0031-6997/02/5401-129–158$7.00
PHARMACOLOGICAL REVIEWS
Vol. 54, No. 1
20104/968732
Printed in U.S.A

Pharmacol Rev 54:129–158, 2002

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

HARALD SCHREM, JÜRGEN KLEMPNAUER, AND JÜRGEN BORLAK

Viszeral und Transplantationschirurgie, Medizinische Hochschule Hannover (H.S., J.K.); and Center of Drug Research and Medical Biotechnology, Fraunhofer Institut für Toxikologie und Aerosolforschung, Hannover, Germany (J.B.)

This paper is available online at http://pharmrev.aspetjournals.org

Address correspondence to: Dr. Jürgen Borlak, Center for Drug Research and Medical Biotechnology, Fraunhofer Institut für Toxikologie und Aerosolforschung, Nicolai Fuchs Str. 1 30625 Hannover, Germany. E-mail: borlak@ita.fhg.de

This article is dedicated to Jemima Ann Schrem.

***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

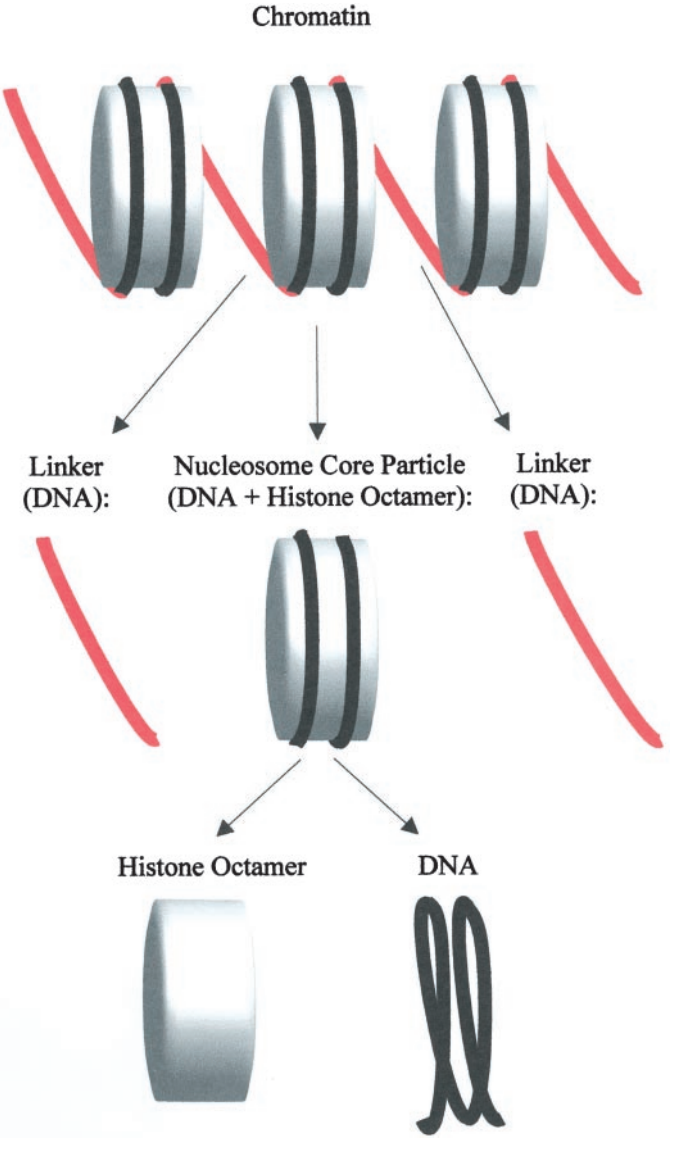

FIG. 1. Schematic drawing of the basic entities that form chromatin. The histone octamer is represented as a disk, the linker DNA as a red ribbon, and the DNA of the nucleosome core particle as a black ribbon.

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

[1] 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; p/CAF, p300/CBP-associated factor; NCoA, nuclear receptor coactivator.

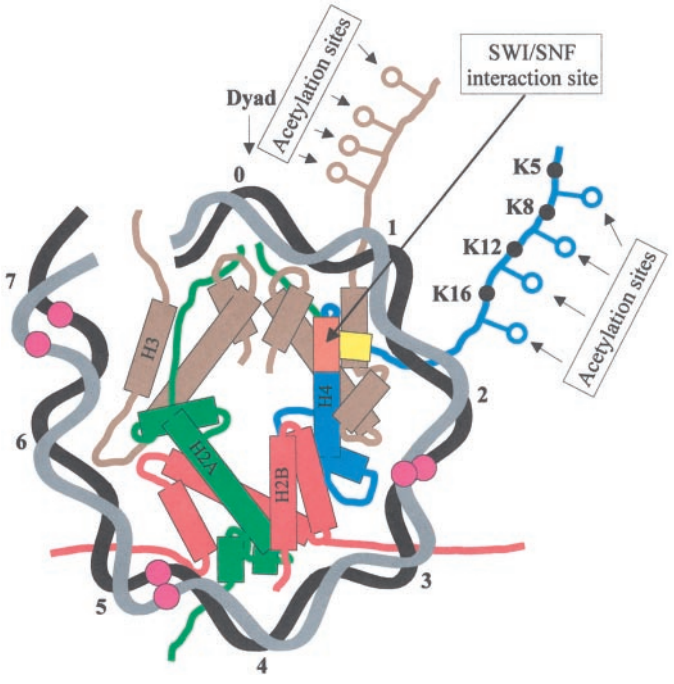

FIG. 2. Depicted is a schematic transection of the nucleosome core particle based on X-ray findings of its crystal structure by Luger et al. (1997) and on findings reviewed by Bird and Wolffe (1999) and by Kornberg and Lorch (1999). Half the core particle is shown with four histone molecules and 73 DNA base pairs. Core histone fold domains for H2A, H2B, H3, and H4 are indicated by green, red, brown, and blue coloring, respectively. DNA is shown wrapped around the histone fold domains with the numbers 0 to 7 indicating turns away from the dyad axis (Dyad). CpGs accessible in the major groove to MBD proteins (MBDPs) that act as transcriptional repressors through DNA methylation are indicated (purple). The N-terminal tail of histone H4 that can be targeted by the ATP-utilizing chromatin remodeling complex NuRD (also called Mi-2 complex) is indicated by K5, K8, K12, and K16. The N-terminal helix of histone H4, which is targeted by the histone-binding protein RbAp48, a subunit of the NuRD complex, is colored yellow. The NuRD complex is able to influence transcriptional activity through ATP-dependent chromatin remodeling, chromatin deacetylation, and DNA methylation (see text for details and references). A key site of interaction between histone H4 and DNA where mutations in the protein relieve the requirement for SWI/SNF activity is shown in orange. "Lollipops" on the tails of histones H3 and H4 indicate acetylation sites.

recent data and hopefully stimulate further investigations on the role of chromatin remodeling and chromatin modifications in liver function, liver regeneration, and liver development. Additionally, the concepts of epigenetics and position-effect variegation (PEV), and their possible impact on gene transcription and expression, are discussed, because these concepts are of fundamental importance in gene transcription but are widely neglected in molecular investigations of liver-specific gene expression.

1. 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 (Coté 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 (Coté 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; Sudarsanam and Winston, 2000).

BRG-1 is capable of remodeling mononucleosomes and nucleosomal arrays as a purified protein in vitro. The addition of further subunits of the human SWI/SNF complex (hSNF5/INI1, BAF155, BAF170) to BRG-1 increases the remodeling activity to a level comparable with that of the whole human SWI/SNF complex. On this basis it was postulated that these proteins define the functional core of the human SWI/SNF complex (Phelan et al., 1999; Phelan et al., 2000).

a. Switch/Sucrose Nonfermenting Subunits and Their Interaction with DNA. Studies with *brm* deletion mutants revealed a region with homology to the AT-hook present in high-mobility-group protein I/Y (HMGI/Y). This region was shown to be required for the tethering of *brm* to chromatin. In vitro this domain is able to mediate binding to the minor groove of DNA with a preference for A+T-rich sequences. Deletion of this sequence in *brm* leads to increased extractability of the protein (Muchardt and Yaniv, 1999).

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 G_2/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).

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/*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 G_1-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

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 (Östlund-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 Wolffe, 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 Wolffe, 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 Wolffe, 1997; Workman and Kingston, 1998). In particular, a number of transcriptional regulatory proteins, including GCN5, PCAF, p300/CBP, TFII250, 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-

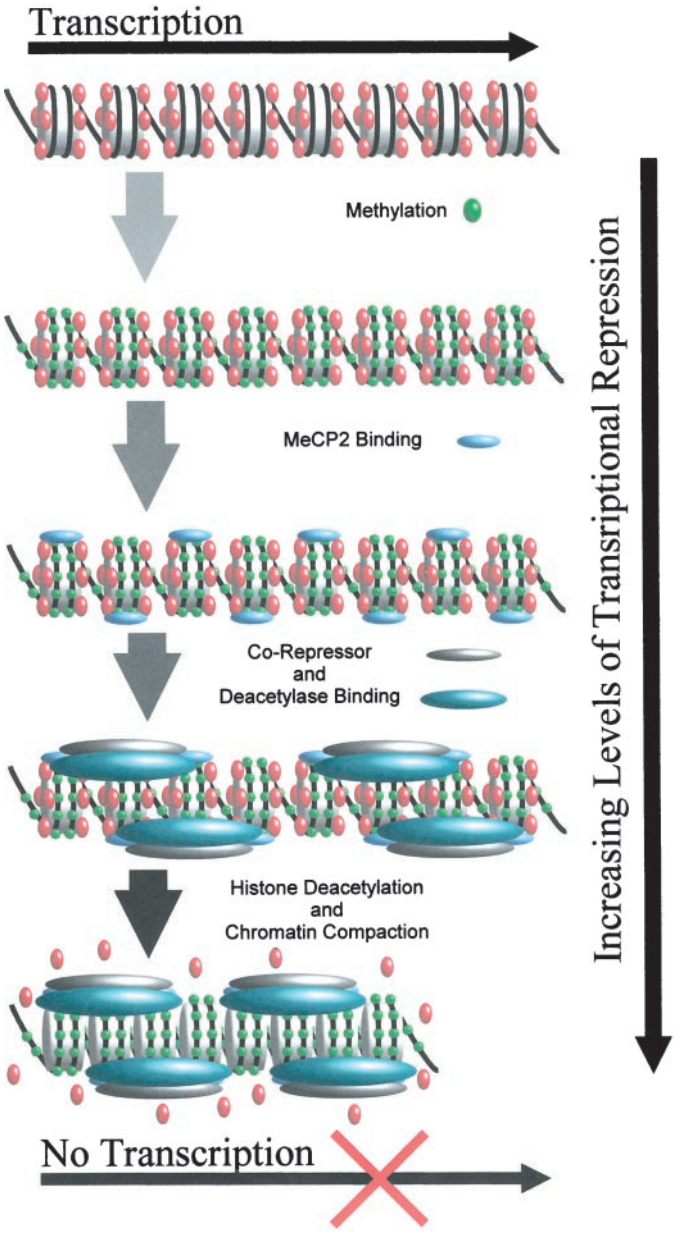

FIG. 3. This schematic model on increasing levels of transcriptional repression induced by DNA methylation and MeCP2 binding followed by corepressor and deacetylase binding with subsequent deacetylation and chromatin compaction was adapted with modifications from Jones and Laird (1999). Nucleosome core particles are shown as gray disks with DNA wrapped around as black ribbon. Acetylation, methylation, MeCP2 binding, corepressor and deacetylase binding are represented by red, green, blue, gray, and dark blue spheres, respectively.

ing for acetylation of histones to increase the accessibility of transcription factors. In addition, it has recently been shown that p300/CBP and p300/CBP-associated factor (p/CAF) are able to acetylate nonhistone proteins, including some transcription factors such as p53 and components of the general transcription machinery such as TFIIE (Gu and Roeder, 1997; Imhof et al., 1997).

