either to homodimers with the same transcription factor or to heterodimers with other members of the same family of transcription factors (Horiuchi et al., 1997).

The bZIP family of proteins is one of the largest and most conserved groups of eukaryotic transcription factors/repressors (Niu et al., 1999). These transcription factors use an atypically simple motif for DNA recognition called the basic region, yet family members discriminate differentially between target sites that differ only in half-site spacing. Two such sites are the cAMP-response element (CRE) and the AP-1 target site (Metallo and Scheutz, 1994). The DNA-binding motif of transcription factors belonging to the bZIP family is bipartite, consisting of a dimerization interface termed “leucine zipper” and a DNA contact surface termed the “basic region”. Specificity of DNA binding has been shown to be imparted by the basic region (Agre et al., 1989).

The CCAAT/enhancer-binding proteins (C/EBPα, C/EBPβ, C/EBPγ, and C/EBPδ) form a subfamily of bZIP transcription factors that display sequence homology within the bZIP domain. The conserved basic region in this subfamily contains two motifs that exhibit significant homology to the bipartite nuclear localization signal promoting nuclear transport of a bZIP transcription factor (Williams et al., 1997).

Further important members of the bZIP family of transcription factors are c-jun, c-fos (AP-1), and CREB. The molecular chaperone bZIP enhancing factor (BEF) has been shown to increase DNA binding of transcription factors that contain a basic region leucine zipper (bZIP) DNA-binding domain. BEF stimulates DNA binding by recognizing the unfolded leucine zipper and promoting the folding of bZIP monomers to dimers. Anti-
sense experiments indicate that BEF is required for efficient transcriptional activation by bZIP proteins in vivo (Virbasius et al., 1999).

III. Molecular Regulation of Liver Function

A. Liver-Specific Gene Expression

The transcription rate of genes encoding liver-specific proteins is distinctly higher in hepatocytes as compared with other cell types (Powell et al., 1984). The transcription of several hepatic genes is activated during liver development and later modulated depending on extracellular stimulation (Schmid and Schulz, 1990; Cascio and Zaret, 1991; Shiojori et al., 1991). Experiments using a cDNA library from mouse liver poly(A)+ RNA that was then differentially screened with poly(A)+ RNA from liver and nonliver cells provided strong evidence that the predominant control of liver-specific gene expression resides at the level of transcription (Derman et al., 1981; Aran et al., 1995). Clones proven to be liver-specific were picked and used as templates for hybridization with radioactive RNA newly transcribed in vitro in nuclei isolated from liver and nonliver tissues. The hybridization signals obtained with RNA synthesized with liver nuclei were at least 10 times more intense than those obtained with nuclei from other tissues. Because the cDNA clones represented an unbiased population of transcripts, the findings led to the conclusion that liver-specific gene expression is primarily a consequence of transcriptional regulation (Derman et al., 1981).

Transient transfection assays in which the introduced gene does not integrate into the genome have been instrumental in identifying the regulatory sequences in DNA that confer liver-specific gene expression. Analyses performed on a wide variety of genes that code for entirely different proteins show shared regulatory sequences. Moreover, characterization of the regulatory sequences of a number of genes has shown that each gene contains a combination of some or all of the liver-specific shared motifs (Benvenisty and Reshef, 1991; Aran et al., 1995). It is this combination of cis-regulatory elements rather than a single element that appears to be required for liver-specific gene expression. Finally, these shared motifs bind distinct cognate liver-enriched transcription factors and have aided in isolating and characterizing these factors (for review see De Simone and Cortese, 1991; Lai and Darnell, 1991; Aran et al., 1995).

B. Liver-Enriched Transcription Factors

Six families of liver-enriched transcription factors have been characterized so far: HNF-1, HNF-3, HNF-4, HNF-6, C/EBP, and D-binding protein (DBP). The analysis of the tissue distribution of these factors and the determination of their hierarchical relations have led to the hypothesis that the cooperation of liver-enriched transcription factors with the ubiquitous transactivating factors is necessary, and possibly even sufficient, for the maintenance of liver-specific gene transcription (Hayashi et al., 1999).

HNFs are a heterogeneous class of evolutionarily conserved transcription factors that contain several families of liver-enriched transcription factors that are required for cellular differentiation and metabolism (Duncan et al., 1998). The liver-enriched transcription factor family containing the C/EBPs was formerly called HNF-2 and will be reviewed separately along with the D-binding protein (DBP).

IV. Hepatocyte Nuclear Factors

The HNF-1, HNF-3, HNF-4, and HNF-6 families of transcription factors contain several members. It should be noted that liver-enriched transcription factors are not exclusively expressed in the liver. For example, HNF-1α, -3α, -3β, -3γ, -4γ, and -6 are also expressed in pancreatic β-cells (Vaisse et al., 1997). HNF-1α and HNF-4α play there a critical role in normal pancreatic β-cell function. Mutations in these liver-enriched transcription factors result in two forms of maturity-onset diabetes of the young (MODY), MODY3 and MODY1, respectively (Yamagata et al., 1996; Vaisse et al., 1997; Chevre et al., 1998). There are many more examples of relevant extrahepatic functions of liver-enriched transcription factors, but it is beyond the scope of this review to provide a complete summary of those extrahepatic functions.

A. The Hepatocyte Nuclear Factor-1 Family

HNF-1 is a transcriptional regulator composed of HNF-1α and HNF-1β hetero- and homodimers. These homeoproteins share identical DNA-binding domains but have different transcriptional activation properties (Kuo et al., 1991; Song et al., 1998).

The HNF-1α gene was assigned by somatic cell hybrids and recombinant inbred strain mapping to mouse chromosome 5 near Bcl-1 and to human chromosome 12 region q22-qter, revealing a different chromosomal region for these two species (Kuo et al., 1990). The HNF-1β gene was assigned to human chromosome 17 and murine chromosome 11. These chromosomal localizations differ from that of the HNF-1α gene, indicating that both genes are not clustered on the genome (Bach et al., 1991).

HNF-1 is one of the most important transactivators of liver-specific albumin transcription (Maire et al., 1989). HNF-1 acts as an accessory factor to enhance the inhibitory action of insulin on mouse glucose-6-phosphatase gene transcription (Streeper et al., 1998). HNF-1α is also an accessory factor required for activation of glucose-6-phosphatase gene transcription by glucocorticoids (Lin et al., 1998). Several lines of evidence point to a direct transactivation of the mouse ferrochelatase promoter by HNF-1α in the liver (Muppala et al., 2000).
Plasma lipoprotein(a) concentrations are highly heritable and predominantly determined by the liver-specific apolipoprotein(a) [apo(a)] gene. Elevated levels of lipoprotein(a) in the plasma are a risk factor for coronary artery disease and stroke. Positive regulation of transcription of the apo(a) gene is dependent on the binding of HNF-1α to a regulatory element located downstream of the mRNA start site (Wade et al., 1994).

HNF-1α is able to repress the transcription of liver-specific genes as demonstrated for the sucrose-isomaltase gene. Glucose repression of transcription of this gene in cooperation with three HNF-1-binding sites in the sucrose-isomaltase promoter. Mutagenesis of the HNF-1-binding sites showed that the two distal HNF-1-binding sites are crucial for the glucose regulation of the sucrose-isomaltase promoter. Mutagenesis of the HNF-1 binding sites showed that the two distal HNF-1-binding sites are crucial for the glucose regulation of the sucrose-isomaltase gene (Rodolosse et al., 1998).

A number of genes that are predominantly expressed in the liver are positively regulated by HNF-1α interacting with the respective cis-acting HNF-1-binding elements in the promoters of these genes (see also Table 1). Serum colloid osmotic pressure is believed to control hepatic output of plasma proteins. Many plasma proteins that are secreted from the liver, including albumin, have a HNF-1-binding site in their promoter. The activity of HNF-1α in highly differentiated hepatoma cells was shown to be modulated by a fluctuation in the level of oncotically active macromolecules like dextran or albumin in the surrounding cell culture medium. Higher oncotic pressures lead to a decrease in HNF-1α mRNA levels (Pietrangelo and Shafritz, 1994).

1. Dimerization Cofactor of Hepatocyte Nuclear Factor-1α and Liver-Specific Gene Expression. Interestingly, HNF-1α, but not HNF-1β, is expressed in the liver. Under physiologic conditions as well as in transfection experiments with HNF-1α and HNF-1β, stable homodimer formation can be found in the liver, whereas in other organs, heterodimers also are detected. From these data it was assumed that the extent of heterodimerization may be regulated in a tissue-specific manner. Furthermore, it could be shown that exclusive expression of HNF-1β is associated with repression of a subset of hepatocyte-specific genes in the dedifferentiated hepatocyte cell line C2, in differentiated F9 cells, in somatic hybrids between hepatocytes and fibroblasts, and in the lung (Mendel et al., 1991a).

HNF-1α is unique among the vertebrate homeodomain-containing proteins in that it dimerizes in the absence of its DNA recognition sequence (Mendel et al., 1991b). A dimerization cofactor of HNF-1α (DCoH) could be identified that displays a restricted tissue distribution and does not bind to DNA, but, rather, selectively stabilizes HNF-1α homodimers. The formation of a stable tetrameric DCoH-HNF-1α complex requires the dimerization domain of HNF-1α and does not change the DNA-binding characteristics of HNF-1α, but enhances its transcriptional activity. DCoH regulates the formation of transcriptionally active tetrameric complexes and thus may contribute to the developmental and tissue specificity of the complex (Mendel et al., 1991b). DCoH plays an important role in liver development and liver-specific gene expression, because HNF-1α is regarded as an important regulator of the transcriptional network in liver development and liver-specific gene expression.

