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Review

Transcriptional control in the mammalian liver: liver development, perinatal repression, and zonal gene regulation

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Abstract. Liver function is crucial for maintaining metabolic homeostasis in mammals. Numerous genes must be properly regulated for the liver to develop and perform a variety of activities. Several recent gene-knockout studies in mice have clarified the roles of GATA6, HNF4α, and Foxa1/Foxa2 in early stages of liver formation. After the liver forms, transcriptional changes continue to occur; during the perinatal period, certain genes such as α-fetoprotein and H19 are silenced, others are activated, and position-dependent (or zonal) regulation is established. Zhx2 was recently identified as one factor involved in postnatal repression of α-fetoprotein and other genes. Furthermore, several studies indicate that negative regulation is involved in the zonal control of glutamine synthetase. Finally, exciting new evidence indicates that signaling through the Wnt/β-catenin pathway is also involved in zonal regulation in the adult liver.

Keywords. Liver development, Zhx2, Wnt/β-catenin, glutamine synthetase, alpha-fetoprotein.

Overview of liver function and structure

The liver is the largest internal organ in mammals. It serves as an endocrine and exocrine organ and carries out numerous functions that are involved in maintaining homeostasis within an organism [1]. These functions include (a) the production of serum proteins, including clotting factors and transport proteins such as albumin and transferrin; (b) the removal and breakdown of serum proteins, red blood cells and microbes; (c) the production or removal of glucose during periods of fasting or eating, respectively; (d) the processing of fatty acids and triglycerides; (e) maintaining cholesterol homeostasis via synthesis or catabolism; (f) the synthesis and interconversion of non-essential amino acids; (g) the breakdown of toxic endogenous compounds such as ammonia; (h) the production and excretion of bile components; (i) the detoxification of xenobiotic agents; and (j) the storage of numerous substances. The liver is well positioned to carry out these functions; it receives blood from two sources, the portal vein and the hepatic artery, and the hepatic architecture facilitates the exchange of materials between the blood and hepatocytes. In addition, the hepatic biliary system enables the liver to transport bile into the intestines.

While not as obvious as other organs such as the lung, kidney, and pancreas, the adult liver is comprised of repeating structural units termed lobules (Fig. 1). The lobule is loosely defined as a hexagonal structure that consists of plates of anastomosing hepatocytes. At the center of the lobule is the central vein, whereas six portal triads ring the hypothetical edge of each lobule. Each portal triad is comprised of an intrahepatic bile duct, portal vein, and hepatic artery. This lobular organization has functional significance. For example, while some liver functions described above can be carried out by all hepatocytes, other functions are limited to a subset of hepatocytes. This compartmentalization of function

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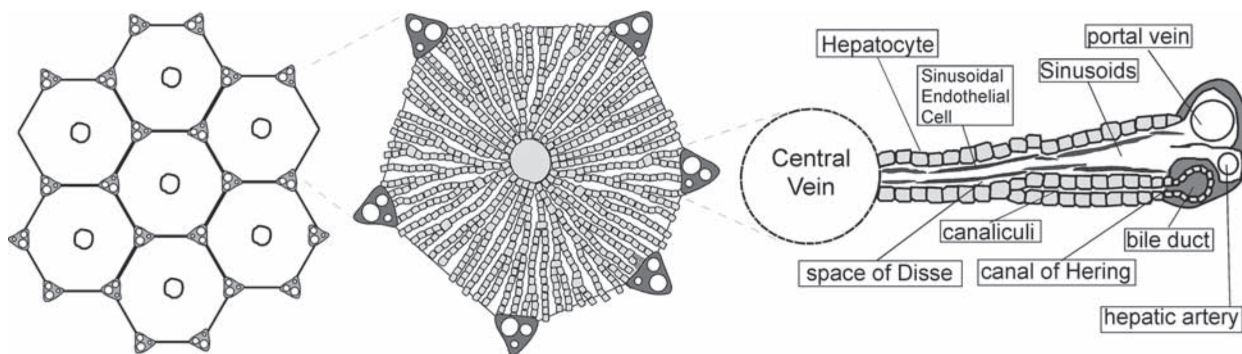

Figure 1. Schematic diagram of the anatomy of the adult mammalian liver. Left: The lobule structure of the adult liver show the repeating hexagonal lobules that are found in the adult mammalian liver. Middle: Each lobule is centered around the central vein. Each corner of the hexagon contains a portal triad which is composed of a portal vein, hepatic artery, and bile duct. Plates of hepatocytes extend outward from the central vein. Right: The portocentral axis of the liver lobule. Blood enters the liver through the portal vein and hepatic artery (periportal region) and flows along sinusoids towards the central vein (pericentral region). Transfer of materials between the blood and hepatocytes occurs in the space of Disse. Canaliculi transport bile from hepatocytes to the bile duct.

is determined by the position of hepatocytes within the liver lobule, a phenomenon called positional (or zonal) heterogeneity or metabolic zonation [2–4]. Some liver enzymes are synthesized in periportal regions (regions around the portal triad), whereas other enzymes are produced in pericentral regions (surrounding the central veins). Some enzymes show gradual zonation, whereas others exhibit highly restricted zonal activity. This zonation allows opposing metabolic pathways to be carried out within distinct, non-overlapping regions of the liver. This zonal heterogeneity is established during the perinatal period, a time when other dramatic changes also occur in the liver, including the transcriptional silencing of genes such as α-fetoprotein (AFP), and the activation of other genes such as phosphoenolpyruvate carboxykinase (PEPCK). While the molecular basis for zonal gene expression is poorly understood, several recent advances have helped elucidate this mode of position-dependent gene regulation (described below in greater detail).

The highly organized liver architecture facilitates liver function (Fig. 1). The vasculature of the liver is quite remarkable. Most of the blood enters the liver through the portal vein and is rich in nutrients and possible toxins from the gastrointestinal tract; oxygen-rich blood also enters the liver from the hepatic artery. Blood travels along plates of hepatocytes through small capillaries termed sinusoids, and then exits through central veins that converge into hepatic veins, which ultimately lead to the vena cava. The sinusoids are lined by epithelial cells that form the barrier between the blood and hepatocytes; the narrow region between these two cell types is termed the space of Disse. In the liver, hepatocytes are arranged in plates of cells that are one to two cells thick. The sinusoidal side of these hepatocytes interfaces the space of Disse, whereas the opposite (apical) side forms the canalicular membrane. The bile canaliculi, small channels that are between adjacent hepatocytes, transport bile to the intrahepatic bile duct in a direction that is opposite to the sinusoidal blood flow. The transition region between the canaliculi and intrahepatic bile ducts is called the canal of Hering, narrow channels that are lined by hepatocytes and bile duct epithelial cells (BECs, also called cholangiocytes). These intrahepatic bile ducts converge into larger extrahepatic ducts, which ultimately join the common bile duct that transports bile either to the gall bladder (for storage, in species that have a gall bladder) or directly to the small intestine. There is considerable interest in the canals of Hering, since these regions contain a small population of cells called Oval cells [5]. Oval cells serve as resident stem cells in the adult liver and can serve as progenitors for both hepatocytes and BECs [6]. Oval cells are thought to play an important role in liver regeneration, a remarkable property of the liver to regain its normal mass in response to parenchymal cell loss [7].