It could be demonstrated that 17β-estradiol treatment of the human breast cancer cell line MCF-7 causes a rapid and dramatic increase of acetylation of histones at the promoter of 17β-estradiol receptor target genes including pS2, cathepsin D, c-Myc, and EB1. Surprisingly this acetylation seems to be a transient phenomenon despite the continuous presence of hormone. It was found that the 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

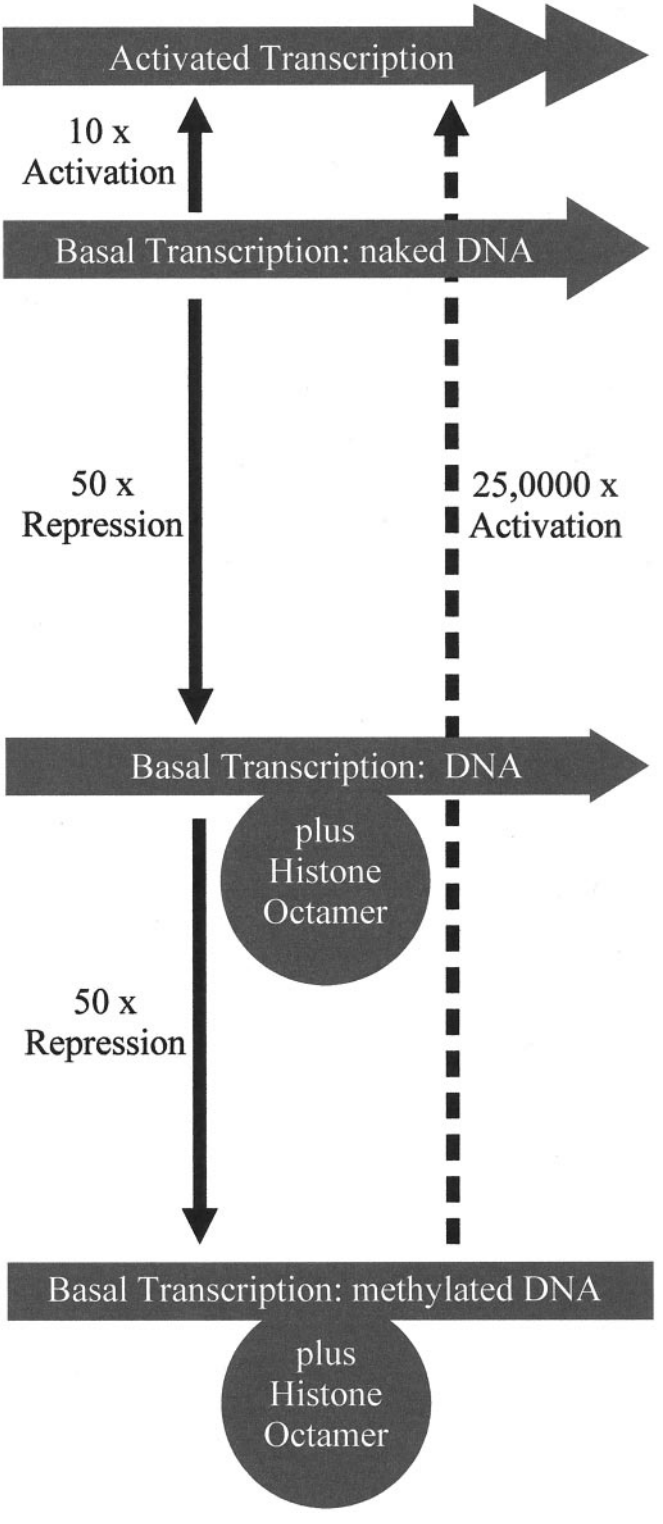

FIG. 4. Shown is a model adapted with modifications from Bird and Wolffe (1999) on different levels of transcriptional activity due to activated, repressed, and derepressed basal DNA transcription with quantitative estimates. It was proposed that the formation of chromatin between a DNA template and a histone octamer leads to a repressive effect on basal transcriptional activity when compared with transcription from a naked DNA template. Further repression results from additional methylation of DNA. It was assumed that DNA methylation may expand the range of transcriptional regulation significantly beyond that which could be achieved by chromatin modification alone.

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-

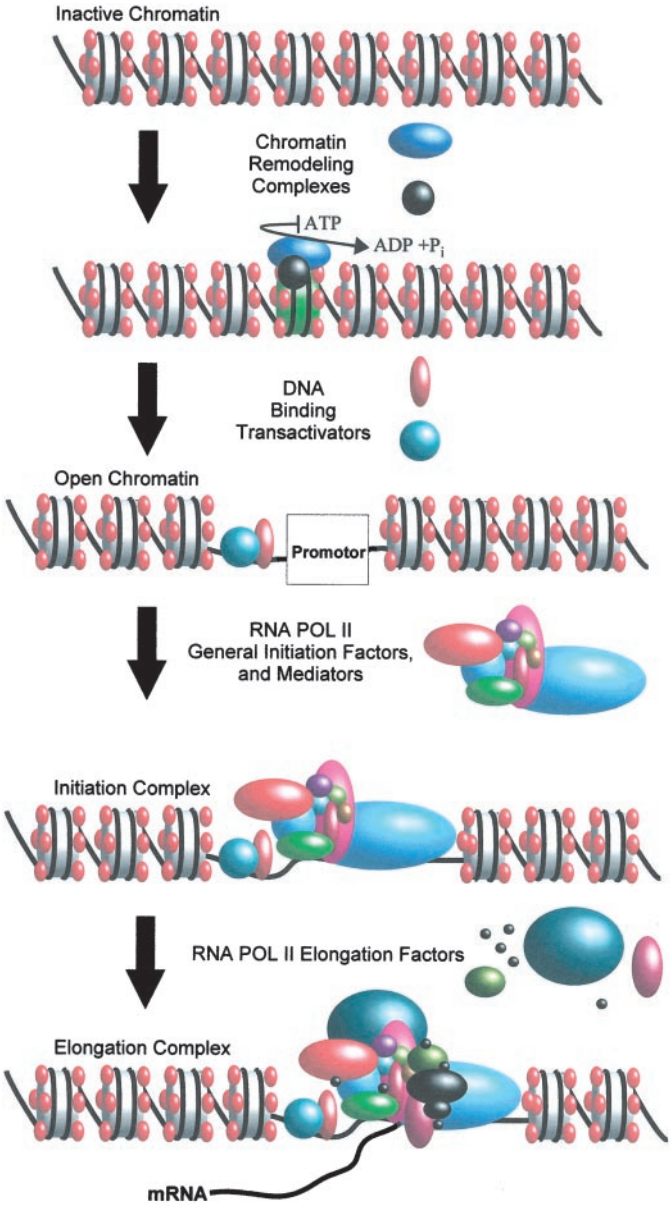

FIG. 5. Formation of the multiprotein complex at the promoter of a gene. The depicted model shows acetylated chromatin (acetylation = red spheres) that is selectively remodeled by ATP-dependent chromatin remodeling complexes (blue sphere and black sphere) and thus allowing the binding of DNA-binding transactivators close to the promoter of the gene. Then the RNA polymerase II, general initiation factors, and mediators bind at the promoter followed by the binding of RNA polymerase II elongation factors to the multiprotein complex, finally leading to mRNA transcription. The proteins that form the multiprotein complex are represented by spheres in different shapes and colors.

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 three-dimensional 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 paralogs (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 superfamily 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 onecut 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 Schepartz, 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 Bcd-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 represses 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 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-4 α-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. → indicates a continuing sequence in the next line.

HNF-1α Binding Sites						
Gene	Regulatory Element	Gene Region	Position of Binding Site	First Position	Species	Reference
Albumin	tGGTTAGtaattactaa		−363 to −338		*Homo sapiens*	Liu et al., 1991
	GTTTGTTCTT	Element eG	528 to 547	*Nhe*I at −11.4 kb	*Mus musculus*	Frain et al., 1990
Aldolase B	CAGAGTTATTGAATAAACACCTC		−126 to −104	t.s.s.	*Rattus norvegicus*	Gregori et al., 1993; Tsutsumi et al., 1989
α1-Antitrypsin	TGGTTAATATTCACCAgc		−86 to −56	t.s.s.	*H. sapiens*	De Simone and Cortese, 1991
α-Fetoprotein	TGTTAATTATTGGCAAATTGCCTAACTTC→A		−128 to −99	t.s.s.	*Rattus rattus*	Jose-Estanyol and Danan, 1988
α-Fibrinogen	AGGACAAAGCCAAT	Promoter	−67 to −54	t.s.s.	*H. sapiens*	Hu et al., 1995
Apolipoprotein AII	GATATCTATTTAACTGATTTCACCC	Distal region I, N	−903 to −879	t.s.s.	*H. sapiens*	Chambaz et al., 1991; Cardot et al., 1993
Apolipoprotein B	GTTTATCAGTGACTAGTCATTGAT	Intron 2, enhancer	835 to 876	Cap	*H. sapiens*	Brooks and Levy-Wilson, 1992
β-Fibrinogen	CAAACTGTCAAATATTAACTAAAGGGAG	β28 element	−103 to −75	t.s.s.	*R. norvegicus*	Kuo et al., 1991; Xanthopoulos et al., 1991
CYP2E1	TGATAGCCAACTGCAGCTAATAATAAACCA		−127 to −93	t.s.s.	*R. norvegicus*	Ueno and Gonzalez, 1990
CRP	TTTGTAATAAATAACTCA		−175 to −133	t.s.s.	*H. sapiens*	Majello et al., 1990; Toniatti et al., 1990
	CAATGTTGGAAAATTATttacat		−80 to −57			
Factor VIII	ATATTTTAGAGAAGAATTAACCTTT	Element A	−59 to −35	ATG	*H. sapiens*	Figueiredo and Brownlee, 1995
IGFBP-1 (insulin-like growth factor binding protein-1)	TGCGGCGCTGCCAATCATTAAC		−79 to −53	t.s.s.	*H. sapiens*	Powell et al., 1995
Large surface protein (HBV)	TAGTTAATCATTACTTC	SPI promoter	−93 to −68	t.s.s.	Human hepatitis B virus	Chang et al., 1989
Prothrombin	GTGTTCCTGCTCTTTGTCC		−941 to −920	t.s.s.	*H. sapiens*	Chow et al., 1991
Surface antigen (HBV)	GTTAATCATTAC	Pre-S1 promoter	−93 to −69	t.s.s.	Human hepatitis B virus	Zhou and Yen, 1991
Vitellogenin A2	TGAGGTAATtgTTTACACAa	AABS element	−124 to −85	t.s.s.	*Xenopus laevis*	Drewes et al., 1991

pachik et al., 1998). 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; Clevidence 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 (Hadzopoulou-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 $TAF_{II}31$ and $TAF_{II}80$; TFIIB; TFIIH-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 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 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. → indicates a continuing sequence in the next line.

