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Review

The Foxa family of transcription factors in development and metabolism

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Abstract. The Foxa subfamily of winged helix/*forkhead* box (*Fox*) transcription factors has been the subject of genetic and biochemical study for over 15 years. During this time its three members, *Foxa1*, *Foxa2* and *Foxa3*, have been found to play important roles in multiple stages of mammalian life, beginning with early development, continuing during organogenesis, and finally in metabolism and homeostasis in the adult. *Foxa2* is required for the formation of the node and notochord, and in its absence severe defects in gastrulation, neural tube patterning, and gut morphogenesis result in embryonic lethality. *Foxa1* and *Foxa2* cooperate to establish competence in foregut endoderm and are required for normal development of endoderm-derived organs such as the liver, pancreas, lungs, and prostate. In post-natal life, members of the *Foxa* family control glucose metabolism through the regulation of multiple target genes in the liver, pancreas, and adipose tissue. Insight into the unique molecular basis of *Foxa* function has been obtained from recent genetic and genomic data, which identify the Foxa proteins as 'pioneer factors' whose binding to promoters and enhancers enable chromatin access for other tissue-specific transcription factors.

Keywords. Transcription factors, development, organogenesis, metabolism, diabetes.

Introduction

The *forkhead* box (*Fox*) gene family is named for the *Drosophila* gene *fork head*, mutations in which cause defects in head fold involution, resulting in a characteristic spiked head appearance [1]. The FORK HEAD protein contains a 110-amino acid motif that is conserved from yeast to man and which functions as a DNA-binding domain (reviewed in [2]). Over 100 *Fox* genes have been identified and classified into subfamilies [3], and many have been shown to have important biological functions by genetic analyses. Several have been identified as genes mutated in human disease, with phenotypes ranging from defective T cell differentiation to speech impediments [2]. The vertebrate Foxa subfamily of Fox transcription factors is the most closely related to the original *Drosophila* protein FORK HEAD and comprises *Foxa1*, *Foxa2*, and *Foxa3*. In the 15 years since its discovery, the *Foxa* family has been proven to be critical in a variety of processes, both during development and in postnatal life. This review summarizes these findings, with a focus on recent discoveries on the roles of *Foxa* family members in organogenesis and glucose metabolism.

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The Foxa protein family: structure and activity

The Foxa protein family was initially discovered on the basis of DNA binding activity present in liver nuclear ex-

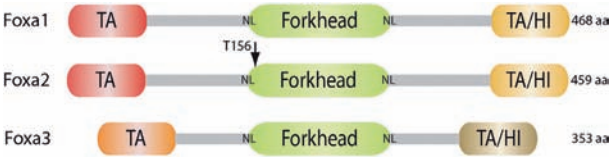

Figure 1. Three-dimensional structure of the *forkhead* domain, showing interaction with DNA [133, 134]. Within helix 3 (H3) and wing/loop 2 (W2), multiple residues make direct and water-mediated contacts with the DNA. H3 interacts within the major groove, while W2 straddles the DNA backbone and makes contacts in both the major and minor grooves.

tract which was specific for the promoters of the *transthyretin* (*Ttr*), *α1-antitrypsin* (*Serpina1)*, and *albumin* (*Alb1*) genes [4, 5]. For this reason, the genes were originally named *hepatocyte nuclear factor-3* (*HNF-3*) α, β, and γ until the nomenclature of all vertebrate *forkhead*-box containing genes was standardized in 2000 [3]. The Foxa1–3 *forkhead* box protein sequences are 95% identical and are flanked by sequences required for nuclear localization (Fig. 1). Foxa1–3 share weaker homology outside the *forkhead* box, with the greatest degree of conservation at the N and C termini; overall Foxa3 is less closely related than Foxa1 and Foxa2. Foxa2 is unique in that only it contains an AKT2/PKB phosphorylation site at the N terminus of the *forkhead* domain; the potential function of this phosphorylation site is controversial and is discussed further below.

