The Fox Genes in the Liver: From Organogenesis to Functional Integration

JOHN LE LAY AND KLAUS H. KAESTNER

Department of Genetics and Institute for Diabetes, Obesity, and Metabolism, University of Pennsylvania
School of Medicine, Philadelphia, Pennsylvania

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Le Lay J, Kaestner KH. The Fox Genes in the Liver: From Organogenesis to Functional Integration. Physiol Rev 90: 1–22, 2010; doi:10.1152/physrev.00018.2009.—Formation and function of the liver are highly controlled, essential processes. Multiple signaling pathways and transcriptional regulatory networks cooperate in this complex system. The evolutionarily conserved FOX, for Forkhead bOX, class of transcriptional regulators is critical to many aspects of liver development and function. The FOX proteins are small, mostly monomeric DNA binding factors containing the so-called winged helix DNA binding motif that distinguishes them from other classes of transcription factors. We discuss the biochemical and genetic roles of Foxa, Foxl1, Foxm1, and Foxo, as these have been shown to regulate many processes throughout the life of the organ, controlling both formation and function of the liver.

I. INTRODUCTION

The liver is the body’s largest internal organ, comprising ~5% of body mass in mammals. As examples of its tremendous functional diversity, the liver secretes essential serum components and clotting factors; regulates glucose, protein, and lipid metabolism; and detoxifies xenobiotics, drugs, and other chemicals. Impaired liver function is associated with multiple disease states. In addition, the development of the vertebrate liver has served as a paradigm for understanding fundamental mechanisms of organogenesis. Initial analyses of liver-specific gene expression in fetal and adult liver and hepatoma cell lines led to the identification of a number of liver-enriched transcription factors containing various structural motifs. Among these are the divergent homeodomain proteins HNF1α and HNF1β; the winged helix proteins Foxa1, Foxa2, and Foxa3 (previously termed HNF-3α, -β, and -γ); the leucine zipper proteins C/EBPα and -β; the orphan nuclear receptor HNF4α; and the PAR protein DBP (reviewed in Refs. 32, 44, 207). While none of these factors is exclusively expressed in the liver, the combinatorial actions of tissue-specific and hormone-dependent transcription factors collaborate to achieve the stringency and dynamic regulation of gene expression required for the proper development and function of the organ.

Twenty years ago, the biochemical characterization of proteins that bind to the promoters of genes expressed in a liver-specific, or at least liver-enriched, fashion led to the discovery of the Foxa or hepatic nuclear factor 3 (HNF-3) transcription factors (42, 95–97). Shortly thereafter, Weigel and Jäckle and coworkers (194, 195) discovered that the central domain of the nuclear protein encoded by the Drosophila melanogaster gene fork head, which is essential for the proper formation of the foregut and hindgut in the fly, is closely related to the Foxa/HNF-3 proteins. In fact, this ~100-amino acid motif, termed Forkhead or winged helix domain, is conserved among all Fox genes and forms the basis of their classification (Fig. 1). The
mutant phenotype of the fork head fly, together with the observation that the Foxa genes are expressed very early during the formation of definite endoderm in the mouse, led to the hypothesis that the Foxa proteins function in mammalian liver development (5, 122, 151, 154), which will be discussed in detail in section II.

The metabolic role of the Fox proteins is the subject of section III. Both Foxa and Foxo proteins have been shown by biochemical and genetic means to play a major role in orchestrating the metabolic functions of the liver. Especially instructive here is the story leading to the discovery that Foxo proteins are major transcriptional mediators of insulin signaling. As it turns out, this discovery might not have been possible without the seminal work in genetic model systems, specifically the nematode Caenorhabditis elegans and the fruit fly Drosophila melanogaster. This section also highlights the remarkable evolutionary conservation of these signaling systems, despite the fact that the final outcome of pathway activation differs between worm, fly, and human.

In section IV, the recent discovery of an important function of another member of the Fox gene family, termed Foxl1, in the liver will be discussed. From genetic lineage tracing experiments, it appears that the Foxl1 gene is expressed specifically in facultative progenitor cells of the liver, the so-called “oval cells.” These cells have been difficult to identify in the normal, quiescent liver but are abundant after certain types of liver injury. Foxl1 appears to be a marker of at least a subset of these bipotential progenitor cells, as cells expressing Foxl1-Cre track to both hepatocytes and cholangiocytes following bile duct ligation.

Finally, section V details the role of Foxm1b in hepatic carcinogenesis. This fascinating protein is expressed in many proliferating cell types but is extinguished upon terminal differentiation. In the liver, Foxm1b is highly induced after partial hepatectomy, when hepatocytes in the remaining liver lobes reenter the cell cycle to restore liver mass. Foxm1b is required for hepatocyte proliferation in these conditions as
shown by loss of function, while gain of function for Foxm1b can restore the regenerative capacity of the aging liver. But, most excitingly, Foxm1b-deficient hepatocytes are resistant to hepatic carcinogenesis, suggesting inhibition of Foxm1b as a potential therapeutic approach for the treatment of liver cancer.

Multiple excellent reviews on the Forkhead Box gene family have appeared over the past decade, and the reader is referred to these for further detailed information on the role of Fox genes in cancer, pancreas development, or in evolution, for instance (1, 7, 8, 57, 81, 82, 91, 98, 134, 141, 189, 197). In addition, two “snapshots” have appeared that contain salient information on all mammalian Fox genes in tabular format (183). Members of this diverse gene family, with 42 members in mammals, have been shown to be expressed in all cell types and organ systems. Fox genes function in speech acquisition in humans and vocal learning in song birds; they control developmental processes from pharynx development in the worm to chordogenesis in zebrafish to iris development in humans, to just name a few examples. While this review is focused on the function of Fox genes in the liver, we would like to point out that this covers only a small part of the impressive range of biology that is influenced by members of this gene family.

II. ORGANOGENESIS OF THE LIVER

A. The Foxa Family in Early Embryogenesis

During mouse development, the first Foxa gene to be activated is Foxa2, whose mRNA and protein gene expression are first detectable at embryonic day 6.5 (E6.5) in the node and the anterior primitive streak (5, 122, 151, 154). The node of the mammalian embryo is critical to gastrulation, the process that first establishes each of the three fundamental germ layers (ectoderm, mesoderm, and endoderm) and is equivalent to the dorsal blastopore lip in Xenopus and Hensen’s node in chicken. By E7.5, Foxa2 expression is found throughout the definitive endoderm and persists into adulthood in endodermal derivatives such as the liver, pancreas, lung, thyroid, and prostate (41, 87, 97, 120, 122, 154, 155, 203). The importance of Foxa2 to early embryogenesis has been demonstrated by targeted gene ablation. Mouse embryos homozygous for a null mutation in Foxa2 die by E11 due to severe defects in structures related to all three germ layers, abnormalities of the neural tube and somites, absence of the notochord, and failure to form a gut tube (4, 196). A summary of this and several other Fox family mutant phenotypes relevant to this review is described in Table 1.