HNF-3 Binding Sites						
Gene	Regulatory Element	Gene Region	Position of Binding Site	First Position	Species	Reference
Albumin	GTTTGTTCTT	Element eG	528 to 547	*Nhe*I at −11.4 kb	*M. musculus*	Liu et al., 1991
Aldolase B	CAGAGTTATTGAATAAACACCTC		−126 to −104	t.s.s.	*R. norvegicus*	Gregori et al., 1993; Tsutsumi et al., 1989
α1-Antitrypsin	AATATTGACTTTG	5′-Region	−378 to −366	t.s.s.	*M. musculus*	Samadani and Costa, 1996
α-Fetoprotein	caAAGTCAATAAag	5′-Region	−6103 to −6090	t.s.s.	*R. norvegicus*	Lannoy et al., 1998; Samadani et al., 1996
α2-Macroglobulin	GGTATTGACTTTA	5′-Region	−452 to −442	t.s.s.	*R. norvegicus*	Samadani and Costa, 1996
Alkaline phosphatase	gaTGTTTgttct		−953 to −942	ATG	*H. sapiens*	Ye et al., 1997
Apolipoprotein B	CTGTCCTGTTTATCAGTGACTAGTCATT	Intron 2, enhancer, element E	839 to 935	t.s.s.	*H. sapiens*	Brooks et al., 1991
	→ATTCGAAGCATGTGAGGGTGAGGAAA →TACTGACTTTAACCTTTGTGAAGAAAT		892 to 904			Samadani and Costa, 1996
	→CGAACCTCCACCCCC					
Insulin-like growth factor binding protein 1	TcacaagcaAAACAAActtattttgaacacgg	IRS (insulin responsive sequence)	−168 to −137	t.s.s.	*R. norvegicus*	Goswami et al., 1994
	cactagCAAAACAaactTATTTTGaacac		−124 to −96		*H. sapiens*	O'Brien et al., 1995
Phosphoenol-pyruvate carboxykinase	GtgacacctcacagctgTGGTGTTTTGacaaccagcag	IRS (insulin-responsive sequence)	−433 to −396	t.s.s.	*R. norvegicus*	O'Brien et al., 1995; Wang et al., 1996
	TGGTGTTTTGACAAC		−416 to −402			
6-Phosphofructo-2-kinase/ fructose-2,6-bisphosphatase	GTCTTTTATTTGCATACTCA	Promoter, site III	−132 to 112	t.s.s.	*R. norvegicus*	Lemaigre et al., 1993
Protein C	agggccAAGCAAATATTTGTGGttatgga		−43 to −15	t.s.s.	*H. sapiens*	Spek et al., 1995
Transferrin	AATCTACAAGTGTTTGCAGCATAGTCAT	Site A	−306 to −297	t.s.s.	*Salmo salar*	Stenson et al., 2000
Tryptophan oxygenase	TCTATTGATTTAT	5′-Region	−220 to −208	t.s.s.	*R. norvegicus*	Samadani and Costa, 1996
TTR (transthyretin, prealbumin)	GtTGACTAAgtcaataatcagaatcag	Element TRE	−111 to −88	t.s.s.	*M. musculus*	Qian and Costa, 1995
	TATTTGTGTAG	HNF3-W	−140 to −131			Costa and Grayson, 1991
	ctgTTCAAACATGtcCTAATACTCTgtctctgc	TTR-2	32 to 46			Samadani et al., 1996
	CTAAGTCAATAAT	HNF3-S	−111 to −82			Samadani and Costa, 1996
Tyrosine amino transferase	CcggacgtttCTCAATATTTGCTCtggcaga		−10514 to −10484	t.s.s.	*R. norvegicus*	Nitsch et al., 1993
	GGCCAcaaataaaaga		−5463 to −5448			Grange et al., 1991; Lemaigre et al., 1993; Rigaud et al., 1991
	GCCACAGTTATGCAAAACACAAAACAA		−5399 to −5364			
	→TAAG		−5377 to −5345			
	CAGTCTGCAAACAGATGAAGATTTT		−5322 to −5291			
	ACTTTATTTGCAATAGAAAATC		−2487 to −2465			
	TAGAACAAACAAGTCCTGCGT		−2440 to −2414			
Vitellogenin A2	TGAGGTAATtgTTTACACAa	AABS element	−124 to −85	t.s.s.	*X. laevis*	Kaling et al., 1991
Vitellogenin B1	TGTGTGC	Promoter	−58 to −52	t.s.s.	*X. laevis*	Cardinaux et al., 1994
	TATTTAC	Promoter	−81 to −75			
	GCAAACA	Promoter	−125 to −119			

TABLE 3

Shown are examples of liver-specific genes that contain a regulatory element with a HNF-4 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-4 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. → indicates a continuing sequence in the next line.

Gene	HNF-4 Binding Sites					
	Regulatory Element	Gene Region	Position of Binding Site	First Position	Species	Reference
α1-Antitrypsin	CTCAGATCCCAGCCAGTGGACTTAGCCC CTGTTTTGC		−134 to −98	t.s.s.	*H. sapiens*	Monaci et al., 1988
Apolipoprotein CIII	TGGGTCCAGAGGGCAAAA CAGGTGACCTTTGCCC	5′-enhancer, element 1 C3P element	−745 to −725 −93 to −71	t.s.s.	*H. sapiens*	Bisaha et al., 1995 Mietus-Snyder et al., 1992
Biliary glycoprotein Factor VIII	CGCCCCAGCACACATGATCAGA AAGGTTCTGATTAAAGCAGACTTATGCC	FP1 element Promoter, element E	−158 to −137 −311 to −279	t.s.s. ATG	*H. sapiens* *H. sapiens*	Hauck et al., 1994 Figueiredo and Brownlee, 1995
Ornithine transcarbamylase	→CCTAC gttaGATGAACTTTAAACCTTTGtgat	Enhancer, element I	76 to 102	*Hin*cII	*R. norvegicus*	Nishiyori et al., 1994; Murakami et al., 1990
Transferrin	cagCTTAACCTCTGAACTTCTAagg	Element IV	161 to 185	*Hin*cII	*H. sapiens*	Schaeffer et al., 1993
TTR (transthyretin, prealbumin)	AACACGGGAGGTCAAAGATTGCGCCC GGCAAGGTTCATattgtgtag	PR I element Element HNF4P	−76 to −48 −156 to −130	t.s.s. t.s.s.	*H. sapiens* *M. musculus*	Schaeffer et al., 1993 Xanthopoulos et al., 1991
	TGCAAGGGTCAT	Element HNF4D	−1 to 10			Sladek et al., 1990

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 TFIID, via direct physical interactions with TFIIB. However, recruitment of TFIIB by HNF-4 was not sufficient for activation, because HNF-4 deletion derivatives lacking AF-2 bound TFIIB. 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 TFIIB to the promoter complex; the second step is AF-2-dependent and entails entry of preinitiation complex components acting downstream of TFIIB (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 transactivation 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 (Viollet 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 acetylate 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 4
This table provides an overview of HNF-4 coactivators and agonistic ligands and their functions

	Substance	HNF-4 Domain	Mode of Action	References
Agonistic ligands	Saturated (C14:0)-CoA	LBD	Binding of ligand leads to increased HNF-4α dimerization	Hertz et al., 1998
			Binding of ligand activates binding of the HNF-4α dimer to it's cognate enhancer element	
	Saturated (C16:0)-CoA	LBD	Binding of ligand activates binding of the HNF-4α dimer to it's cognate enhancer element	
Coactivators	SRC-1 (NCoA-1)	AF-2	Specific H3 and H4 histone acetylation by intrinsic SRC-1 HAT activity	Spencer et al., 1997
			Interaction with p/CAF (with intrinsic HAT activity)	
			Interaction with C terminus of CBP/p300 (with intrinsic HAT activity)	Yao et al., 1996
			Interaction between HNF-4, SRC-1, and SRC-2 leading to increased transactivation activity of HNF-4	Wang et al., 1998a
	SRC-2 (NCoA-2 GRIP1, TIF2)	AF-2	Interaction between HNF-4, SRC-1, and SRC-2 is leading to increased transactivation activity of HNF-4	Wang et al., 1998a
	SRC-3 (NCoA-3, ACTR, AIB1, p/CIP, TRAM-1, RAC3)	AF-2	Intrinsic histone acetylase activity	Chen et al., 1997
			Protein-protein interaction with p/CAF and CBP/p300	Torchia et al., 1997
	CBP/p300	AF-1, AF-2	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	Dell and Hadzopoulou-Cladaras, 1999
			Histone acetylation: intrinsic histone acetylase (HAT) activity	Bannister and Kouzarides, 1996
			Protein-protein interaction: CBP/p300 binds p/CAF that also contains an intrinsic HAT activity	Yang et al., 1996
			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	Soutoglou et al., 2000a
			Binding to the AF-1 and AF-2 domain leads to ligand-independent HNF-4α activation	Ktistaki et al., 1995
			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	Kamei et al., 1996
			Acetylation of general transcription factors TFIIEb and TFIIF by p300 leading to increased transcription	Ogryzko et al., 1996
			Interaction with components of the basal transcription machinery (TBP, TFIIB, RNA helicase) leading to increased transcription	Kamei et al., 1996
	ADA2	AF-1	ADA2-GCN5 complex acetylates histones	Green et al., 1998
			ADA2 interacts with the AF-1 domain of HNF-4 and TBP and TAFs facilitating transcription by the transcriptional machinery	Barlev et al., 1995
	PC4	AF-1	PC4 contains two ssDNA binding domains that might be implicated in the opening of the DNA double helix at a post-initiation step	Green et al., 1998; Brandsen et al., 1997
			PC4 interacts with the AF-1 domain of HNF-4 and TAFs facilitating transcription by the transcriptional machinery	Ge and Roeder, 1994
	COUP-TFI/COUP-TFII	E	Promoters that are recognized by HNF-4a 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	Ktistaki and Talianidis, 1997

TABLE 5
Overview of HNF-4 repressors and antagonistic ligands and their functions

	Substance	HNF-4 Domain	Mode of Action	References
Antagonistic ligands	ω-3 and ω-6 polyunsaturated fatty acyl-CoAs (C18:3, ω-3)-CoA Saturated (C18:0)-CoA		Unknown	Hertz et al., 1998
			Lowers affinity of the HNF-4α dimer to its cognate enhancer	
			Lowers affinity of the HNF-4α dimer to its cognate enhancer	
			Leads to decreased HNF-4α dimerization	
Repressors	SHP	AF-2	Competition with coactivators for the AF-2 binding domain	Lee et al., 2000
		AF-2	Direct inhibition after binding to the AF-2 domain through a SHP repressor domain (unknown mechanism)	
	COUP-TFs	No	Competition with HNF-4α for binding to *cis*-acting DNA sites of many regulatory regions	Kimura et al., 1993; Ktistaki and Talianidis, 1997
	HNF-1α	AF-2	Binding to the AF-2 domain, probably competing with coactivators	

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,

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 onecut 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 6

Shown are examples of liver-specific genes that contain a regulatory element with a HNF-6 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-6 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. → indicates a continuing sequence in the next line.

