Steroidogenic Factor 1: an Essential Mediator of Endocrine Development

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ABSTRACT

The orphan nuclear receptor steroidogenic factor 1 (SF-1, also called Ad4BP and officially designated NR5A1) has emerged as an essential regulator of endocrine development and function. Initially identified as a tissue-specific transcriptional regulator of the cytochrome P450 steroid hydroxylases, SF-1 has considerably broader roles, as evidenced from studies in knockout mice lacking SF-1. The SF-1-knockout mice lacked adrenal glands and gonads and therefore died from adrenal insufficiency within the first week after birth. In addition, SF-1 knockout mice exhibited male-to-female sex reversal of their internal and external genitalia, impaired expression of multiple markers of pituitary gonadotropes, and agenesis of the ventromedial hypothalamic nucleus (VMH). These studies delineated essential roles of SF-1 in regulating endocrine differentiation and function at multiple levels, particularly with respect to reproduction. This chapter will review the experiments that established SF-1 as a pivotal, global determinant of endocrine differentiation and function. We next discuss recent insights into the mechanisms controlling the expression and function of SF-1 as well as the current status of research aimed at delineating its roles in specific tissues. Finally, we highlight areas where additional studies are needed to expand our understanding of SF-1 action.

I. Initial Isolation of Steroidogenic Factor 1

Steroid hormones are essential for fluid and electrolyte balance, intermediary metabolism, sexual differentiation, and reproductive function. Once the pathways of steroid hormone biosynthesis were defined and shown to involve the concerted actions of several cytochrome P450 mixed-function oxidases, attention turned to elucidating the mechanisms that regulate the expression of these enzymes. With the isolation of the bovine 21-hydroxylase cDNA (White *et al.*, 1984b), followed shortly thereafter by the cloning of cDNAs encoding the side-chain cleavage enzyme (Matteson *et al.*, 1984; Morohashi *et al.*, 1984) and 11β-hydroxylase (John *et al.*, 1984), these questions could be addressed at a molecular level. David Chaplin, a postdoctoral fellow in J.G. Seidman's laboratory who previ-

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ously had isolated cosmids spanning the S region of the mouse H-2 complex, cloned a cosmid that contained the mouse 21-hydroxylase gene and ≈ 30 kb of its 5′-flanking region (White *et al.*, 1984a). The Schimmer laboratory had extensive experience with Y1 mouse adrenocortical tumor cells, which expressed several cytochrome P450 steroid hydroxylases – but not steroid 21-hydroxylase — in a hormonally responsive manner and were readily amenable to both stable and transient transfection (Schimmer, 1985). In a collaborative effort, the Schimmer and Seidman laboratories showed that Y1 cells stably transfected with the mouse 21-hydroxylase cosmid recovered hormonally regulated expression of 21-hydroxylase (Parker *et al.*, 1985). Thereafter, 5′-deletion assays localized sequences essential for cell-selective and hormone-regulated expression of the 21-hydroxylase gene to the proximal 330 bp of 5′-flanking DNA (Parker *et al.*, 1986; Handler *et al.*, 1988).

Using similar approaches, a number of groups analyzed the 5′-flanking regions of genes encoding the cytochrome P450 steroid hydroxylases (for reviews of these studies, see Omura and Morohashi, 1995; Parker and Schimmer, 1995). In particular, studies by two groups identified shared AGGTCA promoter elements in the proximal promoter regions of several of the steroid hydroxylases that interacted with the same DNA-binding protein (Rice *et al.*, 1991; Morohashi *et al.*, 1992). This protein, which initially was found only in steroidogenic cell lines, was designated steroidogenic factor 1 (SF-1) or adrenal 4-binding protein (Ad4BP). The selective expression of SF-1 in steroidogenic cells and its regulation of multiple genes encoding steroid hydroxylases provided the first clues that it was an important determinant of the cell-selective expression of the steroidogenic enzymes.