Foxa1 is also broadly expressed in the early embryo, in a very similar pattern to Foxa2, although Foxa1 mRNA is not detectable until E7.0 in the late primitive streak and is not found in the node (5, 122). With some notable exceptions, the expression domains of Foxa1 and Foxa2 largely overlap in adulthood, suggesting likely functional redundancy for these two factors (15, 137). Foxa3 expression does not initiate in the endoderm until E8.5 and is not present in the primitive streak or axial mesoderm (122). However, by E10.5, Foxa3 expression is found in the liver primordium and persists throughout development into the adult liver where it is the most highly expressed member of the Foxa family, at least at the mRNA level (84, 122). In contrast to Foxa2, neither Foxa1 nor Foxa3 is required during early mouse embryogenesis as both Foxa1−/− and Foxa3−/− embryos appear normal and develop to birth (12, 13, 85, 86, 166), likely owing to differences in their spatial and temporal expression domains as well as functional compensation by the remaining Foxa factors.

B. Liver Organogenesis and Developmental Competence

The developing endoderm initially consists of an epithelial sheet that lines the ventral surface of the embryo. Shortly after specification, the endodermal epithelium invaginates anteriorly to form the ventral foregut, which is the region that gives rise to the liver, lung, thyroid, and ventral pancreas (Fig. 2) (206, 208, 213). Posteriorly, a more dorsal domain of the definitive endoderm exists that develops into the intestines and the dorsal bud of the pancreas. The liver primordium is first delineated at approximately E8.5 with the expression of hepatocyte-specific genes in cells termed hepatoblasts (65, 80, 210). These hepatoblasts remain multipotent, as they will further differentiate into the hepatocytes and cholangiocytes (the epithelial cells of the bile duct) that make up the bulk of the mature liver (206). Fate mapping studies have revealed that the liver bud is derived from laterally positioned precursor populations within the foregut endoderm, which migrate toward the ventral midline to meet a spatially distinct domain also harboring hepatic progenitors (181).

The specification and development of these domains are controlled by an array of inductive and inhibitory signals originating from the adjacent mesoderm (100–103). Studies performed nearly 30 years ago showed that cardiac mesoderm from several species maintained the capacity to induce hepatic epithelium when transplanted into the chick endoderm (58). It has since been confirmed, in multiple systems, that fibroblast growth factor (FGF) signaling from the cardiac mesoderm is necessary for both the induction of hepatic cell fate as well as the expansion of these cells once specified and that the mech-
anism involves activation of the mitogen-activated protein kinase (MAPK) pathway (29, 37, 80, 162, 167, 212). FGF signaling simultaneously suppresses the ventral pancreatic program, thus directing the early patterning of the foregut endoderm (51). Bone morphogenetic proteins (BMPs), secreted from the nearby septum transversum mesenchyme, have also been shown to act coordinately as inductive signals mediating both the hepatic and ventral pancreatic fates (149, 167, 212). Interestingly, suppression of other signaling events is also required for the early stages of endoderm specification. Wnt signaling, for instance, must initially be inhibited to allow proper hepatic specification, although its reactivation is required at later stages to support expansion of the liver bud and liver organogenesis (6, 115, 127, 130, 177).

It is now believed that these signals influence cell-autonomous factors such as transcriptional regulators to initiate highly specific gene expression programs. However, it has also been suggested that these inductive signals alone are not sufficient to elicit the desired impact on their target cells in the developing endoderm. Rather, specific molecular events within the receiving cells must first occur before the tissue can become competent to respond to the instruction of the signal (Fig. 3) (205). This model is based on the observation that dorsal endoderm, which does not normally differentiate into liver cells, can be induced to express the liver marker albumin if dissected between E8.5 and E11.5 and cultured in the presence of FGF (23). This competence is lost, however, if the dorsal endoderm is isolated at E13.5 or later, suggesting that factors required for competence are no longer present at this stage or have ceased to disseminate the directive of the signal. Interestingly, there is a direct correlation between the ability of FGF to induce hepatic gene expression in the dorsal endoderm and the binding activity of Foxa and GATA proteins to an albumin gene enhancer region. Thus the loss of competence was accompanied by the loss of Foxa and GATA binding in the more mature dorsal endoderm (23). In support of this model, earlier studies from the Zaret lab had demonstrated selective binding of Foxa and GATA transcription factors to this enhancer in the endoderm, even prior to activation of the albumin gene, further implicating Foxa and GATA proteins as important factors involved in the establishment of developmental competence (22, 65).

Traditionally, transcription factors are thought to promote gene activation via intrinsic transactivation domains or through the recruitment of cofactors that enhance their activation potential. For example, this has been shown for GATA1 and cAMP response element binding protein (CREB) binding protein (CBP), a cofactor with histone acetyltransferase properties that augments

<table>
<thead>
<tr>
<th>Table 1. Targeted deletions of Foxa and Foxo family members in mice</th>
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<tr>
<td><strong>Foxa Genotype</strong></td>
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<tr>
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<tr>
<td>Foxo3−/−</td>
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<tr>
<td><strong>Conditional null alleles and compound mutants</strong></td>
</tr>
<tr>
<td>Foxa2loxP/loxP</td>
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<tr>
<td>Foxa2loxP/loxP</td>
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<td>Foxa2loxP/loxP</td>
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<tr>
<td>Foxo1loxP/loxP</td>
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transcriptional activation by loosening local chromatin and, presumably, facilitating engagement of additional transcription factors (19). Although Foxa proteins may also act in this manner, specific cofactors mediating their activity have not been identified, and the Foxa factors seem to have the additional ability to modulate chromatin structure directly. Upon solution of the crystal structure for the Foxa DNA-binding domain, it was revealed that the so-called winged helix motif is strikingly similar to a protein domain of histone H5 (Fig. 4). This finding suggested that Foxa proteins have the capacity to physically disrupt nucleosome structure through displacement of linker histones that normally keep chromatin compacted and inaccessible to regulatory factors (40, 144). Indeed, it has since been shown that the Foxa1 protein is capable of binding its target sites in vitro even when the DNA is occupied by nucleosomes and that this binding results in the opening of local synthetic chromatin (36, 38, 116). Furthermore, in the absence of Foxa proteins, other transcription factors (C/EBP, NF-1) were unable to access their cognate binding sites within this DNA (38, 39). In combination with its capacity to bind target sequences in advance of gene activation, it is this unique ability to engage target sites compacted in nucleosomal DNA and to enable the subsequent binding of additional regulators that has led to the designation of Foxa proteins as “pioneer” factors (205).