The chromosomal localization of the genes for DCoH was assigned to chromosomes 10 in both humans and mice by Southern blot analyses of somatic cell hybrids (Milatovich et al., 1993). DCoH functions as both a transcriptional coactivator and a pterin dehydratase (Cronk et al., 1996). The human DCoH (also named pterin-4α-carbinolamine dehydratase) is a bifunctional protein proposed to be involved in entirely different biochemical functions. The protein coding region of the gene is about 5 kb long and contains 4 exons. Within the 5′-flanking sequence, potential regulatory regions include consensus binding sites for transcription factor Sp1, an AP-1, and several AP-2-binding sites; however, the 5′ upstream region lacks both a proximal TATA and CAAT box promoter element (Thony et al., 1995).

B. The Hepatocyte Nuclear Factor-3 Subfamily

The mammalian HNF-3/fkh family consists of at least 30 distinct members and is expressed in a variety of different cellular lineages (Qian and Costa, 1995). The HNF-3 gene subfamily is composed of three proteins (α, β, and γ) that mediate hepatocyte-enriched transcription of numerous genes whose expression is necessary for organ function (Samadani and Costa, 1996). All three transcription factors share strong homology in the winged-helix/fork head DNA-binding domain (region I) that overlaps with the nuclear localization signal (Qian and Costa, 1995). HNF-3α, -β, and -γ are able to recognize the same DNA sequence (Samadani and Costa, 1996; Pani et al., 1992a,b). They also possess two similar stretches of amino acids at the carboxyl terminus (regions II and III) and a fourth segment of homology at the amino terminus (region IV) (Pani et al., 1992a,b).

The HNF-3 proteins demonstrate homology with the Drosophila homeotic gene fork head in regions I, II, and III, suggesting that HNF-3 may be its mammalian homolog (Pani et al., 1992a). Experiments using site-directed mutagenesis within regions II and III (amino acids 361–458) of HNF-3β demonstrated their importance for transactivation. In cotransfection assays with expression vectors that produced different truncated HNF-3β proteins, amino-terminal sequences defined by conserved region IV also contributed to transactivation, but region IV activity required the participation of the region II-III domain (Pani et al., 1992a).

HNF-3α and HNF-3β regulate gene expression in endoderm-derived hepatocytes, and intestinal, pancreatic, and bronchiolar epithelium (Rausa et al., 1997; Clevidence et al., 1998). HNF-3α may also play an important role in development and maintenance of urogenital tract epithelial cells (Clevidence et al., 1998; Ko-
TABLE 1

Shown are examples of liver-specific genes that contain a regulatory element with a HNF-1 binding site. The species of the investigated gene with its regulatory sequence as well as the respective references are indicated. The positions of the HNF-1 binding sites have preferably been taken from published DNase I footprinting studies, if available. The next preference is for chemical modifications, and the last for gel retardation assays. In case of different positional information for both DNA strands, the more upstream position has been taken for the 5'-border and the more downstream position for the 3'-border of the site. If not stated otherwise, the position numbers generally refer to the transcription start site (t.s.s.). Occasionally they may refer to the translation start codon stated as ATG or to a defined restriction site. When the authors emphasized a specific motif within the published regulatory sequence, it is written in capitals whereas the rest of the sequence is written in lowercase letters. 3 indicates a continuing sequence in the next line.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Regulatory Element</th>
<th>Gene Region</th>
<th>Position of Binding Site</th>
<th>First Position</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>tGGTTAGaattactaa</td>
<td>Element eG</td>
<td>−363 to −338</td>
<td>NheI at −11.4 kb</td>
<td>Homo sapiens</td>
<td>Liu et al., 1991</td>
</tr>
<tr>
<td>Aldolase B</td>
<td>GTTGTTCCTT</td>
<td>−126 to −104</td>
<td>t.s.s.</td>
<td>Mus musculus</td>
<td>Rattus norvegicus</td>
<td>Frain et al., 1990; Gregori et al., 1993; Tsutsumi et al., 1989</td>
</tr>
<tr>
<td>α1-Antitrypsin</td>
<td>TGGTTAATTTCCagc</td>
<td>86 to 56</td>
<td>t.s.s.</td>
<td>H. sapiens</td>
<td></td>
<td>De Simone and Cortese, 1991</td>
</tr>
<tr>
<td>α-Fetoprotein</td>
<td>TGGTTAATTTCGCTAACTTC</td>
<td>−128 to −99</td>
<td>t.s.s.</td>
<td>Rattus rattus</td>
<td></td>
<td>Jose-Estanyol and Danan, 1988</td>
</tr>
<tr>
<td>α-Fibrinogen Apolipoprotein AI</td>
<td>AGGCAAAAGCCTAT</td>
<td>Promoter</td>
<td>−67 to −54</td>
<td>t.s.s.</td>
<td>H. sapiens</td>
<td>Hu et al., 1995; Chambaz et al., 1991; Cardot et al., 1993</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>GTTTATCAGTGACTAGTTCATTGAT</td>
<td>835 to 876</td>
<td>Cap</td>
<td>H. sapiens</td>
<td></td>
<td>Brooks and Levy-Wilson, 1992</td>
</tr>
<tr>
<td>β-Fibrinogen</td>
<td>CAAACTGCACCAAT</td>
<td>Distal region I, N</td>
<td>−903 to −879</td>
<td>t.s.s.</td>
<td>H. sapiens</td>
<td></td>
</tr>
<tr>
<td>CYP2E1</td>
<td>TGATGCCTTGATATTG TTTGGAATATTTAAACTAAGGGGAG</td>
<td>59 to 35</td>
<td>ATG</td>
<td>H. sapiens</td>
<td></td>
<td>Figueiredo and Brownlee, 1995</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>CAATTTGAAAAATTATGAT</td>
<td>−80 to −57</td>
<td>t.s.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP-1 (insulin-like growth factor binding protein-1)</td>
<td>TGCGGCGCTGCCAATCATTAAC</td>
<td>79 to 53</td>
<td>ATG</td>
<td>H. sapiens</td>
<td></td>
<td>Powell et al., 1995</td>
</tr>
<tr>
<td>Large surface protein (HBV)</td>
<td>TAGTTAATCATATACCTTC</td>
<td>SPI promoter</td>
<td>−93 to −68</td>
<td>t.s.s.</td>
<td>Human hepatitis B virus</td>
<td>Chang et al., 1989</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>GTTTCCCTGTGGTTG TTTGGAATATTTAAACTAAGGGGAG</td>
<td>59 to 35</td>
<td>ATG</td>
<td>H. sapiens</td>
<td></td>
<td>Chow et al., 1991</td>
</tr>
<tr>
<td>Surface antigen (HBV)</td>
<td>GTTTCCCTGTGGTTG TTTGGAATATTTAAACTAAGGGGAG</td>
<td>59 to 35</td>
<td>ATG</td>
<td>Human hepatitis B virus</td>
<td></td>
<td>Zhou and Yen, 1991</td>
</tr>
<tr>
<td>Vitellogenin A2</td>
<td>TGAGGTTAAAGTTTCC</td>
<td>AABS element</td>
<td>−124 to −85</td>
<td>t.s.s.</td>
<td>Xenopus laevis</td>
<td>Drewes et al., 1991</td>
</tr>
</tbody>
</table>
HNF-3α and HNF-3β are members of a large family of developmentally regulated transcription factors that participate in embryonic pattern formation (Rausa et al., 1997; Clevé et al., 1998).

Stimulation of HNF-3α gene transcription upon retinoic acid-induced differentiation of mouse F9 embryonal carcinoma cells can give rise to three distinct differentiated cell types: visceral endoderm, parietal endoderm, and primitive endoderm, which indicates that HNF-3α may play an important role in differentiation during primitive endoderm formation, an extremely early event during murine embryogenesis (Jacob et al., 1994).

A number of liver-specific genes that are predominantly expressed in the liver are positively regulated by HNF-3α, -β, or -γ through interaction with the respective cis-acting HNF-3-binding elements in the promoters of these genes (see also Table 2). In contrast, HNF-3 bound to the HNF-3-binding site of the human aldolase B promoter completely antagonizes transactivation of the liver-specific aldolase B gene by HNF-1 and DBP (Gregori et al., 1993).

Partial hepatectomy produced minimal fluctuation in HNF-3 (α, β, and γ) and transthyretin expression, suggesting that HNF-3α, -β, and -γ expression is not influenced by proliferative signals induced during liver regeneration. In acute-phase livers a dramatic reduction in HNF-3α expression was observed, which correlates with a decrease in the expression of target genes, such as the transthyretin gene (Qian et al., 1995).

C. The Hepatocyte Nuclear Factor-4 Subfamily

The HNF-4 subfamily belongs to the nuclear receptor superfamily, which contains more than 150 proteins that represent nuclear receptors for steroids, retinoids, thyroid hormone, and vitamin D, as well as many related proteins (Mangelsdorf et al., 1995). HNF-4 subfamily members include HNF-4α, HNF-4β, and HNF-4γ and many splice variants. HNF-4 was formerly classified as an orphan member of the steroid/thyroid nuclear receptor superfamily, because HNF-4 had no defined ligand. Hertz et al. (1998) reported that fatty acyl-CoA thioesters are ligands of HNF-4α. Therefore it seems no longer justified to think of the HNF-4 subfamily members as orphan members of the larger nuclear receptor superfamily.

