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Review

Fox tales: Regulation of gonadotropin gene expression by forkhead transcription factors

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ABSTRACT

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are produced by pituitary gonadotrope cells and are required for steroidogenesis, the maturation of ovarian follicles, ovulation, and spermatogenesis. Synthesis of LH and FSH is tightly regulated by a complex network of signaling pathways activated by hormones including gonadotropin-releasing hormone, activin and sex steroids. Members of the forkhead box (FOX) transcription factor family have been shown to act as important regulators of development, homeostasis and reproduction. In this review, we focus on the role of four specific FOX factors (FOXD1, FOXL2, FOXO1 and FOXP3) in gonadotropin hormone production and discuss our current understanding of the molecular function of these factors derived from studies in mouse genetic and cell culture models.

Contents

1. Introduction to forkhead transcription factors

The forkhead box (FOX) gene family of transcription factors consists of over 100 proteins that have been divided into subfamilies ranging from FOXA to FOXS (Hannenhalli and Kaestner, 2009). The family is named after the *forkhead* transcription factor that was identified in *Drosophila melanogaster*, which, when mutated, gave the insect embryo a distinctive spiked or fork-headed appearance (Weigel et al., 1989). FOX proteins have been characterized in eukaryotes such as yeast, tunicates, nematodes, fish, amphibians, birds, and mammals including humans. Expansion of FOX proteins occurred early in eukaryotic evolution with all bilaterans having at least 19 FOX genes and mammals having over 40 (Jackson et al., 2010).

All FOX proteins contain a highly conserved DNA binding domain (DBD) that is ~100 amino acids in length (Jackson et al., 2010). This forkhead DBD has a winged helical structure composed of three alpha helices and two butterfly-like wings when bound to DNA. FOX proteins have similar binding specificity to a core sequence [T(A/G)TT(T/G)] but different subfamilies recognize diverse DNA sequences adjacent to the core sequence (Wijchers et al.,

Abbreviations: ACTH, adrenocorticotropic hormone; AP1, activator protein 1; CGA, chorionic gonadotrophin alpha subunit; DBD, DNA binding domain; EGR1, early growth response protein 1; e, embryonic day; FSH, follicle-stimulating hormone; FBE, forkhead binding element; FOX, forkhead box; FLRE, FOXL2 binding element; GnRH, gonadotropin-releasing hormone; Gnrhr, GnRH receptor; GH, growth hormone; LH, luteinizing hormone; PITX, paired-like homeodomain transcription factor; PRL, prolactin; SF1, steroidogenic factor 1; SBE, SMAD binding element; TSHB, thyroid stimulating hormone beta.

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2006). In contrast to the DBD, the amino and carboxyl-terminal domains of FOX proteins diverge widely, likely reflecting the function of these proteins in a wide variety of key biological processes including development, proliferation, differentiation, stress resistance, apoptosis, metabolism, and reproduction. Although a potential role for FOX proteins in reproduction was suggested by altered fertility in *Caenorhabditis elegans* mutants of DAF-16 (a FOXO homolog) (Tissenbaum and Ruvkun, 1998), it is only in the past decade that we have begun to understand how FOX proteins regulate production of mammalian gonadotropin hormones.

The gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are produced exclusively in the gonadotrope cells of the anterior pituitary and secreted into the blood where they regulate steroidogenesis and gametogenesis in the gonads (Burns and Matzuk, 2002). LH and FSH are synthesized in response to hormones, such as gonadotropin-releasing hormone (GnRH), activin and gonadal steroids (Seeburg et al., 1987; Vale et al., 1977). LH and FSH are dimeric glycoproteins composed of a common chorionic gonadotrophin alpha subunit (CGA) and a unique beta subunit (LHB or FSHB) (Pierce and Parsons, 1981). *Cga* mRNA is first expressed in the developing murine pituitary at embryonic day (e) 11.5, *Lhb* at e16.5, and *Fshb* at e17.5 (Japon et al., 1994). In this review, we discuss the function and molecular mechanisms of four specific FOX factors that have been reported to regulate gonadotropin gene expression: FOXD1, FOXL2, FOXO1, and FOXP3.

2. FOXD1

FOXD1 (FREAC-4) was originally reported to be highly expressed in the kidney and testis while the mouse homolog was identified in the brain as brain-factor-2 (Hatini et al., 1994; Pierrou et al., 1994). *Foxd1* knockout mice have undeveloped kidneys and die within 24 h after birth due to renal failure (Hatini et al., 1996; Levinson et al., 2005). FOXD1 is also expressed in the retina and is necessary for normal development of the retina and optic chiasm (Herrera et al., 2004). While not much is known about the functions of the amino and carboxyl-terminal regions of FOXD1, the forkhead domain of FOXD1 (Fig. 1) was reported to bind to a core consensus RTAAYA motif (Pierrou et al., 1994).