Gene	Regulatory Element	Gene Region	Position of Binding Site	First Position	Species	Reference
HNF-6 Binding Sites						
α1-Antitrypsin	TCCATTGATTTAG	5′-Region	−195 to −184	t.s.s.	*M. musculus*	Samadani and Costa, 1996
α-Fetoprotein	caAAGTCAATAAag	5′-Region	−6103 to −6090	t.s.s.	*R. norvegicus*	Lannoy et al., 1998; Samadani et al., 1996
CYP2C12	gcaaaATATTGATTTtatggtg	Promoter	−53 to −30	t.s.s.	*R. norvegicus*	Lannoy et al., 1998
CYP2C13	AATATTGATTCTG		−53 to −41	t.s.s.	*R. norvegicus*	Samadani and Costa, 1996
Phosphoenolpyruvate carboxykinase	caaagttTAGTCAATCAaacgttg	5′-Region	−263 to −240	t.s.s.	*R. norvegicus*	Lannoy et al., 1998; Jacquemin et al., 1999
6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase	GctttgAAATCAATTTcaaag	Promoter, site IV	−216 to −196	t.s.s.	*R. norvegicus*	Lannoy et al., 1998; Jacquemin et al., 1999; Lemaigre et al., 1996
	agtttcaaacaaacaaaaaAAATCCATAActttca	Intron 1, GRU (glucocorticoid response unit)				
Tryptophan oxygenase	TCTATTGATTTAT	5′-Region	−220 to −208	t.s.s.	*R. norvegicus*	Samadani and Costa, 1996
TTR (transthyretin, prealbumin)	CTAAGTCAATAAT	5′-Region, HNF3S element	−111 to −82	t.s.s.	*M. musculus*	Samadani and Costa, 1996; Costa and Grayson, 1991

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 transcriptional activation 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 Roeder, 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 (Griffo 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 (Griffo et al., 1993). Dedifferen-

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 Apo AI) 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 (Ktistaki 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 [CCl(4)] (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 $\rightarrow$ C mutation in the promoter region of the protein C gene. It could be shown that the -14 T $\rightarrow$ 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 α_1-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-3γ (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 α_1-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 α_1-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 α_1-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.

Acknowledgments. This work was supported in part by research grants from Novartis Pharma GmbH Germany, BU Transplantation and Immunology to H.S. and J.K. and the Lower Saxony and Ministry of Science and Culture to J.B.

REFERENCES

Acampora D, D'Esposito M, Faiella A, Pannese M, Migliaccio E, Morelli F, Stornaiuolo A, Nigro V, Simeone A, and Boncinelli E (1989) The human HOX gene family. *Nucleic Acids Res* **17:**10385–10402

Agre P, Johnson PF, and McKnight SL (1989) Cognate DNA binding specificity retained after leucine zipper exchange between GCN4 and C/EBP. *Science (Wash DC)* **246:**922–926.

Ahringer J (2000) NuRD and Sin3 histone deacetylase complexes in development. *Trends Genet* **16:**351–356.

Aran A, Cassuto H, and Reshef L (1995) Cooperation between transcription factors regulates liver development. *Biol Neonate* **67:**387–396.

Arents G and Moudrianakis EN (1995) The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization. *Proc Natl Acad Sci USA* **93:**11174–11179.

Argyrokastritis A, Kamakari S, Kapsetaki M, Kritis A, Talianidis I, and Moschonas NK (1997) Human hepatocyte nuclear factor-4 (hHNF-4) gene maps to 20q12–q13.1 between PLCG1 and D20S17. *Hum Genet* **99:**233–236.

Asiedu CK, Scotto L, Assoian RK, and Ehrlich M (1994) Binding of AP-1/CREB proteins and of MDBP to contiguous sites downstream of the human TGF-β 1 gene. *Biochim Biophys Acta* **1219:**55–63.
Bach I, Mattei MG, Cereghini S, and Yaniv M (1991) Two members of an HNF1 homeoprotein family are expressed in human liver. *Nucleic Acids Res* **19:**3553–3559.
Bannister AJ and Kouzarides T (1996) The CBP co-activator is a histone acetyltransferase. *Nature (Lond)* **384:**641–643.
Barlev NA, Candau R, Wang L, Darpino P, Silverman N, and Berger SL (1995) Characterization of physical interactions of the putative transcriptional adaptor, ADA2, with acidic activation domains and TATA-binding protein. *J Biol Chem* **33:**19337–19344.
Baumhueter S, Mendel DB, Conley PB, Kuo CJ, Turk C, Graves MK, Edwards CA, Courtois G, and Crabtree GR (1990) HNF-1 shares three sequence motifs with the POU domain proteins and is identical to LF-B1 and APF. *Genes Dev* **4:**372–379.
Bazett-Jones DP, Coté J, Landel CC, Peterson CL, and Workman JL (1999) The SWI/SNF complex creates loop domains in DNA and polynucleosome arrays and can disrupt DNA-histone contacts within these domains. *Mol Cell Biol* **19:**1470–1478.
Benvenisty N and Reshef L (1991) Regulation of tissue- and development-specific gene expression in the liver. *Biol Neonate* **59:**181–189.
Bird AP and Wolffe AP (1999) Methylation-induced repression—belts, braces and chromatin. *Cell* **99:**451–454.
Bisaha JG, Simon TC, Gordon JI, and Breslow JL (1995) Characterization of an enhancer element in the human apolipoprotein C-III gene that regulates human apolipoprotein A-I gene expression in the intestinal epithelium. *J Biol Chem* **270:**19979–19988.
Bradbury EM (1992) Reversible histone modifications and the chromosome cell cycle. *Bioessays* **14:**9–16.
Brandsen J, Werten S, van der Vliet PC, Meisterernst M, Kroon J, and Gros P (1997) C-terminal domain of transcription cofactor-PC4 reveals dimeric ssDNA binding site. *Nat Struct Biol* **4:**900–903.
Brooks AR, Blackhart BD, Haubold K, and Levy-Wilson B (1991) Characterization of tissue-specific enhancer elements in the second intron of the human apolipoprotein B gene. *J Biol Chem* **266:**7848–7859.
Brooks AR and Levy-Wilson B (1992) Hepatocyte nuclear factor 1 and C/EBP are essential for the activity of the human apolipoprotein B gene second-intron enhancer. *Mol Cell Biol* **12:**1134–1148.
Cardinaux JR, Chapel S, and Wahli W (1994) Complex organization of CTF/NF-I, C/EBP, and HNF3 binding sites within the promoter of the liver-specific vitellogenin gene. *J Biol Chem* **269:**32947–32956.
Cardot P, Chambaz J, Kardassis D, Cladaras C, and Zannis VI (1993) Factors participating in the liver-specific expression of the human apolipoprotein A-II gene and their significance for transcription. *Biochemistry* **32:**9080–9093.
Cascio S and Zaret KS (1991) Hepatocyte differentiation initiates during endodermal-mesenchymal interactions prior to liver formation. *Development* **113:**217–225.
Chambaz J, Cardot P, Pastier D, Zannis VI, and Cladaras C (1991) Promoter elements and factors required for hepatic transcription of the human apoA-II gene. *J Biol Chem* **266:**11676–11685.
Chang HK, Wang BY, Yuh CH, Wei CL, and Ting LP (1989) A liver-specific nuclear factor interacts with the promoter region of the large surface protein gene of human hepatitis B virus. *Mol Cell Biol* **9:**5189–5197.
Chang M and Jaehning JA (1997) A multiplicity of mediators: alternative forms of transcription complexes communicate with transcriptional regulators. *Nucleic Acids Res* **25:**4861–4865.
Chariot A, Gielen J, Merville MP, and Bours V (1999) The homeodomain-containing proteins: an update on their interacting partners. *Biochem Pharmacol* **58:**1851–1857.
Chartier FL, Bossu JP, Laudet V, Fruchart JC, and Laine B (1994) Cloning and sequencing of cDNAs encoding the human hepatocyte nuclear factor 4 indicate the presence of two isoforms in human liver. *Gene* **147:**269–272.
Chen H, Lin RJ, Xie W, Wilpitz D, and Evans RM (1999) Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. *Cell* **98:**675–686.