Recently, the function of Foxa proteins has been linked to epigenetic events (201). When Xu et al. (201) investigated the methylation status of CpG dinucleotides of specific genes supposed to be silent in embryonic stem cells, they made the surprising finding that not all CpGs were methylated, thus uncoupling gene activation from methylation status. In the case of the albumin locus, a CpG dinucleotide within a Foxa binding site in the distal enhancer was not methylated, yet the albumin gene was not transcribed. While no Foxa1 protein was present in embryonic stem cells, binding of Foxd3 was shown to occur instead at this Foxa binding site. This finding suggests that maybe Foxd3 is the true “pioneer” factor and correlates binding of Fox factors to CpG methylation status.

C. Foxa1/a2 Deficiency and the Liver-Less Mouse

The Foxa genes were initially cloned based on their ability to bind regulatory regions of the liver-enriched transthyretin (Ttr) and alpha-1-antitrypsin genes (42). They have subsequently been shown to regulate, both directly and indirectly, the expression of a variety of signaling and metabolic proteins expressed in the liver (57). The early activation of the Foxa genes in the hepatogenic region of the foregut endoderm, combined with the abundance of liver-specific Foxa target genes, has been interpreted as evidence that Foxa genes play a key role in regulating hepatogenesis. The early lethal phenotype observed in Foxa2−/− embryos, owing mostly to node and notochord defects (4, 196), precluded analysis of Foxa2 at later stages of development, thus necessitating the derivation of mice harboring a conditional Foxa2 allele to directly assess its role during liver development. Mice bearing a floxed Foxa2 allele have been crossed with mice expressing Cre recombinase under the control of Foxa3 regulatory sequences resulting in deletion of Foxa2 beginning at E8.5 throughout the Foxa3 expression domain, including the region of endoderm that gives rise to the liver bud, but not in the axial mesoderm where
Foa2 is required for notochord development (104). The resulting Foxa2loxP/loxP; Foxa3-Cre mice are viable and show no signs of impaired liver development (105).

**FIG. 3.** The developmental competence model. Schema depicting the proposed dynamics of nucleosome structure at an enhancer region of the liver-specific albumin gene. A: initially, at the time of gastrulation, the gene is transcriptionally silent because of DNA compaction by nucleosomes, thus preventing access of transcription factors to their cognate DNA binding sites. This would also correspond to all cell types in which the albumin gene lacks the potential to be activated. B: in the endoderm, specific regions of chromatin are loosened, and occupancy of select transcription factors, including Foxa and Gata4, can be detected, even prior to transcriptional activation of the gene. This combination of events is proposed to be critical to establish a degree of competence that will eventually permit gene activation. C: finally, during hepatic induction of the endoderm, subsequent binding of additional factors, such as CCAAT/enhancer binding protein-beta (C/EBPβ) and nuclear factor 1 (NF1), results in activation of the albumin gene. [From Zaret (206), with permission from Nature Publishing Group.]

Foxa1−/− and Foxa3−/− mice also have normal liver histology, indicating that either the Foxa family is not required for hepatogenesis or that the Foxa family members

**FIG. 4.** Structural similarity between Foxa3 and linker histone H5. The X-ray crystal structures of linker histone H5 and the Foxa3 DNA-binding domain. Notice the similarity in the orientation of the helices and sheets, shown in blue and green, respectively. [From Clark et al. (40), with permission from Nature Publishing Group.]
act coordinately and thus maintain some degree of functional redundancy (86, 104, 164). To address this possibility, embryos lacking both Foxa1 and Foxa2 in the endoderm were derived (Foxa1\(^{loxP/loxP}\); Foxa2\(^{loxP/loxP}\); Foxa3-Cre) (104). No animals lacking both Foxa1 and Foxa2 in the endoderm were born and no embryos of this genotype were found beyond E10. However, Foxa1\(^{loxP/loxP}\); Foxa2\(^{loxP/loxP}\); Foxa3-Cre embryos were identified at E8.5 and E9.5 and found to be completely deficient in hepatic specification, as neither liver bud development nor expression of the earliest liver marker gene \(\alpha\)-fetoprotein (Afp) was evident (Fig. 5).

Furthermore, while exposure of ventral foregut endoderm explants to FGF2 in culture normally results in the induction of liver marker genes such as Alb1 and Ttr, the Foxa1/Foxa2 mutant endoderm lacks this capacity, demonstrating that Foxa1 and Foxa2 are required for the initiation of liver specification by inductive signals (104).

At present, the Foxa1/Foxa2-deficient mouse is the only known model of a completely “liver-less” vertebrate (83). Although these results provided genetic support for the competency model, direct analysis of chromatin structure and nucleosome positioning in the absence of Foxa proteins will be required to confirm their role as “pioneer factors.” We do not know, for instance, how the absence of Foxa proteins affects chromatin structure in the foregut endoderm. If the “pioneer factor” model is correct, one would expect that the albumin enhancer, as well as all other genes activated in the liver bud, remain in an inaccessible chromatin status, similar to the situation in tissues that never activate “liver genes” such as the heart or kidney. Addressing this issue experimentally is hampered by limited size of the foregut endoderm, making biochemical assays such as chromatin immunoprecipitation or nucleosome structure analysis quite challenging.
In response to elevated blood glucose, insulin is secreted from pancreatic β-cells. The result of insulin signaling in the liver is decreased gluconeogenesis and glycogenolysis, with increased glycogen storage. Collectively, this combination of activities results in increased hepatic glucose uptake and storage along with decreased hepatic glucose production. In times of low plasma glucose levels, glucagon and glucocorticoids function coordinately to increase gluconeogenesis and glycogenolysis while suppressing glycogen storage. The net result of this is increased hepatic glucose production and secretion.

III. Foxa AND Foxo: INTEGRATING TRANSCRIPTION AND METABOLISM IN THE MATURE HEPATOCYTE

A. Fox Genes and Glucose Homeostasis

The severe morbidity and mortality of metabolic diseases such as diabetes and familial hyperinsulinism attest to the importance of proper glycemic control. One of the principal functions of the liver is to contribute to the maintenance of blood glucose levels within a relatively tight physiological range (Fig. 6). When nutrient availability is limited and circulating glucose levels fall, the liver becomes the main center for glucose production, via gluconeogenesis or glycogenolysis, as a means to help oppose the hypoglycemic state. These processes are largely governed by the combined actions of glucagon and glucocorticoids. On the other hand, when energy sources are in abundance, the liver transitions to become a pivotal location for glucose uptake and storage, while the genes involved in glucose production are simultaneously suppressed. In this case, insulin is the primary signal that directs the hepatic response to deal with periods of hyperglycemia. Thus the dynamic actions of the liver, as a hormone-responsive target organ, are indispensable to the overall goal of regulating glucose homeostasis.