Although *Foxd1* was reported in an expression library derived from e14.5 pituitary, β-galactosidase was not observed in the developing pituitary gland of mice in which *Foxd1* was replaced with *lacZ* (Gumbel et al., 2012). On the other hand, β-galactosidase was detected in the mesenchyme surrounding the pituitary at e10.5 and e14.5 (Gumbel et al., 2012). This discrepancy may be explained by the presence of mesenchyme in the dissected e14.5 pituitaries in the expression library. Gumbel et al. also asked whether FOXD1 was important for gonadotropin gene expression (Gumbel et al., 2012). In contrast to *Cga* and *Fshb* mRNA levels, levels of *Lhb* were significantly decreased in *Foxd1* knockout mice at e18.5 compared to wild-type littermates. In addition, the intensity of LHB staining was reduced in the *Foxd1* knockout mice while the number of LHB-positive cells remained the same, indicating that decreased *Lhb* expression was not due to impaired gonadotrope differentiation. Since FOXD1 is not expressed in the pituitary, rather in the mesenchyme surrounding the pituitary, the reduction in *Lhb* expression may be due to loss of signaling factors from the mesenchyme. Factors, such as fibroblast growth factor or bone morphogenetic protein, are expressed in the mesenchyme and have been reported to regulate the amount of CGA and adrenocorticotropic hormone (ACTH) (Ericson et al., 1998). It will be interesting to determine in future studies what factors in the pituitary mesenchyme are regulated by FOXD1 and how they, in turn, regulate *Lhb* gene expression.

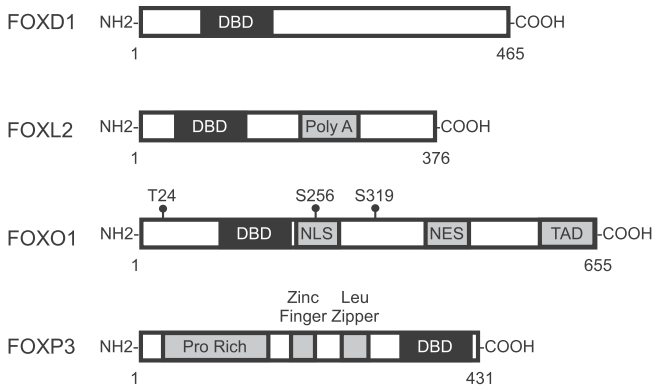

Fig. 1. Structural organization of the FOXD1, FOXL2, FOXO1, and FOXP3 proteins. Numbering of the amino acids is relevant to the human proteins. Abbreviations: DBD, DNA-binding domain; Poly A, polyalanine tract; NLS, nuclear localization signal; NES, nuclear export signal; TAD, transactivation domain; Pro Rich, proline rich domain; Leu, leucine.

3. FOXL2

FOXL2 is a single exon gene expressed in the developing eyelid, pituitary and ovary. Humans with mutations in *FOXL2* develop Blepharophimosis Ptosis Epicanthus Inversus Syndrome (BPES) which is an autosomal dominant disorder characterized by distinctive eyelid abnormalities. Two clinical subtypes have been described; type I is associated with premature ovarian failure (Crisponi et al., 2001). Knockout of *Foxl2* in mice recapitulated the human syndrome and demonstrated that *Foxl2* is required for ovarian granulosa cell differentiation and proliferation as well as female sex determination (Uhlenhaut and Treier, 2011). Like other FOX proteins, FOXL2 contains a forkhead DBD (Fig. 1) that recognizes a conserved core sequence or a specific high-affinity FOXL2 binding element (FLRE) (Benayoun et al., 2008b). FOXL2 also has a unique 14 amino acid polyalanine tract in the carboxyl-terminal region which is a mutational hotspot in BPES patients (Verdin and De Baere, 2012). Interestingly, a somatic C402G mutation in the FOXL2 DBD has been found in over 95% of adult granulosa cell tumors (Verdin and De Baere, 2012).

In mice, *FOXL2* has been reported to be expressed relatively early in pituitary gland development at e10.5 and e12.5 (Dasen et al., 1999; Treier et al., 1998) and at e11.5, coincident with CGA (Ellsworth et al., 2006). Once induced, FOXL2 expression in the pituitary is maintained throughout embryonic development and into adulthood. FOXL2 is expressed in gonadotropes and thyrotropes but not in corticotropes, somatotropes or lactotropes (Blount et al., 2009; Ellsworth et al., 2006). In agreement with the *in vivo* data in mice, FOXL2 is expressed in immortalized cell lines that represent gonadotropes at different stages of development, such as αT3-1 and LβT2 cells (Blount et al., 2009; Ellsworth et al., 2006). FOXL2 is expressed in non-proliferating cells during development (Ellsworth et al., 2006), suggesting that this factor may play a role in cellular differentiation. However, knockout of *Foxl2* in mice results in a hypoplastic pituitary that has a similar proportion of the endocrine cell types in the anterior pituitary (Justice et al., 2011), indicating that FOXL2 is not required for pituitary cell type specification.

Foxl2 knockout mice have a high percentage of embryonic lethality (50–95%) and the majority of surviving mice die at 3–5 weeks of age. Not surprisingly, given the role of *Foxl2* in the ovary (Schmidt et al., 2004; Uda et al., 2004), the surviving female mice have severe ovarian defects as well as impaired gonadotropin hormone production (Justice et al., 2011). To test the hypothesis that FOXL2 is required for FSH synthesis, Tran et al. generated a