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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.

Keywords

Forkhead; Follicle-stimulating Hormone; Luteinizing Hormone; Pituitary; Gonadotrope; Transcription

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

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different subfamilies recognize diverse DNA sequences adjacent to the core sequence (Wijchers et al., 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 hours 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.

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 conditional knockout of *Foxl2* in the pituitary using a cre recombinase knocked in to *Gnrhr* (GRIC-Cre) (Wen et al., 2008) crossed to a floxed *Foxl2* mouse (Uhlenhaut et al., 2009). In this model, female mice were subfertile with decreased ovarian weight and ovulation rates while males were subfertile with decreased testis size and sperm counts (Tran et al., 2013) Both models had substantially decreased *Fshb* mRNA and serum FSH levels (Justice et al., 2011; Tran et al., 2013).

Dispersed primary pituitary cells from both *Foxl2* knockout models also had significantly reduced activin induction of *Fshb* (Justice et al., 2011; Tran et al., 2013). Activin is a critical regulatory component of *Fshb* synthesis and the amount of bioavailable activin fluctuates during the estrous cycle due to changes in intrapituitary follistatin and ovarian inhibin levels (Besecke et al., 1996; Besecke et al., 1997; Woodruff et al., 1996). Activin signaling in gonadotrope cells through type II and type I receptors (ActRII A/B and activin receptor-like kinases 4/7) results in the phosphorylation of receptor-associated SMAD2/3 (Bernard, 2004; Dupont et al., 2003; Norwitz et al., 2002). Upon phosphorylation, SMAD2/3 bind to SMAD4 and translocate into the nucleus of gonadotropes (Norwitz et al., 2002) where they regulate gene expression as a heterodimer or in combination with other transcription factors. SMAD3/4 can bind DNA directly through a defined Smad-binding element (SBE)

(GTCTAG[N]C) or a SMAD half site (GTCT). In addition to SMAD proteins, analysis of mammalian *FSHB* promoters in LβT2 cells demonstrated that the FOXL2 transcription factor is essential for activin induction of murine, porcine and human *FSHB* gene expression [recently reviewed in (Bernard et al., 2010; Bernard and Tran, 2013; Coss et al., 2010)]. SiRNA knockdown of *Foxl2* in LβT2 cells substantially decreased activin induction of a luciferase reporter linked to the *Fshb* promoter as well as endogenous *Fshb* mRNA levels (Lamba et al., 2009; Tran et al., 2011). Mutation of the FOXL2 DNA binding domain also resulted in reduced activin induction of the *Fshb* promoter, indicating that FOXL2 DNA binding was required for its effect (Tran et al., 2011).

Several FOXL2 binding elements (FBE) in the *Fshb* promoter have been characterized to date (Fig. 2). Mutation of a SMAD half site at −116/−113 in the murine *Fshb* promoter resulted in almost a complete lack of activin responsiveness (Bailey et al., 2004). This SBE was shown to bind SMAD2/3/4 (Bailey et al., 2004; McGillivray et al., 2007). FOXL2 binds to a FBE overlapping this site at −113/−108 (Fig. 2) and mutation of A-108G resulted in a profound reduction in activin response (Lamba et al., 2009; Tran et al., 2011). Mutation of a SMAD half site at −149/−146 also resulted in decreased activin responsiveness (Bailey et al., 2004). SMAD binding at this site was only detected using overexpression of the MH1 domain of SMADs in a gel-shift assay, suggesting that this is a low affinity site (Tran et al., 2011). An overlapping FBE site at −154/−149 was reported to bind FOXL2 with a much higher affinity in the porcine promoter than the human or murine promoters due to one or two base-pair changes, respectively (Fig. 2) (Corpuz et al., 2010; Lamba et al., 2009). Although mutation of the SBE disrupted FOXL2 binding (Corpuz et al., 2010), it is uncertain whether this FBE is necessary for activin responsiveness on the murine promoter since a TTT-142GGG mutation just outside this site significantly reduced activin induction (Corpuz et al., 2010) while a TT-152GG mutation within this site only had a modest effect (Tran et al., 2011).

In contrast to the two FBEs described above that are conserved amongst different mammalian species, the murine promoter contains another FBE at −350/−341 that binds FOXL2 with high affinity (Fig. 2) (Corpuz et al., 2010). Mutation of this element had a substantial effect on activin responsiveness (Corpuz et al., 2010; Tran et al., 2011) as well as synergy between activin and progestins (Ghochani et al., 2012). Interestingly, there is also a rodent-specific consensus SBE at −267/−260 that is required for maximal activin responsiveness (Gregory et al., 2005; Lamba et al., 2006; Suszko et al., 2003) and a putative SBE at −355/−352. Mutation of the −355/−352 SBE altered the responsiveness of the murine *Fshb* promoter to SMAD3 overexpression and synergy between activin and progestins (Corpuz et al., 2010; Ghochani et al., 2012). Since the murine, porcine and human promoters all contain the proximal FBE and SBE, the sensitivity of the murine *Fshb* promoter to activin may be due to the rodent-specific distal FBE and SBEs while the porcine promoter may be highly responsive to activin due to the central high affinity FBE.

In addition to regulating Fshb gene expression, FOXL2 may also regulate expression of the GnRH receptor (*Gnrhr*) and *Cga*. Activin induction of murine *Gnrhr* in αT3-1 cells mapped to a composite GnRH activating sequence (GRAS) in the proximal promoter that contains SMAD, activator protein 1 (AP1) and FOXL2 binding sites (Duval et al., 1999; Ellsworth et al., 2003). Mutation of the 3’ end of GRAS had little effect on SMAD or AP1 binding to the *Gnrhr* promoter but had an effect on activin responsiveness. Although the specific base pairs necessary for FOXL2 to bind this element remain to be determined, overexpression of a FOXL2-VP16 fusion protein induced a multimer of the GRAS element and required the FBE to do so (Ellsworth et al., 2003). In regards to CGA, overexpression of FOXL2 or FOXL2-VP16 was shown to stimulate *Cga* gene expression in LβT2 cells (Ellsworth et al., 2006). It is unknown whether this occurs through binding of FOXL2 to the *Cga* promoter. A

FOXL2-VP16 transgenic mouse also expressed CGA in a similar spatial pattern to the fusion protein (Ellsworth et al.). However, it is not clear whether FOXL2 is necessary for regulation of *Gnrhr* or *Cga* transcription *in vivo* since *Gnrhr* and *Cga* mRNA levels were decreased in female Foxl2 KO (Justice et al.) but were unchanged in the gonadotrope-specific *Foxl2* KO mouse model (Tran et al., 2013).

FOXL2 has also been reported to regulate transcription of follistatin, an activin bioneutralizing protein, through a FBE located in the first intron of the follistatin gene (Blount et al., 2009) that is quite similar in sequence to the FLRE (Benayoun et al., 2008b). Although it remains to be demonstrated what base pairs are required for FOXL2 binding to this element, this FBE and the adjacent SBE in the intronic enhancer are required for activin induction of follistatin transcription since mutation of either reduced activin responsiveness (Blount et al., 2009; Blount et al., 2008). SiRNA knockdown of FOXL2 in αT3-1 cells also significantly decreased activin induction of follistatin (Blount et al., 2009). In agreement with the cell culture studies, follistatin mRNA levels were decreased in both the global and gonadotrope-specific Foxl2 knockout mice levels (Justice et al., 2011; Tran et al., 2013). It is interesting to note that FOXL2 does not appear to be expressed in S100-positive folliculostellate cells which also express follistatin (Blount et al., 2009). This may be a mechanism to restrict the effects of activin on follistatin production to gonadotrope cells.