Based on evidence that SF-1 was a key determinant of the expression of the cytochrome P450 steroid hydroxylases, the Parker and Morohashi laboratories independently cloned cDNAs encoding SF-1. Morohashi and colleagues used an oligonucleotide affinity column to purify the protein from bovine adrenal glands, ultimately allowing them to obtain amino acid sequence and clone a bovine cDNA with an oligonucleotide probe (Honda *et al.*, 1993). In contrast, Douglas Rice, a postdoctoral fellow in the Parker laboratory, reasoned that the AGGTCA DNA recognition motif represented a binding site for an atypical member of the nuclear hormone receptor family. Using a hybridization probe comprising the DNA-binding region of retinoid X receptor, the Parker laboratory isolated a cDNA clone that was expressed in adrenal gland, testes, and ovaries, but not in a variety of other tissues (Lala *et al.*, 1992).

Subsequent studies established that the mouse and bovine cDNAs encoded orthologs of a protein that transactivated the steroid hydroxylase promoters in steroidogenic and nonsteroidogenic cells. As predicted from the cloning strategy used by the Parker group, the sequences of these cDNAs confirmed that SF-1

belonged to the nuclear hormone receptor family, with striking homology to the *Drosophila* nuclear receptor fushi tarazu factor 1 (Ftz-F1) and the mouse nuclear receptor embryonal long terminal repeat-binding protein (Tsukiyama *et al.*, 1992). SF-1 homologs have been identified in a diverse group of species that includes humans, marmosets, cows, sheep, horses, mice, rats, pigs, tamarind wallabies, chickens, turtles, salmon, trout, zebrafish, flies, and worms.

II. Developmental Profile of SF-1 Expression

To address the potential roles of SF-1 during mammalian development, Yayoi Ikeda in the Parker laboratory used *in situ* hybridization to analyze its spatial and temporal profiles of expression in mouse embryos (Ikeda *et al.*, 1994). As anticipated, SF-1 transcripts were detected in the adrenal primordium from very early stages of its development (approximately embryonic day (E) 10.5). As the chromaffin cell precursors migrated into the adrenal primordium at ≈ E12.5 to E13.5, SF-1 expression was restricted to the steroidogenic cells in the cortex. The initiation of SF-1 expression before the onset of steroidogenesis supported its key role in steroid hydroxylase expression and suggested additional roles in adrenal development.

In mice, gonadal development first becomes apparent at ≈ E9, when the intermediate mesoderm condenses into the urogenital ridge, which ultimately contributes cell lineages to the gonads, adrenal cortex, and kidneys. At this time, developing testes and ovaries are indistinguishable histologically and thus are termed indifferent or bipotential. By ≈ E12.5, the fetal testes have organized into the testicular cords, which contain fetal Sertoli cells and primordial germ cells, and the surrounding interstitial region, which contains the Leydig cells. Faint expression of SF-1 was seen in both male and female embryos from the inception of the indifferent stage (E9.0–E9.5), persisting thereafter throughout the indifferent gonad stage. Coincident with formation of the testicular cords at E12.5, SF-1 expression persisted in the testes but diminished in ovaries (Hatano *et al.*, 1994; Ikeda *et al.*, 1994). In addition, SF-1 transcripts were detected in both the interstitial region, where Leydig cells produce steroid hormones, and the testicular cords, where fetal Sertoli cells produce anti-Müllerian hormone. SF-1 transcripts also were detected in the embryonic diencephalon — the precursor to the endocrine hypothalamus – and the anterior pituitary gland (Ikeda *et al.*, 1994). Taken together, these findings suggested roles for SF-1 in gonadal development that extended beyond its effects on the expression of the steroidogenic enzymes and actions to regulate multiple levels of the hypothalamic-pituitary-steroidogenic organ axis.

