

Steroidogenic factor 1 (SF1) is essential for pituitary gonadotrope function

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SUMMARY

Knockout mice lacking the orphan nuclear receptor steroidogenic factor 1 (SF1) exhibit a complex endocrine phenotype that includes adrenal and gonadal agenesis, impaired expression of pituitary gonadotropins, and absence of the ventromedial hypothalamic nucleus (VMH). These multiple defects complicate efforts to delineate primary versus secondary effects of SF1 deficiency in different tissues, such that its direct role in gonadotropes remains uncertain. To define this role, we have expressed Cre recombinase driven by the promoter region of the common α subunit of glycoprotein hormones (α GSU), thereby inactivating a loxP-modified SF1 locus in the anterior pituitary gland. Although pituitary-specific SF1 knockout mice were fully viable, they were sterile and failed to develop normal secondary sexual characteristics.

Their adrenal glands and VMH appeared normal histologically, but their testes and ovaries were severely hypoplastic. α GSU-Cre, loxP mice had normal levels of most pituitary hormones, but had markedly decreased expression of LH and FSH. Treatment with exogenous gonadotropins stimulated gonadal steroidogenesis, inducing germ cell maturation in males and follicular and uterine maturation in females – establishing that the gonads can respond to gonadotropins. The pituitary-specific SF1 knockout mice are a novel genetic model of hypogonadotropic hypogonadism that establishes essential role(s) of SF1 in pituitary gonadotropes.

Key words: Hypogonadotropic hypogonadism, Orphan nuclear receptor, Gonadotropins, Tissue-specific knockout, Mouse

INTRODUCTION

Mammalian reproduction requires complex, reciprocal interactions among gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus, gonadotropes in the anterior pituitary, and the testes or ovaries. Because of this complexity, mutations in many genes can impair reproductive function (reviewed by Achermann and Jameson, 1999; Adashi and Hennebold, 1999). One such gene encodes the orphan nuclear receptor steroidogenic factor 1 (SF1; Nr5a1 – Mouse Genome Informatics), which plays essential roles at multiple levels of the reproductive axis (reviewed by Parker and Schimmer, 1997; Morohashi and Omura, 1996). SF1 knockout mice have adrenal and gonadal agenesis (Luo et al., 1994; Sadovsky et al., 1995) and ablation of the VMH (Ikeda et al., 1995; Shinoda et al., 1995), establishing essential roles of SF1 in these sites.

The role of SF1 in the pituitary is less clear. The pituitaries of SF1 knockout mice have markedly decreased expression of both pituitary gonadotropins – luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Ingraham et al., 1994; Shinoda et al., 1995). Moreover, promoter analyses in transfected cells and transgenic mice suggest that SF1 directly regulates the expression of α GSU (Barnhart and Mellon, 1994; Ingraham et al., 1994), LH β (Halvorson et al., 1996; Keri and Nilson, 1996; Le Drean et al., 1997; Halvorson et al., 1998;

Tremblay and Drouin, 1999; Wolfe, 1999; Dorn et al., 1999), FSH β (Brown and McNeilly 1997) and the GnRH receptor (Duval et al., 1997; Ngan et al., 1999). These results argue that SF1 is an important mediator of gonadotrope function. However, GnRH treatment of SF1 knockout mice induced pituitary expression of gonadotropins (Ikeda et al., 1995), indicating that gonadotropes can express presumptive SF1 target genes in the absence of SF1. Moreover, a human with adrenal insufficiency and 46, XY sex reversal due to a mutation of SF1 had elevated – rather than decreased – gonadotropin levels (Achermann et al., 1999).

Thus, we still do not know if SF1 plays direct roles in pituitary gonadotropes. To resolve this matter, we have used the Cre-loxP system to inactivate SF1 selectively in pituitary gonadotropes and thyrotropes. Our results establish definitively that pituitary expression of SF1 is essential for normal reproductive development and provide a novel genetic model of hypogonadotropic hypogonadism.

MATERIALS AND METHODS

Generation of mice

All experiments involving mice were approved by the Institutional Animal Care Research Advisory Committee at UT Southwestern. The

α GSU-Cre transgene contained 4.6 kb of 5'-flanking region from the mouse α GSU gene (Kendall et al., 1994) cloned upstream of the bacteriophage P1 Cre recombinase with a nuclear localization signal (Cushman et al., 2001). Histochemical analyses of β -galactosidase activity in newborn mice carrying a Cre-dependent *lacZ* reporter gene revealed strong blue staining in the anterior and intermediate lobes of the pituitary gland, with weaker staining in the heart, lungs and skeletal muscle (Cushman et al., 2001). These findings support the ability of the α GSU-Cre transgene to target Cre expression to the anterior pituitary, but suggest that caution must be taken in interpreting potential effects in other tissues that express Cre recombinase at lower levels.