Chen HW, Lin RJ, Schlitz D, Chakravarti A, Nash A, Nagy L, Privalsky ML, Nakatani Y, and Evans RM (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90:**569–580.
Chen WS, Manova K, Weinstein DC, Duncan SA, Plump AS, Prezioso VR, Bachvarova RF, and Darnell JE Jr (1994) Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes Dev* **8:**2466–2477.
Chevre JC, Hani EH, Boutin P, Vaxillaire M, Blanche H, Vionnet N, Pardini VC, Timsit J, Larger E, Charpentier G, et al. (1998) Mutation screening in 18 Caucasian families suggest the existence of other MODY genes. *Diabetologia* **41:**1017–1023.
Chiba H, Muramatsu M, Nomoto A, and Kato H (1994) Two human homologues of *Saccharomyces cerevisiae* SWI2/SNF2 and *Drosophila brahma* are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucl Acids Res* **22:**1815–1820.
Chow BKC, Ting V, Tufaro F, and MacGillivray RTA (1991) Characterization of a novel liver-specific enhancer in the human prothrombin gene. *J Biol Chem* **266:**18927–18933.
Chowdhury S, Gotoh T, Mori M, and Takiguchi M (1996) CCAAT/enhancer-binding protein β (C/EBP β) binds and activates while hepatocyte nuclear factor-4 (HNF-4) does not bind but represses the liver-type arginase promoter. *Eur J Biochem* **236:**500–509.
Cillo C, Faiella A, Cantile M, and Boncinelli E (1999) Homeobox genes and cancer. *Exp Cell Res* **248:**1–9.
Clark SJ, Harrison J, and Molloy PL (1997) Sp 1 binding is inhibited by (m)Cp(m-)CpG methylation. *Gene* **195:**67–71.
Clevidence DE, Zhou H, Lau LF, and Costa RH (1998) The −4 kilobase promoter region of the winged helix transcription factor HNF-3α gene elicits transgene expression in mouse embryonic hepatic and intestinal diverticula. *Int J Dev Biol* **42:**741–746.
Costa RH and Grayson DR (1991) Site-directed mutagenesis of hepatocyte nuclear factor (HNF) binding sites in the mouse transthyretin (TTR) promoter reveal synergistic interactions with its enhancer region. *Nucleic Acids Res* **19:**4139–4145.
Coté J, Peterson CL, and Workman JL (1998) Perturbation of nucleosome core structure by the SWI/SNF complex persists after its detachment, enhancing subsequent transcription factor binding. *Proc Natl Acad Sci USA* **95:**4947–4952.
Cronk JD, Endrizzi JA, and Alber T (1996) High-resolution structures of the bifunctional enzyme and transcriptional coactivator DCoH and its complex with a product analogue. *Protein Sci* **5:**1963–1972.
Dell H and Hadzopoulou-Cladaras M (1999) CREB-binding protein is a transcriptional coactivator for hepatocyte nuclear factor-4 and enhances apolipoprotein gene expression. *J Biol Chem* **274:**9013–9021.
Derman E, Krauter K, Walling L, Weinberger C, Ray M, and Darnell JE Jr (1981) Transcriptional control in the production of liver-specific mRNAs. *Cell* **23:**731–739.
Descharette J and Weiss MC (1974) Characterization of differentiated and dedifferentiated clones from rat hepatoma. *Biochimie* **56:**1603–1611.
De Simone V and Cortese R (1991) Transcriptional regulation of liver-specific gene expression. *Curr Opin Cell Biol* **3:**960–965.
Devine JH, Hewetson A, Lee VH, and Chilton BS (1999) After chromatin is SWItched-on can it be RUSHed? *Mol Cell Endocrinol* **151:**49–56.
Dorer DR and Henikoff SS (1994) Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila*. *Cell* **77:**993–1002.
Dorer DR and Henikoff S (1997) Transgene repeat arrays interact with distant heterochromatin and cause silencing in cis and trans. *Genetics* **147:**1181–1190.
Drewes T, Klein-Hitpass L, and Ryffel GU (1991) Liver specific transcription factors of the HNF3-, C/EBP- and LFB1-families interact with the A-activator binding site. *Nucleic Acids Res* **19:**6383–6389.
Drewes T, Senkel S, Holewa B, and Ryffel GU (1996) Human hepatocyte nuclear factor 4 isoforms are encoded by distinct and differentially expressed genes. *Mol Cell Biol* **16:**925–931.
Dunaief JL, Strober BE, Guha S, Khavari PA, Alin K, Luban J, Begemann M, Crabtree GR, and Goff SP (1994) The retinoblastoma protein and BRG-1 form a complex and cooperate to induce cell cycle arrest. *Cell* **79:**119–130.
Duncan SA, Navas MA, Dufort D, Rossant J, and Stoffel M (1998) Regulation of a transcription factor network required for differentiation and metabolism. *Science (Wash DC)* **281:**692–695.
Faust DM, Boshart M, Imaizumi-Scherrer T, Schutz G, and Weiss MC (1994) Constancy of expression of the protein kinase A regulatory subunit R1 α in hepatoma cell lines of different phenotypes. *Cell Growth Differ* **5:**47–53.
Feil R and Khosla S (1999) Genomic imprinting in mammals: an interplay between chromatin and DNA methylation? *Trends Genet* **15:**431–435.
Figueiredo MS and Brownlee GG (1995) cis-Acting elements and transcription factors involved in the promoter activity of the human factor VIII gene. *J Biol Chem* **270:**11828–11838.
Finch JT, Lutter LC, Rhodes D, Brown AS, Rushton B, Levitt M, and Klug A (1977) Structure of nucleosome core particles of chromatin. *Nature (Lond)* **269:**29–36.
Frain M, Hardon E, Ciliberto G, and Sala-Trepat JM (1990) Binding of a liver-specific factor to the human albumin gene promotor and enhancer. *Mol Cell Biol* **10:**991–999.
Fryer CJ and Archer TK (1998) Chromatin remodelling by the glucocorticoid receptor requires the BRG-1 complex. *Nature (Lond)* **393:**88–91.
Furuta H, Iwasaki N, Oda N, Hinokio Y, Horikawa Y, Yamagata K, Yano N, Sugahiro J, Ogata M, Ohgawara H, et al. (1997) Organization and partial sequence of the hepatocyte nuclear factor-4 α/MODY1 gene and identification of a missense mutation, R127W, in a Japanese family with MODY. *Diabetes* **46:**1652–1657.
Garrick D, Fiering S, Martin DI, and Whitelaw E (1998) Repeat-induced gene silencing in mammals. *Nat Genet* **18:**56–59.
Ge H and Roeder RG (1994) Purification, cloning, and characterization of a human coactivator, PC4, that mediates transcriptional activation of class II genes. *Cell* **78:**513–523.
Gehring WJ, Affolter M, and Bürglin T (1994) Homeodomain proteins. *Annu Rev Biochem* **63:**487–529.
Giordano A and Avantaggiati ML (1999) p300 and CBP: partners for life and death. *J Cell Physiol* **181:**218–230.
Glass CK, Rose DW, and Rosenfeld MG (1997) Nuclear receptor coactivators. *Curr Opin Cell Biol* **9:**222–232.
Goodman RH and Smolik S (2000) Cbp/p300 in cell growth, transformation, and development. *Genes Dev* **14:**1553–1577.
Goswami RG, Lacson RG, Yang E, Sam R, and Unterman TG (1994) Functional analysis of glucocorticoid and insulin response sequences in the rat Insulin-like Growth Factor-Binding Protein-1 promoter. *Endocrinology* **134:**736–743.
Grange T, Roux J, Rigaud G, and Pictet R (1991) Cell-type specific activity of two glucocorticoid responsive units of rat tyrosine aminotransferase gene is associated with multiple binding sites for C/EBP and a novel liver-specific nuclear factor. *Nucleic Acids Res* **19:**131–139.
Gray SG, Eriksson T, and Ekstrom TJ (1999) Methylation, gene expression and the chromatin connection in cancer. *Int J Mol Med* **4:**333–350.
Green VJ, Kokkotou E, and Ladias JA (1998) Critical structural elements and multitarget protein interactions of the transcriptional activator AF-1 of hepatocyte nuclear factor 4. *J Biol Chem* **273:**29950–29957.
Gregori C, Kahn A, and Pichard AL (1993) Competition between transcription factors HNF1 and HNF3, and alternative cell-specific activation by DBP and C/EBP contribute to the regulation of the liver-specific aldolase B promoter. *Nucleic Acids Res* **21:**897–903.
Griffo G, Hamon-Benais C, Angrand PO, Fox M, West L, Lecoq O, Povey S, Cassio D, and Weiss M (1993) HNF4 and HNF1 as well as a panel of hepatic functions are