The adult liver is comprised of numerous cell types. Hepatocytes carry out most of the functions associated with the liver and are the predominant cell type in this organ. These polygonal, metabolically active parenchymal cells comprise 60% of the cells and 80% of the cellular mass within the liver [8]. Hepatocytes are polarized cells and, as such, share properties with other polarized epithelial cell types. The apical surface, or canalicular membrane, of hepatocytes is involved in the unidirectional transfer of components into the bile. Transporters required for these activities are found exclusively on this surface [9]. The intercellular domains are regions of hepatocyte-hepatocyte contact and contain junctions involved with intercellular communication. Of particular significance are the tight junctions that occur between canalicular and intercellular domains; these structures insure that components of the bile, which are destined for transport to the small intestine, do not leak into the bloodstream [10]. The si-

nusoidal membrane interfaces with the space of Disse, and is involved in the bidirectional exchange of materials between hepatocytes and the bloodstream. The sinusoidal membrane has numerous microvilli and contains a variety of receptors, channels, and other proteins involved with the vigorous and efficacious exchange of a wide variety of compounds [11].

While hepatocytes comprise the bulk of the liver, they are by no means the only cells in this organ. The adult liver is comprised of numerous cell types [11]. BECs line the bile ducts. BECs share a common lineage with hepatocytes, since both cell types arise from bipotential hepatoblasts. The endothelial cells surrounding the sinusoids are important for the exchange of materials between blood and the space of Disse. The highly fenestrated nature of the sinusoidal epithelial cells facilitates the active transfer of materials between the bloodstream and space of Disse and, ultimately, hepatocytes. Bone marrow-derived Kupffer cells are hepatic macrophages and also comprise part of the sinusoidal lining. These phagocytic cells can eliminate aged red blood cells and microbes, can present antigens and therefore influence immune function, and can produce a variety of cytokines and chemokines, some of which can have a dramatic effect on hepatocytes. Hepatic stellate cells (HSCs) are another peri-sinusoidal cell type. These cells are the major reservoir for vitamin A in the body. HSCs produce a variety of extracellular matrix (ECM) proteins and can also synthesize numerous cytokines and chemokines. HSCs have a major role in the hepatic response to injury, and activation of HSCs due to chronic liver damage leads to fibrosis [12]. Pit cells are resident natural killer cells in the liver and are important in immune function.

For the liver to carry out its myriad of functions, numerous genes must be activated as it develops [13, 14]. Some genes are activated as soon as the liver forms, whereas others are activated at later developmental stages. Once activated, expression of many of these genes changes in response to subsequent developmental signals and various extracellular stimuli. The perinatal period is particularly active for changes in liver gene expression, with some genes being activated, other genes being repressed, and zonal gene expression being established. These changes enable the liver to maintain metabolic homeostasis during the dramatic transition that occurs at birth. Here, we review transcriptional control of early liver development and changes in gene expression that occur in the perinatal liver, and highlight recent advances in these areas. We emphasize mouse model systems, although it is clear that other experimental systems, notably xenopus and zebrafish, are proving to be extremely valuable in the study of liver development and function [15, 16]. Elucidating the genetic basis of hereditary defects in the human hepatobiliary system also has provided important insight into liver development and function [17].

Transcriptional control of early liver development

As in other examples of organogenesis, liver development can be separated into several overlapping stages (Fig. 2). In the first stage, when specification is established, cells become ‘competent’ and are capable of taking a certain fate but do not show any overt change. Competent cells subsequently become ‘committed’ to a particular lineage and exhibit morphological changes and express genes associated with commitment. Cells then ‘differentiate’ along that lineage and are ultimately capable of carrying out the functions of a terminally differentiated cell. While liver development has been studied in a variety of genetically tractable and non-tractable organisms, much of our recent understanding of competency, commitment, and differentiation in liver development has come from mouse studies. This is due to the relative ease in which

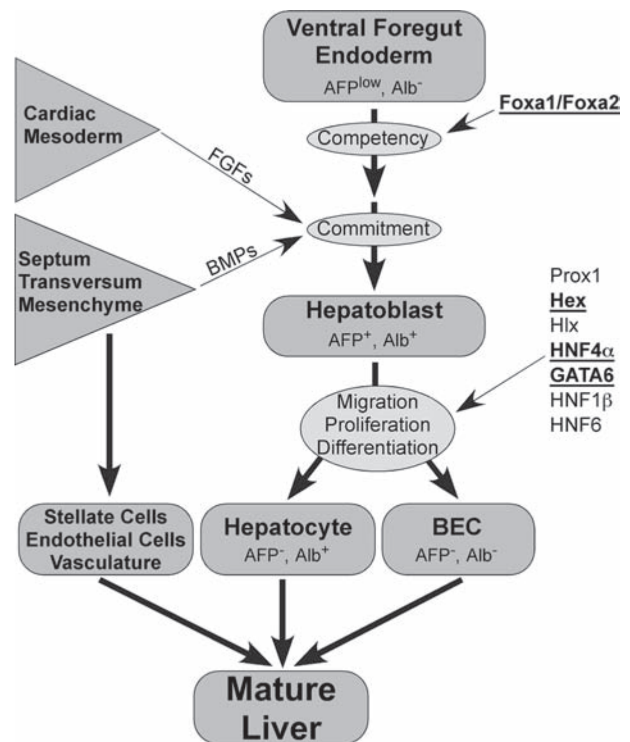

Figure 2. Schematic model of early liver development. The liver arises from the foregut endoderm, which expresses AFP at low levels and does not express albumin (Alb). Specification requires the sequential competency and then commitment of the foregut endoderm to become bipotential hepatoblasts, which express AFP and Alb. Hepatoblasts proliferate and migrate into the septum transversum, and differentiate into hepatocytes or bile duct epithelial cells (BECs). Hepatocytes, BECs, septum transversum-derived cells and other cell types (Kupffer cell and pit cells) form the mature liver. FGFs and BMPs, synthesized by cardiac mesoderm and septum transversum mesenchyme, respectively, facilitate the commitment of competent foregut endoderm cells to become hepatoblasts. Transcription factors listed on the right are involved in early development events as shown; those shown in bold are emphasized in the text.

mice can be maintained, our ability to genetically modify mice by transgenic and gene knockout approaches, and the completion of the mouse genome sequencing project. *In vitro* co-culture systems have also proved invaluable for elucidating the factors and cellular interactions required for liver formation [18]. In mice, the cells in the foregut endoderm form a columnar epithelium at roughly 8.5 days of gestation (7–8 somite pairs); this occurs shortly after the ventral foregut is positioned adjacent to the developing cardiac mesoderm [19]. Early liver genes, such as albumin and AFP, are activated at this time and indicate commitment to the hepatic fate. Around day 9 (10–12 somite pairs), these epithelial cells proliferate and migrate into the septum transversum, and a liver bud can be seen as an outgrowth of hepatoblasts [18]. The hepatoblasts in the liver bud are bipotential, giving rise to both hepatocytes and bile duct epithelial cells. The septum transversum mesenchyme contributes endothelial cells and stellate cells that form and line the sinusoids [19, 20]. The hepatic vasculature develops as the liver bud grows and will ultimately help establish the cellular architecture that is important for normal liver function. By day 10.5, hematopoiesis occurs in the liver and ultimately comprises well over 50% of the liver mass by mid-gestation [21].