Homologous recombination in E14TG2a mouse embryonic stem (ES) cells was used to place loxP sites 5' and 3' of the last exon of the *Ftzf1* (*Nr5a1* – Mouse Genome Informatics) gene encoding SF1, also incorporating a neomycin resistance cassette 3' to the structural gene (see Fig. 1 for a schematic diagram of the targeting strategy). This exon encodes the C-terminal region of SF1 – including the AF-2 motif that is essential for SF1 transcriptional activation (Ito et al., 1998; Crawford et al., 1997) – and also contains the transcription termination/polyadenylation signals. Following blastocyst injection of targeted ES clones, chimeric mice transmitted the floxed SF1 allele to produce mice that were heterozygous at the *Nr5a1* allele (i.e. +/F). These mice exhibited no apparent phenotypic abnormalities and were fully fertile, allowing us to cross them with mice carrying the α GSU-Cre transgene. Presumably due to ectopic Cre expression in germ cells, the floxed SF1 locus occasionally underwent generalized recombination, yielding a restriction fragment of altered size in Southern blotting analyses (Fig. 1). Mice homozygous for this recombined allele (i.e. R/R) died in the perinatal period with adrenal and gonadal agenesis (L. Z., unpublished observation), establishing the severe effect of globally deleting the last exon of SF1.

In the studies described here, we have analyzed mice carrying one floxed allele (F) and one recombined (R) allele and the α GSU-Cre transgene (i.e. F/R, α GSU-Cre mice), which consistently exhibited a severe reproductive phenotype. In preliminary studies, F/F, α GSU-Cre mice had a reproductive phenotype intermediate between wild-type, α GSU-Cre mice and F/R, α GSU-Cre mice, suggesting a dosage effect for Cre inactivation of SF1 (L. Z., unpublished observations).

Tissue analyses, hormone assays and gonadotropin treatment of tissue-specific knockout mice

Levels of SF1 protein were determined by immunohistochemical analyses with rabbit anti-bovine SF1 antiserum (a generous gift from Dr Ken Morohashi), biotinylated goat anti-rabbit secondary antiserum and the Vectastain ABC kit (Vector Laboratories, Burlingame, CA), according to the recommended protocol. Immunohistochemical analyses of pituitary trophic hormones were performed with antibodies from the National Hormone and Pituitary Program (NIDDK). Immunohistochemical analyses of GnRH used the LR-1 antibody (a generous gift from Dr Robert Benoit) at a 1:10,000 dilution. Serum levels of FSH and LH were measured by radioimmunoassay using reagents from the National Hormone and Pituitary Program. With the LH assay, only values for males clearly exceeded the lower limit of sensitivity of the assay.

For gonadotropin rescue experiments, mice at 6 weeks of age were injected intraperitoneally with 5 IU pregnant mare's serum gonadotropins (PMSG) daily for 10 days, and then the relevant organs were harvested, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned for histological analyses. For GnRH rescue experiments, adult F/R, α GSU-Cre mice were treated with GnRH (60 ng intraperitoneally every 2 hours for 2 days) and then the pituitary glands were harvested and used for immunohistochemical analyses as previously described (Ikeda et al., 1995).

Gene expression analysis

After genotyping, tissues were harvested, pooled to provide sufficient

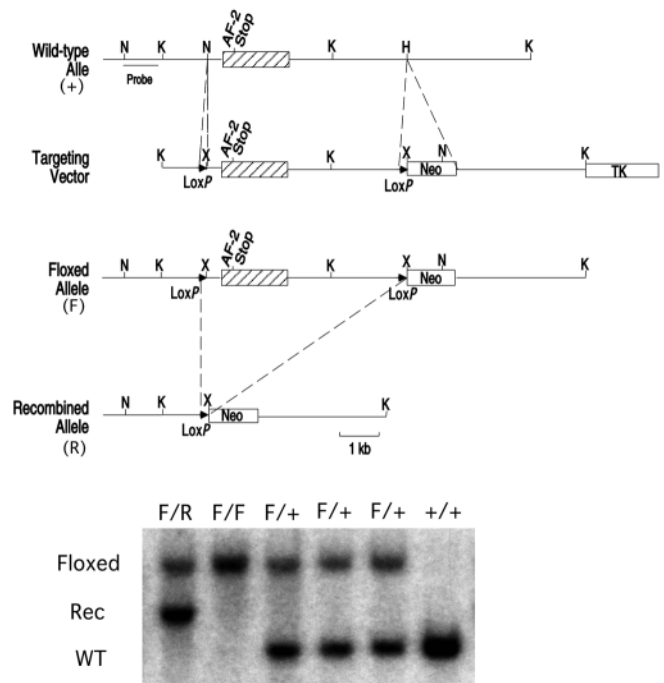


Fig. 1. Pituitary-specific inactivation of SF1. (A) Introduction of loxP sites within *Ftzf1* locus by homologous recombination in ES cells. The wild-type allele, targeting vector, floxed allele (F), and recombined allele (R) are indicated, as is the probe used for Southern blotting analyses. H, *HindIII*; K, *KpnI*; N, *NcoI*. (B) Southern blotting analysis showing different alleles in mice of different genotypes.

material and total RNA was prepared using the RNazol method according to the manufacturer's protocol. Levels of gene expression were determined by semi-quantitative RT-PCR assay as described (Lee et al., 1996), using the following specific primer pairs: SF1 (5'-AAATTCCTGAACAACCACAGC-3' forward and 5'-GCATCTCAATGAGAAGGTTG-3' reverse); LH β (5'-GCCGGCCTGTCAACGCAACC-3' forward and 5'-GAGGGCCACAGGGAAGGAGA-3' reverse); FSH β (5'-TGAAGTACCAACATCACC-3' forward and 5'-ACTATCACACTTGCCACAGT-3' reverse); TSH β (5'-CTCGGATCCGTGCTTTTTGCTCTTGC-3' forward and 5'-AAAAGCTTCCAGATAGAAAGACTGCGG-3' reverse); the cholesterol side chain cleavage enzyme (5'-AGTGGCAGTCGTGGGGACAGT-3' forward and 5'-TAATACTGGTGATAGGCCGCC-3' reverse). Control reactions analyzed the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (5'-ACCACAGTCCATGCCATCAC-3' forward and 5'-TCCACCACCCTGTTGCTGTA-3' reverse).