So how does FOXL2 function in the activin induction of gonadotropin genes? One possibility is that activin signaling results in phosphorylation and translocation of SMAD proteins into the nucleus, where they partner with FOXL2 to regulate transcription of specific target genes. Presently, it is unclear whether FOXL2 is constitutively bound to DNA or recruited following activin treatment. DNA pulldown and chromatin immunoprecipitation experiments showed that FOXL2 can bind to the *Fshb* proximal promoter or the follistatin intronic enhancer without activin treatment, although FOXL2 binding to the follistatin enhancer was enhanced with activin (Blount et al., 2009; Corpuz et al., 2010). It is also becoming apparent that FOXL2 interaction with SMAD proteins may be necessary for activin transcriptional regulation. As noted previously, FOXL2 binding sites in the *Fshb*, follistatin and *Gnrhr* promoters are adjacent to a SBE. Additionally, a FBE and SBE multimer is sufficient for activin responsiveness, in contrast to a FBE multimer on its own (Corpuz et al., 2010). FOXL2 was also reported to interact with SMAD3 in a mammalian two-hybrid system and in co-immunoprecipitation experiments in HEK293 cells (Blount et al., 2009; Ellsworth et al., 2003). FOXL2 was also shown to associate with endogenous SMAD2/3 in gonadotrope cells (Lamba et al., 2010). Although the FOXL2 DBD and the SMAD3 carboxyl-terminal MH2 domain are required for interaction, it is not known how interactions between these two proteins facilitate DNA binding and transcriptional activation. Since FOXL2 has been reported to be post-translationally modified (Benayoun et al., 2008a) and phosphorylated by a serine/threonine kinase, LATS1 in ovarian granulosa cells (Pisarska et al., 2010), it is also possible that activin signaling in gonadotropes may regulate FOXL2 activity through an as yet unidentified kinase. Thus, a decade of investigating the role of FOXL2 in gonadotropin hormone regulation has provided many insights and left us with new questions regarding FOXL2 action in gonadotropes.

4. FOXO1

The FOXO subfamily of transcription factors consists of 4 genes in mammals: FOXO1 (alternatively known as FKHR), FOXO3 (FKHRL1), FOXO4 (AFX) and FOXO6 (Burgering, 2008; Jacobs et al., 2003). FOXO1 was originally identified as a chromosomal translocation in human alveolar rhabdomyosarcomas (Anderson et al., 1998). FOXOs have been shown to be key regulators of cellular pathways involved in apoptosis, stress resistance, cell cycle arrest and DNA damage repair (Accili and Arden, 2004; Greer and

Brunet, 2005). They also have important roles in metabolism, homeostasis and reproduction. Knockout of Foxo4 in mice had no overt phenotype, suggesting functional redundancy between FOXO4 and FOXO1/3 (Hosaka et al., 2004). *Foxo3* knockout mice have an age-dependent reduction in fertility caused by defective ovarian follicular growth, similar to premature ovarian failure in women (Castrillon et al., 2003). *Foxo1* knockout mice die at e10.5 due to a lack of vascularization (Hosaka et al., 2004). However, conditional knockouts of *Foxo1* have demonstrated that FOXO1 plays a role in ovarian granulosa cell proliferation and apoptosis, along with FOXO3 and that FOXO1 is essential for maintenance and differentiation of spermatogonial stem cells in the testis (Goertz et al., 2011; Liu et al., 2013).

Like the other FOX proteins, FOXOs contain a highly conserved forkhead DBD (Fig. 1). The structures of FOXO1, FOXO3 or FOXO4 bound to DNA have been solved (Boura et al., 2010; Brent et al., 2008; Tsai et al., 2007). These structures indicate that FOXO target gene expression is probably regulated in a differential manner due to variations in the affinity for different DNA response elements. FOXO proteins recognize two 8 base pair sequences distinct from a core forkhead consensus sequence: the insulin response element present in the IGFBP1 promoter [TT(A/G)TTTTG] and the DAF-16 family binding element [TT(G/A)TTTAC] (Furuyama et al., 2000; Tang et al., 1999). FOXOs also contain a nuclear localization signal (NLS), a nuclear export sequence (NES) and a carboxyl-terminal transactivation domain (Obsil and Obsilova, 2008; Tzivion et al., 2011).

The activity of FOXOs is tightly controlled by post-translational modifications including phosphorylation, acetylation and ubiquitination (Calnan and Brunet, 2008). Activation of insulin and growth factor signaling pathways negatively regulate FOXOs through phosphorylation of three conserved residues by the AKT serine/threonine kinase, resulting in their active nuclear export and inhibition of their transcriptional activities (Van Der Heide et al., 2004). Phosphorylation of FOXOs by other kinases, such as c-jun N-terminal kinase, in response to stress, results in their translocation to the nucleus (Essers et al., 2004; Kops et al., 2002). Studies have also demonstrated that FOXOs can be acetylated by CBP/p300 and deacetylated by sirtuins such as SIRT1 (Brunet et al., 2004; van der Horst et al., 2004).

Intriguingly, deletion of *Foxo1* and *Foxo3* in the somatic tissues of adult female mice resulted in pituitary adenomas, suggesting that FOXO proteins may play important roles within the pituitary gland (Paik et al., 2007). Although the expression of FOXOs in human pituitary has not yet been characterized, FOXO1 expression was reported to be down regulated in human pituitary null cell and gonadotrope adenomas (Michaelis et al., 2011). FOXO1 is also expressed in the developing and adult murine pituitary (Nakae et al., 2008; Villarejo-Balcells et al., 2011). More recently, Arriola et al. showed FOXO1 expression in adult murine gonadotropes and thyrotropes as well as in immortalized cell lines that represent these cell types such as the αT3-1, LβT2 and TαT-1 cell lines (Arriola et al., 2012). Dual label immunofluorescence was performed to determine whether other hormone-producing cell types in the anterior pituitary express FOXO1 and FOXO3 (Fig. 3). As previously reported, FOXO1 colocalized with >95% LHB-containing gonadotropes and thyroid stimulating hormone beta (TSHB)-containing thyrotropes (Fig. 3A). FOXO1 was not expressed in somatotropes, lactotropes or corticotropes containing growth hormone (GH), prolactin (PRL) or ACTH, respectively. FOXO1 expression was also not observed in AtT20 cells, derived from an ACTH-secreting mouse pituitary tumor (data not shown). In contrast, FOXO3 colocalized with TSHB-containing thyrotropes and ACTH-containing corticotropes but not with gonadotropes, somatotropes or lactotropes containing LHB, GH or PRL, respectively (Fig. 3B).

It should be noted that the restriction of FOXO1 expression to adult murine gonadotrope cells was not observed in another study. In this report, FOXO1 was detected in a subset of cells within the anterior pituitary (7% of adult murine gonadotropes, 9% thyrotropes, 15% lactotropes, 30% corticotropes and 63% somatotropes) (Majumdar et al., 2012). At this time, it is not clear why there is a discrepancy between the two reports. It seems unlikely that mouse strain differences account for the discrepancy since both studies used C57BL/6 mice. Whether the sex or age of the mice influences FOXO1 expression in the anterior pituitary remains to be determined. We have determined that both FOXO1 antibodies used in the immunofluorescence studies (11350, Santa Cruz Biotechnology and 2880, Cell Signaling Technology) detect the same full-length FOXO1 protein (~82 kDa) in western blot analysis of proteins derived from LβT2 cells as well as pituitaries from male and female C57BL/6 mice (data not shown). One difference we have noted is that the signal from the FOXO1 2880 antibody is extremely faint in immunofluorescence experiments using paraffin-embedded pituitary sections.

Since the Foxo1 mouse knockout is embryonic lethal, it is not known whether FOXO1 is required for gonadotropin gene expression *in vivo*. Analysis of a *Foxo1* conditional knockout in pituitary gonadotropes in my laboratory using the GRIC-Cre mouse (Wen et al., 2008) crossed to a floxed FOXO1 mouse (Paik et al., 2007) should help answer this question. In the meantime, we have used immortalized LβT2 cells to study the function of FOXO1 in gonadotropes. We found that insulin signaling via PI3K resulted in phosphorylation of FOXO1 and export of FOXO1 from the nucleus to the cytoplasm (Arriola et al., 2012), indicating that the canonical PI3K/AKT/FOXO1 signaling pathway is intact in gonadotropes. We also demonstrated that overexpression of FOXO1, or a constitutively active FOXO1 that cannot be phosphorylated and exported into the cytoplasm, in LβT2 cells resulted in significantly decreased basal and GnRH-induced *Lhb* mRNA (Arriola et al., 2012). The suppressive effect of FOXO1 was shown to occur on both the rat 1.8 kb and human 1 kb LHB promoters, suggesting that the effect may be conserved in mammals.