HNF-4 participates in the regulation of several genes involved in diverse metabolic pathways (e.g., glucose, cholesterol, and fatty acid metabolism), in the synthesis of blood coagulation factors, and in developmental processes determining the hepatic phenotype (see also Table 3) (Sladek et al., 1990; Jiang et al., 1995; Yamagata et al., 1996; Hadzopoulou-Cladaras et al., 1997).

HNF-4α (gene symbol, TCF14) is an upstream regulator of HNF-1α expression (Yamagata et al., 1996) and is expressed in the mammalian liver, kidney, and digestive tract (Sladek et al., 1990; Holewa et al., 1997). The human HNF-4α gene was mapped to chromosome 20q in a region syngenic with mouse chromosome 2, to which the HNF-4 ortholog has been assigned (Argyrokastritis et al., 1997; Chevre et al., 1998).

HNF-4β was first identified in Xenopus and showed distinct activation and expression profiles in oogenesis and embryogenesis of Xenopus laevis (Holewa et al., 1997).

A novel HNF-4 subtype called HNF-4γ could be located on human chromosome 8. Northern blot analysis revealed that HNF-4γ is expressed in the kidney, pancreas, small intestine, testis, and colon but not in the liver, whereas HNF-4α RNA was found in all of these tissues (Drewes et al., 1996).

An example of negative HNF-4 regulation is the mitochondrial HMG-CoA synthase gene. HNF-4 binds to the mitochondrial HMG-CoA synthase nuclear receptor response element and represses peroxisome proliferator-activated receptor (PPAR)-dependent activation of reporter gene linked to the mitochondrial HMG-CoA synthase gene promoter (Rodriguez et al., 1998). Another example of negative regulation by HNF-4 is the acyl-CoA oxidase gene. Both PPARα and HNF-4 efficiently bind to the acyl-CoA oxidase gene enhancer element, but PPARα exhibits much stronger transactivation than HNF-4. As a result, HNF-4 suppressed the gene-activating function of PPARα, when they were expressed together, due to competition for a common binding site (Nishiyama et al., 1998). An example of repression by HNF-4 could be found in studies of the rat arginase promoter activity that is stimulated by C/EBPs and DBP (Chowdhury et al., 1996).

1. The Structure and Domains of Hepatocyte Nuclear Factor-4. HNF-4 contains two transactivation domains, designated AF-1 and AF-2, which activate transcription in a cell type-independent manner. Deletion of AF-1 results in 40% reduction of the HNF-4-mediated activation. AF-1 consists of the extreme N-terminal 24 amino acids and functions as a constitutive autonomous activator of transcription. This short transactivator belongs to the class of acidic activators, and it is predicted to adopt an amphipathic α-helical structure. In contrast, the AF-2 transactivator is complex, spanning the 128–366 region of HNF-4, and it cannot be further dissected without impairing activity (Hadjopoulos-Cladaras et al., 1997). AF-1 shares common structural motifs and molecular targets with the activation domains of p53, NF-κB-p65, and VP-16 (a herpes simplex virus-1 virion protein), implying that these activators may function through common mechanisms (Green et al., 1998). Remarkably, AF-1 interacts with multiple proteins that act at distinct steps during transcription (including TBP; the TBP-associated factors TAF1131 and TAF1130; TFIIB; TFIH; p62; and the coactivators CBP, ADA2, and PC4) providing a possible mechanism for the functional synergy exhibited by this activator in vivo (Green et al., 1998).
**TABLE 2**

Shown are examples of liver-specific genes that contain a regulatory element with a HNF-3 binding site. The species of the investigated gene with its regulatory sequence as well as the respective references are indicated. The positions of the HNF-3 binding sites have preferably been taken from published DNase I footprinting studies, if available. The next preference is for chemical modifications, and the last for gel retardation assays. In case of different positional information for both DNA strands, the more upstream position has been taken for the 5'-border of the site. If not stated otherwise, the position numbers generally refer to the transcription start site (t.s.s.). Occasionally they may refer to the translation downstream position for the 3'-start codon stated as ATG or to a defined restriction site. When the authors emphasize a specific motif within the published regulatory sequence it is written in capitals whereas the rest of the sequence is written in lowercase letters.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Regulatory Element</th>
<th>Gene Region</th>
<th>Position of Binding Site</th>
<th>First Position</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>GTTTGTCTTT</td>
<td>Element eG</td>
<td>528 to 547</td>
<td>Nhe1 at –11.4 kb</td>
<td>M. musculus</td>
<td>Liu et al., 1991</td>
</tr>
<tr>
<td>Aldolase B</td>
<td>CATGGATTGAGAATAACACCTC</td>
<td>5'-Region</td>
<td>–126 to –104</td>
<td>t.s.s.</td>
<td>R. norvegicus</td>
<td>Gregori et al., 1993; Tsubumi et al., 1989</td>
</tr>
<tr>
<td>α-1-Antitrypsin</td>
<td>AAATTGACTTTA</td>
<td>5'-Region</td>
<td>–378 to –366</td>
<td>t.s.s.</td>
<td>M. musculus</td>
<td>Samadani and Costa, 1996</td>
</tr>
<tr>
<td>α-Fetoprotein</td>
<td>caAGTGCAATAAg</td>
<td>5'-Region</td>
<td>–6103 to –6090</td>
<td>t.s.s.</td>
<td>R. norvegicus</td>
<td>Lannoy et al., 1998; Samadani et al., 1996</td>
</tr>
<tr>
<td>α2-Macroglobulin</td>
<td>GGTATTGACTTTG</td>
<td>5'-Region</td>
<td>–452 to –442</td>
<td>t.s.s.</td>
<td>R. norvegicus</td>
<td>Samadani and Costa, 1996</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>CTGCTCATTTAGTCTAGTCATT</td>
<td>Intron 2, enhancer, element E</td>
<td>839 to 935</td>
<td>t.s.s.</td>
<td>H. sapiens</td>
<td>Ye et al., 1997</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>CTGGTTTTGCTAC</td>
<td>892 to 904</td>
<td>Samadani and Costa, 1996</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin growth factor binding protein 1</td>
<td>TggacacctcacagctgTGGTGATTGacaaccagcag</td>
<td>IRS (insulin responsive sequence)</td>
<td>–168 to –137</td>
<td>t.s.s.</td>
<td>R. norvegicus</td>
<td>Goswami et al., 1994</td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxykinase</td>
<td>TGGTGTTTTGACAAC</td>
<td>IRS (insulin-responsive sequence)</td>
<td>–124 to –96</td>
<td>t.s.s.</td>
<td>H. sapiens</td>
<td>O'Brien et al., 1995</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>TGTTTATTTGCATACT</td>
<td>Promoter, site III</td>
<td>–416 to –402</td>
<td>t.s.s.</td>
<td>R. norvegicus</td>
<td>Lemaigre et al., 1996</td>
</tr>
<tr>
<td>Protein C</td>
<td>agggecAAGGAAATTTTGTGTTGgaaa</td>
<td>–43 to –15</td>
<td>t.s.s.</td>
<td>H. sapiens</td>
<td>Spek et al., 1995</td>
<td></td>
</tr>
<tr>
<td>Transferrin</td>
<td>AAGTTGAGTACTGCAATG</td>
<td>Site A</td>
<td>–306 to –297</td>
<td>t.s.s.</td>
<td>Salmo salar</td>
<td>Stenson et al., 2000</td>
</tr>
<tr>
<td>Tryptophan oxygenase</td>
<td>CgtTAAGGATTTG</td>
<td>5'-Region</td>
<td>–220 to –208</td>
<td>t.s.s.</td>
<td>R. norvegicus</td>
<td>Samadani and Costa, 1996</td>
</tr>
<tr>
<td>TTR</td>
<td>GGTGTTTTGCTAC</td>
<td>81 to 52 t.s.s.</td>
<td>X. laevis</td>
<td>Cardinaux et al., 1994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine aminotransferase</td>
<td>GGGGATTTTCTGTTGTCAG</td>
<td>10514 to 10484</td>
<td>t.s.s.</td>
<td>R. norvegicus</td>
<td>Nitsch et al., 1993</td>
<td></td>
</tr>
<tr>
<td>Vitellogenin A2</td>
<td>TGAGGTTAGTACCATCACAAa</td>
<td>AABS element</td>
<td>–124 to –85</td>
<td>t.s.s.</td>
<td>X. laevis</td>
<td>Kaling et al., 1991</td>
</tr>
<tr>
<td>Vitellogenin B1</td>
<td>CTGTTGTC</td>
<td>Promoter</td>
<td>–58 to –52</td>
<td>t.s.s.</td>
<td>X. laevis</td>
<td>Cardinaux et al., 1994</td>
</tr>
</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th>Gene Region</th>
<th>Gene</th>
<th>Regulatory Element</th>
<th>Species</th>
<th>Position of Binding Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-enhancer, element 1</td>
<td>Apolipoprotein CIII</td>
<td>CAGGTGACCTTTGCCC</td>
<td>H. sapiens</td>
<td>93 to 1992</td>
</tr>
<tr>
<td>3'-enhancer, element 1</td>
<td>Factor VIII</td>
<td>CTCAGATCCCAGCCAGTGGACTTAGCCC</td>
<td>H. sapiens</td>
<td>1-Antitrypsin</td>
</tr>
<tr>
<td>Enhancer, element I</td>
<td>TTR (transthyretin)</td>
<td>GCAGCTTAACCTCTGAACTTCTAagg</td>
<td>R. norvegicus</td>
<td>156 to 185</td>
</tr>
<tr>
<td>Promoter, element E</td>
<td>Transferrin</td>
<td>AACACGGGAGGTCAAAGATTGCC</td>
<td>H. sapiens</td>
<td>PR I element</td>
</tr>
<tr>
<td>Enhancer, element I</td>
<td>TTR (transthyretin)</td>
<td>CCAAGGTTCATatttgtgtag</td>
<td>M. musculus</td>
<td>1 to 10</td>
</tr>
</tbody>
</table>