RESULTS

SF1 expression is selectively impaired in the anterior pituitaries of F/R, α GSU-Cre mice

To begin to define specific roles of SF1 at different levels of the reproductive axis, we used the Cre-loxP strategy (Marth, 1996; Sauer, 1998) to inactivate SF1 selectively in cells expressing Cre recombinase driven by the regulatory sequences of mouse α GSU. This promoter normally is active in gonadotropes – where SF1 is proposed to play important roles – and in thyrotropes – which do not normally express SF1 (Kendall et al., 1994). As noted above, a Cre-dependent *lacZ*

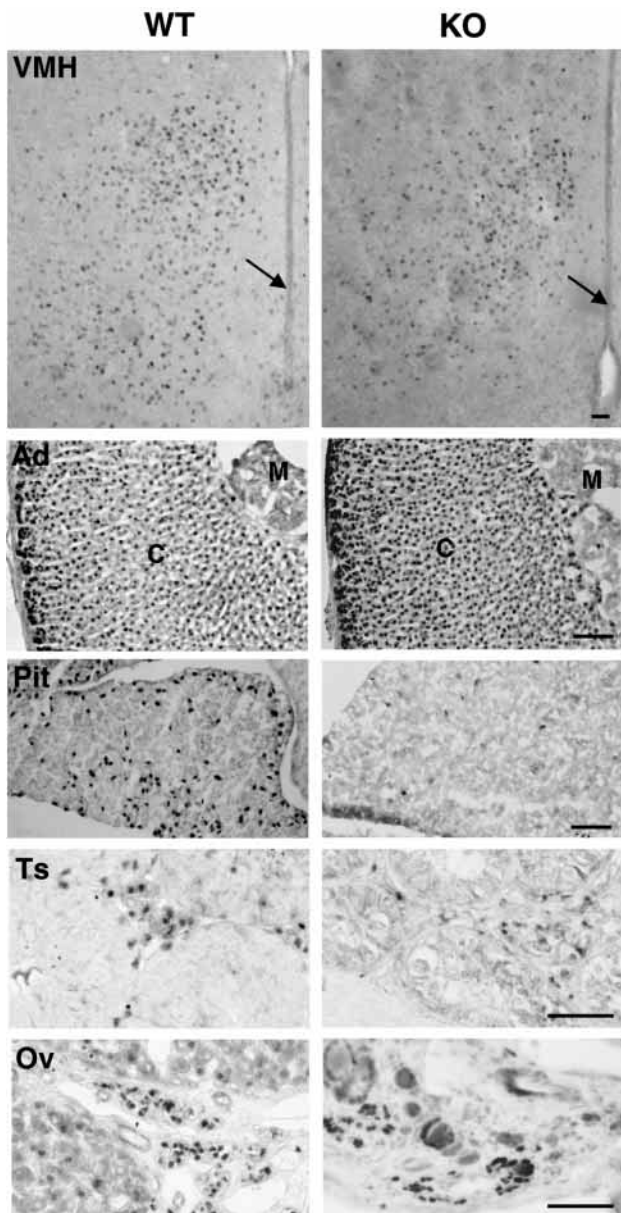


Fig. 2. SF1 expression is impaired specifically in the pituitary of pituitary-specific SF1 knockout mice. Tissues were obtained from wild-type (WT) or F/R, α GSU-Cre (KO) mice and SF1 immunoreactivity was determined as described in Materials and Methods. The arrows indicate the third ventricle. Ad, adrenal gland; C, cortex; M, medulla; Ov, ovary; Pit, pituitary gland; Ts, testis; VMH, ventromedial hypothalamic nucleus; Scale bars: 50 μ m.

reporter gene also was expressed in other sites (e.g., heart, lung, skeletal muscle), suggesting that the α GSU-Cre transgene also targeted Cre expression to sites where the α GSU promoter normally is not active. Because these sites do not express SF1, it is highly unlikely that the phenotype described below reflects Cre-mediated modification of SF1 in these other sites.

Based on in situ hybridization analyses, expression of α GSU commences at approximately embryonic day (E) 12.5 (Japon et al., 1994), preceding the onset of pituitary expression of SF1 at \sim E13.5 (Ingraham et al., 1994). Moreover, expression of a