III. The Roles of SF-1 *in Vivo*

A. KNOCKOUT MOUSE STUDIES

To address the role of SF-1 *in vivo*, three groups (Parker, Morohashi, and Milbrandt) used targeted gene disruption in embryonic stem cells to generate SF-1 knockout mice. In the Parker laboratory, Xunrong Luo generated the SF-1 knockout mice in collaboration with Drs. Beverly Koller and Ann Latour (Luo *et al.*, 1994). Consistent with the model that SF-1 was required for adrenal and gonadal steroidogenesis, SF-1 knockout mice died shortly after birth from adrenocortical insufficiency and exhibited male-to-female sex reversal of the external genitalia (Luo *et al.*, 1994; Sadovsky *et al.*, 1995). By analogy with human subjects with impaired expression of the steroid hydroxylases, the Parker laboratory anticipated that the adrenal glands of SF-1 knockout mice would be hyperplastic due to their inability to make glucocorticoids and the consequent exposure to high levels of corticotropin (ACTH). In a major surprise, shown in Figure 1, the adrenal glands and gonads were completely absent in newborn SF-1 knockout mice (Luo *et al.*, 1994; Sadovsky *et al.*, 1995). Subsequent studies showed that the initial stages of adrenal and gonadal development occurred in the absence of SF-1, followed by their regression at a specific stage of development. Because their gonads regressed before male sexual differentiation normally occurs, the internal and external urogenital tracts of SF-1 knockout mice were female, irrespective of genetic sex.

The gonadotropes of SF-1 knockout mice also had impaired expression of a number of genes that regulate reproduction, including luteinizing hormone β (LH-β), follicle-stimulating hormone β (FSH-β), the α-subunit of glycoprotein hormones (αGSU), and the receptor for gonadotropin-releasing hormone (Ingraham *et al.*, 1994; Shinoda *et al.*, 1995). As shown in Figure 2, these knockout mice also lacked the ventromedial hypothalamic nucleus (VMH), a hypothalamic region linked to feeding and appetite regulation and female reproductive behavior (Ikeda *et al.*, 1995; Shinoda *et al.*, 1995). Finally, although the functional consequences remain to be defined, the SF-1 knockout mice had defects in their splenic parenchyma (Morohashi *et al.*, 1999).

Guided in part by studies in human subjects with clinical disorders due to haploinsufficiency of SF-1 or other genes involved in gonadal development (see below), Ingraham and colleagues examined more carefully the phenotype of heterozygous SF-1 knockout mice. These studies, which revealed decreased adrenal volume associated with impaired corticosterone production in response to stress (Bland *et al.*, 2000), suggested that the level of SF-1 expression may be very important for optimal adrenal development.

Because the original SF-1 knockout mice are globally deficient in SF-1, they cannot be used to delineate the roles of SF-1 at specific sites of expression. For