Classic chick embryo transplantation studies by LeDouarin [19] indicated that the cardiac mesoderm signals the cells in ventral foregut endoderm to differentiate into hepatoblasts. *In vitro* co-culture studies in mice have confirmed the importance of cardiac mesoderm signals for the induction of hepatic genes such as AFP and albumin [18]. Subsequent studies indicated that fibroblast growth factor 1 (FGF1) and FGF2, by themselves, could initiate hepatic development of ventral endoderm [22]. Interestingly, the concentration of FGFs influenced the fate of ventral endoderm; these endoderm cells expressed hepatic markers in the presence of low FGF2 levels and lung markers in the presence of high FGF2 levels [23]. This indicates that the local concentration of FGFs, which would depend on the distance between ventral endoderm and cardiac mesoderm, would determine the fate of these endoderm cells. Other FGFs are likely to also induce the hepatic cell fate of ventral endoderm, since liver development appears normal in *Fgf1*$^{-/-}$*, Fgf2*$^{-/-}$ mice [24]. In addition to FGFs, bone morphogenic proteins (BMPs, members of the TGF-β superfamily) also contribute to the induction of hepatogenesis. BMPs are produced at high levels in the septum transversum, suggesting that these mesenchymal cells are also required for the commitment of foregut endoderm to a hepatic fate [25].

A number of transcription factors have been identified that are involved in liver development (Table 1), many of which were originally identified as regulators of liver-specific genes in the adult liver [26]. It turns out that many of these factors have roles in the development of multiple cell types and organs, which has made it difficult to determine their roles specifically during liver formation. Furthermore, regulatory networks exist in which liver-enriched transcription factors regulate each other, again complicating efforts to unravel mechanisms of liver gene regulation [27]. However, due in large part to studies in gene knockout mice, a picture is emerging of the transcriptional circuits that initiate hepatogenesis. Many of the transcription factors found to affect early

Table 1. Transcription factors involved in early liver development and liver phenotype in knockout mice.

Gene Knockout	Description/phenotype
Foxa1$^{-/-}$, *Foxa2*$^{loxP/loxP}$, *Foxa3-Cre*	Complete loss of Foxa1, loss of Foxa2 in foregut endoderm due to Cre recombinase expression: Liver bud fails to form *in vivo*, liver genes fail to activate when endoderm is co-cultured with FGF2. First identification of factors required for competency for hepatogenesis [70].
Prox1$^{-/-}$	Complete loss of Prox1: Hepatoblasts form as judged by Alb$^+$ cells, but these cells fail to migrate into the septum transversum and proliferate [29].
Hex$^{-/-}$	Complete loss of Hex: Alb$^+$ hepatoblasts are present and presumptive liver bud forms. Foregut endoderm changes to columnar epithelium but fail to change further to pseudostratified epithelium, resulting in hepatoblasts that are unable to migrate into septum transversum and differentiate further [30–32].
Hlx$^{-/-}$	Complete loss of Hlx: Liver bud forms but hepatoblast proliferation is severely diminished. Hlx is expressed primarily in mesenchymal cells, indicating a role for these mesodermal cells in liver development [34].
Hnf4$\alpha^{loxP/loxP}$*, Alfp-Cre*	Loss of HNF4α in hepatoblasts due to Cre recombinase expression: Liver develops, but loss of expression of numerous liver genes. Liver architecture is severely affected; expression of genes involved in polarized epithelial morphology and cell-cell interactions is diminished [52, 53].
Gata6$^{-/-}$	Aggregation of GATA6$^{-/-}$ ES cells with wild-type tetraploid embryos: The liver forms and Alb$^+$ hepatoblasts are present, but these cells fail to expand and differentiate further [58].
Hnf6$^{-/-}$	Complete loss of HNF6: Liver forms but differentiation of BECs occurs prematurely; impaired formation of bile ducts and lack of gall bladder [45].
Hnf1$\beta^{loxP/loxP}$*, Alfp-Cre*	Loss of HNF1β in hepatoblasts due to Cre recombinase expression: Liver forms but differentiation of bile ducts and gall bladder is impaired [46].

liver development function after specification occurs, *i.e.* after liver genes such as albumin and AFP are activated. *Prox1* is the vertebrate orthologue of the *Drosophila prospero* gene [28]. In mice that are deficient in *Prox1*, hepatoblasts formed, as judged by the presence of albumin-positive cells [29]. However, these cells failed to migrate into the septum transversum. Thus, hepatoblasts developed but fail to expand. Interestingly, livers did form in these mice, but were devoid of hepatocytes [29]. In mice lacking the *Hex* homeodomain gene, a presumptive liver bud was formed and liver genes such as albumin were expressed, but hepatoblasts failed to proliferate so that expansion of the liver bud did not occur [30, 31]. Recently, Bolt et al. [32] showed that foregut endoderm cells morphologically changed to columnar epithelial cells in $Hex^{-/-}$ embryos, but that these cells failed to change further into pseudostratified epithelial cells. Because of this block, the hepatoblasts were unable to migrate into the septum transversum and differentiate further [32]. The *Hlx* gene encodes a divergent homeodomain protein and is expressed primarily in the visceral mesenchyme of the developing gut [33]. The liver bud formed but hepatoblast proliferation was severely diminished in *Hlx*-deficient mouse embryos, providing genetic evidence for the importance of mesodermal tissue in liver development [34].

Once the liver bud forms, rapid proliferation during the mid-gestational period is needed for expansion of the liver. This period of rapid expansion is severely diminished in mice lacking Xbp1 [35], c-Jun [36] and the NF-κB p65 subunit [37] (similar liver phenotypes are seen in mice lacking IKK-β and IKK-γ, which are involved in NF-κB signaling pathways [38, 39]). In these cases, proliferation is highly diminished and apoptosis is increased.

Fetal liver development appears normal but liver function is severely affected in mice that lack other liver enriched transcription factors [26]. For example, C/EBPα-deficient mice are born but die from severe hypoglycemia soon after birth; glycogen storage, gluconeogenesis and lipid storage were greatly diminished in these mice [40]. Expression of a number of liver-enriched genes, including phenylalanine hydroxylase, is dramatically reduced in hepatocytes lacking HNF1α [41]. COUP-TF I and COUP-TF II can both act in a positive or negative manner to regulate many liver-enriched genes [42–44]. Although there is no obvious liver phenotype in mice deficient for either of these proteins [42–44], their ability to regulate genes in the liver and to interact with liver-enriched factors demonstrate an important role in hepatocyte gene regulation.

While much attention has focused on hepatocyte development, recent advances have furthered our understanding of the development of the biliary system. Deletion of HNF6 and targeted disruption of HNF1β in the fetal liver both resulted in abnormal biliary development; BECs differentiated earlier than normal, the biliary tree was disorganized, and extrahepatic bile duct and gall bladder development was diminished in both these animal models [45, 46]. Alagille syndrome, a disorder in humans that results in impaired bile secretion due to diminished bile ducts, is frequently due to mutations in the *Jagged1* gene [47]. Since Jagged1 is a ligand of the Notch transmembrane receptor, this indicates a role for the Notch signaling pathway in biliary formation. This phenotype is consistent with mutagenesis studies in zebrafish that have also implicated notch signaling in bile duct formation [48].