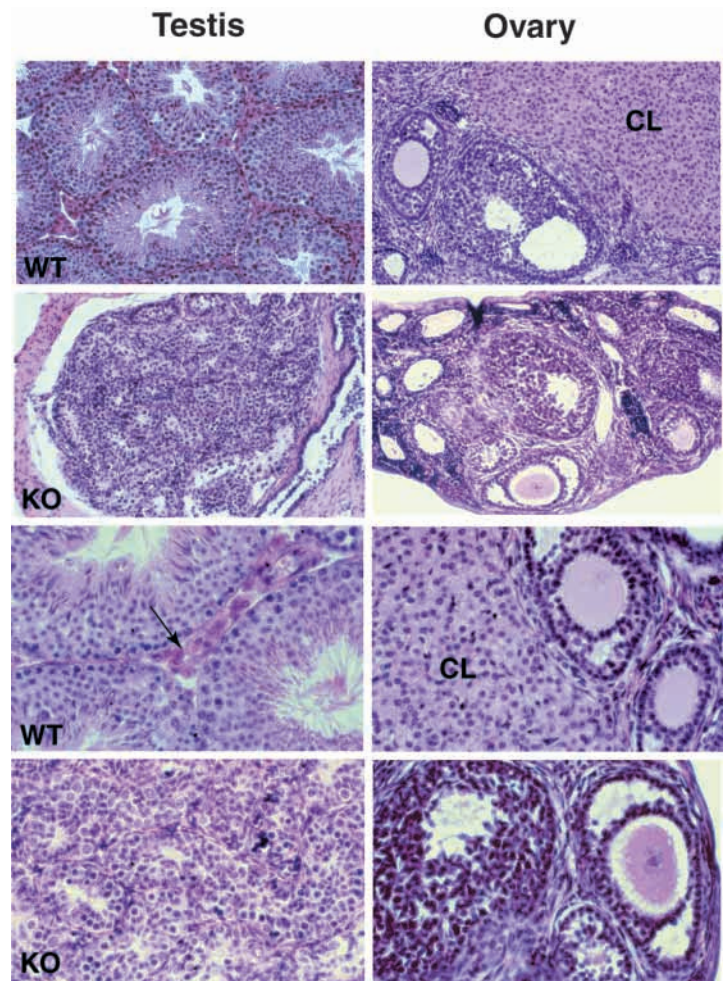


Fig. 3. Pituitary-specific knockout mice have hypoplastic gonads. Tissues obtained from wild-type (WT) or F/R, α GSU-Cre (KO) mice were processed for histological analysis as described in Materials and Methods. The top two rows show lower magnification photomicrographs and the lower two rows show higher power photomicrographs. CL, corpus luteum. The arrow indicates Leydig cells in the interstitial region.

Cre-dependent β -galactosidase reporter gene was detected in α GSU-Cre embryos at E12.5 (Cushman et al., 2001). Thus, although we have not yet determined the precise time when the *Ftzh1* locus is recombined in the pituitary, SF1 inactivation very likely commences in utero.

Mice homozygous for the loxP-modified (i.e. 'floxed') *Ftzh1* allele appeared normal and were fertile, as were mice carrying the α GSU-Cre transgene. In crosses of mice carrying the F and R *Ftzh1* alleles and the α GSU-Cre transgene, offspring with the F/R, α GSU-Cre genotype were born at the expected frequency and initially were indistinguishable from wild-type littermates (data not shown).

To assess the effect of this targeted genetic modification on SF1 expression in various tissues, we performed immunohistochemical analyses with an antibody raised against full-length bovine SF1. As shown in Fig. 2, SF1 immunoreactivity was normal in the VMH, adrenal cortex, testes and ovaries. In contrast, SF1 immunoreactivity in the anterior pituitary gland – the intended site of tissue-specific knockout – was reduced to background levels. Based on our

targeting strategy, we anticipated that the Cre-recombined *Ftzf1* allele would encode a truncated SF1 protein lacking 82 amino acids at its C terminus (Fig. 1); we further expected that this protein would still react with this antiserum raised against full-length SF1. The absence of SF1 immunoreactivity suggests that Cre-mediated recombination decreases either the stability of SF1 protein or the transcription or stability of SF1 transcripts.

Pituitary-specific SF1 knockout mice are sexually infantile

At the normal age of puberty, the vaginas in F/R, α GSU-Cre females did not open, while males had hypoplastic external and internal genitalia and cryptorchid testes. Significantly, both male and female F/R, α GSU-Cre mice were infertile and, even at 6 months of age, never exhibited signs of secondary sexual development. In addition, sex steroid-dependent tissues (e.g., seminal vesicles and prostate in males and uterus in females) were markedly hypoplastic (data not shown). These findings suggest that their synthesis of sex steroids is severely impaired.

To determine the basis for this sexual infantilism, we examined SF1-expressing tissues in F/R, α GSU-Cre mice. Consistent with their postnatal survival, the adrenal glands had well-defined cortex and medulla (Fig. 2). Similarly, the VMH and anterior pituitary of pituitary-specific SF1 knockout mice appeared grossly intact (Fig. 2). In contrast, the gonads were markedly hypoplastic (Fig. 3). The testes were organized into primitive seminiferous tubules containing Sertoli cells and immature spermatogonia, indicating that testis determination, germ cell migration, and sex cord formation were unimpaired. The germ cells were markedly diminished in number and failed to progress through the normal stages of spermatogenesis: occasional pachytene spermatocytes were seen, but mature spermatids were absent. In addition, Leydig cells in the interstitial region were severely decreased in number and showed none of the histological features characteristic of steroidogenic cells.

In the ovaries (Fig. 3, right panels), follicles formed and developed through the primary, secondary, and antral stages; however, neither large preovulatory follicles nor corpora lutea were observed. These findings – coupled with sexual infantilism of the accessory sex organs – strongly suggest that sex steroid production is impaired in the pituitary-specific SF1 knockout mice.