So how does FOXO1 act as a repressor of *Lhb* gene expression? Several basal transcription factors including specific protein 1, steroidogenic factor 1 (SF1) and paired-like homeodomain transcription factor (PITX1) synergize with early growth response protein 1 (EGR1) induced by GnRH signaling to up-regulate *Lhb* transcription (Halvorson et al., 1996; Kaiser et al., 2000; Keri and Nilson, 1996; Rosenberg and Mellon, 2002; Weck et al., 2000). FOXO1 repression of basal and GnRH-induced *Lhb* transcription mapped to the proximal *Lhb* promoter that contains PITX1, SF1 and EGR1 binding elements (Arriola et al., 2012). While the FOXO1 DBD appears to be required for the suppressive effect, there was no evidence that recombinant FOXO1 bound the proximal *Lhb* promoter. Further analysis with the native protein in gel-shift and chromatin immunoprecipitation assays may provide more insight into the mechanism of FOXO1 repression. We also found that induction of *Lhb* due EGR1 overexpression was repressed by FOXO1 in LβT2 cells and induction of *Lhb* due to EGR1 plus PITX1 or SF1 expression was repressed by FOXO1 in CV-1 cells. Thus far, the data suggests that FOXO1 elicits a suppressive effect via protein-protein interactions with transcription factors necessary for *Lhb* synthesis.

Since some of the transcription factors necessary for *Lhb* synthesis also regulate *Fshb* transcription, we determined whether FOXO1 modulates *Fshb* gene expression. In a recent report, we demonstrated that overexpression of constitutively active FOXO1 or PI3K inhibition, which increases FOXO1 nuclear localization, reduced basal and GnRH-induced *Fshb* transcription in LβT2 or dispersed primary pituitary cells, respectively (Skarra et al., 2013). Similarly to its action on the *Lhb* promoter, FOXO1 repression of Fshb mapped to the proximal promoter containing a PITX1 binding element and required the FOXO1 DBD

although there was no evidence that FOXO1 bound to the proximal *Fshb* promoter (Skarra et al., 2013). Additional results indicating that the mechanism of FOXO1 repression of basal Fshb transcription involves PITX1 include a physical interaction between FOXO1 and PITX1 in a GST pull-down assay that required the FOXO1 DBD as well as FOXO1 repression of PITX1 induction of the *Fshb* promoter in CV-1 cells (Skarra et al., 2013). Interestingly, constitutively active FOXO1 overexpression also resulted in suppression of *Pitx1* mRNA and PITX1 protein levels, indicating another potential mechanism for FOXO1 suppression of *Fshb* transcription. Furthermore, GnRH induction of an *Fshb* promoter containing a deletion at −50/−41 or −30/−21 was not repressed by FOXO1, suggesting that these two regions, one of which overlaps the PITX1 binding element, may be involved in FOXO1 suppression of GnRH-induced *Fshb* synthesis.

Our initial reports demonstrating FOXO1 regulation of *Lhb* and *Fshb* gene expression raise many questions. Most importantly, what is the physiological role of FOXO1 in gonadotropes? As a repressor, does it play a role in the response to pulsatile GnRH, as suggested for the Ngfi-A-binding protein family of EGR corepressors (Lawson et al., 2007)? Is it important for the alterations in gonadotropin production observed in situations of metabolic stress? And mechanistically, how does FOXO1 act as a repressor? Are there other FOXO1 gene targets in gonadotrope cells? And is there cross-talk between GnRH and insulin signaling pathways at the level of FOXO1?

5. FOXP3

FOXP3 is essential for normal immune function because of its regulation of the differentiation and function of $CD4^+CD25^+$ regulatory T cells (Lowther and Hafler, 2012). It is expressed in the thymus and spleen as well as epithelial cells of the lung, mammary gland and prostate (Chen et al., 2008; Hori et al., 2003). *Foxp3* is located on the X chromosome in humans and mice. Mutations in human *Foxp3* result in an autoimmune syndrome called immunodysregulation, polyendocrinopathy and enteropathy, X-linked (IPEX) that primarily affects males. IPEX results in severe autoimmunity, characterized by hypothyroidism, diabetes mellitus and failure to thrive that is often lethal within the first year of life (Wildin et al., 2002). Male *Foxp3* knockout mice and mice with a spontaneous mutation in *Foxp3* that results in a truncated protein lacking the DBD called scurfy also have an IPEX-like syndrome (Khattri et al., 2003). The FOXP3 forkhead domain is different from other FOX family members in that it is located near the carboxyl-terminus of the protein instead of the amino terminus (Fig. 1). It also contains a proline rich domain in the amino terminus which is responsible for transcriptional repression as well as a centrally located zinc finger and leucine zipper domain which facilitates the formation of FOXP3 dimers or association with other transcription factors (Deng et al., 2012).

In addition to the IPEX-like syndrome, male scurfy mice are hypogonadal and infertile (Godfrey et al., 1991). One recent study investigated whether FOXP3 is necessary for gonadotropin gene expression in adult male scurfy mice. Although scurfy mice die early, they can be kept alive for 6-9 weeks if they are not weaned. Jung et al. found that male scurfy mice had decreased *Lhb*, *Fshb*, *Cga* and *Gnrhr* mRNA levels (Jung et al., 2012). LHB and CGA protein levels were also reduced, suggesting that these mice are hypogonadotropic. Since *Cga* was reduced but *Tshb* mRNA was increased, it would be interesting to determine if changes in the pituitary translated into altered LH, FSH and TSH serum levels.

Jung et al. then looked to see if FOXP3 is expressed in the hypothalamus or pituitary but could not detect any *Foxp3* mRNA using RT-PCR in either tissue (Jung et al., 2012). Even though *Foxp3* was not detected in the hypothalamus, it is possible that GnRH production is

affected indirectly by FoxP3 so the authors examined whether *Gnrh* expression was altered in the scurfy mice. Although there was no statistical difference in *Gnrh* expression was detected, this may be due to the high degree of variability in GnRH expression observed in the control animals. A trend towards decreased *Gnrh* could indicate that GnRH production was impaired in the scurfy mice. To further address this question, Jung et al. tested whether *Fshb* and *Lhb* expression were rescued after treatment with D-ala-6-GnRH for 2 days (Jung et al., 2012). GnRH treatment did not restore *Fshb* and *Lhb* mRNA levels, suggesting that GnRH production was not altered in the scurfy mice and indicating that the defect may be at the level of the pituitary. A caveat to this experiment is that a longer treatment paradigm may be necessary to see an effect. On the other hand, a significant decrease in pituitary expression of *Gnrhr* was observed, indicating that GnRH production could be impaired in the scurfy animals since GnRH has been reported to positively regulate synthesis of its receptor in gonadotrope cells (McCue et al., 1997; Norwitz et al., 1999; White et al., 1999).

Since scurfy mice suffer from autoimmunity, it is possible that the pituitary gonadotrope cells were destroyed by the immune system, similarly to the pancreatic beta cells (Lahl et al., 2007). However, equivalent levels of SF1 mRNA and protein were detected in scurfy mice compared to controls, indicating that normal numbers of gonadotropes were present in the scurfy mice (Jung et al., 2012). Since *Tshb* mRNA levels were increased in the scurfy mice but *Cga* was decreased, it is possible that TSH levels in the scurfy mice were lower due to reduced CGA production in the pituitary which would result in a lack of negative T4 feedback and increased *Tshb* gene expression. Hypothyroidism can negatively impact gonadotropin hormone production. However, treatment of the scurfy mice with exogenous thyroid hormone resulted in decreased *Tshb* mRNA levels but did not restore gonadotropin gene expression (Buffy Ellsworth, personal communication), suggesting that another mechanism is responsible for the infertility.