The three-dimensional structure of the Foxa *forkhead* box has been determined by X-ray crystallography (Fig. 2) [6]. It comprises three α-helices arranged in a helix-turn-helix core which is flanked by loops; the structure is thus referred to as a 'winged helix' or *forkhead* box. It has been noted that the *forkhead* box structure is similar to that of the linker histone H1 [6]. The importance of this relatedness is underscored by the discovery of Zaret and colleagues [7, 8] that the Foxa proteins are able to open highly compacted chromatin *in vitro* in a manner not requiring the SWI/SNF chromatin remodeling complex. The mechanism of this activity is almost certainly due to the ability of the C-terminal domain of Foxa to interact with the core histones H3 and H4 [7]. Consequently, the Foxa proteins have been proposed to act as 'pioneer' transcription factors, displacing linker histones from compacted chromatin and facilitating the binding of other transcription factors. Recent genetic and genomic studies (discussed below) have provided evidence that enabling chromatin access for other transcription factors is indeed a function of the Foxa proteins *in vivo*.

Outside of the *forkhead* box, regions at both the N and C termini of Foxa1–3 are conserved, and functional analysis of these domains (from Foxa2) via transcriptional reporter assays has revealed that they act as transcriptional activators [9, 10]. Many subsequent studies have confirmed that the Foxa proteins function as transcription factors, as defined by the presence of sequence-specific DNA-binding activity and the ability to regulate the transcription of target genes. A partial list of Foxa target genes is provided in Table 1; these genes have been identified as Foxa targets through DNA-binding studies [electrophoretic mobility shift assay; chromatin immunoprecipitation (ChIP)] plus either transcriptional reporter assays in cultured cells or via measurements of gene expression in Foxa-deficient genetic models. While it is certain that the genes listed in Table 1 represent only a small fraction of the complete set of Foxa targets, one generalization suggests itself. The majority of known Foxa target genes are involved in the function of terminally differentiated cell types, such as *Alb1* and *protein C* (hepatocytes), glucose transporters and enzymes related to glucose metabolism (hepatocytes and pancreatic islets), and surfactant proteins (lung epithelium). Foxa targets during embryological stages are likely to be under-represented in this list because of the greater technical challenges involved in their identification. In the future, large-scale techniques such as orthogonal analysis of ChIP/promoter microarray and gene expression data will be utilized to provide information regarding many more Foxa target genes in a variety of tissues and developmental stages.

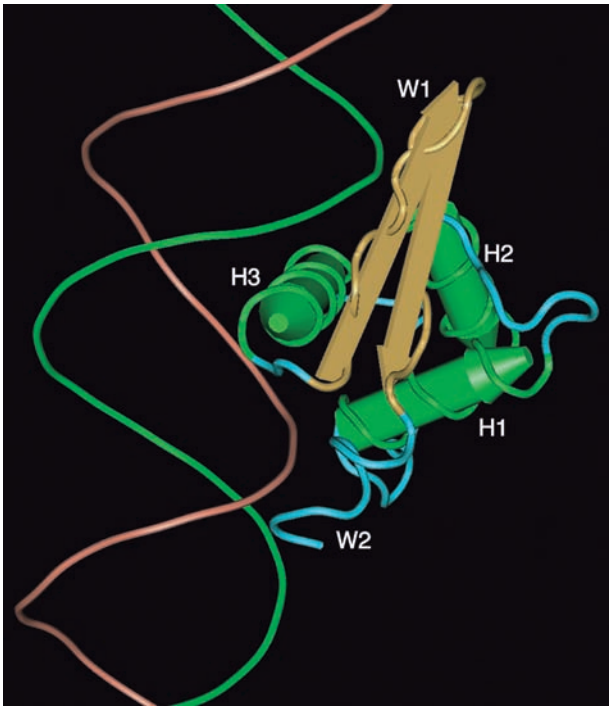

Figure 2. Schematic representation of functional domains present in Foxa1–3. Amino acid numbering is for the mouse proteins. All three family members share 95% identity within the *forkhead* domain, while outside this domain Foxa1 and Foxa2 are 39% identical and 51% similar. Outside of the *forkhead* domain, Foxa3 is only weakly similar to Foxa1 and Foxa2, with the greatest homology in the N-terminal and C-terminal transactivation domains. The C-terminal region has also been shown to interact with the core histones H3 and H4 [7]. Only Foxa2 has a potential AKT2/PKB phosphorylation site at T156. TA, transactivation domain; HI, histone interaction domain; NL, nuclear localization.