The molecular mechanisms responsible for the appropriate metabolic adaptations of the liver to both hypoglycemia and hyperglycemia are controlled in large part at the level of transcription. For example, in times of prolonged energy deprivation, hepatic gluconeogenesis is the primary source of glucose production. To sustain the requisite level of glucose synthesis, the expression levels of several gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK, encoded by the Pck1 gene) and glucose-6-phosphatase (G6Pase), must be stimulated. PEPCK is generally considered the rate-limiting enzyme of gluconeogenesis, while G6Pase catalyzes the final step in generation of free glucose that can be exported from the hepatocyte, a process equally important to glycogenolysis. However, recent data on hepatocyte-specific ablation of the Pck1 gene have called its importance to gluconeogenesis into question, while revealing a more complex role for this enzyme in regulating hepatic energy metabolism (27, 163). Transcriptional control of these enzymes is achieved through the binding of specific transcription factors to their cognate target sites found within promoter and enhancer regions of these genes (68, 69, 112, 121, 131, 170, 173, 190, 191). Multiple genes encoding hepatic enzymes and serum proteins have been found to contain Foxa and Foxo binding sites, leading to the hypothesis that these factors play critical roles in the hepatic response to fasting (Table 2).

While upregulation of certain liver genes is critical to the mission of glucose production in response to hypoglycemia, mechanisms must also be in place to rapidly suppress these same genes in times of nutrient abundance. The latter are largely under the control of insulin signaling. In fact, persistent hepatic glucose production due to impaired insulin signaling is a hallmark of diabetes, further demonstrating the need for strict transcriptional regulation of this process. This has been demonstrated quite clearly in multiple mouse models of insulin resist-

### Table 2. Selected Foxa and/or Foxo target genes

<table>
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<tr>
<td>Ucp2</td>
<td>ChIP, CTA</td>
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EMSA, electrophoretic mobility shift assay; CTA, cotransfection assay; ChIP, chromatin immunoprecipitation; KO, genetic models involving absent or reduced Fox expression.
tance, including the liver-specific insulin receptor and IRS1/IRS2 double knockouts, each of which develops severe diabetic phenotypes (52, 117, 178). Binding of insulin to its receptor on the cell surface initiates a series of phosphorylation events involving the sequential recruitment of IRS proteins, induction of phosphoinositol (PI)-3-kinase, and finally the activation of AKT, a kinase which can, in turn, directly modify regulatory molecules such as transcription factors (108, 178). Insulin signaling affects the expression of many genes, both positively and negatively, including PEPCK and G6Pase through modulation of factors bound to cis-acting insulin response elements (IRE). A multitude of in vitro and in vivo DNA binding assays have demonstrated the ability of Foxo proteins to directly engage IREs within these genes (10, 11, 63, 72, 125). The Foxo proteins have since proven to be unifying end points in insulin signaling, as targets of both activation and suppression of hepatic genes involved in the appropriate and timely response to hormonal stimulation.

The contribution of the Foxo and Foxa subfamilies of transcription factors to hepatic glucose metabolism has been studied by genetic means. Surprisingly, selective deletion of Foxa2 in hepatocytes during late fetal development appeared to have little impact on liver function (174). Upon initial inspection, these mice were viable and had morphologically normal livers. Furthermore, they were euglycemic and had remarkably few alterations to the hepatic gene expression program. However, upon fasting, these mice failed to fully activate a series of genes including PEPCK, tyrosine aminotransferase (TAT), and insulin-like growth factor binding protein 1 (Igfbp1) that are involved in the reciprocal response to produce glucose. It was subsequently shown that Foxa2 was required for the full stimulatory activity of both glucocorticoids and cAMP, the second messenger downstream of glucagon and epinephrine signaling, through its ability to promote CREB and glucocorticoid receptor (GR) binding to their targets in vivo (211). Nevertheless, the overall impact of Foxa2 deletion in hepatocytes was relatively mild, likely owing to the compensatory activities of other Foxa family members, considering their potential to bind to and transactivate the same complement of cis-regulatory elements in a number of crucial target genes (86, 97). Foxa3−/− mice display a similarly mild hepatic phenotype, but uniquely develop moderate hypoglycemia with prolonged fasting due in part to an inability to properly activate the GLUT2 glucose transporter, which equilibrates glucose levels between hepatocytes and circulation (85). Thus, although these phenotypes demonstrate the importance of Foxa2 and Foxa3 to proper liver function, as was the case for their role during development, it will likely require the simultaneous and inducible liver-specific deletion of multiple Foxa genes in combination to fully assess their contribution to hepatic glucose metabolism. Lai et al. (97) showed nearly two decades ago using electrophoretic mobility shift assays that the three Foxa proteins can have different affinities for various target sites in vitro. With the advent of ChIP-Seq technology, this hypothesis can now be tested on a genome-wide scale to determine if certain sites are preferentially bound by Foxa1 or Foxa2 in vivo.

The Foxo transcription factors have also long been considered key regulators of energy metabolism. In fact, a factor, termed daf-16, later identified to correspond to the mammalian Foxo class, was first described nearly 30 years ago in a screen for mutations aimed at identifying mediators of Dauer formation in C. elegans larvae. Dauer formation is an adaptation of the nematode where development is temporarily arrested in response to environmental cues (147). Energy deprivation, for example, can provoke Dauer formation, which generally results in the synthesis of a protective cuticle, cessation of feeding, and dramatic reductions in metabolic rate. Interestingly, this process is reversible as larvae can exit the Dauer stage and reinitiate the normal developmental process if nutrients become more abundant. Mutations in the C. elegans orthologs of the insulin receptor (daf-2), PI3-kinase (age-1), and protein kinase B (akt1/2) all result in premature and permanent entry into the Dauer state, clearly demonstrating a role for the insulin signal transduction pathway in mediating this process. Mutations in daf-16, the gene more recently identified as the C. elegans ortholog of Foxo, suppress these Dauer formation phenotypes, thus providing the first indication that the Foxo proteins may play an important role in managing the utilization of energy resources (132, 147). The role of daf-16/Foxo in insulin signaling was quickly found to represent an evolutionarily conserved pathway. For instance, flies deficient for dFOXO, the Drosophila melanogaster or fruit fly homolog of Daf-16, are incapable of appropriately upregulating key genes, including the insulin receptor, in response to starvation (142).

Immediately following the discovery that daf-16 encodes a transcription factor orthologous to the vertebrate Foxo proteins, multiple labs pursued the question whether mammalian Foxo proteins play a role in insulin signaling transduction, and by what mechanism and to which degree (63). Foxo proteins directly engage IREs within insulin-responsive genes, implicating them as important directors of the transcriptional response to insulin (9, 35, 69, 94, 182, 204). Biochemical and overexpression studies showed that Foxo1, as well as Foxo3a and Foxo4, two closely related Foxo family members, can indeed be phosphorylated by protein kinase B, also known as AKT, a downstream effector of the activated insulin receptor (17, 25, 67, 146). So-called “kinase-dead” and phosphomimetic mutant versions of Foxo subsequently confirmed the involvement of specific AKT target residues (Thr24, Ser256, and Ser319 in Foxo1) in mediating their activity. In fact, phosphorylation of these sites results in a loss of
Foxo DNA binding affinity, possibly due to conformational changes in the DNA-binding domain, and subsequent nuclear exclusion (25), thus providing a strategy for suppression of target genes that are counterproductive to the directive of insulin signaling (Fig. 7).