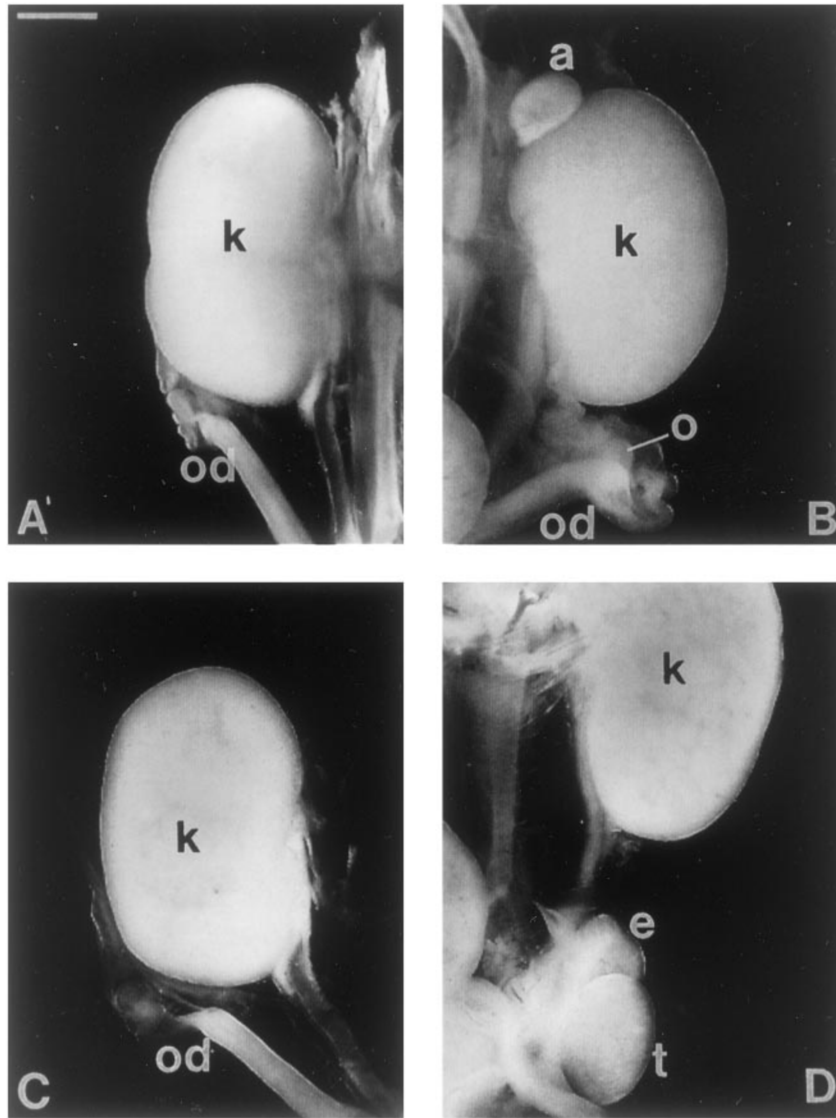

FIG. 1. SF-1 knockout mice lack adrenal glands and gonads and have female internal genitalia. The dissected genitourinary tracts of wild-type female (B) and male (D) and SF-1 knockout female (A) and male (C) mice are shown. Note the absence of adrenal glands and gonads in SF-1-deficient mice and the presence of oviducts in both males and females. a, adrenal gland; k, kidney; o, ovary; t, testis; e, epididymis; od, oviduct. [Reprinted from Luo X, Ikeda Y, Parker KL 1994 A cell specific nuclear receptor is required for adrenal and gonadal development and for male sexual differentiation. Cell 77:481–490, with permission from Elsevier Science.]

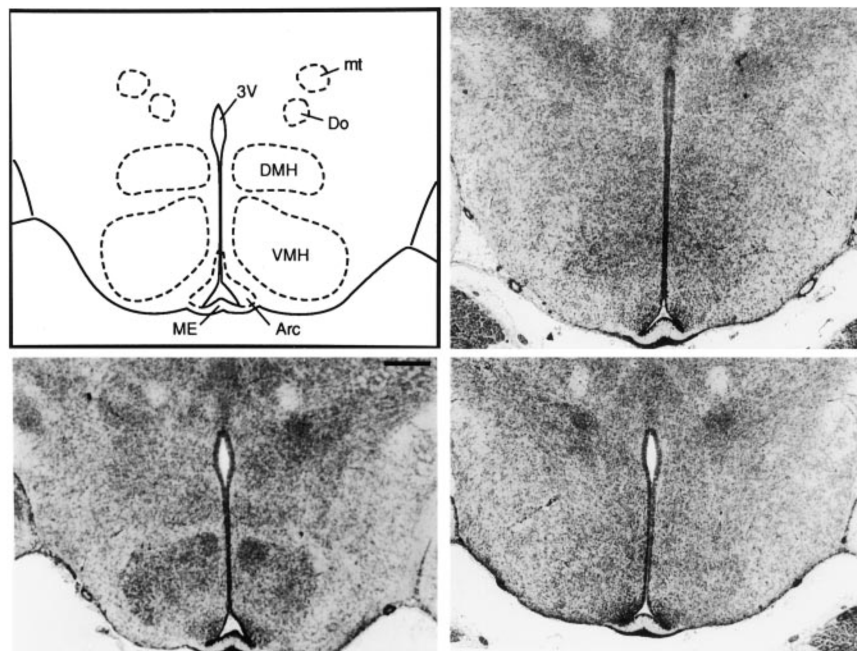

FIG. 2. SF-1 knockout mice lack the ventromedial hypothalamic nucleus (VMH). Serial coronal sections from wild-type (lower left) and -/- male (upper right) and female (lower right) mice were stained and analyzed histologically. Shown at the upper left is a schematic diagram of anatomical regions found within these sections. VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; ME, median eminance; Arc, arcuate nucleus; Do, dorsal hypothalamic nucleus; 3V, 3rd ventricle; mt, mammilothalamic tract. [Modified with permission from Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL 1995 The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. Mol Endocrinol 9:478–486. Copyright The Endocrine Society.]