extinguished and reexpressed in parallel in chromosomally reduced rat hepatoma-human fibroblast hybrids. *J Cell Biol* **121:**887–898.
Grunstein M (1997) Histone acetylation in chromatin structure and transcription. *Nature (Lond)* **389:**349–352.
Gu W and Roeder RG (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90:**595–606.
Guschin D, Wade PA, Kikyo N, and Wolffe AP (2000) ATP-dependent histone octamer mobilization and histone deacetylation mediated by the Mi-2 chromatin remodeling complex. *Biochemistry* **39:**5238–5245.
Hadzopoulou-Cladaras M, Kistanova E, Evagelopoulou C, Zeng S, Cladaras C, and Ladias JA (1997) Functional domains of the nuclear receptor hepatocyte nuclear factor 4. *J Biol Chem* **272:**539–550.
Hampsey M (1998) Molecular genetics of the RNA polymerase II general transcriptional machinery. *Microbiol Mol Biol Rev* **62:**465–503.
Hata S, Inoue T, Kosuga K, Nakashima T, Tsukamoto T, and Osumi T (1995) Identification of two splice isoforms of mRNA for mouse hepatocyte nuclear factor 4 (HNF-4). *Biochim Biophys Acta* **1260:**55–61.
Hata S, Tsukamoto T, and Osumi T (1992) A novel isoform of rat hepatocyte nuclear factor 4 (HNF-4). *Biochim Biophys Acta* **1131:**211–213.
Hauck W, Nedellec P, Turbide C, Stanners CP, Barnett TR, and Beauchemin N (1994) Transcriptional control of the human biliary glycoprotein gene, a CEA gene family member down-regulated in colorectal carcinomas. *Eur J Biochem.* **223:**529–541.
Hayashi Y, Wang W, Ninomiya T, Nagano H, Ohta K, and Itoh H (1999) Liver enriched transcription factors and differentiation of hepatocellular carcinoma. *Mol Pathol* **52:**19–24.
Hertz R, Magenheim J, Berman I, and Bar-Tana J (1998) Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4a. *Nature (Lond)* **392:**512–516.
Hiemisch H, Schutz G, and Kaestner KH (1997) Transcriptional regulation in endoderm development: characterization of an enhancer controlling Hnf3g expression by transgenesis and targeted mutagenesis. *EMBO J* **16:**3995–4006.
Holewa B, Zapp D, Drewes T, Senkel S, and Ryffel GU (1997) HNF4β, a new gene of the HNF4 family with distinct activation and expression profiles in oogenesis and embryogenesis of *Xenopus laevis*. *Mol Cell Biol* **17:**687–694.
Holstege FC, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, and Young RA (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95:**717–728.
Horiuchi M, Kurihara Y, Katahira M, Maeda T, Saito T, and Uesugi S (1997) Dimerization and DNA binding facilitate α-helix formation of Max in solution. *J Biochem (Tokyo)* **122:**711–716.
Hu CH, Harris JE, Davie EW, and Chung DW (1995) Characterization of the 5′-flanking region of the gene for the α chain of human fibrinogen. *J Biol Chem* **270:**28342–28349.
Ichinose H, Garnier JM, Chambon P, and Losson R (1997) Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. *Gene* **188:**95–100.
Imbalzano AN, Kwon H, Green MR, and Kingston RE (1994) Facilitated binding of TATA-binding protein to nucleosomal DNA. *Nature (Lond)* **370:**481–485.
Imhof A, Yang XJ, Ogryzko VV, Nakatani Y, Wolffe AP, and Ge H (1997) Acetylation of general transcription factors by histone acetyltransferases. *Curr Biol* **7:**689–692.
Issa JPJ, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, and Baylin SB (1994) Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* **7:**536–540.
Issa JPJ, Vertino PM, Boehm CD, Newsham IF, and Baylin SB (1996) Switch from monoallelic to biallelic human IGF2 promotor methylation during aging and carcinogenesis. *Proc Natl Acad Sci USA* **93:**11757–11762.
Iyemere VP, Davies NH, and Brownlee GG (1998) The activation function 2 domain of hepatic nuclear factor 4 is regulated by a short C-terminal proline-rich repressor domain. *Nucleic Acids Res* **26:**2098–2104**.**
Jacob A, Budhiraja S, Qian X, Clevidence D, Costa RH, and Reichel RR (1994) Retinoic acid-mediated activation of HNF-3 α during EC stem cell differentiation. *Nucleic Acids Res* **22(11):**2126–2133.
Jacquemin P, Lannoy VJ, Rousseau GG, and Lemaigre FP (1999) OC-2, a novel mammalian member of the ONECUT class of homeodomain transcription factors whose function in liver partially overlaps with that of hepatocyte nuclear factor-6. *J Biol Chem* **274:**2665–2671.
Jaenisch R (1997) DNA methylation and imprinting: why bother? *Trends Genet* **13:**323–329.
Jiang G, Nepomuceno L, Hopkins K, and Sladek FM (1995) Exclusive homodimerization of the orphan receptor hepatocyte nuclear factor 4 defines a new subclass of nuclear receptors. *Mol Cell Biol* **15(9):**5131–5143.
Jin C, Marsden I, Chen X, and Liao X (1999) Dynamic DNA contacts observed in the NMR structure of winged helix protein-DNA complex. *J Mol Biol* **289:**683–690.
Jones PA and Laird PW (1999) Cancer epigenetics comes of age. *Nat Genet* **21(2):**163–167.
Jose-Estanyol M and Danan JL (1988) A liver-specific factor and nuclear factor I bind to the rat α-fetoprotein promoter. *J Biol Chem* **263:**10865–10871.
Kaling M, Kugler W, Ross K, Zoidl C, and Ryffel GU (1991) Liver-specific gene expression: A-activator-binding site, a promoter module present in vitellogenin and acute-phase genes. *Mol Cell Biol* **11:**93–101.
Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK, and Rosenfeld MG (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85:**403–414.
Kimura A, Nishiyori A, Murakami T, Tsukamoto T, Hata S, Osumi T, Okamura R, Mori M, and Takiguchi M (1993) Chicken ovalbumin upstream promoter-transcription factor (COUP-TF) represses transcription from the promoter of the gene for ornithine transcarbamylase in a manner antagonistic to hepatocyte nuclear factor-4 (HNF-4). *J Biol Chem* **268:**11125–11133.
Klug A (1999) Zinc finger peptides for the regulation of gene expression. *J Mol Biol* **293:**215–218.
Klug A, Rhodes D, Smith J, Finch JT, and Thomas JO (1980) A low resolution structure for the histone core of the nucleosome. *Nature (Lond)* **287:**509–516.
Kohler JJ, Metallo SJ, Schneider TL, and Schepartz A (1999) DNA specificity enhanced by sequential binding of protein monomers. *Proc Natl Acad Sci USA* **96:**11735–17739.
Kopachik W, Hayward SW, and Cunha GR (1998) Expression of hepatocyte nuclear factor-3α in rat prostate, seminal vesicle, and bladder. *Dev Dyn* **211:**131–140.
Kornberg RD and Lorch Y (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98:**285–294.
Kornberg RD and Thomas JO (1974) Chromatin structure: oligomers of the histones. *Science (Wash DC)* **184:**865–868.
Kritis AA, Argyrokastritis A, Moschonas NK, Power S, Katrakili N, Zannis VI, Cereghini S, and Talianidis I (1996) Isolation and characterization of a third isoform of human hepatocyte nuclear factor 4. *Gene* **173:**275–280.
Kritis AA, Ktistaki E, Barda D, Zannis VI, and Talianidis I (1993) An indirect negative autoregulatory mechanism involved in hepatocyte nuclear factor-1 gene expression. *Nucleic Acids Res* **21:**5882–5889.
Krokan HE, Nilsen H, Skorpen F, Otterlei M, and Slupphaug G (2000) Base excision repair of DNA in mammalian cells. *FEBS Lett* **476:**73–77.
Ktistaki E, Ktistakis NT, Papadogeorgaki E, and Talianidis I (1995) Recruitment of hepatocyte nuclear factor 4 into specific intranuclear compartments depends on tyrosine phosphorylation that affects its DNA-binding and transactivation potential. *Proc Natl Acad Sci USA* **92:**9876–9880.
Ktistaki E and Talianidis I (1997a) Modulation of hepatic gene expression by hepatocyte nuclear factor 1. *Science (Wash DC)* **277:**109–112.
Ktistaki E and Talianidis I (1997b) Chicken ovalbumin upstream promoter transcription factors act as auxiliary cofactors for hepatocyte nuclear factor 4 and enhance hepatic gene expression. *Mol Cell Biol* **17:**2790–2797.
Kuo CJ, Conley PB, Hsieh CL, Francke U, and Crabtree GR (1990) Molecular cloning, functional expression, and chromosomal localization of mouse hepatocyte nuclear factor 1. *Proc Natl Acad Sci USA* **87:**9838–9842.
Kuo CJ, Mendel DB, Hansen LP, and Crabtree GR (1991) Independent regulation of HNF-1 α and HNF-1 β by retinoic acid in F9 teratocarcinoma cells. *EMBO J* **10:**2231–2236.
Kuo MH, Zhou J, Jambeck P, Churchill ME, and Allis CD (1998) Histone acetyltransferase activity of yeast Gcn5 is required for the activation of target genes in vivo. *Genes Dev* **12:**627–639.
Lahuna O, Fernandez L, Karlsson H, Maiter D, Lemaigre FP, Rousseau GG, Gustafsson J, and Mode A (1997) Expression of hepatocyte nuclear factor 6 in rat liver is sex-dependent and regulated by growth hormone. *Proc Natl Acad Sci USA* **94:**12309–12313.
Lahuna O, Rastegar M, Maiter D, Thissen JP, Lemaigre FP, and Rousseau GG (2000) Involvement of Stat5 (signal transducer and activator of transcription 5) and HNF-4 (hepatocyte nuclear factor 4) in the transcriptional control of the hnf6 gene by growth hormone. *Mol Endocrinol* **14:**285–294.
Lai E and Darnell JE Jr (1991) Transcriptional control in hepatocytes: A window on development. *Trends Biochem Sci* **16:**427–435.
Landry C, Clotman F, Hioki T, Oda H, Picard JJ, Lemaigre FP, and Rousseau GG (1997) HNF-6 is expressed in endoderm derivatives and nervous system of the mouse embryo and participates to the cross-regulatory network of liver-enriched transcription factors. *Dev Biol* **192:**247–257.
Lannoy VJ, Burglin TR, Rousseau GG, and Lemaigre FP (1998) Isoforms of hepatocyte nuclear factor-6 differ in DNA-binding properties, contain a bifunctional homeodomain, and define the new ONECUT class of homeodomain proteins. *J Biol Chem* **273:**13552–13562.
Laurent BC, Treitel MA, and Carlson M (1991) Functional interdependence of the yeast SNF2, SNF5, and SNF6 proteins in transcriptional activation. *Proc Natl Acad Sci USA* **88:**2687–2691.
Lee YH, Sauer B, and Gonzalez FJ (1998) Laron dwarfism and non-insulin-dependent diabetes mellitus in the Hnf-1α knockout mouse. *Mol Cell Biol* **18:**3059–3068.
Lee YK, Dell H, Dowhan DH, Hadzopoulou-Cladaras M, and Moore DD (2000) The orphan nuclear receptor SHP inhibits hepatocyte nuclear factor 4 and retinoid X receptor transactivation: two mechanisms for repression. *Mol Cell Biol* **20:**187–195.
Lemaigre FP, Durviaux SM, and Rousseau GG (1993) Liver-specific factor binding to the liver promoter of a 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene. *J Biol Chem* **268:**19896–199905.
Lemaigre FP, Durviaux SM, Truong O, Lannoy VJ, Hsuan JJ, and Rousseau GG (1996) Hepatocyte nuclear factor 6, a transcription factor that contains a novel type of homeodomain and a single cut domain. *Proc Natl Acad Sci USA* **93:**9460–9464.
Lewin B (1994) *Genes V*. Oxford University Press, New York.
Lin B, Morris DW, and Chou JY (1998) Hepatocyte nuclear factor 1α is an accessory factor required for activation of glucose-6-phosphatase gene transcription by glucocorticoids. *DNA Cell Biol* **17:**967–974.
Lindner T, Gragnoli C, Furuta H, Cockburn BN, Petzold C, Rietzsch H, Weiss U, Schulze J, and Bell GI (1997) Hepatic function in a family with a nonsense mutation (R154X) in the hepatocyte nuclear factor-4α/MODY1 gene. *J Clin Invest* **100:**1400–1405.
Liu JK, DiPersio CM, and Zaret KS (1991) Extracellular signals that regulate liver transcription factors during hepatic differentiation in vitro. *Mol Cell Biol* **11:**773–784.
Luger K, Mäder AW, Richmond RK, Sargent DF, and Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature (Lond)* **389:**251–260.
Maire P, Wuarin J, and Schibler U (1989) The role of cis-acting promoter elements in tissue-specific albumin gene expression. *Science (Wash DC)* **244:**343–346.
Majello B, Arcone R, Toniatti C, and Ciliberto G (1990) Constitutive and IL-6-