Expression of *Foxa1–3* in the embryo and adult

The *Foxa2* gene is the first in the family to be activated during embryogenesis and its expression is detected in the anterior primitive streak and the node at embryonic day 6.5 (E6.5) in the mouse [11–16]. The node in the mammalian embryo is homologous to the dorsal blastopore lip in *Xenopus*, a structure that has been known for many decades to be crucial in gastrulation. During gastrulation, the three germ layers (ectoderm, mesoderm and

Table 1. A partial list of Foxa target genes, as defined by evidence of both DNA binding and gene regulation by Foxa family members.

Gene	Technique	References
Acadm	ChIP, CTA	[48]
Acadvl	ChIP, CTA	[48]
Afp	EMSA, CTA, KO	[30, 60–63]
Alas1	EMSA, CTA	[64, 65]
Alb1	EMSA, KO	[4, 30, 66, 67]
Aldob	CTA	[67–70]
Apoa1	EMSA, CTA	[71, 72]
Apob	EMSA, CTA	[73]
Bdh1	ChIP, CTA	[48]
C4bp	EMSA, CTA	[74]
Cdkn1b	EMSA, CTA	[75]
Cps1	EMSA, CTA	[76, 77]
Cpt1	ChIP, CTA	[48]
Cyp2a2	EMSA, CTA	[78]
Cyp2c11	EMSA, CTA	[79]
Cyp2c12	EMSA, CTA	[80]
Cyp2c6	DF, CTA	[81]
Cyp2h1	DF, CTA	[82]
Dlk1	EMSA, CTA	[47]
F2	EMSA, CTA	[83]
G6pc	EMSA, CTA	[84]
Gcg	EMSA, CTA, KO	[29]
Gck	EMSA, CTA, ChIP, KO	[48, 85–87]
Hadhsc	ChIP, KO	[41]
Hepatitis B enhancers/promoters	EMSA, CTA	[88–92]
HMGCS	ChIP, CTA	[48]
Hnf4a	ChIP, CTA	[48]
Igfbp1	EMSA, CTA	[93, 94]
Ipf1	EMSA, CTA, KO, ChIP	[95–97]
Klkb1	EMSA, CTA, ChIP	[37]
Lpl	EMSA, CTA	[47]
Pbsn	EMSA, CTA	[37]
Pck1	EMSA, CTA, KO	[40, 67, 98–100]
Pklr	ChIP, CTA	[48]
Proc	EMSA, CTA	[101]
Scgb1a1	EMSA, CTA	[102–105]
Serpina1	EMSA, CTA	[66, 106–109]
Sftpa-d	EMSA, CTA, KO	[23, 34, 36, 110–112]
Shh	KO, TG	[34, 113, 114]
Slc10a1	EMSA, CTA	[115, 116]
Slc2a2	EMSA, CTA, KO	[28, 85, 117]
Tat	EMSA, CTA, KO	[40, 118–120]
Tff1	EMSA, CTA	[121]
Titf1	EMSA, CTA	[122]
Trf	EMSA, CTA, KO	[40, 67, 123]
Ttr	EMSA, CTA, ChIP	[30, 67, 109, 124, 125]
Ucp2	ChIP, CTA	[48]
Vtg b1	EMSA, DF, CTA	[126–129]
Vtn	EMSA, CTA	[130, 131]

EMSA, electrophoretic mobility shift assay; CTA, cotransfection assay; ChIP, chromatin immunoprecipitation; DF, DNase I footprinting; KO, genetic models involving absent or reduced *Foxa* expression; TG, transgenic models involving elevated *Foxa* expression.

endoderm) are established, and all organs are subsequently derived from these layers. Newly formed mesoderm and definitive endoderm cells migrating from the node at this stage express *Foxa2*, and at E7.5 *Foxa2* expression is maintained in the notochord and throughout the definitive endoderm [13]. *Foxa2* mRNA is also present in the ventral neural plate at this time and subsequently in the floor plate region of the neural tube. The *Foxa2* gene is active throughout the definitive endoderm posterior to the oral plate and in endoderm-derived structures such as the lung, thyroid, liver, pancreas, and prostatic buds, where its expression persists through development and adulthood [12–14, 17–20]. Weak expression of *Foxa2* has also been noted in developing skeleton [21]. In the developing nervous system, *Foxa2* can be detected in the floor plate of the spinal cord and in periventricular areas of the midbrain and diencephalon. By E15.5, *Foxa2* expression in the nervous system is restricted to an area surrounding the fourth ventricle and to specific midbrain nuclei and motor neurons [13, 16, 21].