Dissection of the transcription cycle revealed that HNF-4 activated transcription by facilitating assembly of a preinitiation complex intermediate consisting of TBP, the TATA box-binding protein component of TFIID and TFIIID, via direct physical interactions with TFIIIA. However, recruitment of TFIIIA by HNF-4 was not sufficient for activation, because HNF-4 deletion derivatives lacking AF-2 bound TFIIIA. On the basis of these results, HNF-4 appears to activate transcription at two distinct levels. The first step involves AF-2-independent recruitment of TFIIIA to the promoter complex; the second step is AF-2-dependent and entails entry of preinitiation complex components acting downstream of TFIIIA (Malik and Karathanasis, 1996). The 360–366 region of HNF-4 contains a motif that is highly conserved among transcriptionally active nuclear receptors, and it is essential for AF-2 activity, but it is not necessary for dimerization and DNA binding of HNF-4. Thus, HNF-4 deletion mutants lacking the 361–465 region bind efficiently to DNA as homo- and heterodimers and behave as dominant negative mutants (Hadzopoulou-Cladaras et al., 1997). Remarkably, the full transcriptional activation potential of AF-2 is inhibited by the region spanning residues 371–465 (region F). The inhibitory effect of region F on the HNF-4 AF-2 activity is a unique feature among members of the nuclear receptor superfamily, and it has been proposed that it defines a distinct regulatory mechanism of transcriptional activation by HNF-4 (Hadzopoulou-Cladaras et al., 1997). In later studies a repressor domain has been localized to residues 428–441 in region F of HNF-4 that is sufficient by itself to repress the activity of the AF-2 domain. Multiple mutations within this repressor domain enhance activity (Iyemere et al., 1998).

2. The Relevance of Hepatocyte Nuclear Factor-4 Splice Variants. Further complexity of gene control by HNF-4α transcription factors can be anticipated by the differential splicing of the 10 initially identified exons of the HNF-4α gene (Nakhei et al., 1998). Thus, so far, seven distinct splice variants have been identified in human and murine cDNA samples. HNF-4α1 represents the initially identified transcript, whereas HNF-4α2 through HNF-4α7 are the splice variants identified subsequently (Sladek et al., 1990; Hata et al., 1992, 1995; Chartier et al., 1994; Drewes et al., 1996; Kritis et al., 1996; Furuta et al., 1997; Nakhei et al., 1998). HNF-4α1, HNF-4α2, and HNF-4α3 were initially referred to as HNF-4A, HNF-4B, and HNF-4C, respectively (Hata et al., 1992, 1995; Kritis et al., 1996). In all HNF-4α splice variants the DNA-binding domain remains unchanged (Violet et al., 1997; Nakhei et al., 1998). The impact of these different splice variants on the regulation of downstream target gene regulation remains largely to be determined. The consequences of the existence of different splice variants on the regulation of gene transcription are still not fully understood.
Within the 5′-untranslated region of HNF-4β, the two splice variants HNF4β2 and HNF4β3 with additional exons were detected. Both HNF-4β splice variants share HNF-4-binding sites with HNF-4α but have lower DNA-binding activities and weaker transactivation potential than HNF-4α (Holewa et al., 1997).

In cotransfection experiments evidence was obtained that HNF-4γ is significantly less active than HNF-4α2 and that the HNF-4α splice variant HNF-4α4 has no detectable transactivation potential. Therefore, the differential expression of distinct HNF-4 proteins may play a key role in the differential transcriptional regulation of HNF-4-dependent genes (Drewes et al., 1996).

3. Homo- and Heterodimerization of Hepatocyte Nuclear Factor-4 Proteins. Studies with in vitro translated HNF-4 protein show that it binds to its recognition site as a dimer, and cotransfection assays indicate that it activates transcription in a sequence-specific fashion in nonhepatic (HeLa) cells (Sladek et al., 1990). It has been proposed that HNF-4 forms homodimers in contrast to other members of the nuclear receptor superfamily that also form heterodimers with other members of the nuclear receptor superfamily like retinoid X receptor α (RXR-α) (Jiang et al., 1995). Later, it could be demonstrated that another orphan member of the nuclear hormone receptor superfamily called SHP (short heterodimer partner), which contains the dimerization and ligand-binding domain found in other family members but lacks the conserved DNA-binding domain (Seol et al., 1996), specifically inhibits transactivation by HNF-4 and other hormone receptor superfamily members with which it interacts (Seol et al., 1996; Lee et al., 2000). Therefore, it has been suggested that SHP functions as a negative regulator of receptor-dependent signaling pathways (Seol et al., 1996; Lee et al., 2000). SHP represses nuclear hormone receptor-mediated transactivation via two separate steps: first by competition with coactivators and second by direct effects of its transcriptional repressor function (Lee et al., 2000).

4. Regulation of Hepatocyte Nuclear Factor-4 Function by Phosphorylation. HNF-4 DNA-binding activity is modulated post-translationally by phosphorylation (Ktistaki et al., 1995; Viollet et al., 1997). Phosphorylated HNF-4 is concentrated in distinct nuclear compartments within the cell, as evidenced by in situ immunofluorescence and electron microscopy. Inhibition of HNF-4 phosphorylation with genistein results in a loss of the nuclear compartmentalization of HNF-4 associated with a significantly decreased ability to activate endogenous target genes (Ktistaki et al., 1995).

In cell-free systems and in cultured cells, phosphorylation at tyrosine residue(s) is important for the DNA-binding activity of HNF-4 and, consequently, for its transactivation potential (Ktistaki et al., 1995). Further experiments demonstrated that phosphorylation of HNF-4 by cAMP-dependent protein kinase A at serine residues leads to a reduced DNA-binding affinity of HNF-4 in vitro (Viollet et al., 1997). It could be demonstrated that in vivo phosphorylation of HNF-4 depends on the diet; it is decreased by a carbohydrate-rich diet and is increased by fasting or in refed animals given glucagon or isoproterenol and phosphodiesterase inhibitors (Viollet et al., 1997). Phosphorylation of HNF-4 by cAMP-dependent protein kinase A at serine residues might be involved in the transcriptional inhibition of liver genes by cAMP inducers (Viollet et al., 1997).

5. Agonistic and Antagonistic Ligands for the Nuclear Receptor Hepatocyte Nuclear Factor-4α. In 1998 Hertz and coworkers published the discovery of several ligands for HNF-4 with agonistic and antagonistic effects on HNF-4α transcriptional activity (see also Tables 4 and 5). It could be demonstrated that long-chain fatty acids directly modulate the transcriptional activity of HNF-4α by binding as their acyl-CoA thioesters to the ligand-binding domain of HNF-4α. This binding shifts the oligomeric-dimeric equilibrium of HNF-4α, because it could be shown that the binding of saturated (C14:0)-CoA to the ligand-binding domain of HNF-4α leads to increased HNF-4α dimerization and activates binding of the HNF-4α dimer to its cognate enhancer element, whereas saturated (C16:0)-CoA only activates binding of the HNF-4α dimer to its cis-acting element. In contrast, the antagonistic ligands ω-3 and ω-6 polyunsaturated fatty acyl-CoAs, (C18:3, w-3)-CoA, and saturated (C18:0)-CoA decrease the transcriptional activity of HNF-4α. (C18:3, w-3)-CoA and saturated (C18:0)-CoA were shown to lower the affinity of HNF-4α to its cognate enhancer element. Furthermore, it could be demonstrated that saturated (C18:0)-CoA leads to decreased HNF-4α dimerization (Hertz et al., 1998).

6. Acetylation of Nucleosomal Histones and Hepatocyte Nuclear Factor-4 by cAMP Response Element-Binding Protein. CBP possesses an intrinsic acetyltransferase activity capable of acetylating nucleosomal histones as well as several nonhistone proteins. It could be demonstrated that CBP can acylate HNF-4 at lysine residues within the nuclear localization sequence. CBP-mediated acetylation is crucial for the proper nuclear retention of HNF-4, which is otherwise transported out to the cytoplasm via the CRM1 pathway. Acetylation also increases HNF-4 DNA-binding activity and its affinity of interaction with CBP itself, and is required for target gene activation. Acetylation is a key post-translational modification that may affect several properties of a transcription factor critical for the execution of its biological functions (Soutoglou et al., 2000a).