FOXN1 is another forkhead transcription factor that is also important for immune function. A spontaneous mutation in *Foxn1* called nude results in athymic, immunocompromised mice (Flanagan, 1966; Pantelouris and Hair, 1970). It is intriguing that the infertility in the scurfy mice is rescued when the scurfy mice are bred with the nude mice (Godfrey et al., 1991), suggesting that the infertility in the scurfy mice is due to autoimmunity. Additional evidence for this idea comes from the fact that scurfy mice have high cytokines levels due to decreased action of the regulatory T cells (Lin et al., 2005). Since cytokines have been shown to inhibit gonadotropin hormone production (Savino et al., 1999; Wu and Wolfe, 2012), it is possible that the infertility of the scurfy mice is secondary to their autoimmunity. Further investigation should reveal whether the hypogonadism observed in the scurfy mice is due to defects at the hypothalamic, pituitary level and/or gonadal levels as well as the molecular mechanisms that are involved.

6. Summary

Although the first members of the FOX transcription factor family were identified over 20 years ago, it is only in the past decade that investigators have focused on the role of FOX transcription factors in mammalian reproduction. Like the large nuclear receptor family of transcription factors, we are now beginning to appreciate the important functions of FOX transcription factors in the regulation of the hypothalamic-pituitary-gonadal axis. In this review, we focused on four FOX factors that have been reported to regulate gonadotropin hormone synthesis. Since additional FOX factors are expressed in the pituitary or regulate pituitary function, including FOXA1, FOXE1, FOXF1, and FOXG1 (Kalinichenko et al., 2003; Norquay et al., 2006; Wang et al., 2010; Zannini et al., 1997), we anticipate many more studies concerning the regulation of gonadotropin gene expression by FOX transcription factors in the years to come.

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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

References

Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell. 2004; 117:421–426. [PubMed: 15137936]

Anderson MJ, Viars CS, Czekay S, Cavenee WK, Arden KC. Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. Genomics. 1998; 47:187–199. [PubMed: 9479491]

Arriola DJ, Mayo SL, Skarra DV, Benson CA, Thackray VG. FOXO1 transcription factor inhibits luteinizing hormone beta gene expression in pituitary gonadotrope cells. J Biol Chem. 2012; 287:33424–33435. [PubMed: 22865884]

Bailey JS, Rave-Harel N, Coss D, McGillivray SM, Mellon PL. Activin regulation of the follicle-stimulating hormone β-subunit gene involves Smads and the TALE homeodomain proteins Pbx1 and Prep1. Mol. Endocrinol. 2004; 18:1158–1170. [PubMed: 14764653]

Benayoun BA, Auer J, Caburet S, Veitia RA. The post-translational modification profile of the forkhead transcription factor FOXL2 suggests the existence of parallel processive/concerted modification pathways. Proteomics. 2008a; 8:3118–3123. [PubMed: 18604817]

Benayoun BA, Caburet S, Dipietromaria A, Bailly-Bechet M, Batista F, Fellous M, Vaiman D, Veitia RA. The identification and characterization of a FOXL2 response element provides insights into the pathogenesis of mutant alleles. Hum. Mol. Genet. 2008b; 17:3118–3127. [PubMed: 18635577]

Bernard DJ. Both SMAD2 and SMAD3 mediate activin-stimulated expression of the follicle-stimulating hormone beta subunit in mouse gonadotrope cells. Mol. Endocrinol. 2004; 18:606–623. [PubMed: 14701940]

Bernard DJ, Fortin J, Wang Y, Lamba P. Mechanisms of FSH synthesis: what we know, what we don't, and why you should care. Fertil. Steril. 2010; 93:2465–2485. [PubMed: 20403589]

Bernard DJ, Tran S. Mechanisms of activin-stimulated FSH synthesis: the story of a pig and a FOX. Biol Reprod. 2013; 88:78. [PubMed: 23426431]

Besecke LM, Guendner MJ, Schneyer AL, Bauer-Dantoin AC, Jameson JL, Weiss J. Gonadotropin-releasing hormone regulates follicle-stimulating hormone-beta gene expression through an activin/follistatin autocrine or paracrine loop. Endocrinology. 1996; 137:3667–3673. [PubMed: 8756531]

Besecke LM, Guendner MJ, Sluss PA, Polak AG, Woodruff TK, Jameson JL, Bauer-Dantoin AC, Weiss J. Pituitary follistatin regulates activin-mediated production of follicle-stimulating hormone during the rat estrous cycle. Endocrinology. 1997; 138:2841–2848. [PubMed: 9202226]

Blount AL, Schmidt K, Justice NJ, Vale WW, Fischer WH, Bilezikjian LM. FoxL2 and Smad3 coordinately regulate follistatin gene transcription. J. Biol. Chem. 2009; 284:7631–7645. [PubMed: 19106105]

Blount AL, Vaughan JM, Vale WW, Bilezikjian LM. A Smad-binding element in intron 1 participates in activin-dependent regulation of the follistatin gene. J Biol Chem. 2008; 283:7016–7026. [PubMed: 18184649]

Boura E, Rezabkova L, Brynda J, Obsilova V, Obsil T. Structure of the human FOXO4-DBD-DNA complex at 1.9 A resolution reveals new details of FOXO binding to the DNA. Acta Crystallogr D Biol Crystallogr. 2010; 66:1351–1357. [PubMed: 21123876]

Brent MM, Anand R, Marmorstein R. Structural basis for DNA recognition by FoxO1 and its regulation by posttranslational modification. Structure. 2008; 16:1407–1416. [PubMed: 18786403]

Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science. 2004; 303:2011–2015. [PubMed: 14976264]

Burgering BM. A brief introduction to FOXOlogy. Oncogene. 2008; 27:2258–2262. [PubMed: 18391968]

Burns KH, Matzuk MM. Minireview: genetic models for the study of gonadotropin actions. Endocrinology. 2002; 143:2823–2835. [PubMed: 12130545]

Calnan DR, Brunet A. The FoxO code. Oncogene. 2008; 27:2276–2288. [PubMed: 18391970]

Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. Science. 2003; 301:215–218. [PubMed: 12855809]

Chen GY, Chen C, Wang L, Chang X, Zheng P, Liu Y. Cutting edge: Broad expression of the FoxP3 locus in epithelial cells: a caution against early interpretation of fatal inflammatory diseases following in vivo depletion of FoxP3-expressing cells. J Immunol. 2008; 180:5163–5166. [PubMed: 18390696]

Corpuz PS, Lindaman LL, Mellon PL, Coss D. FoxL2 is required for activin induction of the mouse and human follicle-stimulating hormone β-subunit genes. Mol. Endocrinol. 2010; 24:1037–1051. [PubMed: 20233786]

Coss D, Mellon PL, Thackray VG. A FoxL in the Smad house: activin regulation of FSH. Trends Endocrinol. Metab. 2010; 21:562–568. [PubMed: 20598900]

Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, Bisceglia L, Zelante L, Nagaraja R, Porcu S, Ristaldi MS, Marzella R, Rocchi M, Nicolino M, Lienhardt-Roussie A, Nivelon A, Verloes A, Schlessinger D, Gasparini P, Bonneau D, Cao A, Pilia G. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat. Genet. 2001; 27:159–166. [PubMed: 11175783]

Dasen JS, O'Connell SM, Flynn SE, Treier M, Gleiberman AS, Szeto DP, Hooshmand F, Aggarwal AK, Rosenfeld MG. Reciprocal interactions of Pit1 and GATA2 mediate signaling gradient-induced determination of pituitary cell types. Cell. 1999; 97:587–598. [PubMed: 10367888]

Deng G, Xiao Y, Zhou Z, Nagai Y, Zhang H, Li B, Greene MI. Molecular and biological role of the FOXP3 N-terminal domain in immune regulation by T regulatory/suppressor cells. Exp Mol Pathol. 2012; 93:334–338. [PubMed: 23041265]