The expression of *Foxa1* follows that of *Foxa2*, with *Foxa1* mRNA first detectable at E7.0 in the late primitive streak and then in the notochord, neural plate and floor plate of the neural tube [13, 22]. Overall, the expression of *Foxa1* parallels that of *Foxa2*, with few notable differences (a detailed comparison is provided in [23]). The range of *Foxa1* expression in the adult respiratory and gastrointestinal tracts is slightly more extensive than that of *Foxa2*, and *Foxa1* is expressed in the renal pelvis, ureters, bladder, and male reproductive organs, whereas *Foxa2* is not [23, 24]. *Foxa1* expression in the brain is also more extensive than that of *Foxa2*.

Foxa3 expression differs from that of *Foxa1* and *Foxa2* in a number of ways. It is not present in the primitive streak, axial mesoderm, or neural structures in the embryo or adult. It is first detected in the embryo at E8.5 in a region extending from the hindgut to the midgut-foregut boundary. *Foxa3* expression in this region (and in organs derived from this segment of the embryonic endoderm) persists through embryogenesis and adulthood; indeed, *Foxa3* is the most highly-expressed member of the *Foxa* family in the adult liver. *Foxa3* is also the only *Foxa* gene to be expressed in the long bones, although this expression is limited to E13–16 and is not maintained in the adult [13].

Table 2. Targeted deletions of Foxa family members in mice.

Null alleles

Foxa genotype	Phenotype	References
Foxa1$^{-/-}$	Death at P2-P12 Hypoglycemia Abnormal prostate morphology Delayed respiratory cell maturation and alveolar morphogenesis	[23, 29, 35, 38, 86]
Foxa2$^{+/-}$	Normal lifespan and fertility if on favorable genetic background Increased adiposity on a high-fat diet; decreased adipocyte glucose uptake and glycolysis	[47]
Foxa2$^{-/-}$	Death at E10-11 Severe defects in node, notochord, neural tube, and gut tube	[25]
Foxa3$^{-/-}$	Normal morphology and lifespan Hypoglycemia after prolonged fast	[28]

Conditional null alleles and compound mutants

Foxa genotype	Cre transgene	Phenotype	References
Foxa2$^{loxP/loxP}$	*Ins.Cre* (*β*-cells)	Death at P9-P12 Hypoglycemia Hyperinsulinism	[41, 132]
Foxa2$^{loxP/loxP}$	*Alfp.Cre* (hepatocytes)	Diminished induction of gluconeogenic enzymes during fasting	[46]
Foxa2$^{loxP/loxP}$	*Alb.Cre* (hepatocytes)	Normal	[31]
Foxa2$^{loxP/loxP}$	*Foxa3-Cre* (endoderm)	Death at P0-P5 Hypoglycemia Hypoglucagonemia	[33]
Foxa2$^{loxP/loxP}$	*SP-C-rtTA; (tetO)*$_7$*Cre* (respiratory epithelium)	Death at P0-P1 Respiratory distress syndrome	[111]
Foxa1$^{-/-}$; *Foxa2*$^{loxP/loxP}$	Foxa3-Cre	Death at E9.5-10.5 Loss of liver specification	[30]
Foxa1$^{-/-}$; *Foxa2*$^{loxP/loxP}$	SP-C-rtTA; (tetO)$_7$Cre	Abnormal branching morphogenesis and epithelial differentiation of lung	[23]

The role of the *Foxa* family in early development

Mouse embryos homozygous for a null mutation of *Foxa2* die by E11 and show severe defects in structures related to all three germ layers, with abnormalities of the neural tube and somites, absence of the notochord, and failure to form the gut tube, although endoderm cells are present (this and all other *Foxa* family mutant phenotypes are described in Table 2) [25]. Some of these defects, particularly those of the neural tube and notochord, can be attributed to a loss of expression of *Sonic hedgehog* (*Shh*) [26, 27], as *Foxa2* cooperates with the homeobox gene *Goosecoid* (*Gsc*) in the activation of this gene [27]. In contrast to *Foxa2*, neither *Foxa1* nor *Foxa3* is required during early mouse development, although in zebrafish 'knockdown' of both *foxA3* and *gsc* results in anterior neural plate patterning defects [27]. Thus, while both *Foxa1*$^{-/-}$ and *Foxa3*$^{-/-}$ mice have postnatal phenotypes, both appear normal at birth [28, 29].