example, the apparent defect in gonadotrope function could merely reflect the absence of gonadal steroids. Another limitation of the original SF-1 knockout mice is their early postnatal death from adrenocortical insufficiency, which precludes efforts to examine the roles of SF-1 after differentiation has occurred. Finally, the need to administer exogenous corticosteroids to keep SF-1 knockout mice alive complicates considerably our ability to use these mice to assess the role of the VMH in feeding and weight regulation. To obviate such limitations, the Parker laboratory has begun to use the Cre/loxP system to produce tissue-specific knockouts of SF-1. Marit Bakke initiated this process by modifying the SF-1 locus in embryonic stem cells to insert recognition sites for the bacteriophage Cre recombinase (termed loxP sites) around the last exon of SF-1, which encodes an essential domain for

transcriptional activation and transcription termination sequences. The second essential step in the tissue-specific knockout was to generate a Cre transgene selectively active in a subset of SF-1-expressing cells. Lisa Cushman in the Camper laboratory generated a transgenic mouse line in which Cre expression was directed to the anterior pituitary gland by the 5′-flanking sequences of the α subunit of glycoprotein hormones (Cushman *et al.*, 2000). Liping Zhao bred the loxP-modified SF-1 line with the Cre transgenic mice, ultimately generating mice with pituitary-specific disruption of SF-1.

As shown in Figure 3, the αGSU-Cre/loxP mice selectively lacked SF-1 immunoreactivity in the anterior pituitary (Zhao *et al.*, 2001) but had normal levels at other sites, including the adrenal cortex and VMH. These mice had markedly diminished levels of pituitary gonadotropins and exhibited severe gonadal hypoplasia secondary to impaired gonadotropin stimulation. These pituitary-specific SF-1 knockout mice demonstrated that the local production of SF-1 in mice is essential for normal gonadotrope function, strongly supporting a direct role for SF-1 in gonadotropin gene expression.

B. SF-1 AND HUMAN DISEASE

The sequence of the human gene encoding SF-1 closely resembled that of the mouse gene (Oba *et al.*, 1996; Wong *et al.*, 1996) and SF-1 expression during human embryological development closely paralleled that in mice (Hanley *et al.*, 1999; de Santa Barbara *et al.*, 2000). Thus, it was plausible that mutations in the human SF-1 gene on chromosome 9q33 (Taketo *et al.*, 1995) might cause endocrine disease. Many groups looked for SF-1 mutations in patients with clinical disorders of adrenocortical development and/or sexual differentiation. To date, only two subjects with diseases associated with SF-1 mutations have been described, suggesting that SF-1 mutations occur only rarely. The first subject with a SF-1 mutation presented with adrenocortical insufficiency and 46,XY gonadal dysgenesis (Achermann *et al.*, 1999). In contrast to SF-1 knockout mice, which had diminished gonadotropin levels, this subject had elevated levels of gonadotropins. The second subject also presented with adrenocortical insufficiency, but had a 46,XX karyotype with apparently normal prepubertal ovaries (Biason-Lauber and Schoenle, 2000). Surprisingly, each patient had one apparently normal SF-1 allele and different mutated alleles that resulted in loss-of-function rather than dominant-negative effects. This apparent haploinsufficiency raises the intriguing possibility that gene dosage may be a critical component of SF-1 function in humans. These results further suggest, as seen in other knockout mouse models, that the phenotypes in patients with SF-1 mutations need not correlate precisely with observations in SF-1 knockout mice.