Initial loss-of-function mouse models, however, contributed little toward determining the importance of Foxo factors to energy homeostasis in vivo. Foxo1−/− embryos die at approximately E10.5 as a consequence of incomplete vascular formation (75). Conversely, mice harboring homozygous null mutations in Foxo3a and Foxo4 are viable and grossly indistinguishable from littersmates (31, 133). Foxo3a mutant females eventually became sterile with age, while Foxo4-null mice display no significant abnormalities, although neither of these mice has been properly assessed for metabolic phenotypes. In mouse experiments analogous to those initially performed in nematodes, haploinsufficiency of Foxo1 was capable of partially rescuing the severe diabetic phenotypes of both insulin receptor and insulin receptor substrate 2 (Irs2) global null mutations (125). Importantly, the hyperglycemia associated with each of these mutant mouse models is due to defects in insulin signaling, thus implicating a mechanism where Foxo activity is inhibited by the cascade initiated by insulin receptor activation.

More recently, mice lacking Foxo1 specifically in hepatocytes using Cre/loxP technology have been derived to bypass the lethality of the global null mutation (113). These mice are mildly hypoglycemic following prolonged fasting due to defects in hepatic glucose production, as a consequence of deficiencies in the activation of many key genes including PEPCK, G6Pase, and peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α). PGC1α, a transcriptional cofactor, is another key regulatory gene in the liver, although its specific role in metabolic control remains unresolved. While originally described primarily as a direct mediator of the gluconeogenic program (28, 70, 107, 111), it is now appreciated that PGC1α is critical to mitochondrial functions, such as fatty acid oxidation and flux through the tricarboxylic acid cycle, that provide energy for execution of the metabolically taxing processes that lead to hepatic glucose production (28). Some aspect of PGC1α-directed gene regulation has been proposed to involve direct interactions with Foxo1 that synergistically stimulate expression of genes such as PEPCK and G6Pase (143). However, recent experiments demonstrating that PGC1α can fully stimulate the G6Pase promoter even in the absence of Foxo1 binding have cast doubt on this model into question (157). While the link to PGC1α seems tenuous, the evidence for direct cooperative interactions between Foxo1 and C/EBPα, a transcription factor particularly critical to the initiation of the gluconeogenic program postnatally, is more compelling. In fact, it appears that Foxo1 binding to the PEPCK promoter, in particular, is absolutely dependent on C/EBPα as Foxo1 recruitment to this region was undetectable in C/EBPα−/− hepatocytes (159), further cementing its role as a key regulator of metabolic genes.

B. Foxa Factors Control Bile Acid Metabolism and Biliary Development

The liver has a major role in the detoxification of xenobiotics, metabolism of nutrients, and glucose homeostasis. The liver also plays a major role in the digestion of lipids through the synthesis of bile acids that are subsequently secreted into the bile and transported to the duodenum via the biliary tree, which connects each hepatocyte to the common bile duct by a ductal system of increasing diameter. Once in the gut lumen, bile acids aid in the aqueous solubilization of lipophilic molecules such as triglycerides, cholesterol, and vitamins. In addition, bile acids are very important in the regulation of cholesterol levels, as bile acid synthesis is a major pathway to decrease cholesterol. More recently, bile acids have been shown to be critical regulators of glucose metabolism and energy expenditure (179). However, levels of bile acids have to be tightly regulated. If concentrations of bile acids within hepatocytes become too high, their strong detergent properties lead to hepatocyte toxicity, liver injury, and intrahepatic cholestasis (74). Thus synthesis, degradation, and export of bile acids have to be tightly controlled to maintain health of the hepatocyte as well as the whole body. Several complementary mechanisms serve to prevent bile acid toxicity in the liver, including reduced
synthesis and uptake, increased intracellular detoxification, and active bile acid secretion.

The first suggestion for a link of Foxa transcription factors to bile acid homeostasis came from a study by Costa and colleagues, employing transgenic overexpression of Foxa2 in hepatocytes in mice (145). These mice developed jaundice and had extremely elevated serum bile acid levels. This phenotype was attributed to a decrease in Na\(^+\)/taurocholate transport protein (Ntcp) expression that preceded the rise in serum bile acid levels. Ntcp is a bile acid transporter on the serosal side of the hepatocyte that mediates reuptake of bile acids from the bloodstream. Without this transporter, the ileal-hepatic recirculation of bile acids is interrupted, and bile acids accumulate in the serum. In this model, intrahepatocyte bile acid levels are normal or reduced.

Given the repression of Ntcp levels by transgenic overexpression of Foxa2 in the liver, one would have expected that in Foxa2 deficiency, such as hepatocyte-specific gene ablation using the Cre/loxP system, Ntcp levels would increase. However, the opposite is true, and in fact, Ntcp regulation does not seem to play a major role in Foxa2 deficiency (21, 174). Thus it seems likely that the Ntcp repression seen in the initial transgenic model was the result of the dramatic and nonphysiological overexpression of Foxa2 by the powerful transthyretin promoter.

A different role for Foxa2 in bile acid homeostasis was, however, established using global location analysis of Foxa2 targets and conditional gene ablation (21, 123). In this study, the binding sites of Foxa2 in the genome were determined by ChIP-on-Chip technology, or chromatin immunoprecipitation with a Foxa2-specific antibody followed by hybridization of the recovered target DNA fragments to microarrays containing thousands of promoters and enhancers. Chromatin immunoprecipitation samples were prepared from livers of both wild-type and liver-conditional Foxa2 null mice to ensure that spurious signals resulting from the immunoprecipitation procedure could be filtered out, as true binding events should only be present in the wild-type liver tissue. Close to 500 genes were found to have a Foxa2 binding site in either their promoters or enhancers. Surprisingly, when these Foxa2 targets were analyzed for their functional annotation, genes involved in glucose homeostasis were not at the top of the list; rather, a cluster of categories of genes encoding enzymes and transporters controlling steroid, cholesterol, and lipid metabolism was identified as most enriched for Foxa2 occupancy.