HNF4α was originally identified as a transcriptional regulator in the adult liver and is a member of the nuclear hormone receptor family [49]. Deletion of HNF4α resulted in embryonic death prior to liver development due to failure of extra-embryonic tissue development [50]. To overcome this, Duncan and colleagues [51] used tetraploid aggregation, in which HNF4$\alpha^{-/-}$ ES cells were aggregated with wild-type tetraploid embryos. The tetraploid cells give rise only to extraembryonic tissues, providing the means to analyze the role of HNF4α in embryonic development. Liver specification, but not full differentiation, occurred in these chimeric mice; livers from HNF4$\alpha^{-/-}$ embryos appeared normal at day e12, but failed to express numerous liver genes. In a different experiment, conditional deletion of HNF4α in the fetal liver demonstrated a role for HNF4α in both the transition of hepatoblasts to parenchymal cells and in the coordinated expression of cell adhesion molecules required for polarized epithelial morphology [52]. A more thorough analysis, include microarray comparisons, reveals the importance of HNF4α in the expression of numerous genes involved in cell-cell interactions, including those controlling cell adhesion and tight junctions [53]. These studies emphasize the role of HNF4α not only in the direct regulation of hepatic genes, but also in the formation of parenchymal morphology.

The GATA factors comprise a family of transcriptional regulators that, in vertebrates, contain two zinc-finger motifs [54]. Of the six vertebrate GATA factors, GATA4 and GATA6 are of particular interest in regards to liver gene regulation. GATA 4 has been shown to bind the albumin enhancer prior to the induction of albumin expression in hepatoblasts, demonstrating a possible role for this factor in potentiating the albumin gene for subsequent activation in the liver bud, and suggesting that GATA4 has a role in liver specification [55]. GATA6 regulates HNF4α, suggesting a role for this factor in liver development [56]. Embryos deficient in GATA6 fail to develop past gastrulation, similarly to HNF4$\alpha^{-/-}$ embryos [56, 57]. To overcome this problem, Zhao, Duncan, and colleagues [58] again employed tetraploid aggregates using wild-type tetraploid embryos and GATA6-deficient ES cells. Liver specification occurred, but livers failed

to develop in these chimeric embryos, demonstrating a role for GATA6 in hepatocyte differentiation. While it appeared somewhat surprising that GATA4 could not compensate for the loss of GATA6 in the developing liver, careful analysis revealed that GATA4 is expressed transiently during hepatic specification [58]. Thus, GATA4 might compensate for the absence of GATA6 during specification, but not hepatocyte differentiation. The conditional double knockout of GATA4 and GATA6 in foregut endoderm might elucidate whether these factors have redundant roles in hepatocyte specification.

The Foxa family is comprised of three members, Foxa1, Foxa2 and Foxa3 (formerly HNF3α, HNF3β and HNF3γ). These three proteins were originally identified, purified and cloned by their *in vitro* binding to the transthyretin and α-1-antitrypsin promoters [59, 60]. These factors have C-terminal and N-terminal activation domains and a highly conserved internal 'winged-helix' DNA binding domain [61]. Foxa proteins comprise a subfamily of the Fox family, which contains roughly 40 members [62]. While the Foxa proteins were originally identified in liver, subsequent studies indicated that they are expressed in many tissues and early in development. Foxa1 and Foxa2 are expressed in the definitive embryonic endoderm, well before the liver forms, suggesting that these factors play critical roles in early developmental events. Indeed, Foxa2-deficient embryos die during gastrulation [63, 64]. Foxa1-deficient embryos develop to term and die several weeks after birth; functional redundancy could explain the ability of Foxa1$^{-/-}$ embryos to develop further than Foxa2$^{-/-}$ embryos [65, 66]. Evidence for a role for Foxa proteins in liver specification came from a series of elegant studies by Zaret and colleagues [55, 67, 68], in which binding of Foxa proteins to their cognate binding site in the albumin enhancer was examined by *in vivo* footprinting. They found that Foxa proteins bound the albumin enhancer prior to the onset of albumin expression. This led to the idea of genetic potentiation, in which the binding of Foxa made the albumin enhancer accessible to other factors and competent for subsequent activation of the albumin gene [69]. However, a direct role for Foxa factors in early liver development was hampered by the gastrulation defects in the absence of Foxa2 and the problem of functional redundancy. Recently, Lee and colleagues [70] provided evidence that Foxa factors are required for liver specification. They generated mice that were completely deficient in Foxa1, and lacked Foxa2 only in the foregut endoderm by using Cre-lox technology in which Cre was expressed from the *Foxa3* promoter. Liver buds failed to form, and no AFP expression was detected in the foregut endoderm, in mice that were *Foxa1*$^{-/-}$, *Foxa2*$^{LoxP/LoxP}$, *FoxA3-Cre* [70]. Furthermore, foregut endoderm from these embryos that was co-cultured with FGF2 and heparan sulfate failed to express albumin or transthyretin, two markers of early hepatoblasts [70]. The lack of expression of these early markers indicates that liver specification failed to occur in the absence of Foxa1 and Foxa2. Whereas mutations in other transcription factors affect liver development after specification, as described above, this is the first example of factors that are required for hepatic specification.

While much is known about the transcriptional changes involved in the specification and development of the early fetal liver, considerably less is known about the changes in gene regulation that occur during the perinatal period. However, numerous changes take place in the liver during this time. Hematopoiesis, which is a major function of the fetal liver, declines dramatically as hematopoietic stem cells migrate elsewhere. Hepatocytes, which are highly proliferative in the fetal period, become quiescent. As expected, many cell cycle regulated genes are silenced during this period. In addition, a number of liver-enriched genes are silenced at birth. In contrast, many enzymes, including those involved in metabolism and detoxification, must be induced. The lobular architecture is established during this period, along with zonal control of gene expression. In the subsequent sections, recent advances in our understanding of perinatal gene regulation, particularly transcriptional repression and zonation of gene expression, are reviewed.

Transcription repression at birth

Afr1 and postnatal liver gene repression

Control of AFP serves as an important model of liver-specific gene expression during liver development and disease. AFP is expressed abundantly in the fetal liver, at low levels in the fetal gut and kidney, and like a number of other liver-derived serum proteins, is also expressed at high levels in the visceral yolk sac [71–75]. AFP transcription is dramatically repressed perinatally, resulting in a 10^4-fold reduction in liver AFP mRNA levels within a few weeks after birth [76]. The AFP gene normally remains silent in the adult liver, but can be reactivated during liver regeneration and in hepatocellular carcinoma [77, 78].

The 15-exon mouse AFP gene encodes a 590-amino acid, highly glycosylated serum protein of roughly 67–69 kDa that serves as a transport protein for a number of molecules, and its high concentration contributes to the osmolarity of fetal serum [79]. AFP is a member of the albumin gene family, which also includes albumin, α-albumin (afamin) and vitamin D binding protein [80]. These four genes have evolved from a common ancestor by a series of duplication events and are tightly linked in all mammalian species that have been studied [81, 82].

The regulatory regions that control rodent AFP transcription have been well-characterized in cultured cells and transgenic mice [83–89]. The AFP promoter comprises

roughly 200 bp upstream of exon 1. This region contains binding sites for numerous factors, including HNF1, NF1, C/EBP, Nkx2.8, and FTF [87, 90–97]. Further upstream, a region centered around –850 binds to Foxa and p53 [98, 99]. Further upstream are three distinct enhancer elements, E1, E2, and E3, that are each roughly 300 bp in length [83, 98–101]. E1 and E2 have similar sequences, suggesting that they arose by duplication of a common ancestral enhancer [83]. E1 is present in rodents but is absent in other species that have been analyzed [101]. Of these three enhancers, E3 is the best characterized. It binds several factors, including C/EBP, Foxa, ROR and COUP-TF [101–104].