Dupont J, McNeilly J, Vaiman A, Canepa S, Combarnous Y, Taragnat C. Activin signaling pathways in ovine pituitary and LbetaT2 gonadotrope cells. Biol. Reprod. 2003; 68:1877–1887. [PubMed: 12606325]

Duval DL, Ellsworth BS, Clay CM. Is gonadotrope expression of the gonadotropin releasing hormone receptor gene mediated by autocrine/prarcrine stimulation of an activin response element? Endocrinology. 1999; 140:1949–1952. [PubMed: 10098536]

Ellsworth BS, Burns AT, Escudero KW, Duval DL, Nelson SE, Clay CM. The gonadotropin releasing hormone (GnRH) receptor activating sequence (GRAS) is a composite regulatory element that interacts with multiple classes of transcription factors including Smads, AP-1 and a forkhead DNA binding protein. Mol. Cell. Endocrinol. 2003; 206:93–111. [PubMed: 12943993]

Ellsworth BS, Egashira N, Haller JL, Butts DL, Cocquet J, Clay CM, Osamura RY, Camper SA. FOXL2 in the pituitary: molecular, genetic, and developmental analysis. Mol. Endocrinol. 2006; 20:2796–2805. [PubMed: 16840539]

Ericson J, Norlin S, Jessell TM, Edlund T. Integrated FGF and BMP signaling controls the progression of progenitor cell differentiation and the emergence of pattern in the embryonic anterior pituitary. Development. 1998; 125:1005–1015. [PubMed: 9463347]

Essers MA, Weijzen S, de Vries-Smits AM, Saarloos I, de Ruiter ND, Bos JL, Burgering BM. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. EMBO J. 2004; 23:4802–4812. [PubMed: 15538382]

Flanagan SP. 'Nude', a new hairless gene with pleiotropic effects in the mouse. Genet Res. 1966; 8:295–309. [PubMed: 5980117]

Furuyama T, Nakazawa T, Nakano I, Mori N. Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. Biochem. J. 2000; 349:629–634. [PubMed: 10880363]

Ghochani Y, Saini JK, Mellon PL, Thackray VG. FOXL2 is involved in the synergy between activin and progestins on the follicle-stimulating hormone beta-subunit promoter. Endocrinology. 2012; 153:2023–2033. [PubMed: 22294749]

Godfrey VL, Wilkinson JE, Rinchik EM, Russell LB. Fatal lymphoreticular disease in the scurfy (sf) mouse requires T cells that mature in a sf thymic environment: potential model for thymic education. Proc Natl Acad Sci U S A. 1991; 88:5528–5532. [PubMed: 2062835]

Goertz MJ, Wu Z, Gallardo TD, Hamra FK, Castrillon DH. Foxo1 is required in mouse spermatogonial stem cells for their maintenance and the initiation of spermatogenesis. J Clin Invest. 2011; 121:3456–3466. [PubMed: 21865646]

Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene. 2005; 24:7410–7425. [PubMed: 16288288]

Gregory SJ, Lacza CT, Detz AA, Xu S, Petrillo LA, Kaiser UB. Synergy between activin A and gonadotropin-releasing hormone in transcriptional activation of the rat follicle-stimulating hormone-beta gene. Mol. Endocrinol. 2005; 19:237–254. [PubMed: 15375186]

Gumbel JH, Patterson EM, Owusu SA, Kabat BE, Jung DO, Simmons J, Hopkins T, Ellsworth BS. The forkhead transcription factor, Foxd1, is necessary for pituitary luteinizing hormone expression in mice. PLoS One. 2012; 7:e52156. [PubMed: 23284914]

Halvorson LM, Kaiser UB, Chin WW. Stimulation of luteinizing hormone beta gene promoter activity by the orphan nuclear receptor, steroidogenic factor-1. J. Biol. Chem. 1996; 271:6645–6650. [PubMed: 8636081]

Hannenhalli S, Kaestner KH. The evolution of Fox genes and their role in development and disease. Nat Rev Genet. 2009; 10:233–240. [PubMed: 19274050]

Hatini V, Huh SO, Herzlinger D, Soares VC, Lai E. Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of Winged Helix transcription factor BF-2. Genes Dev. 1996; 10:1467–1478. [PubMed: 8666231]

Hatini V, Tao W, Lai E. Expression of winged helix genes, BF-1 and BF-2, define adjacent domains within the developing forebrain and retina. J Neurobiol. 1994; 25:1293–1309. [PubMed: 7815060]

Herrera E, Marcus R, Li S, Williams SE, Erskine L, Lai E, Mason C. Foxd1 is required for proper formation of the optic chiasm. Development. 2004; 131:5727–5739. [PubMed: 15509772]

Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003; 299:1057–1061. [PubMed: 12522256]

Hosaka T, Biggs WH 3rd, Tieu D, Boyer AD, Varki NM, Cavenee WK, Arden KC. Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. Proc. Natl. Acad. Sci. USA. 2004; 101:2975–2980. [PubMed: 14978268]

Jackson BC, Carpenter C, Nebert DW, Vasiliou V. Update of human and mouse forkhead box (FOX) gene families. Hum Genomics. 2010; 4:345–352. [PubMed: 20650821]

Jacobs FM, van der Heide LP, Wijchers PJ, Burbach JP, Hoekman MF, Smidt MP. FoxO6, a novel member of the FoxO class of transcription factors with distinct shuttling dynamics. J. Biol. Chem. 2003; 278:35959–35967. [PubMed: 12857750]

Japon MA, Rubinstein M, Low MJ. In situ hybridization analysis of anterior pituitary hormone gene expression during fetal mouse development. J. Histochem. Cytochem. 1994; 42:1117–1125. [PubMed: 8027530]

Jung DO, Jasurda JS, Egashira N, Ellsworth BS. The forkhead transcription factor, FOXP3, is required for normal pituitary gonadotropin expression in mice. Biol Reprod. 2012; 86:144, 141–149. [PubMed: 22357547]

Justice NJ, Blount AL, Pelosi E, Schlessinger D, Vale W, Bilezikjian LM. Impaired FSHbeta expression in the pituitaries of Foxl2 mutant animals. Mol Endocrinol. 2011; 25:1404–1415. [PubMed: 21700720]

Kaiser UB, Halvorson LM, Chen MT. Sp1, steroidogenic factor 1 (SF-1), and early growth response protein 1 (egr-1) binding sites form a tripartite gonadotropin-releasing hormone response element in the rat luteinizing hormone-beta gene promoter: an integral role for SF-1. Mol. Endocrinol. 2000; 14:1235–1245. [PubMed: 10935547]

Kalinichenko VV, Gusarova GA, Shin B, Costa RH. The forkhead box F1 transcription factor is expressed in brain and head mesenchyme during mouse embryonic development. Gene Expr Patterns. 2003; 3:153–158. [PubMed: 12711542]

Keri RA, Nilson JH. A steroidogenic factor-1 binding site is required for activity of the luteinizing hormone beta subunit promoter in gonadotropes of transgenic mice. J. Biol. Chem. 1996; 271:10782–10785. [PubMed: 8631889]

Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol. 2003; 4:337–342. [PubMed: 12612581]

Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, Huang TT, Bos JL, Medema RH, Burgering BM. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature. 2002; 419:316–321. [PubMed: 12239572]

Lahl K, Loddenkemper C, Drouin C, Freyer J, Arnason J, Eberl G, Hamann A, Wagner H, Huehn J, Sparwasser T. Selective depletion of Foxp3+ regulatory T cells induces a scurfy-like disease. J Exp Med. 2007; 204:57–63. [PubMed: 17200412]

Lamba P, Fortin J, Tran S, Wang Y, Bernard DJ. A novel role for the forkhead transcription factor FOXL2 in activin A-regulated follicle-stimulating hormone beta subunit transcription. Mol. Endocrinol. 2009; 23:1001–1013. [PubMed: 19324968]