Prompted by these predictions from the functional genomics approach, Bochkis et al. (21) set out to test to what extent Foxa2 contributes to bile acid homeostasis. Using mRNA and protein expression studies, they found complex alterations in the levels of multiple transporters and modifying enzymes for bile acids in mice deficient for Foxa2 in hepatocytes, which as a net results increases intrahepatic bile acid levels and the vulnerability of hepatocytes to the toxic side effects of bile acid accumulation (see schema in Fig. 8). The reduced levels of so-called phase I and phase II enzymes caused a diminished ability of the Foxa2 mutant hepatocytes to detoxify bile acids. Thus, when mice were fed a cholic acid-containing diet, which is well-tolerated by control mice, the Foxa2-deficient animals were severely affected, with increased liver injury. In addition, the authors confirmed that many of the genes encoding these enzymes and transporters are indeed direct Foxa2 targets, as had been suggested by the ChIP-on-Chip study. Finally, FOXA2 protein levels were found to be reduced in the livers of patients with cholestasis, raising the possibility that FOXA2 might be important in human disease as well. However, at present, this is only supported by correlative data, and future experiments are needed to address this issue further, as well as to determine if bile flow and total bile acid pools are altered in Foxa2-deficient livers, to differentiate between cholestasis, or altered bile flow, and hepatocellular injury. Another issue is the fact that intestinal bile acid levels are regulated via negative-feedback regulation of hepatic bile acid production, and therefore, it will be interesting to investigate these as well (77).

Most recently, a novel role for the Foxa1 and Foxa2 was discovered that combines their functions as regulators of development and metabolism (109). In this case, two conditional (loxP-flanked) alleles were employed to enable the simultaneous ablation of these two highly related genes. When the two genes were removed using an albumin-\(\alpha\)-fetoprotein-Cre transgene during fetal liver development, the biliary tree was found to be expanded in postnatal mice (Fig. 9). This bile duct hyperplasia was shown to be the result of increased proliferation of biliary epithelial cells, a function not predicted for the Foxa genes. However, an interesting property established for the Foxa factors, that is their ability to interact with and/or mediate the action of nuclear hormone receptors, ultimately gave a clue to explain this surprising phenotype.

The link between Foxa proteins and nuclear hormone receptors, which ultimately explained the hyperproliferative phenotype of the Foxa1/a2-deleted biliary system, was actually first described in the 1990s and came from investigations into the maintenance of glucose homeostasis introduced above. The liver contributes to the control of glucose metabolism by uptake and storage of glucose (as glycogen or converted to triglycerides) after a carbohydrate-rich meal or by the activation of gluconeogenesis (from glycogen, amino acids, and glycerol) under conditions of hypoglycemia. These processes are orchestrated by insulin or glucagon and glucocorticoids, respectively. The activation of genes encoding gluconeogenic enzymes is controlled by
complex sets of cis-regulatory elements and their cognate DNA binding proteins (112, 114).

The gene encoding the enzyme controlling the rate-limiting step of gluconeogenesis, PEPCK, has served as one of the paradigms for studying hormonal regulation of metabolism in the liver (112, 114). Many cis-regulatory elements necessary for both tissue-specific expression and perinatal activation of the gene are contained within

FIG. 8. The role of Foxa2 in hepatic bile acid metabolism. A: in the hepatocyte, there are several transporters that regulate the uptake or the secretion of bile acids. While Oatps and Ntcp are responsible for bile acid uptake, Mrp3, Mrp4, and Osta2/β directly regulate release into the blood, especially during cholestatic conditions. Several enzymes control bile acid synthesis from cholesterol, with Cyp7a1 catalyzing the rate-limiting step. Once inside the hepatocyte, bile acids must undergo a series of modifications to decrease their cellular toxicity. This processing is orchestrated by a number of hepatic enzymes, including Cyp3a11, Gsta1, Gsta2, Gstm2, Sult2a1, Slc27a5, BBAT, and Ugt1b6. In addition, there are several transporters, such as Bsep, Mrp2, and Mdr1, which regulate elimination of bile acids, secreted as bile, from the hepatocyte. B: upon the hepatocyte-specific deletion of Foxa2, bile acid metabolism, processing, and secretion are impaired, resulting in increased intracellular bile acid concentrations culminating in toxicity and liver injury. This phenotype is likely due to the transcriptional downregulation of multiple key Foxa2-dependent enzymes and transporters involved in proper hepatic bile acid metabolism (Oatp2, Mrp3, Mrp4, Mrp2, Cyp3a11, Slc27a5, and Gsts). [From Moschetta (123), with permission from Elsevier.]

FIG. 9. Hepatic deficiency of Foxa1 and Foxa2 results in bile duct hyperplasia. Immunofluorescent staining of proliferating cells in liver sections from adult control (Foxa1loxP/loxP, Foxa2loxP/loxP) and mutant (Foxa1loxP/loxP, Foxa2loxP/loxP; AlfpCre) mice. Mice were injected with BrdU 1 h before death to label cells in S phase. BrdU-positive nuclei are shown in red, and cholangiocytes are labeled with anti-CK19 antibody (green). Gold arrows point to BrdU-positive cholangiocytes that are dramatically increased in compound mutant mice. [From Li et al. (109), with permission from American Society for Clinical Investigation.]
the first 500 bp of 5′-flanking region (114). Largely through promoter analysis and transfection studies in hepatoma cell lines, a complex hormone response unit has been delineated (112, 114). This 110-bp region contains two binding sites for the glucocorticoid receptor (GR1 and GR2) as well as binding sites for “accessory factors” AF1 and AF2. While AF1 is responsible for mediating the effects of retinoic acid on transcription and binds RAR/RXR, the AF2 site is bound by the Foxa proteins and is necessary for eliciting the maximal glucocorticoid response in cultured hepatoma cells (190). These data suggest that the Foxa proteins are necessary for full activation of the Pck1 gene in vivo, which is supported by the finding that PEPCK mRNA levels and transcription rates are reduced in mice lacking Foxa3 (85). In addition, overexpression of a truncated form of Foxa2 that lacks the COOH-terminal activation domain in hepatoma cells leads to a reduction of glucocorticoid stimulation of the Pck1 gene and reduced rate of glucose production from lactate and pyruvate (192).