induced nuclear factors that interact with the human C-reactive protein promoter. *EMBO J* **9:**457–465.
Malik S, Gu W, Wu W, Qin J, and Roeder RG (2000) The USA-derived transcriptional coactivator PC2 is a submodule of TRAP/SMCC and acts synergistically with other PCs. *Mol Cell* **5:**753–760.
Malik S and Karathanasis SK (1996) TFIIB-directed transcriptional activation by the orphan nuclear receptor hepatocyte nuclear factor 4. *Mol Cell Biol* **16:**1824–1831.
Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, and Evans RM (1995) The nuclear receptor superfamily: the second decade. *Cell* **83:**835–839.
Marsden I, Jin C, and Liao X (1998) Structural changes in the region directly adjacent to the DNA-binding helix highlight a possible mechanism to explain the observed changes in the sequence-specific binding of winged helix proteins. *J Mol Biol* **278:**293–299.
McGinnis W and Krumlauf R (1992) Homeobox genes and axial patterning. *Cell* **68:**283–302.
Meier CA (1997) Regulation of gene expression by nuclear hormone receptors. *J Recept Signal Transduct Res* **17(1–3):**319–335.
Mendel DB, Hansen LP, Graves MK, Conley PB, and Crabtree GR (1991a) HNF-1 α and HNF-1 β (vHNF-1) share dimerization and homeo domains, but not activation domains, and form heterodimers in vitro. *Genes Dev* **5:**1042–1056.
Mendel DB, Khavari PA, Conley PB, Graves MK, Hansen LP, Admon A, and Crabtree GR (1991b) Characterization of a cofactor that regulates dimerization of a mammalian homeodomain protein. *Science (Wash DC)* **254:**1762–1767.
Metallo SJ and Schepartz A (1994) Distribution of labor among bZip segments in the control of DNA affinity and specificity. *Chem Biol* **1:**143–151.
Mietus-Snyder M, Sladek FM, Ginsburg GS, Kuo CF, Ladias JA, Darnell JE Jr, and Karathanasis SK (1992) Antagonism between apolipoprotein AI regulatory protein 1, Ear3/COUP-TF, and hepatocyte nuclear factor 4 modulates apolipoprotein CIII gene expression in liver and intestinal cells. *Mol Cell Biol* **12:**1708–1718.
Milatovich A, Mendel DB, Crabtree GR, and Francke U (1993) Genes for the dimerization cofactor of hepatocyte nuclear factor-1 α (DCOH) are on human and murine chromosomes 10. *Genomics* **16:**292–295.
Miura N and Tanaka K (1993) Analysis of the rat hepatocyte nuclear factor (HNF) 1 gene promoter: synergistic activation by HNF4 and HNF1 proteins. *Nucleic Acids Res* **21:**3731–3736.
Monaci P, Nicosia A, and Cortese R (1988) Two different liver-specific factors stimulate in vitro transcription from the human α1-antitrypsin promoter. *EMBO J* **7:**2075–2087.
Morata G and Sanchez-Herrero E (1999) Patterning mechanisms in the body trunk and the appendages of *Drosophila*. *Development* **126:**2823–2828.
Muchardt C, Bourachot B, Reyes JC, and Yaniv M (1998) *ras* transformation is associated with decreased expression of the *brm*/SNF2α ATPase from the mammalian SWI-SNF complex. *EMBO J* **17:**223–231.
Muchardt C, Reyes JC, Bourachot B, Legouy E, and Yaniv M (1996) The h*brm* and BRG-1 proteins, components of the human SNF/SWI complex, are phorphorylated and excluded from the condensed chromosomes during mitosis. *EMBO J* **15:**3394–3402**.**
Muchardt C and Yaniv M (1993) A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J* **12:**4279–4290.
Muchardt C and Yaniv M (1999) ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job. *J Mol Biol* **293:**187–198.
Muppala V, Lin CS, and Lee YH (2000) The role of HNF-1α in controlling hepatic catalase activity. *Mol Pharmacol* **57:**93–100.
Murakami T, Nishiyori A, Takiguchi M, and Mori M (1990) Promoter and 11-kilobase upstream enhancer elements responsible for hepatoma cell-specific expression of the rat ornithine transcarbamylase gene. *Mol Cell Biol* **10:**1180–1191.
Nakamura T, Akiyoshi H, Shiota G, Isono M, Nakamura K, Moriyama M, and Sato K (1999) Hepatoprotective action of adenovirus-transferred HNF-3γ gene in acute liver injury caused by CCl(4). *FEBS Lett* **459:**1–4.
Nakhei H, Lingott A, Lemm I, and Ryffel GU (1998) An alternative splice variant of the tissue specific transcription factor HNF4α predominates in undifferentiated murine cell types. *Nucleic Acids Res* **26:**497–504.
Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, and Bird A (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature (Lond)* **393:**386–389.
Nishiyama C, Hi R, Osada S, and Osumi T (1998) Functional interactions between nuclear receptors recognizing a common sequence element, the direct repeat motif spaced by one nucleotide (DR-1). *J Biochem (Tokyo)* **123:**1174–1179.
Nishiyori A, Tashiro H, Kimura A, Akagi K, Yamamura K, Mori M, and Takiguchi M (1994) Determination of tissue specificity of the enhancer by combinatorial operation of tissue-enriched transcription factors. Both HNF-4 and C/EBP β are required for liver-specific activity of the ornithine transcarbamylase enhancer. *J Biol Chem* **269:**1323–1331.
Nitsch D, Boshart M, and Schutz G (1993) Activation of the tyrosine aminotransferase gene is dependent on synergy between liver-specific and hormone-responsive elements. *Proc Natl Acad Sci U S A* **90:**5479–5483.
Niu X, Renshaw-Gegg L, Millller L, and Guiltinan MJ (1999) Bipartite determinants of DNA-binding specificity of plant basic leucine zipper proteins. *Plant Mol Biol* **41:**1–13.
O'Brien RM, Noisin EL, Suwanichkul A, Yamasaki T, Lucas PC, Wang JC, Powell DR, and Granner DK (1995) Hepatic nuclear factor 3- and hormone-regulated expression of the phosphoenolpyruvate carboxykinase and insulin-like growth factor-binding protein 1 genes. *Mol Cell Biol* **15:**1747–1758.
Ogryzko VV, Schlitz RL, Russanova V, Howard BH, and Nakatani Y (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87:**953–959.
Östlund-Farrants AK, Blomquist P, Kwon H, and Wrange O (1997) Glucocorticoid receptor-glucocorticoid response element binding stimulates nucleosome disruption by the SWI/SNF complex. *Mol Cell Biol* **17:**895–905.
Pabo CO and Sauer RT (1992) Transcription factors: structural families and principles of DNA recognition. *Annu Rev Biochem* **61:**1053–1095.
Pani L, Overdier DG, Porcella A, Qian X, Lai E, and Costa RH (1992a) Hepatocyte nuclear factor 3 β contains two transcriptional activation domains, one of which is novel and conserved with the Drosophila fork head protein. *Mol Cell Biol* **12:**3723–3732.
Pani L, Qian XB, Clevidence D, and Costa RH (1992b) The restricted promoter activity of the liver transcription factor hepatocyte nuclear factor 3 β involves a cell-specific factor and positive autoactivation. *Mol Cell Biol* **12:**552–562.
Peterson CL and Herskowitz I (1992) Characterization of the yeast SWI1, SWI2 and SWI3 genes, which encode a global activator of transcription. *Cell* **68:**573–583.
Petropoulos I, Auge-Gouillou C, and Zakin MM (1991) Characterization of the active part of the human transferrin gene enhancer and purification of two liver nuclear factors interacting with the TGTTTGC motif present in this region. *J Biol Chem* **266:**24220–24225.
Phelan ML, Sif S, Narlikar GJ, and Kingston RE (1999) Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. *Mol Cell* **3:**247–253.
Phelan ML, Schnitzler GR, and Kingston RE (2000) Octamer transfer and creation of stably remodeled nucleosomes by human SWI-SNF and its isolated ATPases. *Mol Cell Biol* **20:**6380–6389.
Pierreux CE, Stafford J, Demonte D, Scott DK, Vandenhaute J, O'Brien RM, Granner DK, Rousseau GG, and Lemaigre FP (1999) Antiglucocorticoid activity of hepatocyte nuclear factor-6. *Proc Natl Acad Sci U SA* **96:**8961–8966.
Pietrangelo A and Shafritz DA (1994) Homeostatic regulation of hepatocyte nuclear transcription factor 1 expression in cultured hepatoma cells. *Proc Natl Acad Sci USA* **91:**182–186.
Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C, and Yaniv M (1996) Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. *Cell* **84:**575–585.
Powell DJ, Friedman JM, Oulette AJ, Krauter KS, and Darnell JE (1984) Transcriptional and post-transcriptional control of specific messenger RNAs in adult and embryonic liver. *J Mol Biol* **179:**21–35.
Powell DR, Allander SV, Scheimann AO, Wasserman RM, Durham SK, and Suwanichkul A (1995) Multiple proteins bind the insulin response element in the human IGFBP-1 promoter. *Prog Growth Factor Res* **6:**93–101.
Prendergast GC and Ziff EB (1991) Methylation-sensitive sequence-specific DNA binding by the c-Myc basic region. *Science (Wash DC)* **251:**186–189.
Qian X and Costa RH (1995) Analysis of hepatocyte nuclear factor-3 β protein domains required for transcriptional activation and nuclear targeting. *Nucleic Acids Res* **23:**1184–1191.
Qian X, Samadani U, Porcella A, and Costa RH (1995) Decreased expression of hepatocyte nuclear factor 3 α during the acute-phase response influences transthyretin gene transcription. *Mol Cell Biol* **15:**1364–1376.
Rachez C, Gamble M, Chang CPB, Atkins CB, Lazar MA, and Freedman LP (2000) The Drip complex and Src-1/p160 coactivators share similar nuclear receptor binding determinants but constitute functionally distinct complexes. *Mol Cell Biol* **20:**2718–2726.
Rastegar M, Rousseau GG, and Lemaigre FP (2000) CCAAT/enhancer-binding protein-α is a component of the growth hormone-regulated network of liver transcription factors. *Endocrinology* **141:**1686–1692.
Rastegar M, Szpirer C, Rousseau GG, and Lemaigre FP (1998) Hepatocyte nuclear factor 6: organization and chromosomal assignment of the rat gene and characterization of its promoter. *Biochem J* **334(Pt 3):**565–569.
Rausa F, Samadani U, Ye H, Lim L, Fletcher CF, Jenkins NA, Copeland NG, and Costa RH (1997) The cut-homeodomain transcriptional activator HNF-6 is coexpressed with its target gene HNF-3 β in the developing murine liver and pancreas. *Dev Biol* **192:**228–246.
Reijnen MJ, Maasdam D, Bertina RM, and Reitsma PH (1994) Haemophilia B Leyden: the effect of mutations at position +13 on the liver-specific transcription of the factor IX gene. *Blood Coagul Fibrinolysis* **5:**341–348.
Reyes JC, Barra J, Muchardt C, Camus A, Babinet C, and Yaniv M (1998) Altered control of cellular proliferation in the absence of mammalian brahma (SNF2a). *EMBO J* **17:**6979–6991.
Rigaud G, Roux J, Pictet R, and Grange T (1991) In vivo footprinting of rat TAT gene: dynamic interplay between the glucocorticoid receptor and a liver-specific factor. *Cell* **67:**977–986.
Robzyk K, Recht J, and Osley MA (2000) Rad6-dependent ubiquitination of histone H2B in yeast. *Science (Wash DC)* **287:**501–504.
Rodolosse A, Carriere V, Rousset M, and Lacasa M (1998) Two HNF-1 binding sites govern the glucose repression of the human sucrase-isomaltase promoter. *Biochem J* **336 (Pt 1):**115–123.
Rodriguez JC, Ortiz JA, Hegardt FG, and Haro D (1998) The hepatocyte nuclear factor 4 (HNF-4) represses the mitochondrial HMG-CoA synthase gene. *Biochem Biophys Res Commun* **242(3):**692–696.
Roeder RG (1996) The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem Sci* **21:**327–335.
Ryan MP, Jones R, and Morse RH (1998) SWI-SNF complex participation in transcriptional activation at a step subsequent to activator binding. *Mol Cell Biol* **18:**1774–1782.
Samadani U and Costa RH (1996) The transcriptional activator hepatocyte nuclear factor 6 regulates liver gene expression. *Mol Cell Biol* **16:**6273–6284.
Samadani U, Porcella A, Pani L, Johnson PF, Burch JB, Pine R, and Costa RH (1995) Cytokine regulation of the liver transcription factor hepatocyte nuclear factor-3 β is mediated by the C/EBP family and interferon regulatory factor 1. *Cell Growth Differ* **6:**879–890.
Samadani U, Qian X, and Costa RH (1996) Identification of a transthyretin enhancer site that selectively binds the hepatocyte nuclear factor-3 β isoform. *Gene Expr* **6:**23–33.
Schaeffer E, Guillou F, Part D, and Zakin MM (1993) A different combination of