Lamba P, Santos MM, Philips DP, Bernard DJ. Acute regulation of murine follicle-stimulating hormone beta subunit transcription by activin A. J. Mol. Endocrinol. 2006; 36:201–220. [PubMed: 16461939]

Lamba P, Wang Y, Tran S, Ouspenskaia T, Libasci V, Hebert TE, Miller GJ, Bernard DJ. Activin A regulates porcine follicle-stimulating hormone {beta}-subunit transcription via cooperative actions of SMADs and FOXL2. Endocrinology in press. 2010

Lawson MA, Tsutsumi R, Zhang H, Talukdar I, Butler BK, Santos SJ, Mellon PL, Webster NJ. Pulse sensitivity of the luteinizing hormone beta promoter is determined by a negative feedback loop Involving early growth response-1 and Ngfi-A binding protein 1 and 2. Mol. Endocrinol. 2007; 21:1175–1191. [PubMed: 17299135]

Levinson RS, Batourina E, Choi C, Vorontchikhina M, Kitajewski J, Mendelsohn CL. Foxd1-dependent signals control cellularity in the renal capsule, a structure required for normal renal development. Development. 2005; 132:529–539. [PubMed: 15634693]

Lin W, Truong N, Grossman WJ, Haribhai D, Williams CB, Wang J, Martin MG, Chatila TA. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. J Allergy Clin Immunol. 2005; 116:1106–1115. [PubMed: 16275384]

Liu Z, Castrillon DH, Zhou W, Richards JS. FOXO1/3 depletion in granulosa cells alters follicle growth, death and regulation of pituitary FSH. Mol Endocrinol. 2013; 27:238–252. [PubMed: 23322722]

Lowther DE, Hafler DA. Regulatory T cells in the central nervous system. Immunol Rev. 2012; 248:156–169. [PubMed: 22725960]

Majumdar S, Farris CL, Kabat BE, Jung DO, Ellsworth BS. Forkhead Box O1 is present in quiescent pituitary cells during development and is increased in the absence of p27 Kip1. PLoS One. 2012; 7:e52136. [PubMed: 23251696]

McCue JM, Quirk CC, Nelson SE, Bowen RA, Clay CM. Expression of a murine gonadotropin-releasing hormone receptor-luciferase fusion gene in transgenic mice is diminished by immunoneutralization of gonadotropin-releasing hormone. Endocrinology. 1997; 138:3154–3160. [PubMed: 9231762]

McGillivray SM, Thackray VG, Coss D, Mellon PL. Activin and glucocorticoids synergistically activate follicle-stimulating hormone β-subunit gene expression in the immortalized LβT2 gonadotrope cell line. Endocrinology. 2007; 148:762–773. [PubMed: 17082263]

Michaelis KA, Knox AJ, Xu M, Kiseljak-Vassiliades K, Edwards MG, Geraci M, Kleinschmidt-DeMasters BK, Lillehei KO, Wierman ME. Identification of growth arrest and DNA-damage-inducible gene beta (GADD45beta) as a novel tumor suppressor in pituitary gonadotrope tumors. Endocrinology. 2011; 152:3603–3613. [PubMed: 21810943]

Nakae J, Oki M, Cao Y. The FoxO transcription factors and metabolic regulation. FEBS Lett. 2008; 582:54–67. [PubMed: 18022395]

Norquay LD, Yang X, Jin Y, Detillieux KA, Cattini PA. Hepatocyte nuclear factor-3alpha binding at P sequences of the human growth hormone locus is associated with pituitary repressor function. Mol Endocrinol. 2006; 20:598–607. [PubMed: 16239259]

Norwitz ER, Cardona GR, Jeong KH, Chin WW. Identification and characterization of the gonadotropin-releasing hormone response elements in the mouse gonadotropin-releasing hormone receptor gene. J. Biol. Chem. 1999; 274:867–880. [PubMed: 9873026]

Norwitz ER, Xu S, Jeong KH, Bedecarrats GY, Winebrenner LD, Chin WW, Kaiser UB. Activin A augments GnRH-mediated transcriptional activation of the mouse GnRH receptor gene. Endocrinology. 2002; 143:985–997. [PubMed: 11861523]

Obsil T, Obsilova V. Structure/function relationships underlying regulation of FOXO transcription factors. Oncogene. 2008; 27:2263–2275. [PubMed: 18391969]

Paik JH, Kollipara R, Chu G, Ji H, Xiao Y, Ding Z, Miao L, Tothova Z, Horner JW, Carrasco DR, Jiang S, Gilliland DG, Chin L, Wong WH, Castrillon DH, DePinho RA. FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. Cell. 2007; 128:309–323. [PubMed: 17254969]

Pantelouris EM, Hair J. Thymus dysgenesis in nude (nu nu) mice. J Embryol Exp Morphol. 1970; 24:615–623. [PubMed: 5493276]

Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. Ann. Rev. Biochem. 1981; 50:465–495. [PubMed: 6267989]

Pierrou S, Hellqvist M, Samuelsson L, Enerback S, Carlsson P. Cloning and characterization of seven human forkhead proteins: binding site specificity and DNA bending. EMBO J. 1994; 13:5002–5012. [PubMed: 7957066]

Pisarska MD, Kuo FT, Bentsi-Barnes IK, Khan S, Barlow GM. LATS1 phosphorylates forkhead L2 and regulates its transcriptional activity. Am. J. Physiol. Endocrinol. Metab. 2010; 299:E101–109. [PubMed: 20407010]

Rosenberg SB, Mellon PL. An Otx-related homeodomain protein binds an LHβ promoter element important for activation during gonadotrope maturation. Mol. Endocrinol. 2002; 16:1280–1298. [PubMed: 12040015]

Savino W, Arzt E, Dardenne M. Immunoneuroendocrine connectivity: the paradigm of the thymus-hypothalamus/pituitary axis. Neuroimmunomodulation. 1999; 6:126–136. [PubMed: 9876243]

Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, Treier AC, Treier M. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development. 2004; 131:933–942. [PubMed: 14736745]

Seeburg PH, Mason AJ, Stewart TA, Nikolics K. The mammalian GnRH gene and its pivotal role in reproduction. Recent Prog. Horm. Res. 1987; 43:69–98. [PubMed: 3306841]

Skarra DV, Arriola DJ, Benson CA, Thackray VG. Forkhead Box O1 Is a Repressor of Basal and GnRH-Induced Fshb Transcription in Gonadotropes. Mol Endocrinol. 2013 In Press.

Suszko MI, Lo DJ, Suh H, Camper SA, Woodruff TK. Regulation of the rat follicle-stimulating hormone beta-subunit promoter by activin. Mol. Endocrinol. 2003; 17:318–332. [PubMed: 12554780]

Tang ED, Nunez G, Barr FG, Guan KL. Negative regulation of the forkhead transcription factor FKHR by Akt. J. Biol. Chem. 1999; 274:16741–16746. [PubMed: 10358014]

Tissenbaum HA, Ruvkun G. An insulin-like signaling pathway affects both longevity and reproduction in Caenorhabditis elegans. Genetics. 1998; 148:703–717. [PubMed: 9504918]

Tran S, Lamba P, Wang Y, Bernard DJ. SMADs and FOXL2 synergistically regulate murine FSHbeta transcription via a conserved proximal promoter element. Mol. Endocrinol. 2011; 25:1170–1183. [PubMed: 21622537]

Tran S, Zhou X, Lafleur C, Calderon MJ, Ellsworth BS, Kimmins S, Boehm U, Treier M, Boerboom D, Bernard DJ. Impaired fertility and FSH synthesis in gonadotrope-specific Foxl2 knockout mice. Mol Endocrinol. 2013; 27:407–421. [PubMed: 23340250]

Treier M, Gleiberman AS, O'Connell SM, Szeto DP, McMahon JA, McMahon AP, Rosenfeld MG. Multistep signaling requirements for pituitary organogenesis in vivo. Genes Dev. 1998; 12:1691–1704. [PubMed: 9620855]