Similar arrangements of Foxa binding sites and glucocorticoid response elements (GREs) in complex glucocorticoid response units (GRUs) have been described for other genes involved in hepatic glucose metabolism, including IGFBP-1 and 6-phosphofructo-2-kinase (PFK-2; Refs. 140, 175). Another interesting example is that of TAT, where a dynamic interplay between Foxa and the glucocorticoid receptor has been described by in vivo footprinting (148). As in the case of the Pck1 gene, disruption of the Foxa binding sites in the GRU of the TAT gene reduces the amplitude of the glucocorticoid response in vitro (60, 150). The Foxa binding sites appear to be especially important during the perinatal activation of gluconeogenesis. Around birth, there is a dramatic change in the hormonal status of the animal. Insulin levels, which are elevated late in gestation, drop dramatically after birth, while glucagon levels rise postnatally. This inversion of the insulin-to-glucagon ratio leads to a mobilization of glycogen stores in the liver and the transcriptional induction of the genes encoding gluconeogenic enzymes. Grange and colleagues (156) developed two transgenic mouse lines to investigate the contribution of the Foxa binding sites in this process. One line carried a dimer of two perfect GREs, while the other contained the complete GRU from the TAT gene including the Foxa binding sites. In the first transgenic line, reporter expression was inducible by glucocorticoids in adult mice, but not during the perinatal period. In contrast, reporter activity in mice carrying the entire GRU construct was induced up to 50-fold in the perinatal period, highlighting the importance of the Foxa binding sites in this process. A similar difference was found during prolonged fasting, where, again, only the complete GRU restored indelibility to the reporter gene. Thus it appears likely that one or several of the Foxa proteins are necessary for the proper regulation of the TAT gene during the perinatal period as well as during a prolonged fast. As discussed above, the full activation of several hepatic genes in response to glucocorticoids was shown to be dependent on the presence of Foxa2 (211).

How does the paradigm of glucocorticoid response units in glucose homeostasis relate to the control of bile duct proliferation by Foxa1 and Foxa2? One of the proliferate signals for cholangiocytes is interleukin-6 (IL-6). In the case of the IL-6 promoter, the glucocorticoid receptor is not an activator as it is for the genes encoding gluconeogenic enzymes, but a negative regulator of gene expression (16, 48). Li et al. (109) showed using chromatin immunoprecipitation of wild-type or Foxa1/a2-deficient liver that the occupancy of the glucocorticoid receptor on the IL-6 promoter is Foxa-dependent, similar to the situation for glucocorticoid receptor binding to the promoter of the Pck1 gene. However, the outcome is different (Fig. 10). Because glucocorticoid receptor binding is reduced, IL-6 transcription is derepressed, and the resulting enhanced IL-6 expression leads to cholangiocyte hyperproliferation. Furthermore, given the temporal increase of Foxa1 and Foxa2 expression as the animal matures, the two genes could act as terminators of bile duct expansion in the adult liver by inhibiting IL-6 expression.

IV. Foxl1: A MARKER OF ADULT HEPATIC STEM CELLS?

For severe chronic liver disease and liver failure, organ transplantation is currently the only therapeutic option. However, its application is limited by the severe shortage of organ donors. Because the liver possesses the capacity to regenerate, at least to some extent, partial

![Diagram](http://physrev.physiology.org/)
liver transplant procedures have had some success. To be able to treat the large number of eligible patients, other avenues, such as the expansion and differentiation of liver stem or progenitor cells, need to be developed. At the present time, however, these efforts are hampered by the lack of truly specific markers for these cell types.

Patients suffering from any of multiple genetic and acquired chronic disorders of the liver could benefit from cell replacement therapy, preferably with committed hepatic stem cells. In the healthy adult liver, turnover of the two major cell types, the biliary epithelial cell or cholangiocyte and the hepatocyte, is very slow. In fact, in the adult liver, only 1 out of every 20,000 hepatocytes is mitotic at a given time, and as a result, the liver replaces itself only every 1–2 years (26, 171). However, when the liver is injured, cholangiocytes and hepatocytes reenter the cell cycle and restore liver mass and homeostasis (55, 61, 73, 172).

When liver injury is severe or chronic, or when hepatocyte proliferation is blocked experimentally in animal models, small bipotential progenitors are activated (2, 18, 49, 50, 56, 118, 168, 169, 180). These cells, termed “oval cells” or “intermediate hepatobiliary cells,” are small and have a high nuclear-to-cytoplasmic ratio, and cannot be detected in the quiescent liver (161). These cells are frequently found in structures known as “ductular reactions” that are present in both experimental models of liver injury and in patients with chronic liver disease (30, 187) (Fig. 11). Because oval cells are often found when the proliferation of differentiated hepatocytes is impaired, it has been suggested that these cells are a reserve population of hepatic progenitors. While these cells are often found in a niche close to the terminal bile duct, called the Canal of Hering (93) (Fig. 11), the origin and fate of these bipotential progenitor cells in vivo is still controversial due to the lack of truly specific markers. Thus the origin of oval cells, their normal location within the liver, and physiological behavior are unknown. Nevertheless, these progenitors hold the potential to be useful for the restoration of liver function in patients with chronic or even acute liver disease (53, 54, 62, 160, 161, 209). Recently, genetic lineage tracing using a Cre-recombinase transgene under the control of the glial fibrillary acidic protein (GFAP) promoter has suggested that even hepatic stellate cells might be progenitors of epithelial cells in the injured liver (202). Yang et al. (202) found that the GFAP-Cre

![Fig. 11. Lobular structure of the liver. Schematic representation of liver lobule histology indicating the position of a portal triad (consisting of the hepatic portal vein, hepatic artery, and bile duct) relative to a central vein. Hepatic progenitors, such as oval cells, are undetectable in the quiescent adult liver, but are often found in response to liver injury that results in impaired hepatocyte proliferation. While these bipotential progenitor cells are commonly located in the terminal branches of the biliary tree, within the canal of Hering, the origin and fate of these cells in vivo are still disputed due to the lack of suitable markers. [Modified from Bloom and Fawcett. *A Textbook of Histology* (10th ed.) Philadelphia, PA: Saunders, 1975.]
transgene is expressed in stellate cells and cholangiocytes in the quiescent liver, as analyzed by a cross with the Cre responsive Rosa-GFP reporter strain. When mice were fed a methionine/choline-deficient diet supplemented with ethionine (MCDE diet), which causes severe liver injury and loss of liver mass, GFAP-Cre marked cells were found to differentiate into hepatocytes. Thus a subset of either stellate cells or cholangiocytes functioned as hepatocyte progenitors in this model.

While there is substantial evidence supporting the existence of resident bipotential hepatic progenitors, the contribution of bone marrow-derived stem cells, capable of differentiating into hepatocytes and cholangiocytes, is controversial. In the majority of studies, the presence of bone marrow-derived cells in the liver is rare and is the result of cell fusion (reviewed in Refs. 30, 187). However, recent studies suggest the existence of bone marrow-derived multipotent epithelial or mesenchymal cells able to differentiate towards a hepatocytic phenotype (30, 187). A subpopulation of bone marrow cells expresses hepatic antigens, and oval cells express several markers associated with the hematopoietic lineage including CD34, c-kit, and thy-1 (110, 135, 136). These conflicting data regarding the potential for bone marrow-derived cells to restore liver mass and function may reflect differences in the type and extent of liver injury, timing of hematopoietic cell transplantation, and the lack of appropriate markers.