7. Chicken Ovalbumin Upstream Promoter-Transcription Factors and Hepatocyte Nuclear Factor-4: Cooperation and Competition. Chicken ovalbumin upstream promoter-transcription factor (COUP-TF) and HNF-4 were both frequently called orphan members of the steroid/thyroid receptor superfamily and exhibit ubiquitous and liver-enriched tissue distribution, respectively (Kimura et al., 1993). COUP-TFs strongly inhibit tran-
<table>
<thead>
<tr>
<th>Substance</th>
<th>HNF-4 Domain</th>
<th>Mode of Action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated (C14:0)-CoA</td>
<td>LBD</td>
<td>Binding of ligand leads to increased HNF-4α dimerization</td>
<td>Hertz et al., 1998</td>
</tr>
<tr>
<td>Saturated (C16:0)-CoA</td>
<td>LBD</td>
<td>Binding of ligand activates binding of the HNF-4α dimer to its cognate enhancer element</td>
<td></td>
</tr>
<tr>
<td>SRC-1 (NCoA-1)</td>
<td>AF-2</td>
<td>Binding of ligand activates binding of the HNF-4α dimer to its cognate enhancer element</td>
<td></td>
</tr>
<tr>
<td>SRC-2 (NCoA-2, GRIP1, TIF2)</td>
<td>AF-2</td>
<td>Specific H3 and H4 histone acetylation by intrinsic SRC-1 HAT activity</td>
<td>Spencer et al., 1997</td>
</tr>
<tr>
<td>SRC-3 (NCoA-3, ACTR, AIB1, p/CIP, TRAM-1, RAC3)</td>
<td>AF-2</td>
<td>Interaction with C terminus of CBP/p300 (with intrinsic HAT activity)</td>
<td>Yao et al., 1996</td>
</tr>
<tr>
<td>SRC-2 (NCoA-2, GRIP1, TIF2)</td>
<td>AF-2</td>
<td>Interaction between HNF-4, SRC-1, and SRC-2 leading to increased transactivation activity of HNF-4</td>
<td>Wang et al., 1998a</td>
</tr>
<tr>
<td>SRC-3 (NCoA-3, ACTR, AIB1, p/CIP, TRAM-1, RAC3)</td>
<td>AF-2</td>
<td>Interaction between HNF-4, SRC-1, and SRC-2 is leading to increased transactivation activity of HNF-4</td>
<td>Wang et al., 1998a</td>
</tr>
<tr>
<td>CBP/p300</td>
<td>AF-1, AF-2</td>
<td>Protein-protein interaction with p/CAF and CBP/p300</td>
<td>Torchia et al., 1997</td>
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<tr>
<td></td>
<td></td>
<td>Interaction between HNF-4 and CBP is ligand-independent. CBP binds to the HNF-4 AF-1 and AF-2 domains with the N terminus and the N and C terminus, respectively</td>
<td>Dell and Hadzopoulou-Cladaras, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Histone acetylation: intrinsic histone acetylase (HAT) activity</td>
<td>Bannister and Kouzarides, 1996</td>
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<tr>
<td></td>
<td></td>
<td>Protein-protein interaction: CBP/p300 binds p/CAF that also contains an intrinsic HAT activity</td>
<td>Yang et al., 1996</td>
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<td></td>
<td></td>
<td>Acetylation of HNF-4α at lysine residues by CBP/p300 enhances HNF-4α/DNA binding affinity, as well as CBP/p300 binding affinity to HNF-4 and is essential for proper nuclear retention of HNF-4α</td>
<td>Soutoglou et al., 2000a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Binding to the AF-1 and AF-2 domain leads to ligand-independent HNF-4α activation</td>
<td>Ktistaki et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CBP/p300 interacts with its N-terminal fragment directly with nuclear receptors, with its C-terminal fragment directly with SRC-1, and with distinct internal regions with CREB, Fos, TFIIB, and E1A</td>
<td>Kamei et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetylation of general transcription factors TFIIEb and TFIIF by p300 leading to increased transcription</td>
<td>Ogrzyko et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interaction with components of the basal transcription machinery (TBP, TFIIB, RNA helicase) leading to increased transcription</td>
<td>Kamei et al., 1996</td>
</tr>
<tr>
<td>ADA2</td>
<td>AF-1</td>
<td>ADA2-GCN5 complex acetylates histones</td>
<td>Green et al., 1998</td>
</tr>
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<td>ADA2 interacts with the AF-1 domain of HNF-4 and TBP and TAFs facilitating transcription by the transcriptional machinery</td>
<td>Barlev et al., 1995</td>
</tr>
<tr>
<td>PC4</td>
<td>AF-1</td>
<td>PC4 contains two ssDNA binding domains that might be implicated in the opening of the DNA double helix at a post-initiation step</td>
<td>Green et al., 1998; Brandsen et al., 1997</td>
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<tr>
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<td></td>
<td>PC4 interacts with the AF-1 domain of HNF-4 and TAFs facilitating transcription by the transcriptional machinery</td>
<td>Ge and Roeder, 1994</td>
</tr>
<tr>
<td>COUP-TFI/COUP-TFII</td>
<td>E</td>
<td>Promoters that are recognized by HNF-4α and not by COUP-TFs lead to a coactivator function of COUP-TFs through a protein/protein interaction between HNF-4 and COUP-TFs at the HNF-4 E domain</td>
<td>Ktistaki and Talianidis, 1997</td>
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</tbody>
</table>
scriptional activation mediated by nuclear hormone receptors, including HNF-4. COUP-TFs repress HNF-4-dependent gene expression by competition with HNF-4 for common binding sites found in several regulatory regions (Kimura et al., 1993; Ktistaki and Talianidis, 1997b). In contrast, promoters, such as the HNF-1 promoter, which are recognized by HNF-4 but not by COUP-TFs, are activated by COUP-TFI and COUP-TFII in conjunction with HNF-4 more than 100-fold above basal levels, as opposed to about 8-fold activation by HNF-4 alone (Ktistaki and Talianidis, 1997b). This enhancement was strictly dependent on an intact HNF-4 E domain. In vitro and in vivo evidence suggests that COUP-TFs enhance HNF-4 activity by a mechanism that involves their physical interaction with the amino acid 227–271 region of HNF-4 (see also Fig. 6) (Ktistaki and Talianidis, 1997b). Therefore, in certain promoters,

**TABLE 5**

<table>
<thead>
<tr>
<th>Substance</th>
<th>HNF-4 Domain</th>
<th>Mode of Action</th>
<th>Antagonistic ligands</th>
</tr>
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<tbody>
<tr>
<td>SHP</td>
<td>AF-2</td>
<td>No</td>
<td>COUP-TFs</td>
</tr>
<tr>
<td>HNF-1α</td>
<td>AF-2</td>
<td>Direct inhibition after binding to the AF-2 domain, probably competing with coactivators</td>
<td></td>
</tr>
<tr>
<td>Receptors</td>
<td>AF-2</td>
<td>No</td>
<td>COUP-TFs</td>
</tr>
<tr>
<td>SHP</td>
<td>AF-2</td>
<td>Direct inhibition after binding to the AF-2 domain, probably competing with coactivators</td>
<td></td>
</tr>
<tr>
<td>HNF-4</td>
<td>AF-2</td>
<td>No</td>
<td>COUP-TFs</td>
</tr>
<tr>
<td>Domain</td>
<td>AF-2</td>
<td>Direct inhibition after binding to the AF-2 domain, probably competing with coactivators</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 6.** Here we propose a model on the various interactions at the HNF-1α promoter involving the transcription factors HNF-1α, HNF-4α, and their respective coactivators as well as the ligands for HNF-4. Biochemical functions of the coactivators are outlined by arrows in different shapes and colors representing acetylations of various molecules as indicated. Phosphorylation of HNF-4α is also indicated by an arrow. It is thought that the depicted events and interactions are necessary for optimal transcription of HNF-1α, which plays a major role in the hepatocyte nuclear factor network in liver function and during liver development (for more details and references see text).
COUP-TFs act as auxiliary cofactors for HNF-4, orienting the HNF-4 activation domain in a more efficient configuration to achieve enhanced transcriptional activity (Kimura et al., 1993; Ktistaki and Talianidis, 1997b). An example of COUP-TF-associated repression of a liver-specific gene provides the gene for rat ornithine transcarbamylase, an ornithine cycle enzyme (Kimura et al., 1993). Therefore, COUP-TF plays a dual regulatory role depending on the promoter context. Repression of a tissue-specific promoter by a ubiquitous transactivator and derepression by a related tissue-enriched transactivator is potentially an important mechanism for tissue-specific activation of a gene (Kimura et al., 1993; Ktistaki and Talianidis, 1997b).

D. Hepatocyte Nuclear Factor-6

In contrast to HNF-5 there is plenty of evidence for the existence of HNF-6 (see Table 6). The human gene for HNF-6 has been mapped to chromosome bands 15q21.1–21.2 and the rat gene to chromosome 8q24–q31 by Southern blotting of DNA from somatic cell hybrids and by fluorescence in situ hybridization (Vaisse et al., 1997; Rastegar et al., 1998). Interspecific backcross analysis determined that the murine HNF-6 gene is located in the middle of mouse chromosome 9 (Rausa et al., 1997).

Transcription factors of the one-cut class, whose prototype is HNF-6, are characterized by the presence of a single-cut domain and by a peculiar homeodomain. Human OC-2, the second mammalian member of this class, is located on human chromosome 18. The distribution of OC-2 mRNA in humans is tissue-restricted, the strongest expression being detected in the liver and skin. The amino acid sequence of OC-2 contains several regions of high similarity to HNF-6. The recognition properties of OC-2 for binding sites present in regulatory regions of liver-expressed genes differ from, but overlap with, those of HNF-6 (Jacquemin et al., 1999). It might be that in the future, HNF-6 and OC-2 will be regarded as two members of a bigger family.

1. Splice Variants of Hepatocyte Nuclear Factor-6

Two rat cDNA species coding for two isoforms, HNF-6α (465 residues) and HNF-6β (491 residues) could be identified, which differ only by the length of the spacer between the two DNA-binding domains. The two HNF-6 isoforms are generated by alternative splicing of three exons that are more than 10 kb apart from each other. exon 1 codes for the N-terminal part and the cut domain, exon 2 codes for the 26 HNF-6β-specific amino acids, and exon 3 codes for the homeodomain and the C-terminal amino acids (Rastegar et al., 1998). Both isoforms stimulate transcription. The affinity of HNF-6α and HNF-6β for DNA depends on the target sequence. Binding of HNF-6 to DNA involves the cut domain and the homeodomain, but the latter is not required for binding to a subset of sites (Lannoy et al., 1998).

