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Steroidogenic factor 1 (SF-1) is essential for endocrine development and function☆

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Abstract

Steroidogenic factor 1 (SF-1), an orphan nuclear receptor, initially was isolated as a key regulator of the tissue-specific expression of the cytochrome P450 steroid hydroxylases. Thereafter, analyses of sites of SF-1 expression during mouse embryological development hinted at considerably expanded roles for SF-1, roles that were strikingly confirmed through the analyses of SF-1 knockout mice. These SF-1 knockout mice exhibited adrenal and gonadal agenesis, associated with male-to-female sex reversal of their internal and external genitalia and death from adrenocortical insufficiency. These findings showed unequivocally that SF-1 is essential for the embryonic survival of the primary steroidogenic organs. SF-1 knockout mice also had impaired pituitary expression of gonadotropins and agenesis of the ventromedial hypothalamic nucleus (VMH), establishing that SF-1 regulates reproductive function at all three levels of the hypothalamic–pituitary–gonadal axis. This article reviews the experiments that have defined these essential roles of SF-1 in endocrine development and highlights important areas for future studies.

1. Introduction

Because of their essential roles in fluid and electrolyte balance, intermediary metabolism, resistance to stress and sexual differentiation and reproductive function, a number of groups have studied the mechanisms that regulate steroidogenesis. One approach has been to study the gene regulation of the cytochrome P450 steroid hydroxylases (reviewed in Ref. [1]), which catalyze most of the conversions within the steroidogenic pathways [2]. These studies ultimately identified a transcription factor — steroidogenic factor 1 [(SF-1), also called adrenal 4-binding protein (Ad4BP)] — that interacts with conserved AGGTCA promoter elements to regulate the coordinate expression of the steroid hydroxylases within steroidogenic cells [3, 4]. The isolation and characterization of cDNAs encoding this protein revealed that this critical regulator of the steroidogenic enzymes belongs to the nuclear hormone receptor superfamily: transcription factors that mediate transcriptional activation by steroid hormones, thyroid hormone, Vitamin D and retinoids.

2. Insights into SF's function are revealed by its sites of expression

To evaluate whether SF-1 played important endocrine roles in vivo, we first defined the sites where it is expressed. Consistent with its postulated role in steroidogenesis, SF-1 transcripts in adult mice are detected in the steroidogenic compartments of the adrenal gland and gonads (i.e. adrenocortical, testicular Leydig and ovarian theca and granulosa cells [5, 6]). Surprisingly, SF-1 also is expressed in the anterior pituitary gland [7, 8] and in a hypothalamic region called the ventromedial hypothalamic nucleus (VMH) [8, 9]. The expression of SF-1 in steroidogenic cells is consistent with important roles in steroidogenesis, whereas its expression in the pituitary and hypothalamus suggest additional actions within the endocrine axis.

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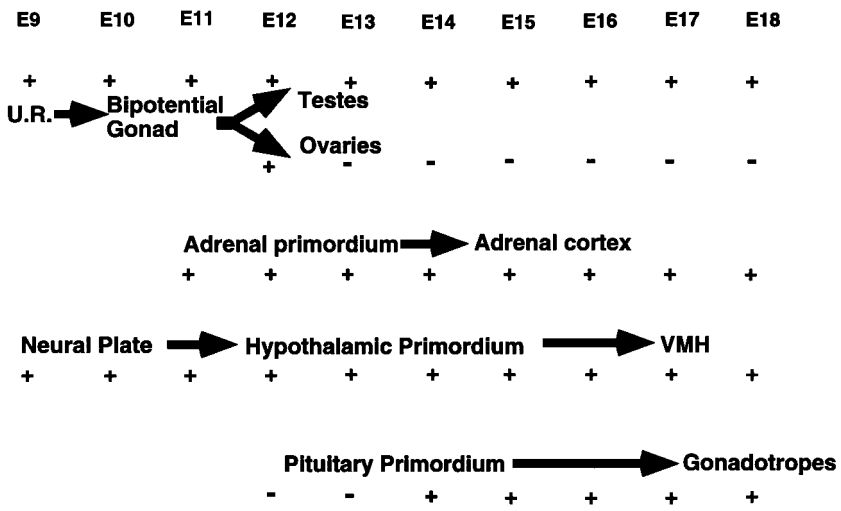

Fig. 1. Ontogeny of SF-1 expression in mouse embryos. The profile of expression of SF-1 transcripts in developing mouse embryos from embryonic day 9 (E9) to E18 is schematically summarized. (+) indicates that SF-1 mRNA was present, (–) indicates that transcripts were absent. The arrows depict the approximate transition times between the different stages of development. U.R. means urogenital ridge, VMH, ventromedial hypothalamic nucleus. Reprinted with permission from Ref. [15].