Very recently, results of genetic lineage tracing experiments provided strong evidence for an epithelial bipotential adult progenitor cell in the liver (109). Greenbaum and colleagues showed using mouse genetics that the Fox11-Cre transgene, which is normally silent in the liver, is activated in cells of the portal triad following liver injury. Activity of the Foxl1-Cre transgene was visualized using the Rosa26R lacZ reporter line, in which Cre-excision of the Foxl1-Cre transgene is expressed in stellate cells and cholangiocytes (30, 187). A subpopulation of bone marrow cells expresses hepatic antigens, and oval cells express several markers associated with the hematopoietic lineage including CD34, c-kit, and thy-1 (110, 135, 136). These conflicting data regarding the potential for bone marrow-derived cells to restore liver mass and function may reflect differences in the type and extent of liver injury, timing of hematopoietic cell transplantation, and the lack of appropriate markers.

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Why is this finding important? First, the Fox11-Cre transgene will allow for the isolation of these bipotential progenitors by simply replacing the Rosa26R lacZ reporter with one in which Cre activates the expression of a fluorescent protein, allowing for fluorescent activated cell sorting. Sorted cells can then be cultured in vitro in attempts to expand their number and characterized using biochemical and molecular means. Second, the Fox11-Cre lineage tracing approach will allow investigators to address the question whether liver tumor cells are the descendants of oval cells, as is currently hypothesized (161).

V. Foxm1b AND LIVER CANCER

Human hepatocellular cancer is the third-leading cause of cancer-related deaths in the world, even though it is only the fifth most frequent (20, 186). This relatively high mortality has been attributed to late detection of the disease that hampers the efficacy of current treatment options. Thus finding new pathways and targets for treatment remains an essential pursuit. One of the Forkhead Box genes, termed Foxm1b, has emerged over the past decade as an interesting player.

When Foxm1b was cloned in multiple laboratories, it was shown to be active in proliferating, but silent in terminally differentiated cells. More specifically, Foxm1b...
expression is induced during the G1/S phase transition and persists throughout the remainder of the cell cycle. Using both in vitro and in vivo studies, Foxm1b has been shown to inhibit the small Cdk inhibitors p21 and p27, and activate both S and G2/M phase cyclins (for review, see Ref. 44). In the context of the liver, two aspects of Foxm1b biology are of particular interest.

While the liver has an astounding and well-known regenerative capacity, allowing for small-for-size and living-related donor organ transplantation, the regenerative capacity is not limitless, and decreases with age. Costa and colleagues (193) discovered that, in rodents at least, the proliferative potency of hepatocytes correlates with Foxm1b levels, which also decrease dramatically with age. Remarkably, when Foxm1b levels were forced to remain high by transgenic overexpression, the liver retained its ability to regenerate, with both DNA synthesis and the rate of mitosis comparable to levels found in young mice. This “fountain of youth” treatment of the liver could also be accomplished by adenoviral expression of Foxm1b in old mice. The increased proliferative capacity of the Foxm1b-treated liver correlated with elevated levels of S-phase-promoting cyclins such as cyclin D1 and A2, and reduced expression of p21, an important inhibitor of cell cycle progression. Guidotti and colleagues (24) took this concept a step further and showed that cultured hepatocytes that overexpress Foxm1b were more efficient at repopulating injured liver in an experimental transplant setting than control cells, again due to increased proliferative potential (24). These overexpression studies were finally confirmed by loss-of-function genetic studies, which once more required the utilization of the conditional gene ablation approach described above. Mice lacking Foxm1b in hepatocytes showed a severe impairment in the regenerative capacity following partial hepatectomy, with both DNA replication and mitosis being affected. In a mirror image of the gain-of-function experiments, loss of Foxm1b led to increased p21 levels and decreased Cdk2 activation and S-phase progression.

Increased hepatocyte proliferation is beneficial after liver injury or in the transplantation setting, but of course has to be controlled to prevent neoplasms. As one might have guessed from the preceding, hepatocytes lacking Foxm1b due to genetic manipulation are resistant to hepatic carcinogens (88). Mice treated with diethylnitrosamine (DEN)/phenobarbitol (PB) develop hepatic adenomas and carcinomas at 23 and 33 wk, respectively (89, 153, 176). Mice deficient for Foxm1b in hepatocytes have guessed from the preceding, hepatocytes lacking Foxm1b due to genetic manipulation are resistant to hepatic carcinogens (88). Mice treated with diethylnitrosamine (DEN)/phenobarbitol (PB) develop hepatic adenomas and carcinomas at 23 and 33 wk, respectively (89, 153, 176). Mice deficient for Foxm1b in hepatocytes (Foxm1b<sup>lox/lox</sup> P Foxo3f<sup>−/−</sup>; AlbuminCre) were remarkably resistant to these hepatic carcinogens. While all control mice developed adenomas or hepatocellular carcinomas, none of the Foxm1b mutant mice did, even when the follow-up time was increased to 50 wk. This carcinogen resistance was accompanied by increased p27 levels in the mutants. Kalinichenko et al. (88) also discovered an exciting link between Foxm1b and the tumor suppressor p19ARF that leads to the following model of hepatic carcinogenesis. At early stages of chemical carcinogenesis, p19ARF levels are elevated, and p19ARF targets Foxm1b to the nucleus, diminishing its efficacy as a transcription factor. At later stages of carcinogenesis, p19ARF is extinguished, allowing full activation of Foxm1b and its pro-proliferative targets, and faster tumor progression (45). The authors go a step further and show that a small peptide derived from p19ARF is able to repress Foxm1b activity, suggesting, at least in principle, an exciting new target for
the treatment of hepatocellular carcinoma. While thus far these studies have been limited to the rodent model, the fact that Foxn1b expression in human liver cancer correlates with poor outcome encourages further investigation into this pro-proliferative Fox gene. Given the results from the rodent studies, it seems likely, however, that any beneficial antitumor effect of inhibiting Foxn1b activity might come at the cost of a reduced ability of the liver to respond to injury.

VI. CONCLUSIONS

Since their discovery more than 20 years ago, the Fox genes have risen to prominence among the families of transcriptional regulators. A comparison to the nuclear receptor gene family, which in mammals is of similar size, is instructive. While the nuclear receptors are of obvious clinical importance due to their “drugability” as ligand-regulated transcription factors, it could be argued the Fox genes even exceed this class in terms of importance in developmental processes. It is satisfying that over the past 5 years, many examples have appeared that demonstrate how the two classes of transcriptional regulators cooperate to fine-tune gene expression. It is clear that in the liver, no process occurs, from induction of the hepaticogenic program to the metabolic and proliferative responses of the adult liver, that is not touched by one Fox gene or another.

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Address for reprint requests and other correspondence: K. H. Kaestner, Dept. of Genetics, Univ. of Pennsylvania School of Medicine, 415 Curie Blvd, Philadelphia, PA 19104-6145 (e-mail: kaestner@mail.med.upenn.edu).

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