2. Hepatocyte Nuclear Factor-6 in Development

Observations that HNF-6 contributes to the control of the

<table>
<thead>
<tr>
<th>Table 6</th>
<th>LIVER-ENRICHED TRANSCRIPTION FACTORS 149</th>
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<tr>
<td>Gene Regulatory Element</td>
<td>Gene Region</td>
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<td>---</td>
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<tr>
<td>α1-Antitrypsin TCCATTGATTTAG</td>
<td>5'-Region</td>
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<tr>
<td>Fetoprotein caAAGTCAATAAag</td>
<td>5'-Region</td>
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<tr>
<td>Phosphoenolpyruvate carboxykinase cagaaagttTAGTCAATCAaacgttg</td>
<td>5'-Region</td>
</tr>
<tr>
<td>6-Phosphofructo-2-omycinase TCTATTGATTTAT</td>
<td>5'-Region</td>
</tr>
<tr>
<td>TTR (transthyretin, prealbumin) CTAAGTCAATAAT</td>
<td>Promoter site IV</td>
</tr>
<tr>
<td>Intein 1, GRU (glucocorticoid responsive unit)</td>
<td>5'-Region</td>
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<td>CYP2C12</td>
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<td>CYP2C13</td>
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<td>CYP2D11</td>
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expression of transcription factors and is expressed at early stages of liver, pancreas, and neuronal differentiation suggest that HNF-6 participates in several developmental programs (Landry et al., 1997). HNF-6 recognizes the −138 to −126 region of the HNF-3β promoter. Site-directed mutagenesis of this HNF-6 site diminishes reporter gene expression, suggesting that HNF-6 activates transcription of this promoter and may thus play a role in epithelial cell differentiation of gut endoderm via regulation of HNF-3β (Samadani and Costa, 1996).

Later, it was recognized that HNF-6 is required for HNF-3β promoter activity and that HNF-6 also recognizes the regulatory region of numerous liver-specific genes (Rausa et al., 1997). In situ hybridization studies of staged specific embryos demonstrate that HNF-6 and its potential target gene, HNF-3β, are coexpressed in the pancreatic and hepatic diverticulum. More detailed analysis of the developmental expression patterns of HNF-6 and HNF-3β provides evidence of colocalization in hepatocytes, intestinal epithelial, and pancreatic ductal epithelial and exocrine acinar cells. The expression patterns of these two transcription factors do not overlap in other endoderm-derived tissues or the neurotube (Rausa et al., 1997).

3. Regulation of Hepatocyte Nuclear Factor-6 Expression by Growth Hormone. HNF-6 expression can be regulated and modulated by growth hormone (GH) (Lahuna et al., 1997, 2000). In hypophysectomized rats, HNF-6 mRNAs increase within 1 h after a single injection of GH. The same GH-dependent induction could be reproduced on isolated hepatocytes. DNA binding experiments showed that the transcription factors STAT5 (signal transducer and activator of transcription 5) and HNF-4 bind to sites located around −110 and −650 of the hnf-6 gene, respectively. Furthermore, it could be demonstrated that STAT5 binding is induced and HNF-4-binding affinity is increased in the liver within 1 h after GH injection to hypophysectomized rats (Rastegar et al., 2000). Using transfection experiments and site-directed mutagenesis, it could be found that STAT5 and HNF-4 stimulated transcription of an hnf-6 gene promoter-reporter construct. Consistent with earlier findings that HNF-6 stimulates the hnf-4 and hnf-3β gene promoters, GH treatment of hypophysectomized rats increased the liver concentration of HNF-4 and HNF-3β mRNAs. Together, these data demonstrate that GH stimulates transcription of the hnf-6 gene by a mechanism involving STAT5 and HNF-4. They show that HNF-6 participates not only as an effector, but also as a target, to the regulatory network of liver transcription factors, and that several members of this network are GH-regulated (Lahuna et al., 2000). In protein-DNA interaction studies and in transfection experiments, it could be found that the liver-enriched transcription factor C/EBPα binds to the hnf-6 gene and inhibits its expression. This inhibitory effect involved an N-terminal subdomain of C/EBPα and two sites in the hnf-6 gene promoter. Using liver nuclear extracts from GH-treated hypophysectomized rats, it was found that GH induces a rapid, transient decrease in the amount of C/EBPα protein. This GH-induced change is concomitant with the transient stimulatory effect of GH on the hnf-6 gene. Stimulation of the hnf-6 gene by GH therefore involves lifting of the repression exerted by C/EBPα in addition to the GH-induced stimulatory effects of STAT5 and HNF-4 on that gene (Pierreux et al., 1999).

4. Inhibitory Protein-Protein Interaction between Hepatocyte Nuclear Factor-6 and a Nuclear Receptor. HNF-6 inhibits the glucocorticoid-induced stimulation of two genes coding for enzymes of liver glucose metabolism, 6-phosphofructo-2-kinase and phosphoenolpyruvate carboxykinase. Binding of HNF-6 to DNA is required for inhibition of glucocorticoid receptor activity. In vitro and in vivo experiments suggest that this inhibition is mediated by a direct HNF-6/glucocorticoid receptor interaction involving the amino-terminal domain of HNF-6 and the DNA-binding domain of the receptor (Pierreux et al., 1999).

E. Coactivators for Hepatocyte Nuclear Factor-1 and Hepatocyte Nuclear Factor-4

Multiple coactivators of HNF-1 and HNF-4 could be identified, including CBP, p300, p/CAF, and a series of factors that have been identified biochemically and by expression cloning (Kamei et al., 1996; Torchia et al., 1997; Yoshida et al., 1997; Dell and Hadzopoulou-Cladaras, 1999; Rachez et al., 2000; Soutoglou et al., 2000a,b). These factors, with a molecular mass around 160 kDa, are members of the p160 protein family and have been shown to exhibit an intrinsic HAT (Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Chen et al., 1997; Glass et al., 1997; Imhof et al., 1997; Spencer et al., 1997). Furthermore, a nuclear receptor coactivator (NCoA) gene family within the p160 protein family has been proposed that includes the homologous factors SRC-1 (also called NCoA-1), SRC-2 (also called NCoA-2, TIF2, GRIP1) and SRC-3 (also called NCoA-3, ACTR, AIB1, p/CIP, TRAM-1, RAC3) (Torchia et al., 1997; Rachez et al., 2000). The NcoA family members SRC-1, SRC-2, and SRC-3 share a conserved N-terminal bHLH, PAS A domain, a serine/threonine-rich region, and a C-terminal glutamine-rich region (Torchia et al., 1997).

SRC-1, SRC-3, and CBP all contain several related leucine-rich, charged helical interaction motifs (also termed LCDs) with a consensus core LXXLL sequence motif that is required for the assembly of coactivator complex, which provides receptor-specific mechanisms of gene activation and allows the selective inhibition of distinct signal-transduction pathways. Mutation of this consensus core motif leads to abolished interaction with nuclear receptors (Torchia et al., 1997). This leads to the inevitable question whether mutations in these LCD domains may lead to disturbances in liver development.
or liver function due to reduced HNF-1 and HNF-4 transactivation potential.

Possibly, conformational changes in the CBP holoprotein, perhaps in part contributed by SRC-3 by forming the coactivator complex, modulate interactions with transcription factors and associated regulatory proteins, including protein kinases and histone acetylases (Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Torchia et al., 1997).

SRC-1 contains a histone acetylase domain between amino acid residues 1107 and 1216 with intrinsic HAT activity specific for histones H3 and H4 (Spencer et al., 1997). Furthermore, SRC-1 also contains two p/CAF-interacting domains between amino acid residues 1207 and 1250 that bind p/CAF, another factor, with intrinsic histone acetylase activity (Yang et al., 1996; Spencer et al., 1997). SRC-1 interacts also with CBP/p300 through a conserved C-terminal domain of CBP/p300 and probably gets involved in a three-way interaction with CBP/p300 and an interacting nuclear receptor or transcription factor (Kamei et al., 1996; Yao et al., 1996).

SRC-3 and CBP are a functional complex, necessary for the activity of several CBP-dependent transcription factors as well as nuclear receptors (Torchia et al., 1997). Whether SRC-3 is required for transactivation by HNF-1 or HNF-4 remains to be determined. SRC-3 forms complexes with significant portions of CBP in the cell and is required for transcriptional activity of nuclear receptors and other CBP/p300-dependent transcription factors (Torchia et al., 1997). The major CBP interaction domain of SRC-3 could be mapped to amino acid residues 758-1115, with an internal 200-amino acid domain that could still interact, whereas a minimal nuclear receptor interaction domain could be mapped N-terminal of the CBP interaction domain to amino acid residues 680-740, which were sufficient for binding of a liganded nuclear receptor (Torchia et al., 1997).

It could be demonstrated that HNF-1 can physically interact with CBP, p/CAF, SRC-1, and SRC-3 and that these interactions lead to increased HNF-1-dependent transcription in functional assays using a genome-integrated promoter. The transactivation potential of HNF-1 was strictly dependent on the synergistic action of CBP and p/CAF. It could be shown that CBP and p/CAF can independently interact with the N-terminal and the C-terminal domain of HNF-1, respectively (see also Fig. 6) (Soutoglou et al., 2000b).