Developmental studies in mouse embryos (Fig. 1) also implicated SF-1 in the function of the primary steroidogenic organs [10]. SF-1 transcripts are detected in the adrenal primordium, the precursor to the adrenal cortex, from the earliest stages of its development [~embryonic day 10.5 (E10.5)]. At E13–E13.5, when the chromaffin cell precursors migrate into the adrenal primordium to form the adrenal medulla, SF-1 expression localizes to the cortical region where steroid hormones are produced.

The onset of gonadogenesis occurs at approximately E9, when the intermediate mesoderm condenses to form a structure called the urogenital ridge. Testes and ovaries are indistinguishable at this time, and thus are called indifferent or bipotential gonads. As in the adrenal gland, SF-1 was expressed in embryos of both sexes from the very earliest stages of gonadogenesis [10–12]. Subsequently, the Y chromosome-encoded gene *Sry* directs development along a sexually dimorphic pathway, such that testes are formed. In contrast, in the absence of *Sry*, ovaries develop and female sexual differentiation ensues. Coincident with sexual differentiation, SF-1 expression increases in testes, with expression in both functional compartments: the testicular cords where fetal Sertoli cells synthesize Müllerian-inhibiting substance (MIS), and the interstitial region where Leydig cells synthesize androgens. The expression of SF-1 in the nonsteroidogenic Sertoli cells suggests that SF-1 may have additional roles in gonadal development that extend beyond regulating steroidogenesis. In ovaries, in contrast, there is a distinct decrease in levels of SF-1 transcripts coincident with sexual differentiation, suggesting that SF-1 — if persistently expressed — may impede normal female sexual differentiation.

Consistent with the results in adult mice, SF-1 also is expressed in the embryonic diencephalon [9] — which is the precursor to the endocrine hypothalamus — and anterior pituitary [7]. These findings, like the expression of SF-1 by Sertoli cells, suggest that SF-1 has additional functions in development beyond its roles in steroidogenesis.

3. SF-1 knockout mice reveal multiple essential roles in endocrine development and function

Using homologous recombination in embryonic stem cells, we produced SF-1 knockout mice, providing a novel system to study its roles in vivo. These SF-1 knockout mice are born at a frequency of 25%, establishing that SF-1 is not essential for prenatal survival. Consistent with their predicted inability to produce testicular androgens, all SF-1 knockout mice have female external genitalia irrespective of genetic sex. Consistent with their predicted inability to make corticosteroids, they die within 1 week of birth and have depressed corticosterone and elevated ACTH levels [13, 14]. These findings dramatically support the essential role of SF-1 in the biosynthesis of steroid hormones. What was not anticipated (Fig. 2) is the absence of adrenal glands and gonads and male-to-female sex reversal of the internal genitalia. These findings indicate that SF-1 is absolutely essential for the development of the primary steroidogenic organs. When examined histologically in timed pregnancies, the earliest stages of urogenital ridge development

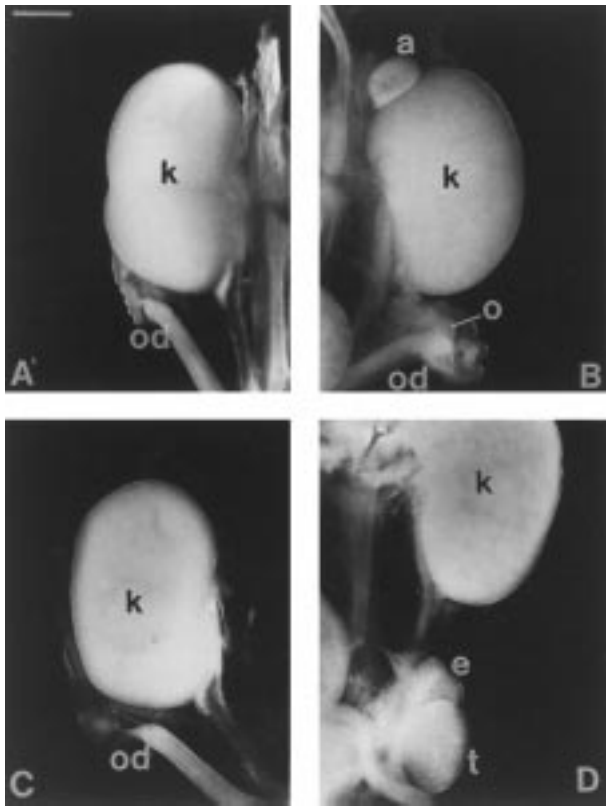

Fig. 2. Newborn SF-1 knockout mice lack adrenal glands and gonads and have female internal genitalia. SF-1 knockout mice (left) and wild-type littermates (right) were sacrificed and the genitourinary tracts were dissected. (A) SF-1 knockout female, (B) wild-type female, (C) SF-1 knockout male and (D) wild-type male. The scale bar = 1 mm. Reprinted with permission from Ref. [13]. k means kidney; a, adrenal; o, ovary; t, testis; e, epididymis; and od, oviduct.

occur relatively normally in SF-1 knockout embryos; at the precise time that sexual differentiation normally occurs, the adrenal glands and gonads regress via a pathway of programmed cell death.