Tsai KL, Sun YJ, Huang CY, Yang JY, Hung MC, Hsiao CD. Crystal structure of the human FOXO3a-DBD/DNA complex suggests the effects of post-translational modification. Nucleic Acids Res. 2007; 35:6984–6994. [PubMed: 17940099]

Tzivion G, Dobson M, Ramakrishnan G. FoxO transcription factors; Regulation by AKT and 14-3-3 proteins. Biochim Biophys Acta. 2011; 1813:1938–1945. [PubMed: 21708191]

Uda M, Ottolenghi C, Crisponi L, Garcia JE, Deiana M, Kimber W, Forabosco A, Cao A, Schlessinger D, Pilia G. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum. Mol. Genet. 2004; 13:1171–1181. [PubMed: 15056605]

Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, Treier AC, Klugmann C, Klasen C, Holter NI, Riethmacher D, Schutz G, Cooney AJ, Lovell-Badge R, Treier M. Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell. 2009; 139:1130–1142. [PubMed: 20005806]

Uhlenhaut NH, Treier M. Forkhead transcription factors in ovarian function. Reproduction. 2011; 142:489–495. [PubMed: 21810859]

Vale W, Rivier C, Brown M. Regulatory peptides of the hypothalamus. Ann. Rev. Physiol. 1977; 39:473–527. [PubMed: 15499]

Van Der Heide LP, Hoekman MF, Smidt MP. The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. Biochem. J. 2004; 380:297–309. [PubMed: 15005655]

van der Horst A, Tertoolen LG, de Vries-Smits LM, Frye RA, Medema RH, Burgering BM. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). J. Biol. Chem. 2004; 279:28873–28879. [PubMed: 15126506]

Verdin H, De Baere E. FOXL2 impairment in human disease. Horm Res Paediatr. 2012; 77:2–11. [PubMed: 22248822]

Villarejo-Balcells B, Guichard S, Rigby PW, Carvajal JJ. Expression pattern of the FoxO1 gene during mouse embryonic development. Gene Expr Patterns. 2011; 11:299–308. [PubMed: 21397048]

Wang Y, Martin JF, Bai CB. Direct and indirect requirements of Shh/Gli signaling in early pituitary development. Dev Biol. 2010; 348:199–209. [PubMed: 20934421]

Weck J, Anderson AC, Jenkins S, Fallest PC, Shupnik MA. Divergent and composite gonadotropin-releasing hormone-responsive elements in the rat luteinizing hormone subunit genes. Mol. Endocrinol. 2000; 14:472–485. [PubMed: 10770486]

Weigel D, Jurgens G, Kuttner F, Seifert E, Jackle H. The homeotic gene fork head encodes a nuclear protein and is expressed in the terminal regions of the Drosophila embryo. Cell. 1989; 57:645–658. [PubMed: 2566386]

Wen S, Schwarz JR, Niculescu D, Dinu C, Bauer CK, Hirdes W, Boehm U. Functional characterization of genetically labeled gonadotropes. Endocrinology. 2008; 149:2701–2711. [PubMed: 18325995]

White BR, Duval DL, Mulvaney JM, Roberson MS, Clay CM. Homologous regulation of the gonadotropin-releasing hormone receptor gene is partially mediated by protein kinase C activation of an Activator Protein-1 element. Mol. Endorinol. 1999; 13:566–577.

Wijchers PJ, Burbach JP, Smidt MP. In control of biology: of mice, men and Foxes. Biochem. J. 2006; 397:233–246. [PubMed: 16792526]

Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet. 2002; 39:537–545. [PubMed: 12161590]

Woodruff TK, Besecke LM, Groome N, Draper LB, Schwartz NB, Weiss J. Inhibin A and inhibin B are inversely correlated to follicle-stimulating hormone, yet are discordant during the follicular phase of the rat estrous cycle, and inhibin A is expressed in a sexually dimorphic manner. Endocrinology. 1996; 137:5463–5467. [PubMed: 8940372]

Wu S, Wolfe A. Signaling of cytokines is important in regulation of GnRH neurons. Mol Neurobiol. 2012; 45:119–125. [PubMed: 22161498]

Zannini M, Avantaggiato V, Biffali E, Arnone MI, Sato K, Pischetola M, Taylor BA, Phillips SJ, Simeone A, Di Lauro R. TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is consistent with a role in controlling the onset of differentiation. EMBO J. 1997; 16:3185–3197. [PubMed: 9214635]

Highlights

- Loss of FOXD1 in the pituitary mesenchyme results in decreased *Lhb* transcription
- FOXL2 is essential for activin-induced *Fshb* transcription and FSH production
- FOXL2 also regulates follistatin, *Gnrhr* and *Cga* gene expression
- FOXO1 suppresses *Lhb* and *Fshb* transcription, potentially via PITX1, SF1 and EGR1
- FOXP3 is required for gonadotropin hormone synthesis and fertility in male mice

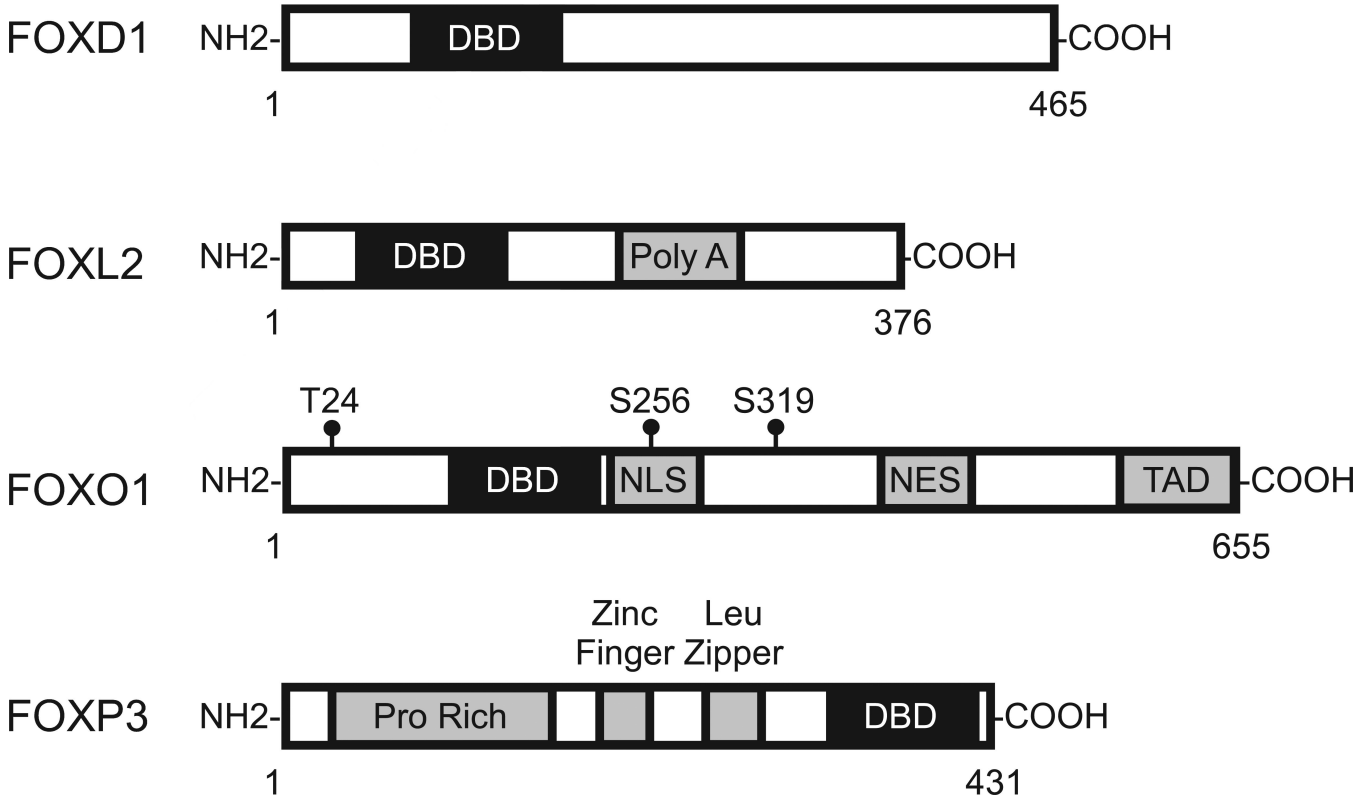

Figure 1.
Structural organization of the FOXD1, FOXL2, FOXO1, and FOXP3 proteins. Numbering of the amino acids is relevant to the human proteins. Abbreviations: DBD, DNA-binding domain; Poly A, polyalanine tract; NLS, nuclear localization signal; NES, nuclear export signal; TAD, transactivation domain;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