The Foxa family in organogenesis

Liver development. The timing of *Foxa* family gene expression during embryogenesis, combined with the many liver-specific *Foxa* target genes, has been interpreted as evidence that *Foxa* genes regulate hepatogenesis. The early lethal phenotype observed in *Foxa2*$^{-/-}$ embryos precludes analysis of *Foxa2* at later stages, thus necessitating the construction of a conditional *Foxa2* allele. Mice bearing a floxed *Foxa2* allele have been crossed with mice expressing the Cre recombinase under the control of the *Foxa3* promoter [30]. The *Foxa3-Cre* transgene results in deletion of *Foxa2* at E8.5 throughout the territory of *Foxa3* expression, including the endoderm at the foregut/midgut border that gives rise to the liver bud. The resulting *Foxa2*$^{loxP/loxP}$; *Foxa3-Cre* mice are born live, with normal liver morphology [31]. Because *Foxa1*$^{-/-}$ and *Foxa3*$^{-/-}$ mice also have normal liver histology, this result indicates that either the Foxa family is not required for hepatogenesis or that the *Foxa* family members act coordinately to establish the liver field. To address this possibility, embryos lacking both *Foxa1* and *Foxa2* (in the endoderm) have been derived (*Foxa1*$^{-/-}$; *Foxa2*$^{loxP/loxP}$; *Foxa3-Cre*) [30]. These embryos are completely deficient in hepatic specification, as neither liver bud development nor expression of the earliest liver marker gene *alpha-fetoprotein* (*Afp*) is evident. While exposure of ventral foregut endoderm to FGF2 in explant 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 induction of liver specification by inductive signals [30]. At present, the *Foxa1*/*Foxa2*-deficient mouse is the only known model of a completely 'liver-less' vertebrate.

Pancreas development. Like the liver, the pancreas develops from the foregut endoderm, and *Foxa1* and *Foxa2* are expressed in this tissue prior to the onset of pancreatic specification [13]. Furthermore, *Foxa1* and *Foxa2* have been shown to regulate several pancreas-specific genes, including *Pdx-1 (Ipf1)*, a transcription factor which is required for expansion of the pancreatic buds and maintenance of differentiated pancreatic cell types (reviewed in [32]). Nevertheless, the gross morphology of the pancreas is normal in *Foxa1*$^{-/-}$, and *Foxa3*$^{-/-}$ neonates. (The physiology of the endocrine pancreas, however, is abnormal in these mutants; see below.) In *Foxa2*$^{loxP/loxP}$; *Foxa3-Cre* mutant mice, the initial steps of pancreatic bud formation and endocrine cell differentiation occur normally, but the α-cell lineage fails to reach its final differentiated state, resulting in hypoglucagonemia and hypoglycemia [33]. Because the *Foxa* family members may compensate for each other during pancreas development, it will be important to determine if the *Foxa1*$^{-/-}$; *Foxa2*$^{loxP/loxP}$; *Foxa3-Cre* model evinces a more severe pancreatic phenotype than the single *Foxa1-3* mutations.

Lung development. *Foxa1* and *Foxa2*, but not *Foxa3*, are expressed in the endoderm which gives rise to the lung buds, where they continue to be expressed in the pulmonary epithelium to adulthood [23, 34]. *Foxa1*$^{-/-}$ mice show some delay in lung epithelial maturation during embryogenesis, but this is resolved by postnatal day 13 (P13), at which time they have normal-appearing lungs [23, 29, 35]. Using the floxed *Foxa2* allele, Whitsett and colleagues [34] studied mice in which *Foxa2* is conditionally deleted in the respiratory epithelium. Lung morphogenesis is normal in these mice, although there are defects in terminal alveolar differentiation, with goblet cell hyperplasia, excess mucin production, and alveolar dilatation [36]. The possibility of redundancy between *Foxa1* and *Foxa2* was explored in embryos homozygous for the *Foxa1* null allele and for the lung epithelium-specific *Foxa2* deletion (*Foxa1*$^{-/-}$/*Foxa2*$^{\Delta/\Delta}$) [34]. These embryos have severe defects in branching morphogenesis of the lungs evident as early as E12.5. The respiratory epithelium of *Foxa1*$^{-/-}$/*Foxa2*$^{\Delta/\Delta}$ embryos retains an immature columnar morphology and fails to express several markers of terminal differentiation [34]. This phenotype is due at least in part to the loss of *Shh* expression in the developing lung. Although these studies did not address the roles of *Foxa1* and *Foxa2* in initial lung specification because the conditional *Foxa2* deletion occurred after this stage, they confirm that the Foxa family plays a critical role in the formation and function of the lung.