AFP represents the best-studied example of perinatal gene silencing. Early insight into possible mechanisms of AFP repression came from a study in which adult serum AFP levels were measured in different strains of inbred mice. This survey found that AFP levels were up to 20-fold higher in BALB/cJ mice than in other strains tested [105]. Low serum AFP levels were seen in BALB/cJ X DBA/2 F1 mice, indicating that the continued AFP expression in BALB/cJ was a recessive trait. The gene that controlled AFP repression was called *raf*, for regulator of AFP, and was later renamed *Afr1* (alpha-fetoprotein regulator 1). Having cloned the AFP gene, Tilghman and colleagues subsequently showed that adult BALB/cJ mice also had higher steady-state liver AFP mRNA levels [106]. A molecular genetic screen by Pachnis et al. [107] identified the H19 gene as an additional target of Afr1-mediated regulation. Like AFP, H19 was repressed at birth in the liver in most strains but continued to be expressed in the adult liver of BALB/cJ mice. H19 has turned out to be an unusual gene; it encodes a 2.4-kb polyadenylated transcript that does not appear to encode a protein, suggesting that the mRNA is the functional product of the H19 gene [108, 109]. In addition, H19 has been extensively studied as a model of genomic imprinting, the phenomenon of parent-of-origin allele-specific expression [110].

Levels of both AFP and H19 are the same in the fetal liver of BALB/cJ and other strains of mice. Both genes are also developmentally repressed in tissues other than the liver and do not show strain-specific differences in adult non-liver tissues. Thus, the Afr1 phenotype in BALB/cJ mice is restricted to the adult liver. Linkage studies mapped the *Afr1* gene to mouse chromosome 15, different than AFP (Chr 5) and H19 (Chr 7) [111]. While transgenic studies indicated that Afr1 acted through the AFP promoter and, therefore, controlled gene expression at the level of transcription [112, 113], nuclear run-on analysis suggested that Afr1 functioned at the post-transcriptional level [114]. Taken together, these studies are consistent with a model in which Afr1 is a liver-specific repressor that acts in a manner that couples transcriptional and post-transcriptional mechanisms. In this model, it is predicted that the mutation of this repressor in BALB/cJ mice leads to incomplete silencing of target genes in the postnatal liver.

The apparent complexity of Afr1 regulation, and the uncertain nature of the Afr1 mutation in BALB/cJ mice, suggested that it might be difficult to identify Afr1 by standard biochemical or molecular genetic strategies. Therefore, Perincheri et al. [115] chose positional cloning as an unbiased approach to identify Afr1. This strategy utilized high-resolution mapping of Afr1 to a narrow chromosomal interval, followed by the analysis of candidate genes within this region. This strategy identified Afr1 as the Zinc-fingers and homeoboxes 2 (Zhx2) gene. The insertion of an endogenous retroviral element in intron 1 accounts for the mutation in the BALB/cJ Zhx2 allele. Transcription initiation of the BALB/cJ Zhx2 allele occurs normally, but a majority of the primary transcripts are spliced from exon 1 to the retroviral element with only a small percentage of the primary transcript being properly spliced to generate a wild-type transcript. Thus, BALB/cJ mice contain a hypomorphic allele that dramatically reduces, but does not eliminate, wild-type Zhx2 transcripts. Liver-specific overexpression of a Zhx2 transgene was able to restore wild-type H19 repression in the livers of adult BALB/cJ mice, confirming that this gene is responsible for the Afr1 phenotype [115].

Zhx2 is a member of a small gene family that includes Zhx1, which is tightly linked to Zhx2 on mouse chromosome 15, and Zhx3, which is found on chromosome 2. In humans, Zhx1 and Zhx2 are on chromosome 8, whereas Zhx3 is on chromosome 20. Zhx1 was originally identified independently in two labs by a yeast two-hybrid screen for proteins that bound NF-YA and immunoscreening of a bone marrow stromal cell cDNA library [116, 117]. A yeast two-hybrid screen for partners of Zhx1 identified both Zhx2 and Zhx3 [118, 119]. All three Zhx proteins can form homodimers and heterodimers with each other and with NF-YA. Zhx2 functions as a transcriptional repressor in *in vitro* assays [118], consistent with the idea that Zhx2 is involved in the postnatal repression of AFP and H19. These three genes have unusual structures, in that the entire protein coding regions are found on an unusually large internal exon (Zhx3 has a short coding region in the 3′ exon; Fig. 3). The proteins coded by these three genes all are predicted to contain two zinc fingers and four homeodomains (a fifth homeodomain may exist in Zhx1; Fig. 3). These three genes are found in all vertebrates analyzed, including humans, dogs, rodents, and zebrafish, but appear to be restricted to the vertebrate lineage as there are no apparent homologues in non-vertebrates such as *Drosophila*, *Caenorhabditis elegans*, and yeast.

The liver-specific Afr1 phenotype in BALB/cJ mice originally led to the suggestion that Zhx2 expression would be limited to this organ. However, Zhx2 is ubiquitously expressed; in fact, Zhx2 mRNA levels are

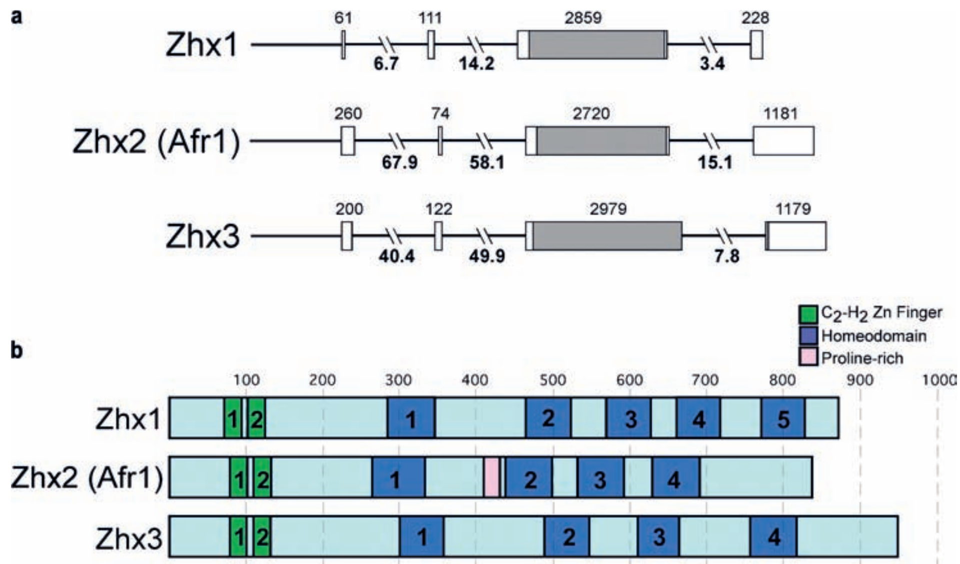

Figure 3. The mouse Zhx family. (*a*) The three mouse Zhx genes show a similar structure. Each gene contains two small 5′ non-coding exons (boxes, numbers above boxes indicate size of exons in bp), followed by an unusually large internal exon 3. The entire coding region of Zhx1 and Zhx2 is contained in this exon; 8 bp of coding sequence is found in the 3′ exon of Zhx3. Numbers below the lines indicate the size of the introns (in kb). (*b*) The three Zhx3 proteins contain conserved C2-H2 zinc fingers in their amino end (green boxes) and four conserved homeodomains (dark blue boxes). Zhx1 is predicted to contain a fifth homeodomain. Zhx2 contains a predicted proline-rich region (pink box) adjacent to homeodomain 2. Numbers above the dashed lines represent amino acids.