CBP binds to the HNF-4 AF-1 and AF-2 domains with the N terminus and the N and C termini, respectively (see also Fig. 6) (Dell and Hadzopoulou-Cladaras, 1999). Interestingly, in contrast to the other nuclear hormone receptors the interaction between HNF-4 and CBP is ligand-independent and leads to enhanced HNF-4 transcriptional activity for liver-specific apolipoprotein CIII gene expression (Dell and Hadzopoulou-Cladaras, 1999). Recruitment of CBP by HNF-4 results in an enhancement of the transcriptional activity of the latter (Yoshida et al., 1997; Dell and Hadzopoulou-Cladaras, 1999). CBP does not activate gene expression in the absence of HNF-4, and dominant negative forms of HNF-4 prevent transcriptional activation by CBP, suggesting that the mere recruitment of CBP by HNF-4 is not sufficient for enhancement of gene expression (Dell and Hadzopoulou-Cladaras, 1999).

As expected, it could be demonstrated, that p300 acts as a HNF-4 coactivator in a manner similar to that of CBP and that p300 and SRC-1 together are able to enhance the transcriptional activity of HNF-4 more than SRC-1 or p300 alone (Wang et al., 1998a,b).

The acidic AF-1 domain of the activator HNF-4 interacts specifically with the coactivators CBP, PC4, and ADA2 (see also Fig. 6). It was speculated that AF-1 could affect the preinitiation step through interaction with CBP and/or the ADA2-GCN5 complex by increasing acetylation of histones and rendering the chromatin more accessible to the transcription machinery (Green et al., 1998). Furthermore, it was hypothesized that AF-1 could act also at a postinitiation step, promoting the opening of the DNA double helix through its interaction with PC4 (Brandsen et al., 1997; Green et al., 1998). PC4 and ADA2 are general coactivators that function cooperatively with TBP-associated factors (TAFs) and mediate functional interactions between upstream activators and the general transcriptional machinery (Ge and Roder, 1994; Barlev et al., 1995). PC4 was shown to possess two ssDNA-binding domains that might be implicated in the opening of the DNA double helix during gene transcription (Brandsen et al., 1997). It could be demonstrated that affinity-purified PC2, which lacks independent activity, acts in synergy with the upstream stimulatory activity (USA)-derived coactivator PC4 to mediate the effects of HNF-4 (Malik et al., 2000). ADA2 was shown to display specific interactions with acidic domains of activators such as the HNF-4 AF-1 domain and with the TBP (Barlev et al., 1995).

Table 4 provides an overview of the HNF-4 coactivators and agonistic ligands, while Fig. 6 provides a model of protein-protein and protein-DNA interactions including the players HNF-1, HNF-4, and their cofactors at the HNF-1α promoter.

**F. The Hepatocyte Nuclear Factor Network and Tissue-Specific Gene Expression**

The presence of HNF-4 protein has been correlated with the expression of the liver phenotype in vitro: intertypic rat hepatoma-human fibroblast hybrids that show extinction of liver-specific gene expression are deficient for the expression of HNF-4 and HNF-1, and reexpression of liver-specific genes in revertants (or hybrid cell segregants) correlates with the reexpression of both genes (Griffio et al., 1993). Because HNF-4 is an upstream regulator of HNF-1 expression, it was proposed that the HNF-4 gene is the primary target of the pleiotropic extinguisher (Griffio et al., 1993).
tiated H5 variant cells of a rat hepatoma cell line that show a pleiotropic loss of hepatic functions and fail to express both HNF-1 and HNF-4 (Descharette and Weiss, 1974; Faust et al., 1994) could be directed toward redifferentiation by stable transfection of epitope-tagged HNF-4 cDNA (Späth and Weiss, 1997). The forced expression of only HNF-4 in these H5 variant cells lead to the activation of a subset of liver-specific genes including α1-antitrypsin, β-fibrinogen, and transthyretin, but not of the endogenous HNF-4 gene. Treatment of the HNF-4tag-expressing cells with dexamethasone induced expression of the transgene by 10-fold, resulting in enhanced expression of target genes of both glucocorticoid hormones and HNF-4 (Späth and Weiss, 1997). The set of activated hepatic genes was extended by treatment of cells with the demethylating agent 5-azacytidine followed by selection in dexamethasone-containing glucose-free medium. Some of the colonies that developed reexpressed the entire set of hepatic functions tested (Späth and Weiss, 1997). In dedifferentiated rat hepatoma H5 cells, the effects of HNF-4 expression extend to the reestablishment of differentiated epithelial cell morphology and simple epithelial polarity. The acquisition of epithelial morphology occurs in two steps. First, expression of HNF-4 results in reexpression of cytokeratin proteins and partial reestablishment of E-cadherin production. Only the transfectants are competent to respond to the synthetic glucocorticoid dexamethasone, which induces the second step of morphogenesis, including formation of the junctional complex and expression of a polarized cell phenotype (Späth and Weiss, 1998).

1. Hepatocyte Nuclear Factor-1 Regulates Hepatocyte Nuclear Factor-4α Expression. Liver-specific expression of the mouse HNF-4α gene was studied by analyzing the promoter region for required DNA elements. Experiments with reporter constructs in transient transfection assays and in transgenic mice revealed distal enhancer elements at kb −5.5 and −6.5 that were sufficient to drive liver-specific expression of the mouse HNF-4α gene in animals (Zhong et al., 1994). A HNF-1-binding site between bp −98 and −68 played an important role in the hepatoma-specific promoter activity of HNF-4 in transient transfection assays but was not sufficient to drive liver-specific expression of a reporter gene in transgenic mice (Zhong et al., 1994).

2. Hepatocyte Nuclear Factor-1α and Hepatocyte Nuclear Factor-4 Regulate Hepatocyte Nuclear Factor-1α Expression. The HNF-1α gene contains a relatively short promoter segment located between positions −82 and −40 to direct cell type-specific HNF-1α transcription. This region contains a single site for HNF-4α (Tian and Schibler, 1991). Transfection experiments revealed that a short region between −118 and −8 is crucial for cell type-specific expression of the HNF-1α gene in HepG2 cells. This region contains two positive cis-elements called site A, a HNF-4α-binding site, and site B, a HNF-1α-binding site. Mutational analyses of these sites and cotransfection assays showed that HNF-4 and HNF-1α can transactivate the HNF-1α gene (Miura and Tanaka, 1993).

It could be demonstrated that HNF-1α negatively regulates its own expression in transient transfection experiments as well as the expression of HNF-4-dependent genes (ApoCIII and ApoAI) that lack HNF-1α-binding sites in their promoter region. DNA binding and cell-free transcription experiments failed to demonstrate any direct or indirect interaction of HNF-1α with the regulatory regions of ApoCIII or ApoAI. From these observations it was assumed that HNF-1α is able to impede HNF-4 binding or activity. An indirect negative autoregulatory mechanism for HNF-1α expression was described, which in turn may affect HNF-4-dependent transcription of other liver-specific genes (Kritis et al., 1993). Later, it could be found that this repression is exerted by a direct interaction of HNF-1α with AF-2, the main activation domain of HNF-4. The dual functions of gene activation and repression suggest that HNF-1α is a global regulator of the transcriptional network involved in the maintenance of the hepatocyte-specific phenotype (Kitstaki and Talianidis, 1997a).

Figure 6 shows a model of the complex molecular interactions that are involved in the regulation of the HNF-1α gene. Numerous coactivators as well as the positive HNF-4 ligands appear to be necessary for optimal HNF-1α expression. In this context it is interesting to note that the HNF-4 coactivators p300/CBP as well as SRC-1 and SRC-3 bind to the activation domain AF-2 of HNF-4. It may well be that HNF-1α competes with coactivator binding at the activation domain AF-2 of HNF-4 and thus exerts its indirect negative autoregulation. Additionally, it might be that this hypothetical competition is further modulated by tissue-specific coactivator availability.

3. Hepatocyte Nuclear Factor-6, OC-2, Hepatocyte Nuclear Factor-3β, and CCAAT/Enhancer-Binding Proteins Regulate Hepatocyte Nuclear Factor-3β Expression. The liver-enriched transcription factor HNF-6 recognizes the −138 to −126 region of the HNF-3β promoter and is required for HNF-3β promoter activity (Samadani and Costa, 1996). Similar to HNF-6, another member of the onecut class of transcription factors called OC-2, with tissue-restricted expression in liver and skin, stimulates transcription of the HNF-3β gene in transient transfection experiments, suggesting that OC-2 participates in the network of transcription factors required for liver differentiation and metabolism (Jacquemin et al., 1999).

Earlier studies showed that promoter activity of HNF-3β requires −134 bp of HNF-3β proximal sequences and binds four nuclear proteins, including two ubiquitous factors. One of these promoter sites interacts with a cell-specific factor, LF/H3 β, whose binding activity correlates with the HNF-3β tissue expression pattern. Furthermore, there is a binding site for the
HNF-3β protein within its own promoter, suggesting that an autoactivation mechanism is involved in the establishment of HNF-3β expression. It has been proposed that both the LF-H3 β and HNF-3 sites play an important role in the cell type-specific expression of the HNF-3β transcription factor (Pani et al., 1992b).