Gonadotropin expression is impaired in pituitary-specific SF1 knockout mice

Although the pituitaries of F/R, α GSU-Cre

mice appeared grossly intact in Hematoxylin and Eosin stained sections (Fig. 2), immunoreactive FSH and LH were virtually absent (Fig. 4), and serum FSH (33 ± 8.5 ng/ml wild-type males versus 2.8 ± 2.8 ng/ml F/R, α GSU-Cre males; 11.6 ± 7 ng/ml wild-type females versus 1.9 ± 1.1 ng/ml F/R, α GSU-Cre females) and LH (1.2 ng/ml wild-type males versus 0.29 ng/ml F/R, α GSU-Cre males) were markedly decreased. This effect was specific, as levels of corticotropin (ACTH) and thyroid-stimulating hormone (TSH) (Fig. 4), and growth hormone and prolactin (data not shown) all were comparable with those in wild-type pituitaries. The normal expression of TSH is especially significant, because it demonstrates a specific defect in gonadotropes rather than a nonspecific, toxic effect of Cre expression in both gonadotropes and thyrotropes. To confirm that the decreased gonadotropin expression in F/R, α GSU-Cre mice reflects decreased mRNA levels of SF1-responsive genes, we performed RT-PCR analyses. As shown in Fig. 5, transcripts for gonadotropins (LH β and FSH β) and the GnRH receptor were decreased markedly relative to wild-type mice. In contrast, normal levels of TSH β transcripts were observed, again establishing the gonadotrope-specific effect of the genetic manipulation of the *Ftzf1* locus. Although we cannot exclude an effect on mRNA stability, these results suggest strongly that the decreased gonadotropin expression in F/R, α GSU-Cre mice results from decreased transcription of multiple SF1 target genes in the pituitary. Testes expression of the cholesterol side chain cleavage enzyme also was decreased relative to wild-type levels; this diminution likely represents a secondary effect of gonadotropin deficiency, as described previously in the hypogonadal mice (O'Shaughnessy et al., 1998).

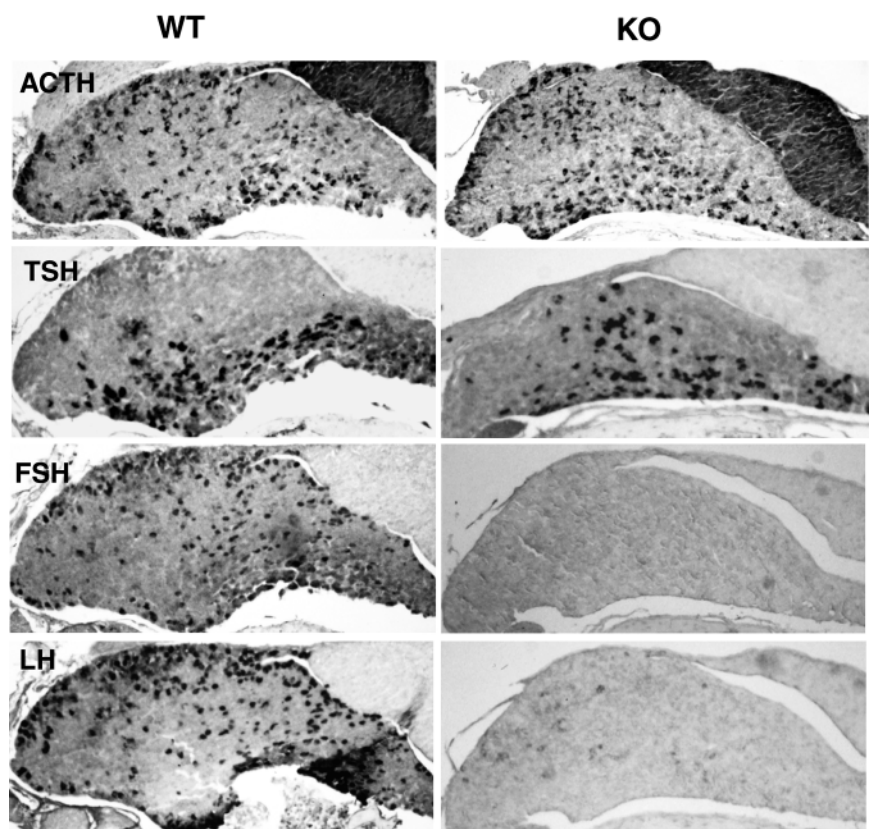


Fig. 4. Pituitary expression of gonadotropins is selectively impaired. Pituitary glands were harvested from wild-type (WT) and F/R, α GSU-Cre (KO) mice and immunoreactivities for mouse pituitary hormones were measured as described in Materials and Methods. ACTH, corticotropin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

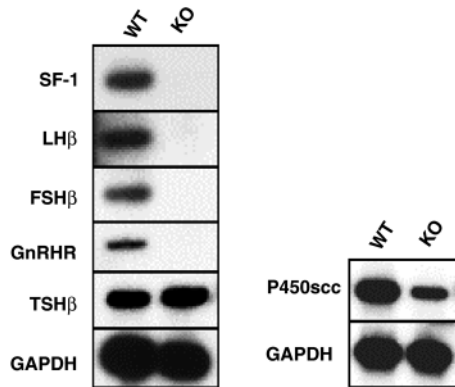


Fig. 5. RT-PCR analysis of gene expression. RNA samples were obtained and used for semi-quantitative RT-PCR analyses of gene expression as described in Materials and Methods. (Left) RT-PCR assays using pituitary RNA. (Right) RT-PCR assays with testes RNA.