lower in the liver than in most other organs [115]. Thus, other mechanisms must account for the liver-specific Afr1 phenotype. It may be that Zhx2 interacts with other liver-specific factors to repress AFP and H19 at birth, or that other Zhx proteins can compensate for the reduction of Zhx2 in organs other than the liver. A third model is that Zhx2 levels in BALB/cJ livers are below a threshold that is needed for repression of target genes (Fig. 4). In organs where Zhx2 levels are high, the reduction in Zhx2 in BALB/cJ mice results in levels that are still sufficiently high for regulation of target genes. In the liver, where wild-type Zhx2 levels are already low, the further reduction in BALB/cJ results in levels that are below a threshold needed for control of target genes. Further analysis will be needed to distinguish between these models.

AFP and H19 are the only known targets of Zhx2. Since these two genes are developmentally repressed in the liver, it seems reasonable that other similarly regulated genes might be targets of Zhx2. One gene that meets this criteria is glypican-3 (Gpc3). Gpc3 encodes a GPI-anchored cell surface heparin sulfate proteoglycan involved in cell growth, and is expressed abundantly in fetal tissues and repressed at birth [120]. Interestingly, Gpc3 is often reactivated in hepatocellular carcinomas, similarly to AFP and H19. RT-PCR analysis revealed that Gpc3 levels are higher in adult BALB/cJ livers compared with other strains, indicating that Gpc3 is a target of Zhx2 (C. Davis, L. Jin, M. L. Peterson and B. T. Spear, in preparation). It is interesting that the three known targets of Zhx2 (AFP, H19 and Gpc3) are all reactivated in cancers. It will be interesting to determine whether the reactivation of these three genes in tumors is due to decreased Zhx2 activity. Finally, the broad tissue distribution of Zhx2 implies that it would regulate target genes in tissues other than the liver. A knockout of the Zhx2 gene, so that protein levels are completely eliminated, could help in evaluating the role of this protein in controlling target genes in the liver and other organs.

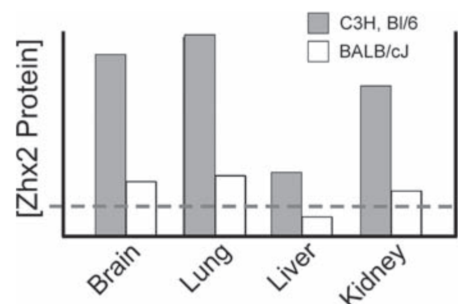

Figure 4. Threshold model to explain the liver-specific Afr1 phenotype that is seen in BALB/cJ mice. The dashed gray horizontal line represents the hypothetical level of Zhx2 (Afr1) protein that would be required for normal function, *i.e.* repress target genes such as AFP and H19. In wild-type mice (Bl/6, C3H), Zhx2 levels are above this threshold in all tissues. In BALB/cJ mice, the hypomorphic mutation reduces Zhx2 levels in all tissues. However, in most tissues (brain, lung, and kidney) levels are still above the threshold required to repress target genes. In BALB/cJ liver, Zhx2 levels are below the threshold and therefore cannot repress AFP and H19.

Chromatin changes and perinatal repression

While BALB/cJ mice provide genetic evidence that Zhx2 is involved in perinatal transcriptional silencing in the liver, other mechanisms are likely involved in repression at birth. A site centered at –850 of the AFP promoter can bind FoxA and p53. While binding of FoxA activates AFP through this site [99], p53 binding can inhibit this FoxA-mediated activation by competing for FoxA binding [98]. This repression was amplified by p300. Confirmation of these *in vitro* studies was demonstrated by delayed AFP repression in p53-deficient mice [121]. Both p53 and p73 can repress AFP expression by altering chromatin in the AFP locus [122]. While these studies suggest that FoxA proteins can positively regulate AFP through the site at –850, other studies suggest that FoxA proteins can inhibit AFP through a site at –165 of the AFP promoter. Since FoxA does not bind this site, its effect might require interactions with other DNA binding proteins [93]. Both ING1b and ING2, members of the inhibitors of growth (ING) family, can repress AFP by mechanisms that might be due to interactions with p53 and/or HNF1 and likely involve chromatin remodeling [123]. Chromatin changes also appear to be involved in perinatal H19 repression. Long et al. [124] used chromatin immunoprecipitation to demonstrate developmental changes in FoxA binding to the H19 enhancer region. Similar analysis of transthyretin, which is regulated by FoxA but is expressed in both the fetal and adult liver, found that FoxA binding to the transthyretin promoter remained unchanged at these two times [124]. Thus, changes in FoxA binding to the H19 enhancers were not simply due to differences in FoxA levels in the fetal and adult liver, but due to changes in the accessibility of FoxA factors to the H19 enhancer.

Hereditary persistence of AFP expression

Human studies have also provided insight into AFP repression. Prenatal screening for maternal AFP levels, a common assay during pregnancy as a diagnostic test for defects in the developing fetus, have identified several cases of hereditary persistence of AFP (HPAFP) in which AFP synthesis continues in the adult liver. In a Scottish kindred, a polymorphism (–119 G $\rightarrow$ A) in the HNF1/NF1 binding site at –120 was associated with HPAFP [125]. This polymorphism resulted in increased HNF1 binding. Two kindreds with HPAFP were characterized in a second study [126]. In the Bengali case, the same –119G $\rightarrow$ A polymorphism was detected. In the second case, an Italian kindred, polymorphisms at –55 (C $\rightarrow$ A) increased affinity for HNF1 [126]. A study of HPAFP in two independent Japanese kindreds also found the same –119G $\rightarrow$ A, with a concomitant increase in HNF1 binding [127]. Taken together, these studies demonstrate a role for HNF1 in AFP promoter activity in the adult liver. In a Taiwanese case of HPAFP, no polymorphisms in the AFP promoter were found [128]. Here, HPAFP could be due to mutations in other AFP regulatory regions or in the trans-acting factors involved in AFP regulation. It should be noted, however, that AFP levels in these cases of HPAFP are still considerably lower than AFP in the fetal liver.

Zonal gene regulation

Establishment of the lobular liver architecture during the perinatal period is accompanied by zonal gene regulation. As mentioned previously, this compartmentalization of function is determined by the position of hepatocytes within the lobule [2, 3, 129, 130]. Some liver enzymes are expressed in periportal regions whereas other enzymes are expressed in pericentral regions. Zonal regulation can either be of the ‘gradient’ type, with expression showing a gradual change in levels of expression across the portocentral axis, or the ‘compartment’ type, in which a highly defined boundary of expression is observed [131]. Carbamoyl phosphate synthetase I (CPS) and glutamine synthetase (GS) are examples of compartment zonation in periportal and pericentral regions, respectively. Certain examples of zonal expression are ‘dynamic’, whereby zonal expression patterns can change based on hormonal or metabolic changes in the liver, or are ‘stable’, in which adaptive changes do not occur [131].