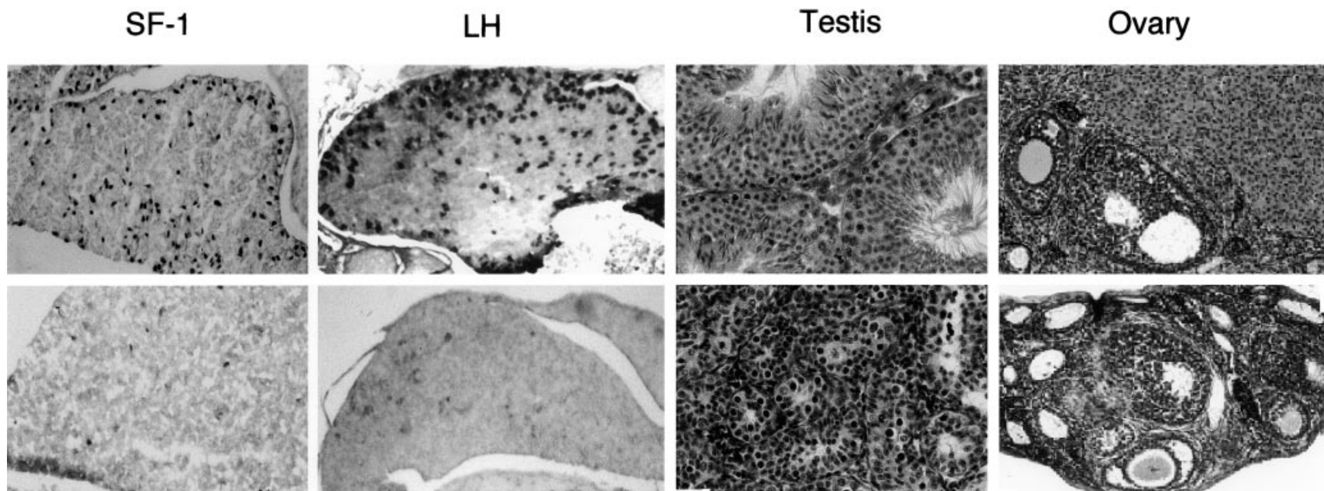

FIG. 3. Pituitary-specific knockout of SF-1 causes hypogonadotropic hypogonadism. The Cre/loxP approach with an αGSU-Cre transgene allowed us to inactivate SF-1 specifically in the anterior pituitary gland. The top panels show wild-type sections and the bottom panels show sections from mice with a pituitary-specific knockout of SF-1. Note that immunoreactivities for SF-1 and LH are both virtually abolished and that the testes and ovaries are markedly hypoplastic secondary to deficient gonadotropin stimulation.

IV. Regulation of SF-1 Expression and Function

A. REGULATION OF SF-1 EXPRESSION

Relatively little is known about the mechanisms that regulate the expression of SF-1 within specific cell lineages. To date, there are no published successes with transgenic promoter analyses, so our insights largely are limited to transfection analyses in cell-culture models. In one published report, a conserved E-box motif in the 5′-flanking region of the rat gene encoding SF-1 was shown to regulate promoter activity in transfected mouse Y1 adrenocortical or I-10 Leydig tumor cells (Nomura *et al.*, 1995). Moreover, a protein that interacted with this E-box motif in gel mobility shift assays was expressed at higher levels in embryonic testes than ovaries. The same E-box motif was implicated as an important regulator of SF-1 promoter activity in Sertoli cells (Daggett *et al.*, 2000) and in Y1 adrenocortical cells and αT3 gonadotropes (Harris and Mellon, 1998). In the latter study, the transcription factor USF-1 was shown to regulate SF-1 expression. In view of the known developmental roles of basic helix-loop-helix proteins that bind these E-box motifs, these studies suggest an important role for basic helix-loop-helix proteins in regulating SF-1 expression in several cell types.