Prostate development. Both *Foxa1* and *Foxa2* are expressed during prostate development, although *Foxa1* is expressed at higher levels and persists postnatally, whereas *Foxa2* does not [20, 21]. *Foxa1* has been shown

to regulate a number of prostate-specific genes, including *prostate specific antigen* (*Klkb1*) and *probasin* (*Pbsn*) [37]. *Foxa1*$^{-/-}$ mouse prostate lacks normal duct structures and the epithelium is arrested in a poorly differentiated embryonic stage [38]. Thus *Foxa1* plays a critical role in prostate development, and neither *Foxa2* nor *Foxa3* can substitute for *Foxa1* in this regard. The phenotypes of the male reproductive system in conditional *Foxa2* mutants and *Foxa3*$^{-/-}$ mice may also reveal distinct roles for these factors, but these have not been described.

Kidney function. *Foxa1* expression in the adult kidney was first described by Costa and colleagues [24]. Prompted by the discovery that the promoter of the rat *Foxa1* gene contains binding sites for a kidney-enriched transcription factor, they re-evaluated the expression domain of *Foxa1* in adult mice and localized *Foxa1* transcripts to the collecting ducts of the kidney. This expression is of functional relevance, as *Foxa1*-deficient mice develop mild diabetes insipidus [39]. *Foxa1* mutants are dehydrated and exhibit electrolyte imbalance due to decreased urine osmolality secondary to renal vasopressin resistance. In summary, the *Foxa* genes play essential and diverse developmental roles in multiple organ systems.

The Foxa family in metabolism

Glucose homeostasis. An evolutionarily conserved function of all Foxa genes appears to be the protection of the organism from hypoglycemia [28, 29, 33, 40, 41]. Mice lacking *Foxa1* have severe hypoglycemia due at least in part to diminished *proglucagon* (*Gcg*) expression. This appears to be a direct regulatory relationship, as in transfection assays *Foxa2* strongly activates the *Gcg* promoter [29]. The *Foxa2*$^{loxP/loxP}$; *Foxa3-Cre* mouse is mildly hypoglycemic and dies during the first week of life [33]. This phenotype reflects requirements for *Foxa2* in both the liver and pancreas, the two organs essential for glucose homeostasis. More focused deletions of *Foxa2* have made it possible to elucidate *Foxa2*′s role in the pancreas. In the *Foxa2*$^{loxP/loxP}$; *Ins.Cre* mouse, *Foxa2* is deleted specifically in pancreatic β-cells, resulting in hypoglycemia and relative hyperinsulinemia. Islets isolated from these mice have two defects: they do not secrete insulin in response to elevated glucose concentrations, and they inappropriately secrete insulin in response to amino acids. The former is explained by diminished expression of the *Foxa2* target genes *Kir6.2* (*Kcnj11*) and *Sur1* (*Abcc8*), which together comprise the K_{ATP} glucose-sensing channel [41]. A novel *Foxa2* target gene, *Hadhsc*, which encodes a short-chain fatty acid dehydrogenase has been identified; it is likely that loss of this gene product also contributes to the *Foxa2*$^{loxP/loxP}$; *Ins.Cre* phenotype because humans with mutations in the orthologous gene *SCHAD* also have hyperinsulinemic hypoglycemia [42–45]. These results indicate that *Foxa1* and *Foxa2* play critical, non-redundant roles in the response of adult islets to glucose and nutrient levels.