transcription factors modulates the expression of the human transferrin promoter in liver and Sertoli cells. *J Biol Chem* **268:**23399–408.
Schmid P and Schulz WA (1990) Coexpression of the c-myc protooncogene with α-fetoprotein and albumin in fetal mouse liver. *Differentiation* **45:**96–102.
Schnitzler G, Sif S, and Kingston RE (1998) Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. *Cell* **94:**17–27.
Schonemann MD, Ryan AK, Erkman L, McEvilly RJ, Bermingham J, and Rosenfeld MG (1998) POU domain factors in neural development. *Adv Exp Med Biol* **449:** 39–53.
Scott MP (1992) Vertebrate homeobox gene nomenclature. *Cell* **71:**551–553.
Seol W, Choi HS, and Moore DD (1996) An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science (Wash DC)* **272:**1336–1339.
Shanahan F, Seghezzi W, Parry D, Mahony D, and Lees E (1999) Cyclin E associates with BAF155 and BRG1, components of the mammalian SWI-SNF complex, and alters the ability of BRG1 to induce growth arrest. *Mol Cell Biol* **19:**1460–1469.
Shilatifard A (1998) Factors regulating the transcriptional elongation activity of RNA polymerase II. *FASEB J* **12:**1437–1446.
Shiojiri N, Lemire JM, and Fausto N (1991) Cell lineages and oval cell progenitors in rat liver development. *Cancer Res* **51:**2611–2620.
Sif S, Stukenberg PT, Kirschner MW, and Kingston RE (1998) Mitotic inactivation of a human SWI/SNF chromatin remodeling complex. *Genes Dev* **12:**2842–2851.
Sladek FM, Zhong WM, Lai E, and Darnell JE Jr (1990) Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes Dev* **4:**2353–2365.
Song CS, Jung MH, Kim SC, Hassan T, Roy AK, and Chatterjee B (1998) Tissue-specific and androgen-repressible regulation of the rat dehydroepiandrosterone sulfotransferase gene promoter. *J Biol Chem* **273:**21856–66.
Soutoglou E, Katrakili N, and Talianidis I (2000a) Acetylation regulates transcription factor activity at multiple levels. Mol Cell **5:**745–751.
Soutoglou E, Papafotiou G, Katrakili N, and Talianidis I (2000b) Transcriptional activation by hepatocyte nuclear factor-1 requires synergism between multiple coactivator proteins. *J Biol Chem* **275:**12515–12520.
Späth GF and Weiss MC (1997) Hepatocyte nuclear factor 4 expression overcomes repression of the hepatic phenotype in dedifferentiated hepatoma cells. *Mol Cell Biol* **17:**1913–1922.
Späth GF and Weiss MC (1998) Hepatocyte nuclear factor 4 provokes expression of epithelial marker genes, acting as a morphogen in dedifferentiated hepatoma cells. *J Cell Biol* **140:**935–946.
Spek CA, Greengard JS, Griffin JH, Bertina RM, and Reitsma PH (1995) Two mutations in the promoter region of the human protein C gene both cause type I protein C deficiency by disruption of two HNF-3 binding sites. *J Biol Chem* **270:**24216–242221.
Spek CA, Lannoy VJ, Lemaigre FP, Rousseau GG, Bertina RM, and Reitsma PH (1998) Type I protein C deficiency caused by disruption of a hepatocyte nuclear factor (HNF)-6/HNF-1 binding site in the human protein C gene promoter. *J Biol Chem* **273:**10168–10173.
Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai M, and O'Malley BW (1997) Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature (Lond)* **389:**194–198.
Spencer VA, and Davie JR (1999) Role of covalent modification of histones in regulating gene expression. *Gene* **240:**1–12.
Stein GS, van Wijnen AJ, Montecino M, Stein JL, and Lian JB (1999) Nuclear structure/gene expression interrelationships. *J Cell Physiol* **181:**240–250.
Stenson C, McNair A, Byrnes L, Murphy M, Smith T, and Gannon F (2000) Atlantic salmon Hnf-3/forkhead cDNA sequence, evolution, expression, and functional analysis. *DNA Cell Biol* **19:**59–68.
Stoffel M and Duncan SA (1997) The maturity-onset diabetes of the young (MODY1) transcription factor HNF4α regulates expression of genes required for glucose transport and metabolism. *Proc Natl Acad Sci USA* **94:**13209–13214.
Streeper RS, Eaton EM, Ebert DH, Chapman SC, Svitek CA, and O'Brien RM (1998) Hepatocyte nuclear factor-1 acts as an accessory factor to enhance the inhibitory action of insulin on mouse glucose-6-phosphatase gene transcription. *Proc Natl Acad Sci USA* **95:**9208–9213.
Strober BE, Dunaief JL, Guha S, and Goff SP (1996) Functional interactions between hBRM/hBRG-1 transcriptional activators and the pRB family of proteins. *Mol Cell Biol* **16(4):**1576–1583.
Sudarsanam P and Winston F (2000) The Swi/Snf family: nucleosome-remodeling complexes and transcriptional control. *Trends Genet* **16:**345–351.
Taraviras S, Monaghan AP, Schutz G, and Kelsey G (1994) Characterization of the mouse HNF-4 gene and its expression during mouse embryogenesis. *Mech Dev* **48:**67–79.
Thompson LH and Schild D (2001) Homologous recombinational repair of DNA ensures mammalian chromosome stability. *Mutat Res* **477:**131–153.
Thomson S, Mahadevan LC, and Clayton AL (1999) MAP kinase-mediated signalling to nucleosomes and immediate-early gene induction. *Semin Cell Dev Biol* **10:**205–214.
Thony B, Neuheiser F, Blau N, and Heizmann CW (1995) Characterization of the human PCBD gene encoding the bifunctional protein pterin-4 α-carbinolamine dehydratase/dimerization cofactor for the transcription factor HNF-1 α. *Biochem Biophys Res Commun* **210:**966–973.
Tian JM and Schibler U (1991) Tissue-specific expression of the gene encoding hepatocyte nuclear factor 1 may involve hepatocyte nuclear factor 4. *Genes Dev* **5:**2225–2234.
Toniatti C, Demartis A, Monaci P, Nicosia A, and Ciliberto G (1990) Synergistic trans-activation of the human C-reactive protein promoter by transcription factor HNF-1 binding at two distinct sites. *EMBO J* **9:**4467–4475.
Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, and Rosenfeld MG (1997) The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature (Lond)* **387:**677–684.
Trautwein C, Will M, Kubicka S, Rakemann T, Flemming P, and Manns MP (1999) 2-Acetaminofluorene blocks cell cycle progression after hepatectomy by p21 induction and lack of cyclin E expression. *Oncogene* **18(47):**6443–6453.
Trouche D, Le Chalony C, Muchardt C, Yaniv M, and Kouzarides T (1997) RB and hbrm cooperate to repress the activation functions of E2F1. *Proc Natl Acad Sci USA* **94:**11268–73.
Tsutsumi K, Ito K, and Ishikawa K (1989) Developmental appearance of transcription factors that regulate liver-specific expression of the aldolase B gene. *Mol Cell Biol* **9(11):**4923–4931.
Tyler JK and Kadonga JT (1999) The „dark side" of chromatin remodeling: repressive effects on transcription. *Cell* **99:**443–446.
Ueno T and Gonzalez FJ (1990) Transcriptional control of the rat hepatic CYP2E1 gene. *Mol Cell Biol* **10:**4495–4505.
Vaisse C, Kim J, Espinosa R 3rd, Le Beau MM, and Stoffel M (1997) Pancreatic islet expression studies and polymorphic DNA markers in the genes encoding hepatocyte nuclear factor-3α, -3β, -3γ, -4γ, and -6. *Diabetes* **46:**1364–1367.
Van Leeuwen HC, Strating MJ, Rensen M, de Laat W, and van der Vliet PC (1997) Linker length and composition influence the flexibility of Oct-1 DNA binding. *EMBO J* **16:**2043–2053.
Venter JC, Adams MD, Myers EW, et al (2001) The sequence of the human genome. *Science (Wash DC)* **291:**1304–1351.
Viollet B, Kahn A, and Raymondjean M (1997) Protein kinase A-dependent phosphorylation modulates DNA-binding activity of hepatocyte nuclear factor 4. *Mol Cell Biol* **17:**4208–4219.
Virbasius CM, Wagner S, and Green MR (1999) A human nuclear-localized chaperone that regulates dimerization, DNA binding, and transcriptional activity of bZIPP proteins. *Mol Cell* **4:**219–228.
Wade DP, Lindahl GE, and Lawn RM (1994) Apolipoprotein(a) gene transcription is regulated by liver-enriched trans-acting factor hepatocyte nuclear factor 1 α. *J Biol Chem* Aug 5;**269(31):**19757–65.
Wade PA and Wolffe AP (1997) Histone acetyltransferases in control. *Curr Biol* **7:**R82–4.
Wade PA and Wolffe AP (1999) Transcriptional regulation: SWItching circuitry. *Curr Biol* **9:**R221–R224.
Wang JC, Stafford JM, and Granner DK (1998a) SRC-1 and GRIP1 coactivate transcription with hepatocyte nuclear factor 4. *J Biol Chem* **273:**30847–30850.
Wang JC, Stromstedt PE, O'Brien RM, and Granner DK (1996) Hepatic nuclear factor 3 is an accessory factor required for the stimulation of phosphoenolpyruvate carboxykinase gene transcription by glucocorticoids. *Mol Endocrinol* **10:**794–800.
Wang L, Liu L, and Berger SL (1998b) Critical residues for histone acetylation by Gcn5, functioning in Ada and SAGA complexes, are also required for transcriptional function in vivo. *Genes Dev* **12:**640–653.
Williams SC, Angerer ND, and Johnson PF (1997) C/EBP proteins contain nuclear localization signals imbedded in their basic regions. *Gene Expr* **6:**371–385.
Wilson CJ, Chao DM, Imbalzano AN, Schnitzler GR, Kingston RE, and Young RA (1996) RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. *Cell* **84:**235–244.
Wintjens R and Rooman M (1996) Structural classification of HTH DNA-binding domains and protein-DNA interaction modes. *J Mol Biol* **262(2):**294–313.
Wolberger C (1999) Multiprotein-DNA complexes in transcriptional regulation. *Annu Rev Biophys Biomol Struct* **28:**29–56.
Wolffe AP and Matzke MA (1999) Epigenetics: regulation through repression. *Science (Wash DC)* **286:**481–486.
Workman JL and Kingston RE (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem* **67:**545–579.
Xanthopoulos KG, Prezioso VR, Chen WS, Sladek FM, Cortese R, and Darnell JE Jr (1991) The different tissue transcription patterns of genes for HNF-1, C/EBP, HNF-3, and HNF-4, protein factors that govern liver-specific transcription. *Proc Natl Acad Sci USA* **88:**3807–3811.
Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, and Bell GI (1996) Mutations in the hepatocyte nuclear factor-4α gene in maturity-onset diabetes of the young. *Nature (Lond)* **384:**458–460.
Yamaguchi Y, Wada T, and Handa H (1998) Interplay between positive and negative elongation factors: drawing a new view of DRB. *Genes Cells* **3:**9–15.
Yang XJ, Ogryzko VV, Nishikawa JI, Howard BH, and Nakatani Y (1996) A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature (Lond)* **382:**319–324.
Yao TP, Ku G, Zhou N, Scully R, and Livingston DM (1996) The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. *Proc Natl Acad Sci USA* **93:**10626–31.
Ye H, Kelly TF, Samadani U, et al (1997) Hepatocyte Nuclear Factor 3/fork head homolog 11 is expressed in proliferating epithelial and mesenchymal cells of embryonic and adult tissues. *Mol Biol Cell* **17:**1626–1641.
Yoshida E, Aratani S, Itou H, Miyagishi M, Takiguchi M, Osumu T, Murakami K, and Fukamizu A (1997) Functional association between CBP and HNF4 in transactivation. *Biochem Biophys Res Commun* **241:**664–669.
Zaret KS, Liu JK, and DiPersio CM (1990) Site-directed mutagenesis reveals a liver transcription factor essential for the albumin transcriptional enhancer. *Proc Natl Acad Sci USA* **87:**5469–5473.
Zhang Y, Huck-Hui N, Erdjument-Bromage H, Tempst P, Bird A, and Reinberg D (1999) Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev* **13:**1924–1935.
Zhang Y, LeRoy G, Seelig HP, Lane WS, and Reinberg D (1998) The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosomal activities. *Cell* **95:**279–289.
Zhong W, Mirkovitch J, and Darnell JE Jr (1994) Tissue-specific regulation of mouse hepatocyte nuclear factor 4 expression. *Mol Cell Biol* **14:**7276–7284.
Zhou DX and Yen TS (1991) The ubiquitous transcription factor Oct-1 and the liver-specific factor HNF-1 are both required to activate transcription of a hepatitis B virus promoter. *Mol Cell Biol* **11:**1353–1359.
Zlatanova J, Caifa P, and Van Holde K (2000) Linker histone binding and displacement: versatile mechanism for transcriptional regulation. *FASEB J* **14:**1697–1704.