Several models have been proposed to account for zonal regulation in the adult liver. The differentiation model proposes that expression of genes is dependent on the developmental status of the hepatocyte [132]. This model suggests that the developmental status of cells along the portocentral axis dictates the gene expression, and is based on evidence that hepatic stem cells exist in portal triads and that hepatocytes adjacent to the portal triads are the least differentiated, whereas hepatocytes around the central veins are terminally differentiated. A variant of the differentiation model is the streaming model [132, 133]. Here, the hepatic stem cells become more differentiated as they migrate from the portal region towards the central veins. While there is evidence that hepatic stem cells exist in the portal regions, strong experimental data argues that neither the developmental nor streaming models can account for zonal gene regulation [134, 135]. It has also been suggested that cell-cell or cell-matrix interactions could account for zonal patterns of expression [136, 137]. For example, interactions between central vein endothelial cells and pericentral hepatocytes could account for GS expression, which is largely limited to a single layer of cells around the central veins. While this model might explain expres-

sion of GS, it is hard to envision how this mechanism could account for gradient examples of zonal gene expression. Portocentral gradients in cell-matrix proteins could account for zonal gene regulation, but there is no evidence for such gradients in matrix proteins. It has also been proposed that the directional flow of blood along the sinusoids in a portocentral direction could govern zonal gene expression [3]. Blood enters the portal triad rich in oxygen, nutrients and hormones. The concentration of these substances changes as blood flows towards the central veins. Although blood-borne molecules that could account for zonal regulation have not yet been firmly identified, this remains the most widely accepted model to account for position-dependent gene expression. As pointed out by Lamers and colleagues [131], gradual changes in regulatory molecules could account for both gradient (gradual) and compartment (well-defined) patterns of expression, depending on how the extracellular stimuli are integrated into the transcriptional machinery that lead to zonal control. Certainly, highly restricted patterns of gene expression are seen in *Drosophila* embryos, even though the levels of extracellular morphogens and transcriptional regulators across the anterior-posterior embryo axis are much more gradual than the target genes that they control [138].

Zonal gene expression is regulated primarily, if not solely, at the level of transcription. Thus, regardless of the extracellular signals that control zonal expression patterns, these differences must ultimately impact the expression or activity of transcriptional regulators. The patterns of numerous liver-enriched transcription factors have been analyzed in the adult liver; most of these do not exhibit any differences in levels across the lobule [131, 139–141]. However, it should be noted that this analysis is not complete, since all factors (liver-enriched and otherwise), as well as co-regulators, have not been analyzed. Certain factors must be post-translationally modified to be fully active, and zonal control of such modifications has not been investigated.

CPS is expressed in the periportal regions of the adult mouse liver and is a well-studied example of periportal expression [142]. CPS transcription is first detected in a subset of hepatocytes at mid-gestation. At birth, all hepatocytes express CPS, and within the first few weeks after birth CPS expression becomes restricted to periportal regions [143]. The CPS gene contains a glucocorticoid response unit (GRU; a combination of GREs and binding sites for other factors), which can confer periportal regulation on a linked transgene [144]. PEPCK, a key enzyme in gluconeogenesis in the liver, and tyrosine aminotransferase (TAT), are also expressed in periportal regions of the adult liver [145]. In contrast to CPS, which is expressed in the fetal liver, PEPCK and TAT are activated at birth. However, these three genes contain GRUs, suggesting that this complex regulatory element is involved in periportal regulation [146–148].

Negative control of GS in periportal hepatocytes

GS is perhaps the most extensively studied gene in regards to zonal control. This enzyme converts glutamate and ammonia into glutamine, and is thus an important enzyme in ammonia detoxification. GS is expressed in many tissues at varying levels. In the fetal liver, GS is expressed in all hepatocytes [149–151]. However, pericentral expression is established perinatally, with GS being highly restricted to a one- to two-cell layer surrounding the central veins of the adult liver [149–151]. This pericentral expression is stable in that it does not change in response to hormones or other stimuli. Two regulatory regions, one at –2.5 (relative to the transcription start site) and a second within the first intron, control GS expression in cultured liver cells. Several recent reports have provided mechanistic insight into how these elements control pericentral GS expression. In a study of GS expression in cultured cells and primary hepatocytes, it was found that GS activity was induced by glucocorticoids in rat hepatoma FAO cells, but that there was no GS induction in primary rat hepatocytes [152]. TAT activity was induced by glucocorticoids in both cell populations [152]. This indicated that the glucocorticoid receptor-dependent pathway was intact in both cell types, but that glucocorticoid activation of GS was being inhibited in primary hepatocytes. Further analysis revealed that an intron 1 fragment contained both a positive element (a GRE that could act as a glucocorticoid-dependent enhancer) as well as distinct 54-bp negative element; this negative element could block glucocorticoid-mediated activation in primary hepatocytes. Analysis by transient transfections indicated that this intronic silencer was more active in periportal-enriched cells than in pericentral-enriched cells [153]. Based on these results, the authors suggest that the lack of GS expression in most hepatocytes (those not encircling the central vein) was due to the repressive action of the intronic silencer element. This paper also indicates that the –2.5-kb enhancer was not involved in zonal control since it was equally active in periportal- and pericentral-enriched hepatocytes. In contrast to this conclusion, Lie-Venema et al. [154] showed that transgenes in which 3 kb of DNA flanking the 5′end of the GS gene could confer pericentral regulation of a linked CAT reporter gene, which would indicate that the region upstream of the GS transcription start site could also confer pericentral regulation. Consistent with this, data from our lab indicate that a 400-bp fragment containing the –2.5-kb rat GS enhancer can confer pericentral transgene expression (B. Spear and L. Long, manuscript in preparation). More experiments will be needed to determine

whether the –2.5-kb enhancer by itself indeed exhibits pericentral activity.

Negative regulation and zonal activity of AFP enhancers

Data from our lab supports the notion that negative regulation might be involved in zonal control. AFP repression in the perinatal liver follows a portal-central gradient, *i.e.* silencing is first seen in portal regions such that the pericentral hepatocytes are the last cells to express AFP until repression is complete [155, 156]. In contrast to the endogenous AFP gene and AFP promoter-containing transgenes, which are repressed at birth, transgenes in which the AFP enhancer region was linked to the heterologous albumin promoter continue to be active in the adult liver [112]. In addition, the three enhancers continue to exhibit DNase hypersensitivity in the adult liver [84]. Taken together, these data suggest that the AFP enhancers continue to be active in the adult liver. In support of this possibility, each enhancer was active in both fetal and adult liver when linked individually to the heterologous *β*-globin promoter [157]. Interestingly, each enhancer-containing transgene exhibit pericentral activity. E1 and E2 showed a gradual decline in activity away from the central vein, whereas E3 was active primarily in a single layer of hepatocytes surrounding the central vein in a manner reminiscent to GS expression (Fig. 5) [157]. The highly restricted E3 activity raised the following question: Is the lack of E3-driven transgene expression in non-pericentral hepatocytes due to the absence of positive factors or due to the presence of negative regulators? To test this, a transgene in which E3 and E2 were both fused to *β*gl-D^d (called E3-E2-*β*gl-D^d) was generated. If the lack of E3 activity in non-pericentral hepatocytes was due to the absence of positive regulators, this transgene would have a pattern of activity similar to E2-*β*gl-D^d (*i.e.* the E2 pattern would be dominant). If, on the other hand, the absence of E3 activity in non-pericentral hepatocytes was due to negative regulation, this transgene would exhibit a pattern of expression similar to E3-*β*gl-D^d (*i.e.* the E3 pattern would be dominant). The E3-E2-*β*gl-D^d transgene clearly showed an E3-like pattern of activity [158]. The 340-bp E3-containing fragment, when linked to E2-*β*gl-D^d, was also sufficient to confer this highly restricted activity [158]. These data argue that the action of E3 is dominant over that of E2 in non-pericentral hepatocytes of the adult liver, and is consistent with the idea that the absence of E3 activity in this population of adult hepatocytes is based on active repression. Since pericentral activity of E3 is established during the perinatal period when AFP mRNA levels are declining, this result also raised the interesting possibility that the negative action of E3 may contribute to postnatal AFP repression.