Later studies demonstrated that members of the C/EBP and proline and acidic amino acid-rich subfamilies of basic region leucine zipper transcription factors bind to the LF-H3 β site, and cotransfection of HepG2 cells showed that these factors are able to activate a HNF-3β promoter reporter construct. The LF-H3 β-C/EBP binding sequence also confers HNF-3β promoter stimulation in response to interleukin (IL)-1 and IL-6. Upstream of this HNF-3β proximal promoter region, an IFN-stimulated response element core sequence (−231 to −210) was found that mediates transcriptional induction by IFN-γ but not IFN-α. Gel mobility supershift assays demonstrated that an IFN-γ-induced protein-DNA complex is disrupted by an antibody specific for interferon-regulatory-factor-1/interferon-stimulated gene factor-2. Surprisingly, the effect of the three cytokines (IL-1, IL-6, and IFN-γ) in combination, as assayed by the same model, is not synergistic. HNF-3β joins the C/EBP family on the list of liver-enriched transcription factors, the expression of which is modulated by cytokines (Samadani et al., 1995).

4. Hepatocyte Nuclear Factor-1α Regulates Hepatocyte Nuclear Factor-3γ in the Liver. HNF-3γ is an important regulator of liver-specific genes, and the expression of this factor is reduced in the liver injured by the toxin carbon tetrachloride (CCl4) (Nakamura et al., 1999). HNF-3γ is thought to be involved in anterior-posterior regionalization of the primitive gut. In the HNF-3γ locus, 170 kb contain all elements important in the regulation of HNF-3γ. A 3′-enhancer could be identified that contains a HNF-1α and -β-binding site that was shown to be crucial for enhancer function in vitro (Hiemisch et al., 1997).

5. Competition and Cooperation (“Coopetition”) between Hepatocyte Nuclear Factor-3α and Hepatocyte Nuclear Factor-3β. Studies using embryoid bodies in which one or both HNF-3α or HNF-3β genes were inactivated showed that HNF-3β was necessary for expression of HNF-3α. HNF-3β positively regulated the expression of HNF-4α/HNF-1α and their downstream targets, implicating a role in diabetes. In these studies HNF-3α acted as a negative regulator of HNF-4α/HNF-1α, demonstrating that HNF-3α and HNF-3β have antagonistic transcriptional regulatory functions in vivo. HNF-3α did not appear to act as a classic biochemical repressor but, rather, exerted its negative effect by competing for HNF-3-binding sites with the more efficient activator HNF-3β. In addition, the HNF-3α/HNF-3β ratio was modulated by the presence of insulin, providing evidence that the HNF network may have important roles in mediating the action of insulin (Duncan et al., 1998).

G. Human Disease Due to Mutations in Hepatocyte Nuclear Factors

Haploinsufficiency of HNF-4α due to a nonsense mutation (Q268X) in exon 7 of the HNF-4α gene leads to an autosomal-dominant, early-onset form of noninsulin-dependent diabetes mellitus (maturity-onset diabetes of the young; gene named MODY1) in humans associated with an abnormal pancreatic β-cell function (Yamagata et al., 1996; Lindner et al., 1997; Stoffel and Duncan, 1997). This mutation deletes 187 C-terminal amino acids of the HNF-4α protein. It has been shown that the mutant HNF-4α protein has lost its transcriptional transactivation activity, and fails to dimerize and bind DNA, implying that the MODY1 phenotype is due to a loss of HNF-4α function (Stoffel and Duncan, 1997). Several genes encoding components of the glucose-dependent insulin secretion pathway (glucose transporter 2, aldolase B, glyceraldehyde-3-phosphate dehydrogenase, and liver pyruvate kinase) as well as fatty acid-binding proteins and cellular retinol-binding protein are dependent upon functional HNF-4α and are down-regulated in embryonic stem cells induced to differentiate into visceral endoderm and lacking proper HNF-4α function (Stoffel and Duncan, 1997). Interestingly, individuals of a family with MODY1 (Dresden-11) and an inherited nonsense mutation, R154X, in the HNF-4α gene showed no abnormalities in lipid metabolism or coagulation except for a paradoxical 3.3-fold increase in serum lipoprotein(a) levels (Lindner et al., 1997).

Hemophilia B Leyden is an X chromosome-linked bleeding disorder characterized by very low plasma levels of blood coagulation factor IX during childhood. After puberty, plasma factor IX levels gradually rise to a maximum of 60% of normal, probably under the influence of testosterone. Single point mutations in the factor IX promoter region of hemophilia B Leyden patients have been reported at −20, −6, −5, +8, and +13. In addition, one promoter mutation (G→C at −26) has been detected that abolishes factor IX expression throughout life. The severity of the hemophilia phenotype appears to be directly related to the degree of disruption of HNF-4 binding to the factor IX promoter and transactivation (Reijnen et al., 1994).

It could be demonstrated that HNF-6 is a major determinant of protein C gene activity. Individuals affected by protein C deficiency are at risk for venous thrombosis. One such affected individual was shown earlier to carry a −14 T→C mutation in the promoter region of the protein C gene. It could be shown that the −14 T→C mutation reduces HNF-6 binding to the protein C promoter. In transient transfection experiments, HNF-6 transactivated the wild-type protein C promoter, and introduction of the mutation abolished transactivation by HNF-6 (Spek et al., 1998). This was
the first report describing the putative involvement of HNF-6 and of a HNF-6-binding site in human pathology.

H. Evidence from Knockout Experiments

Mice lacking HNF-1α fail to thrive and die around weaning after a progressive wasting syndrome with a marked liver enlargement. The transcription rate of genes like albumin and α1-antitrypsin is reduced, whereas the gene coding for phenylalanine hydroxylase is totally silent, giving rise to phenylketonuria. Mutant mice also suffer from severe Fanconi syndrome caused by renal proximal tubular dysfunction. The resulting massive urinary glucose loss leads to energy and water wasting. HNF1-deficient mice may provide a model for human renal Fanconi syndrome (Pontoglio et al., 1996). Mice deficient in HNF-1α develop Laron dwarfism and noninsulin-dependent diabetes mellitus (Lee et al., 1998).

Targeted disruption of the HNF-4α gene, expressed in visceral endoderm, leads to early embryonic death due to malfunction of the yolk sac and impaired gastrulation in HNF-4α−/− mouse embryos (Chen et al., 1994; Stoffel and Duncan, 1997; Duncan et al., 1998).

I. Lack of Confirmation for Existence of Hepatocyte Nuclear Factor-5

Site III of the liver-type promoter of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase contains a TGTTTGC sequence. This TGTTTGC sequence has been called the hepatocyte nuclear factor-5 motif or the HNF-5-TGTTTGC sequence motif, which can be found in several liver-specific genes (Grange et al., 1991; Lemaigre et al., 1993). It has been postulated that a protein named HNF-5 binds to this sequence (Grange et al., 1991, Rigaud et al., 1991). Later, it could be demonstrated that HNF-3 can bind to the putative HNF-5-TGTTTGC sequence motif of the rat tyrosine aminotransferase (TAT) gene promoter, whereas the HNF-5-TGTTTGC sequence motifs from other promoters or enhancers do not bind HNF-3 (Lemaigre et al., 1993). The HNF-5-TGTTTGC sequence motif of the albumin enhancer binds eH-TF (Zaret et al., 1990; Liu et al., 1991), and the HNF-5-TGTTTGC sequence motif of the A domain of the transferrin gene enhancer binds EBP45 and EBP40 (Petropoulos et al., 1991). Although eH-TF, EBP45, and EBP40 produce footprints with typical hypersensitive sites, they differ from HNF-3α, HNF-3β, and HNF-3y (Liu et al., 1991; Petropoulos et al., 1991). Unfortunately the postulated transcription factor HNF-5 that binds to the HNF-5-TGTTTGC sequence motif (Grange et al., 1991) could not be defined in greater detail yet. It is also interesting, that over several years, no further publications on HNF-5 can be found. This is probably due to the lack of confirmation regarding the identity of this postulated transcription factor.

V. Challenges for the Future

The expression of liver-specific genes requires a timely and coordinated expression of different transcription factors from distinct chromosomes. As an example, the α1-antitrypsin gene contains binding sites for HNF-1, HNF-3, HNF-4, and HNF-6, that have been shown to interact with the liver-enriched transcription factors HNF-1α, HNF-3β, HNF-4α1, HNF-4α2, HNF-6α, and HNF-6β (Sladek et al., 1990; De Simone and Cortese, 1991; Samadani and Costa, 1996). Liver-enriched transcription factors that bind to the regulatory sequences of the α1-antitrypsin gene have been assigned to human chromosome 12 region q22-qter, chromosome 20q, and chromosome 15 region q21.1–21.2. Furthermore, the transcription factors that bind to the α1-antitrypsin-regulatory sequence also influence the transcriptional activity of each other. Thus, a considerable challenge for further investigations on the regulation of transcriptional networks will be the understanding of the molecular basis of the orchestration of transcriptional events that are interdependent and at the same time separated on different chromosomes. It can be expected that the chromatin remodeling complexes, as well as biochemical modifications of chromatin, play pivotal roles in liver development and liver-specific gene expression. In the future the exact role of chromatin higher order structure and function in liver development and liver function will need to be determined. Protein-protein interactions between transcription factors and cofactors as well as between components of multiprotein complexes and transcription factors are coming more into focus and illustrate the true complexity of gene transcription. In the posthuman genome era and with the availability of the human DNA sequence, we find ourselves confronted with a plethora of new challenges ahead that will provide newfound knowledge on the origin of life and the molecular basis of disease. There is optimism that new platform technologies in functional genomics will unveil the secrets of gene regulation and phenotype expression. 

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REFERENCES

induced nuclear factors that interact with the human C-reactive protein promoter.