Consistent with their normal numbers and location in the original SF1 knockout mice (Ikeda et al., 1995), GnRH neurons in the preoptic region were normal in number and location and delivered immunoreactive GnRH to the median eminence (data not shown). As observed in the original SF1 knockout mice (Ikeda et al., 1995), treatment with supraphysiological doses of GnRH induced the expression of immunodetectable LH in the pituitary gland of F/R, α GSU-Cre mice (Fig. 6). This finding indicates that very high levels of GnRH can compensate for the effect of SF1 deficiency on LH expression; this compensation may be mediated through *Egr1*, a GnRH-inducible transcription factor that normally synergizes with SF1 to activate the LH β promoter (Lee et al., 1996; Tremblay and Drouin, 1999).

PMSG treatment rescues gonadal steroidogenesis in pituitary-specific SF1 knockout mice

The marked gonadal abnormalities in F/R, α GSU-Cre mice could result from impaired gonadotropin stimulation or from primary defects within the gonads. In the former case, treatment with exogenous gonadotropins should stimulate gonadal steroidogenesis. We administered pregnant mares serum gonadotropins (PMSG) – which contains both LH and FSH bioactivities – to F/R, α GSU-Cre mice and examined the responses of the gonads. As shown in Fig. 7C, PMSG treatment of males caused Leydig cell hypertrophy, inducing luminal opening of the seminiferous tubules and maturation of spermatogonial precursors to the round spermatid stage. As further evidence of sperm maturation, PMSG also induced germ cell expression of

immunoreactive CREM τ – a specific marker of differentiating male germ cells (Fig. 7F). Other effects of PMSG on androgen-responsive tissues included enlargement of the seminal vesicles and prostate (L. Z., unpublished observation).

PMSG treatment of female F/R, α GSU-Cre mice stimulated the maturation of ovarian follicles to the preovulatory stage and induced ovulation – as evidenced by the formation of corpora lutea (Fig. 8). Functionally, PMSG induced sufficient estrogen biosynthesis by the follicles to stimulate growth and maturation of the uterus, with a considerable increase in uterine size and development of endometrial glands. These results establish definitively that the testes and ovaries in F/R, α GSU-Cre mice are functional and that the severe reproductive phenotype results from selective dysfunction of SF1-deficient gonadotropes.

DISCUSSION

Using the Cre/loxP strategy for generating tissue-specific knockouts, we have established essential primary role(s) of SF1 within pituitary gonadotropes. We previously proposed such a role based on impaired gonadotropin expression in SF1 knockout mice and SF1-mediated activation of the α GSU promoter in transfection assays (Ingraham et al., 1994). As described here, the severe reproductive phenotype in the F/R, α GSU-Cre mice proves that SF1 is an essential determinant of gonadotrope function in mice. In contrast, gonadotropin production was unimpaired in an individual with adrenal and gonadal dysfunction caused by a loss-of-function mutation of SF1 (Achermann et al., 1999); perhaps a single functioning SF1 allele in humans maintains gonadotrope function but does not support adrenal or gonadal development. Alternatively, SF1 in gonadotropes may activate the transcription of downstream genes by a mechanism independent of DNA binding.

We deleted the last exon of SF1 based on evidence that the AF-2 motif of SF1 is essential for its transcriptional activation. This exon contains nucleotides encoding the C-terminal 82 amino acids and ~1.6 kb of 3' untranslated region, including

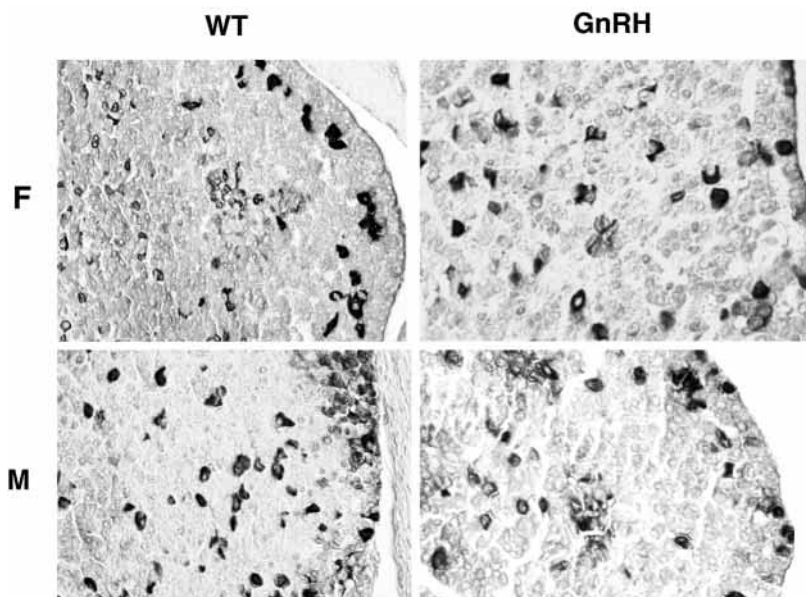
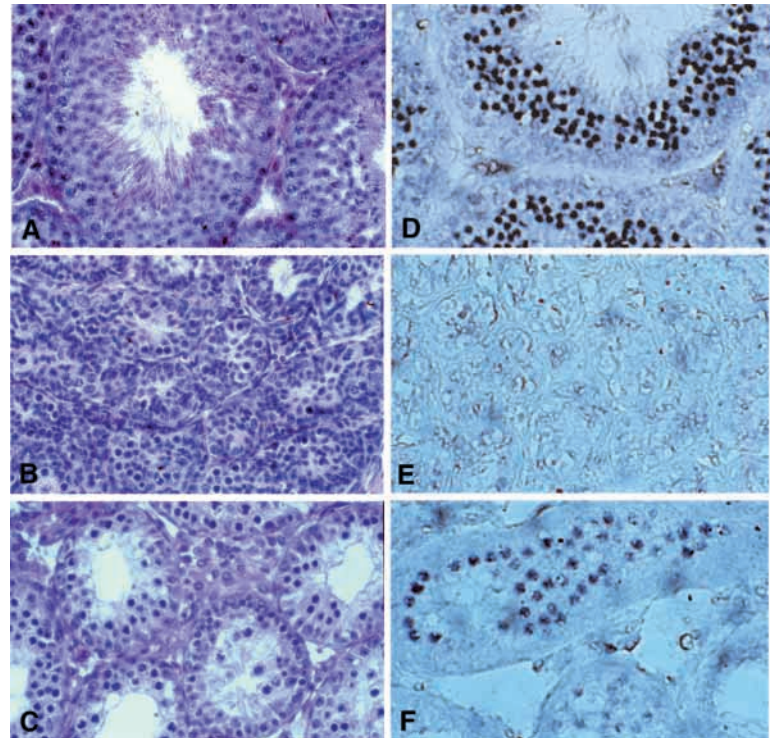