Other promoter elements implicated in SF-1 promoter activity include a GC-rich sequence that may represent a binding site for Sp1 and a CCAAT-box motif (Woodson *et al.*, 1997). The precise roles of these elements in different tissues remain to be defined. Moreover, the lack of success in the reported transgenic expression studies suggests that other elements also play important roles *in vivo*.

One might predict that distinct mechanisms regulate SF-1 expression in the adrenal cortex and gonads, which are believed to arise from the same embryonic lineage (Hatano *et al.*, 1996), versus the anterior pituitary and hypothalamus, which are contiguous structures that interact reciprocally during development (Rosenfeld *et al.*, 1996). In support of this, the pituitary transcripts in some species arise from a transcription initiation site distinct from that used in other sites (Ninomiya *et al.*, 1995; Kimura *et al.*, 2000). In an effort to explore the mechanisms that regulate SF-1 expression *in vivo*, the Parker laboratory recently used a 50-kb fragment derived from a bacterial artificial chromosome to direct expression of a green fluorescent protein (GFP) reporter gene in transgenic mice. As shown in Figure 4, GFP expression in the urogenital ridge was detected at E9.5, closely paralleling the onset of SF expression during gonadogenesis (Ikeda *et al.*, 1994). Although GFP expression in the adrenal primordium and VMH also corresponded to the known expression profile of SF-1, GFP was not expressed in the anterior pituitary. These results document that 50 kb of 5′-flanking region

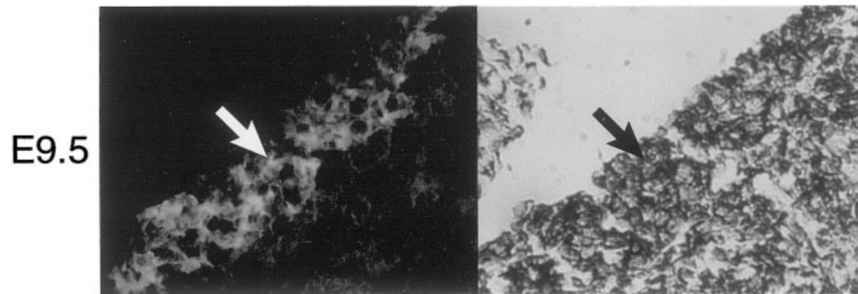

FIG. 4. A green fluorescent protein reporter (GFP) transgene is targeted to the embryonic gonad by SF-1 5′-flanking sequences. A transgene containing 50 kb of 5′-flanking sequences from the mouse locus encoding SF-1 was placed upstream of coding sequences for enhanced green fluorescent protein (eGFP). This construct was microinjected into pronuclei to generate a transgenic founder. A section from an E9.5 embryo was analyzed by fluorescence microscopy, revealing GFP expression in the urogenital ridge (arrows).

suffice to direct regulated expression in most sites but also suggest that additional regulatory elements are required for activation of the pituitary-specific promoter.

B. MODULATION OF SF-1 ACTIVITY

Limited data also are available regarding the regulation of SF-1 activity in cells where it is expressed. As a member of the nuclear hormone receptor family, one obvious possibility is that a ligand regulates SF-1 transcriptional activity in a manner analogous to many former "orphan" members (Kliewer *et al.*, 1999). Using a cotransfection assay, Lala and colleagues noted that hydroxycholesterol derivatives increased SF-1 transcriptional activity by about 10-fold, suggesting that intermediates in the steroidogenic pathway might modulate SF-1 activity (Lala *et al.*, 1997). Others, however, have suggested that the effects of oxysterols are unique to certain nonsteroidogenic cells and may not be relevant to steroidogenic cells (Christenson *et al.*, 1998; Mellon and Bair, 1998). Thus, the precise role of ligands in SF-1 activity remains to be defined.

In the absence of a clear-cut ligand, others have examined the possibility that post-translational modifications alter SF-1 transcriptional activity. In their initial description of the bovine sequence, Morohashi and colleagues noted a potential phosphorylation site for cAMP-dependent protein kinase. Mellon and colleagues subsequently reported that recombinantly expressed SF-1 was phosphorylated *in vitro* by cAMP-dependent protein kinase (Zhang and Mellon, 1996). Mutation of the serine residue in the consensus motif did not impair SF-1 function in transfection assays (Lopez *et al.*, 2001), suggesting that this site is not a key regulator of SF-1 activity.