In the liver, some of the earliest *Foxa* target genes to be identified are involved in glucose homeostasis, particularly the response to fasting. Examples include the gluconeogenic enzymes *phosphoenolpyruvate carboxykinase* (*Pepck*), *glucose-6-phosphatase* (*G6pc*), and *tyrosine aminotransferase* (*Tat*) (see Table 1). The role of *Foxa2* in hepatic glucose homeostasis was evaluated by genetic means in the *Foxa2*$^{loxP/loxP}$; *Alfp.Cre* mouse model, in which *Foxa2* is deleted specifically in hepatocytes. Although these mice are euglycemic, they fail to fully activate *Pepck*, *Tat*, and *Igfbp1* in response to fasting [46]. Not surprisingly, *Foxa3* can also regulate these genes in the liver; in *Foxa3*$^{-/-}$ mice, the expression of gluconeogenic enzymes is diminished as is the expression of *Glut2*, the channel protein used to export glucose from hepatocytes. As a result, *Foxa3*$^{-/-}$ mice are hypoglycemic in response to a prolonged fast [28, 40]. Thus, the Foxa family is an important regulator of the gluconeogenic program in the liver.

Foxa2 also plays a role in energy utilization in fat, as indicated by the *Foxa2*$^{+/-}$ mouse. Although *Foxa2*$^{+/-}$ mice appear normal and have normal fed and fasted blood glucose levels, a phenotype emerges when these mice are fed a high-fat diet [47], at which point they have increased adiposity and decreased energy expenditure. The molecular basis of this phenotype is suggested by the finding that cultured adipocytes overexpressing *Foxa2* have increased expression of genes involved in glucose uptake, glycolysis, lipolysis, and energy dissipation [47]. Conversely, in primary adipocytes harvested from *Foxa2*$^{+/-}$ mice fed a high-fat diet, these same genes are expressed at decreased levels, and the adipocytes have decreased insulin sensitivity, glucose uptake, glycolysis, and lipolysis [47]. Thus, *Foxa2* promotes energy utilization by adipose tissue (rather than triglyceride storage) in the setting of excess caloric intake.

Recent work from Stoffel and colleagues [48, 49] has introduced a new concept regarding *Foxa2* in nutrient metabolism. They have shown that in cultured cells the transcriptional activity of Foxa2 is blocked by treatment with insulin, and that this regulation requires an intact phosphorylation site for the insulin-activated kinase AKT2/PKB at residue T156 of mouse Foxa2. This phosphorylation is reported to result in nuclear exclusion of Foxa2, thereby providing an appealing molecular model in which Foxa2′s gluconeogenic activity is blocked via feeding-induced insulin secretion [48, 49]. Stoffel and colleagues [48] have extended these findings *in vivo* by demonstrating nuclear exclusion of Foxa2 in fed mice and in hyperinsulinemic *ob/ob* mice, *Srebp-1c* transgenic mice, and mice maintained on a high-fat diet. Furthermore, expres-

sion of Foxa2 with a mutated AKT2/PKB site (resulting in constitutive nuclear localization) in these diabetic mice leads to improvements in glucose levels, elevated insulin sensitivity, increased hepatic fat metabolism, and augmented energy expenditure. The authors proposed a model based on these results in which Foxa2 is nuclear only in the starved state, during which it activates multiple genes driving increased hepatic glucose utilization, fatty acid oxidation, and ketogenesis.

However, this model is still controversial. Several labs have attempted to replicate the nuclear exclusion of *Foxa2* in fed wild-type or even *ob/ob* mice, which have greatly elevated insulin levels, but consistently have found Foxa2 protein to be nuclear regardless of metabolic state [46, 50]. Likewise, Foxa2 binding to a number of targets in chromatin was identical in fed and fasted livers [46]. It is also difficult to reconcile the model proposed by Stoffel and colleagues with the normal fasting glucose and glucose tolerance in the livers of *Foxa2*$^{loxP/loxP}$; *Alb.Cre* mice, in which Foxa2 is absent [31]. Experiments are in progress in several laboratories that will address this controversy using genetic means.