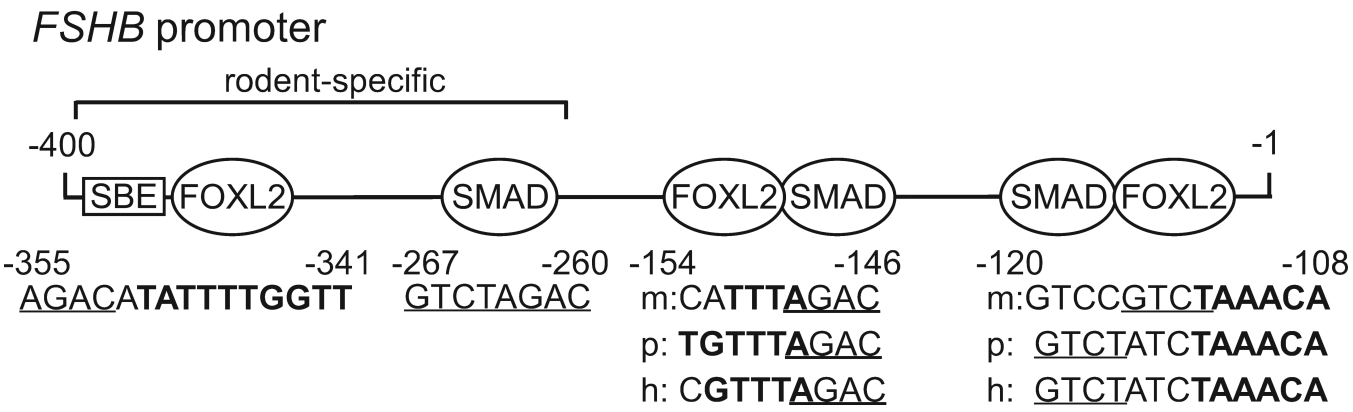

Figure 2.
Schematic of SMAD and FOXL2 binding elements on the *FSHB* promoter. SBE, Smad-binding element; m, murine; p, porcine; h, human. SBEs are underlined and FOXL2 binding elements are in bold.

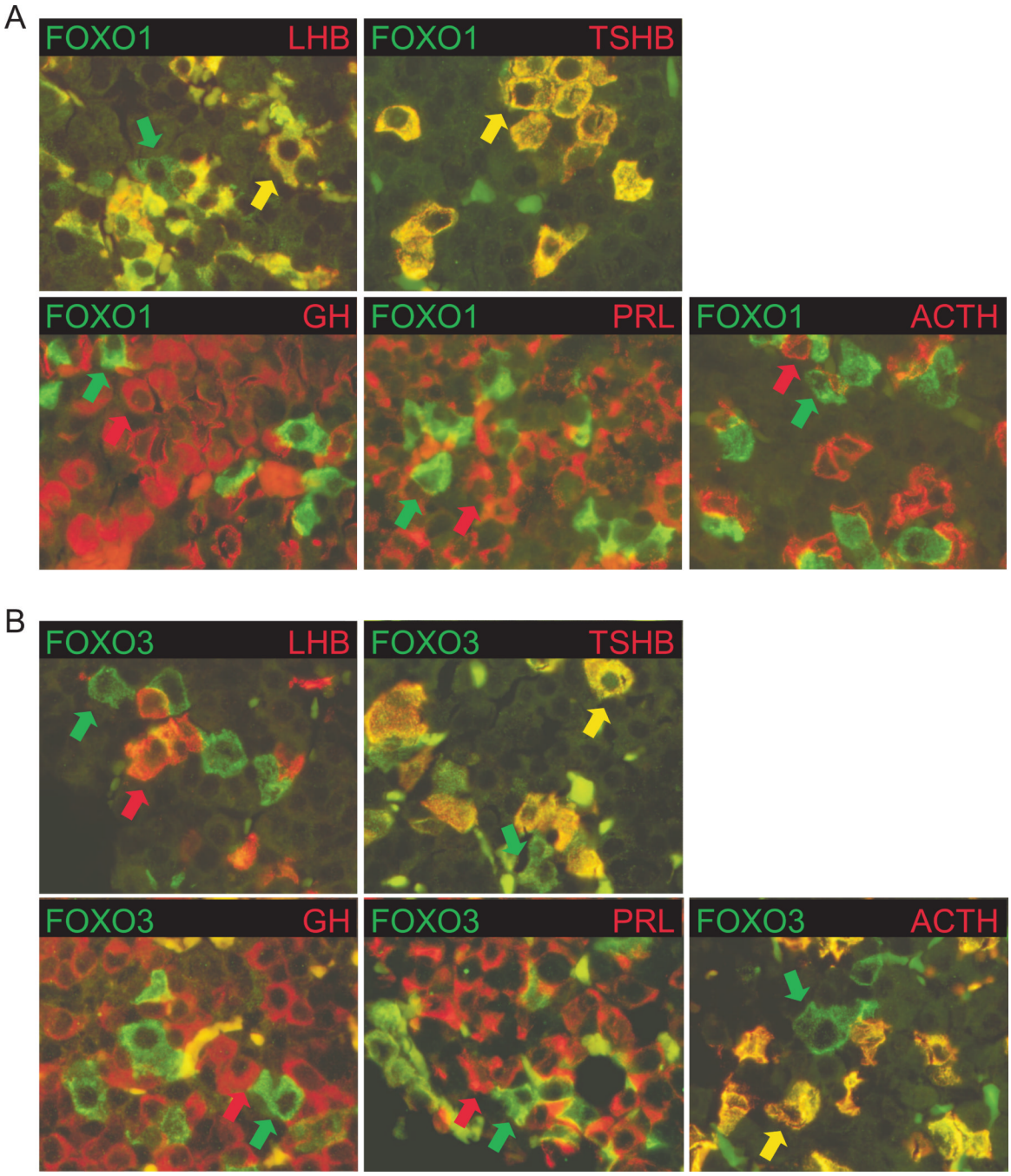

Figure 3.

FOXO1 is expressed in adult murine gonadotropes and thyrotropes while FOXO3 is expressed in thyrotropes and corticotropes. Adult murine pituitary tissue sections were processed and imaged at 40× magnification, as described previously (Arriola et al., 2012). Dual-fluorescence labeling was performed on the same section with A) rabbit anti-human FOXO1 (H-128; Santa Cruz Biotechnology; 1:100 dilution in 10% goat serum/0.3% Triton X-100) or B) anti-human FOXO3 (75D8; Cell Signaling Technology, Inc.; 1:200) and either guinea pig anti-rat LHB (1:200), TSHB (1:200), GH (1:200), PRL (1:10,000), or ACTH (1:10,000) primary antibodies from the NIDDK National Hormone and Pituitary Program for 48 h at 4°C. The sections were then incubated with goat anti-rabbit and anti-guinea pig

Alexa Fluor 488 and 594 (Invitrogen; 1:400) secondary antibodies for 1 h at room temperature. Red arrows indicate LHB, TSHB, GH, PRL or ACTH. Green arrows indicate FOXO1 (A) or FOXO3 (B). Yellow arrows indicate FOXO colocalization with proteins representing the five endocrine cell types in the anterior pituitary. Yellow signal in FOXO3-GH image is due to auto fluorescence of erythrocytes.