As predicted from the expression profile of SF-1 (Fig. 1), the SF-1 knockout mice also have abnormalities in their anterior pituitary and hypothalamus. Immunoreactivities for luteinizing hormone and follicle-stimulating hormone in the anterior pituitary are diminished considerably [7, 8], linking SF-1 to a second level of endocrine function. Finally (Fig. 3), the hypothalamic nucleus where SF-1 normally is expressed — the VMH — is completely absent in SF-1 knockout mice of both sexes [8, 9]. Developmentally, the VMH neurons migrate normally into the hypothalamic primordium, but die right at the end of the prenatal period.

4. Summary and future directions

These studies have established multiple roles for SF-1 within the endocrine axis. As summarized (Table 1), SF-1 regulates a large number of target genes that are essential for steroidogenesis and reproduction (reviewed in [15]). For two of these genes (*LHβ* [16] and *MIS* [17]), transgenic promoter studies have defined *in vivo* roles in regulating target genes. A recent report extends these studies by showing that SF-1 can direct uncommitted embryonic stem cells to differentiate at least partially down the steroidogenic pathway [18]. Future studies seek to identify the target genes through which these developmental effects are mediated. The known target genes of SF-1 are not sufficient to account for the disappearance of the adrenal glands, gonads and VMH. While it formally is possible that the severe phenotype results from the simultaneous loss of multiple SF-1 target genes — none of which by itself can cause the phenotype — it seems more likely that new target genes will be identified that impinge on the cell cycle/proliferation pathways.

Another goal for future studies is to identify transcription factors with which SF-1 cooperates to regulate target genes. Transcription factors that have been reported to interact with SF — either functionally or through direct heterodimerization — include Sp1 [19], estrogen receptor [20], NGFI-A [21], cAMP-responsive element binding protein [22], DAX-1 [23], Wilms tumor 1 (WT1) [24] and PTX-1 [25]. Presumably, these interactions help to determine the differential expression patterns of target genes within SF-1-expressing cells. For example, genes such as steroid 21-hydroxylase and the isozymes of steroid 11β-hydroxylase, well-characterized SF-1-responsive genes, are expressed in the adrenal cortex, but not in the steroi-

Table 1
Target genes for SF-1

VMH	?
Gonadotropes	α-subunit of glycoprotein hormones Luteinizing hormone β GnRH receptor
Adrenal cortex	cytochrome P450 steroid hydroxylases 3β-hydroxysteroid dehydrogenase steroidogenic acute regulatory protein ACTH receptor SR-B1
Gonads	
Leydig cells	cytochrome P450 steroid hydroxylases steroidogenic acute regulatory protein prolactin receptor
Sertoli cells	Müllerian-inhibiting substance
Theca and granulosa cells	cytochrome P450 steroid hydroxylases steroidogenic acute regulatory protein oxytocin