Foxa1–3 as a pioneer factors

The competence hypothesis. As described above, the *Foxa* family regulates the development of a number of organs. This raises the question of how the same factors can play such diverse roles in different endoderm-derived tissues. A related quandary stems from the observation that treatment of either ventral or dorsal endoderm explants with FGF results in the expression of liver marker genes, despite the fact that dorsal endoderm does not normally give rise to liver [51]. It has been proposed that before committing to tissue-specific differentiation, the endoderm must first enter a stage of 'competence', which is characterized by an ability to respond to inductive signals [52]. Studies on the biochemical activity of Foxa proteins by Zaret and colleagues [7, 52–56] have resulted in a model for the molecular basis of competence. They have shown that Foxa proteins interact directly with histones H3 and H4 and are able to bind to their sites within the *Alb1* enhancer even when it is compacted by linker histones. In the absence of Foxa, other transcription factors (C/EBP, NF-1) are unable to access their cognate sites within this DNA, but the binding of Foxa results in relief of chromatin compaction and allows binding of other transcription factors to the enhancer; this activity is not dependent on the SWI/SNF chromatin remodeling complex [7]. Based on these findings, Zaret proposed that competence in the endoderm reflects the activity of Foxa family members. This model also provides an explanation for the question introduced above regarding the involvement of Foxa in the development of diverse endoderm-derived tissues: namely, that Foxa protein expression renders organ-specific promoters competent to respond to other transcription factors whose expression is limited to the appropriate region. The loss of competence to respond to inductive signals in ventral foregut endoderm lacking *Foxa1* and *Foxa2* outlined above [30] provides genetic proof for this hypothesis. To fully confirm this model, it will be necessary to analyze the chromatin structure at Foxa target genes during embryogenesis, which is a challenging task given the very limited amount of tissue obtained from embryos at early stages of organogenesis.

The Foxa proteins as pioneer factors for nuclear hormone receptors. In a remarkable convergence of scientific discoveries, recent studies have suggested that Foxa proteins act as 'pioneer' factors not only in establishing the competence of the ventral foregut endoderm, but to also facilitate the binding of nuclear hormone receptors to their targets in multiple organ systems in the adult mammal. For example, it has been shown that binding of the glucocorticoid receptor (GR) to its targets, which is activated during the fasting response, is dependent in part on *Foxa2* [46]. Similarly, a close apposition of binding sites for *Foxa1* and androgen response elements has been observed in the cis-regulatory elements of several prostate-specific genes, and binding of *Foxa1* precedes that of the androgen receptor [37]. Finally, when location analyses have been carried out for estrogen-response elements, many of these response elements were found in close proximity to binding sites for *Foxa1*, and inhibition of *Foxa1* expression in mammary carcinoma cell lines reduces activation of estrogen-responsive gene [57, 58]. This discovery sheds light on previous findings from expression profiling of cancer cell lines, which had documented induction of *Foxa1* expression in breast cancer samples [59]. Up-regulation of *Foxa1* in these tumors could lead to increased activation of estrogen-responsive genes in the mammary epithelium, resulting in augmented proliferation. It remains to be explored whether *Foxa1-3*, and possibly other *Fox* genes, enable or facilitate the binding of additional nuclear hormone receptors or other transcription factors in other tissues or physiological states. It is also not known if the facilitation of nuclear hormone receptor binding is mediated by alterations in chromatin structure or by another mechanism.

Areas for further study

Although a great deal has been uncovered regarding the biochemical and genetic function of the *Foxa* family, there are several important questions to be addressed. While *Foxa1* and *Foxa2* are required for liver specification, additional genetic models involving tissue-specific deletions of *Foxa1-3* are needed to determine if members

of the family function in a coordinate manner in the genesis of other endoderm-derived organs. To fully confirm the hypothesis that competence is equivalent to relief of chromatin compaction by Foxa1–3, it will be necessary to overcome technical challenges preventing the analysis of chromatin structure in embryonic tissues.

The findings of Stoffel and colleagues [48, 49] are tantalizing because of the implication that *Foxa2* may be critical in the etiology or even treatment of diabetes and/or the metabolic syndrome. Specifically, it may be possible to pharmacologically block phosphorylation of Foxa2, thereby leading to nuclear retention of the protein and activation of genes promoting glucose utilization and improved insulin sensitivity. However, it is first imperative that the central finding of insulin-dependent phosphorylation and nuclear exclusion of Foxa2 be reproduced.

Finally, it will also be essential to characterize the full set of target genes directly regulated by Foxa1–3. The most promising approach to this question is orthogonal analysis, in which ChIP/promoter microarray data is combined with expression microarray data to identify genes both bound and regulated by Foxa1–3. It will be interesting to compare the target genes bound by Foxa1–3 in one tissue to another: is the DNA-binding activity of Foxa tissue specific, or is specificity of gene expression achieved through combinatorial effect of Foxa with other transcription factors?

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