Fig. 6. GnRH induces LH β expression in F/R, α GSU-Cre mice. F/R, α GSU-Cre mice were injected with GnRH and pituitaries were isolated and used for immunohistochemical analyses with anti-LH antiserum as described in Materials and Methods. The top sections are from wild-type (left) and GnRH treated F/R, α GSU-Cre (right) females and the bottom two sections were taken from wild-type (left) and GnRH treated F/R, α GSU-Cre (right) males.

Fig. 7. PMSG rescue of testes function in pituitary-specific SF1 knockout mice. Pituitary specific SF1 knockout mice (6 weeks old) were treated with PMSG as described in Materials and Methods, and then testes were harvested and subjected to histological analyses. (A-C) Hematoxylin and Eosin-stained sections. (D-F) Immunohistochemical detection of CREM τ . (A) WT. (B) F/F, α GSU-Cre. (C) F/F, α GSU-Cre plus PMSG. (D) WT. (E) F/F, α GSU-Cre. (F) F/F, α GSU-Cre plus PMSG.



the 3' polyadenylation/transcription termination signals. A second transcript encoded by the *Ftzfl* allele – termed the embryonic long-terminal repeat binding protein (ELP) – does not contain these sequences (Ninomiya et al., 1993). The perinatal lethality in mice homozygous for the recombinant *Ftzfl* allele establishes again that SF1 is the essential transcript of the *Ftzfl* locus (Luo et al., 1995). The marked diminution of SF1 transcripts in the pituitaries of pituitary-specific SF1 knockout mice (Fig. 5) suggests that deleting the last exon decreases either the transcription or stability of SF1 transcripts. While we cannot draw any conclusions about the functional role of the AF-2 motif of SF1 in vivo, the strategy of targeting the last exon to affect mRNA expression may afford a general approach to inactivate genes whose sequences do not identify important regions for function.

The gonadal hypoplasia in F/R, α GSU-Cre mice is as severe as that seen in mice whose gonadotropes were transgenically ablated (Kendall et al., 1991), arguing that loss of SF1 function in gonadotropes abolishes gonadotropin stimulation of the gonads. In contrast, the phenotype of F/F, α GSU-Cre mice is intermediate between those of +/+, α GSU-Cre mice and F/R, α GSU-Cre mice. This hypomorphic phenotype is illustrated by the testicular weights: the testes of +/+, α GSU-Cre mice weighed 7.9 mg/g body weight, those of F/F, α GSU-Cre mice weighed 3.7 mg/g body weight and those of F/R, α GSU-Cre mice weighed 0.38 mg/g body weight.

The intermediate phenotype in F/F, α GSU-Cre mice points to a gene-dosage effect for Cre-mediated inactivation of the floxed *Ftzfl* allele. Whether this hypomorphic phenotype reflects delayed inactivation of SF1 or incomplete inactivation resulting in mosaicism in gonadotropes remains to be determined. Although this hypomorphic phenotype highlights the need for caution in studies using the Cre-loxP approach, the partial phenotype also may provide an interesting model system to explore the mechanisms of SF1 action in gonadotropes. For example, if the timing of SF1 inactivation is critical for the full effect seen in F/R, α GSU-Cre mice, then comparisons of gene expression