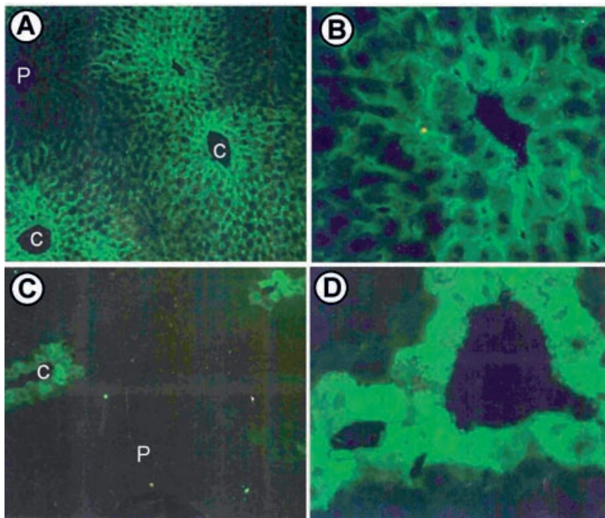

Figure 5. Zonal activity of mouse AFP enhancers in adult liver. Mouse AFP enhancer E2 or enhancer E3 were individually linked to the human *β*-globin promoter that had been fused to the H2-D^d structural gene. The E2-*β*gl-D^d and E3-*β*gl-D^d genes were used to generate transgene mice. Liver sections from 4-week-old adult mice were stained with FITC-labeled antibodies against the H-2D^d protein. (*a*, *b*) Transgene expression in E2-*β*gl-D^d mice (low and high magnification, respectively). E2 has highest activity in pericentral cells, with a gradual reduction in activity towards the portal triad. (*c*, *d*) Transgene expression in E3-*β*gl-D^d mice (low and high magnification, respectively). E3 activity is restricted to a single layer of hepatocytes surrounding the central veins (c: central vein; p: portal triad).

Wnt/*β*-catenin, adenomatous polyposis coli (apc), and pericentral GS expression

Increased GS activity is often seen in hepatocarcinogenesis [159, 160]. It is also known that activation of the Wnt/*β*-catenin signaling pathway is a common occurrence in hepatocellular carcinomas [161, 162]. In mice, GS overexpression is associated with *β*-catenin mutations; such mutations are frequently seen in tumors that arise in mice treated with diethylnitrosamine and phenobarbital [163]. Cadoret et al. [164] used a cDNA subtractive approach to identify genes that were activated in the livers of *β*-catenin-overexpressing transgenic mice. Several induced genes, *e.g.* GS, ornithine aminotransferase and the glutamine transporter GLT1, are all involved in glutamine metabolism and show highly restricted pericentral expression in normal adult liver. In an elegant experiment, adenoviral-mediated gene transfer was performed in mice in which a *lacZ* reporter gene was inserted into the GS locus and thus under the control of the GS control elements. When a constitutively active form of *β*-catenin was expressed off the adenoviral vector, *β*-gal staining was observed throughout the liver lobule; this was not due to adenoviral infection since *β*-gal staining was only observed in pericentral cells when a GFP control adenovirus was used to infect mice [164]. This provides direct evidence

that activated β-catenin can induce GS expression. In a global analysis of gene expression in mouse models of liver tumor formation, Hailfinger et al. [165] found that numerous pericentral genes were induced in tumors that had activating mutations in *β-catenin*, whereas periportal genes were often induced in tumors that had activating mutations in *Ha-ras*. Indeed, several genes that were not known to be zonally regulated, but were significantly upregulated in *Ha-ras*$^+$ or β-catenin$^+$ tumors, were found subsequently to be differentially expressed in periportal and pericentral hepatocytes, respectively. They proposed a model in which zonal control consists of two opposing signals, one delivered by endothelial cells of the central veins activating a β-catenin-dependent pathway, the other by blood-borne molecules activating a Ras-dependent signaling cascade [165]. Thus, gradients of opposing signaling molecules along the portocentral axis would determine expression of certain genes in periportal and pericentral regions of the liver lobule.

The studies described above indicate a role for Wnt/β-catenin signaling in GS activation in liver tumors, suggesting that this signaling pathway could account for pericentral GS expression in normal liver. Direct evidence for this comes from a recent study by Benhamouche and colleagues [166]. Immunohistochemical analysis revealed reciprocal staining of activated (unphosphorylated) β-catenin and the tumor suppressor adenomatous polyposis coli (Apc; an inhibitor of β-catenin activation) across the liver lobule; activated β-catenin was higher in pericentral areas and Apc was higher in periportal areas. Using a liver-specific, tamoxifen-inducible Apc knockout mouse model, the authors go on to show that loss of Apc results in increased GS expression in all hepatocytes across the liver lobule. Further analysis of these mice revealed that other pericentral genes show increased expression in periportal regions after Apc inactivation with a concomitant silencing of periportal genes. In a reciprocal experiment, use of an adenoviral Dickkopf-1 (Dkk1) vector to block β-catenin activation throughout the liver lobule led to the loss of GS expression in pericentral regions and increased pericentral expression of genes that are normally active only in periportal regions. This study demonstrates a role for Wnt/β-catenin and Apc in pericentral and periportal gene regulation in the adult liver.

Summary and future directions

The adult liver is a complex organ that carries out numerous activities. Studies over the past few years have provided new insight into the role of transcription factors in embryonic liver development. It is likely that continued use of genetically modified mice, including conditional knockouts and double knockouts, will further our understanding of liver development. It will be important to further investigate the role of non-endoderm tissue in hepatogenesis, and how cells other than hepatocytes are incorporated into the developing liver. Recent studies have also provided new insight into transcriptional changes that occur during the perinatal period. It will also be important to further characterize the role of chromatin in liver gene regulation in the fetal and postnatal liver. Identification of Zhx2 as a regulator of postnatal gene silencing raises the question of whether changes in the level or activity of Zhx2 are also involved in gene reactivation in the adult liver. The three known targets of Zhx2 – AFP, H19, and glypican-3 – are all activated in regenerating liver and liver cancer. A recent clinical study indicates that Zhx2 expression is often reduced in hepatocellular carcinomas, raising the possibility that this gene is involved in cancer [167]. Signaling pathways and cis-acting elements that govern zonal gene expression have been identified, providing the framework for future studies to further explore this interesting aspect of liver gene regulation. Similarities between pericentral GS activity and AFP enhancer E3 activity raises the possibility that zonal control and postnatal gene silencing are mechanistically linked and warrant further investigation.

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