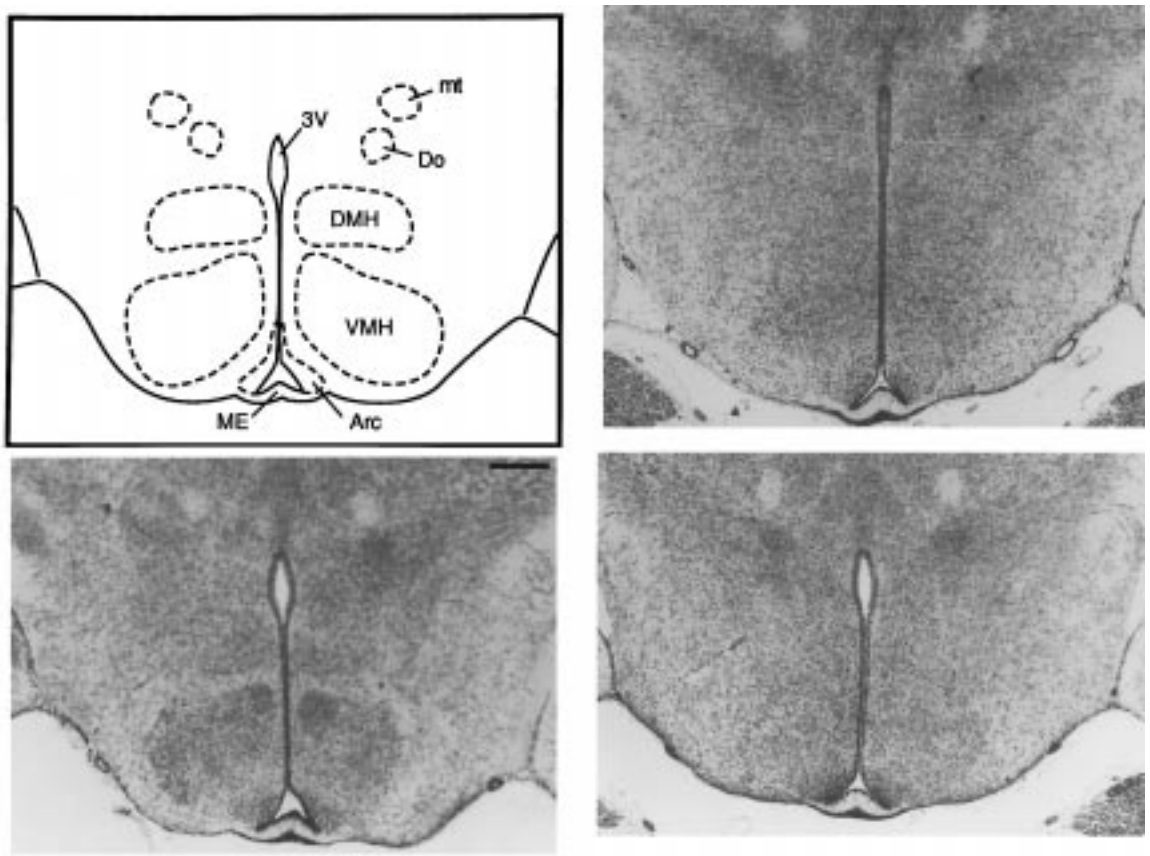

Fig. 3. SF-1 knockout mice lack the ventromedial hypothalamic nucleus. Coronal sections from wild-type (lower left) and SF-1 knockout male (upper right) and female (lower right) mice were stained with cresyl violet and photomicrographs were taken. A schematic diagram of the anatomical regions found within these sections is shown (upper left). The scale bar = 200 μm. mt, mammillothalamic tract; Do, dorsal hypothalamic nucleus; 3V, third ventricle; DMH, dorsomedial hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus; Arc, arcuate nucleus; ME, median eminence. Modified with permission from [9].

dogenic cells of the gonads or the VMH neurons, despite the fact that these cells all express SF-1.

Another important question is the role of ligands in SF-1 action, SF-1, at its extreme carboxy terminus, contains a sequence that closely resembles the AF-2 motif identified in a number of ligand-activated nuclear receptors [26]. This motif has been shown to be essential for SF-1-mediated transcriptional activation [27, 28], as well as for interactions between SF-1 and coactivator proteins [28–30]. If this motif truly correlates with ligand-inducibility, its presence in SF-1 suggests that SF-1 also may be activated by be ligands. Recent studies have shown that oxysterols, particularly 25-hydroxycholestrol, increase SF-1-dependent transcriptional activation in transient transfection assays [27]. Although direct binding of oxysterols to SF-1 has not been shown, and others have reported that oxysterols do not activate SF-1 in mouse MA-10 Leydig cells [31], these findings suggest that oxysterols, or their derivatives, can induce SF-1 transcriptional activation. Given that these compounds exist within steroidogenic cells at concentrations approaching those used in the transient transfection assays, they may contribute to the 'constitutive' actions of SF-1 in steroidogenic cells.

A final question is whether SF-1's role in mice, as defined by the knockout analyses summarized here, also extends to other species. Homologs of SF-1 have been identified in many other species, including vertebrates (e.g. human [32, 33], chicken [34], *Xenopus* [35]) and invertebrates (e.g. *Drosophila* [36] and silkworm [37]). This conservation of sequence implies shared functions, at least in the different mammalian species where the basic principles of steroidogenesis are quite similar. In accord with this model, the expression pattern of SF-1 in human tissues closely corresponds with that seen in mice [38]. Moreover, preliminary analyses of SF-1 expression in human fetuses indicate that SF-1 is expressed in developing adrenal glands and gonads with a profile very similar to that previously defined for mice (N. Hanley and T. Strachan, personal communication). Thus, although mutations in SF-1 have not yet been demonstrated in human patients with abnormalities of sexual differentiation, it seems likely that patients will be found with

clinical disorders resulting from mutations in the human gene on chromosome 9q33.

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