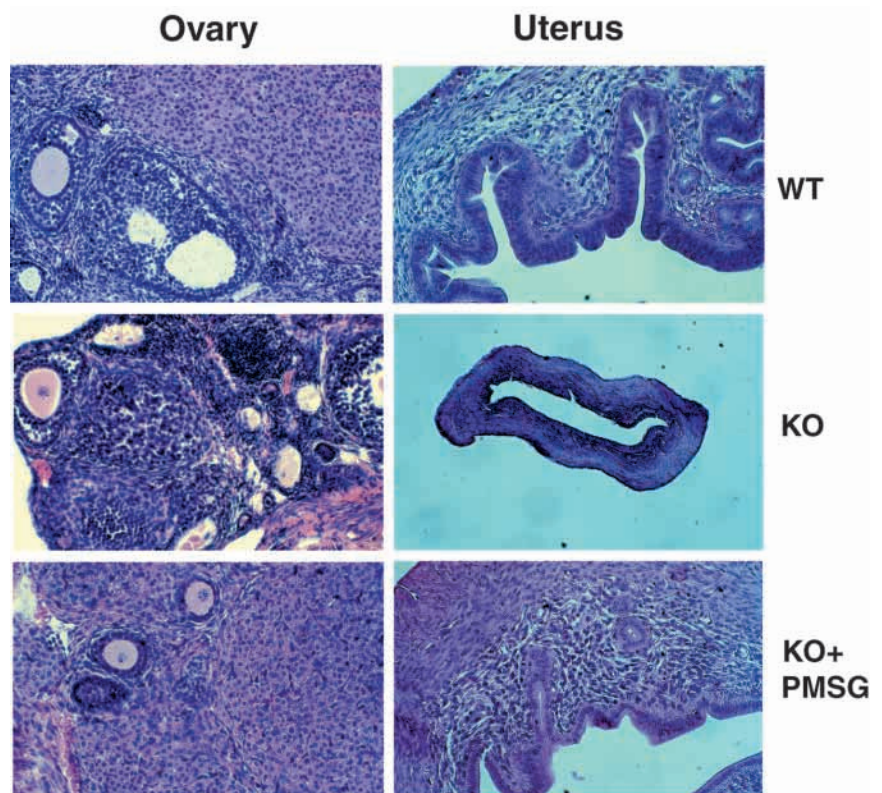


Fig. 8. PMSG rescue of ovarian function in pituitary-specific SF1 knockout mice. Pituitary-specific SF1 knockout (KO) mice (6 weeks old) were treated with PMSG and the ovaries and uterus were harvested, prepared for histological analyses and stained with Hematoxylin and Eosin. Wild type (WT) and untreated are shown for comparison. (Left) ovarian sections. (Right) uterine sections.

patterns in mice with the three genotypes may identify additional SF1 target genes that play important developmental roles in gonadotropes.

A number of transcription factors have been implicated in the development and/or function of gonadotropes. Many of these genes encode homeodomain proteins that play critical roles in the development of multiple pituitary cell lineages (reviewed by Dasen and Rosenfeld, 1999; Burrows et al., 1999). Other transcription factors are linked more selectively to gonadotropes. In the ventral region of the developing pituitary gland, GATA2 induces SF1 expression in gonadotrope precursors (Dasen et al., 1999). SF1 in turn regulates the expression of several downstream genes (e.g. the gonadotropins and the GnRH receptor) that constitute the differentiated gonadotrope phenotype; in the case of LH β , this regulation at least partly is mediated through cooperative interactions with two other transcription factors: Egr1 and Ptx1. (Lee et al., 1996; Halvorson et al., 1998; Dorn et al, 1999; Tremblay and Drouin, 1999). Semi-quantitative RT-PCR assays of pituitary RNA indicated that neither GATA2 nor Egr1 expression was changed in the F/R, α GSU-Cre mice (L. Z., unpublished observations). This probably is not surprising, since GATA2 is proposed to act upstream of SF1 and both transcription factors also are expressed in other cell types within the anterior pituitary gland.

The pituitary-specific SF1 knockout mice join a growing list of natural and induced gene mutations in mice that affect reproduction at different levels of gonadotropin synthesis or action. These models include the *hpg* mice, which carry a deletion in the *Gnrh* gene that precludes its biosynthesis by GnRH neurons, thereby secondarily impairing gonadotropin secretion (Mason et al., 1986); α GSU knockout mice that are deficient in LH, FSH and TSH (Kendall et al., 1995); mice with transgenic ablation of cells expressing α GSU, again causing deficiencies in gonadotropins and TSH (Kendall et al., 1991); and mice with gene knockouts of the β subunit of FSH (Kumar et al., 1997) or its cognate receptor (Abel et al., 2000; Dierich et al., 1998), which impair spermatogenesis in males and cause infertility with impaired follicle maturation in females. Of these models, only the F/R, α GSU-Cre mice provide an experimental system with which to identify additional target genes whose expression constitutes the differentiated gonadotrope phenotype.

Our results establish the utility of using tissue-specific knockouts to dissect the functions of SF1 in the anterior pituitary gland. The same strategy holds considerable promise for expanding our understanding of SF1 action at other sites, such as the primary steroidogenic tissues and the hypothalamus. For example, do the gonads of SF1 knockout mice regress due to a lack of SF1 in Sertoli cells, Leydig cells, or both? The use of cell type-specific Cre transgenes to inactivate SF1 expression selectively in a subset of cells (e.g. mullerian inhibiting substance-Cre in Sertoli cells or steroid 17 α -hydroxylase-Cre in Leydig cells) will allow us to address such issues. Similarly, emerging strategies to induce Cre expression or activity pharmacologically (Nagy, 2000) may allow us to separate developmental roles of SF1 from later functions in maintaining the differentiated phenotype.

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