In a separate line of investigations, the Ingraham laboratory used peptide mapping to define a site of SF-1 phosphorylation (Ser203) that mapped within a consensus motif for phosphorylation by mitogen-activated protein (MAP) kinase (Hammer *et al.*, 1999). They further showed that activating the MAP kinase pathway increased SF-1 transcriptional activation, while mutation of Ser203 diminished SF-1 activity. Collectively, these findings raise the possibility that SF-1 function is modulated by posttranslational modification through extracellular signals that act via the MAP kinase pathway.

C. REGULATION THROUGH PROTEIN-PROTEIN INTERACTIONS

Analyses of human patients and knockout mouse models have identified a number of other genes that play key roles in the development of SF-1-expressing tissues (for a review, see Parker *et al.*, 1999). It is almost certain that these genes interact with SF-1 – either in hierarchical cascades of gene regulation or via protein-protein interactions – to mediate endocrine development. Indeed, a number of factors have been shown to interact directly with SF-1, including the Wilm's tumor related tumor suppressor gene WT-1 (Nachtigal *et al.*, 1998), GATA-4 (Tremblay and Viger, 1999), Ptx1 (Tremblay *et al.*, 1999), SOX9 (de Santa-Barbara *et al.*, 1999), and EGR1 (Halvorson *et al.*, 1998; Dorn *et al.*, 1999). In contrast, SF-1 expression is markedly decreased in the gonads — but not the adrenal glands — of Lhx9 knockout mice, suggesting that Lhx9 regulates SF-1 expression in the gonads (Birk *et al.*, 2000). Finally, DAX-1 and SF-1 apparently interact both hierarchically (i.e., SF-1 regulates DAX1 expression) (Yu *et al.*, 1998; Kawabe *et al.*, 1999) and by direct protein-protein interactions wherein DAX1 inhibits SF-1 transcriptional activation (Ito *et al.*, 1997; Crawford *et al.*, 1998).

Besides interactions with other tissue-specific transcription factors, it is apparent that coactivators and co-repressors are critical modulators of nuclear receptor transcriptional activity (for a review, see Xu *et al.*, 1999). Predictably, a number of co-regulators have been reported to interact with SF-1, including CBP/P300 (Monte *et al.*, 1998), GRIPI (Hammer *et al.*, 1999), SRC-1 (Crawford *et al.*, 1997a), MBP1 (Kabe *et al.*, 1999), SMRT (Hammer *et al.*, 1999), and N-CoR (Crawford *et al.*, 1998; Nachtigal *et al.*, 1998). It is possible that differential interactions with these co-regulators, as well as with the tissue-specific transcription factors described above, specify the differential expression of SF-1 target genes in various tissues. An increased understanding of the ways in which these genes interact to regulate the expression of specific target genes undoubtedly will provide important new insights into processes of endocrine development.

V. Directions for Future Research

The studies summarized here have defined essential roles of SF-1 at multiple levels of endocrine differentiation and function, particularly within the reproductive axis. Specifically, SF-1 is the first transcriptional regulator shown to play key roles at all levels of the hypothalamic-pituitary-steroidogenic organ axis. Despite this considerable progress, a number of important questions remain to be answered. We still do not completely understand the factors that govern the expression of SF-1 or that regulate its activity in different tissues. Nor do we understand the specific roles of SF-1 at the different sites where it is expressed. As noted earlier, the pituitary-specific knockout of SF-1 has helped establish the functional importance of SF-1 within mouse gonadotropes. Similar efforts now are underway to inactivate SF-1 specifically in the VMH (using neuron-specific promoters), in Leydig/theca cells (using steroid 17α-hydroxylase or Mullerian inhibiting substance (MIS) receptor promoters), and in Sertoli/granulosa cells (using the MIS or inhibin promoters). These experiments should provide novel insights into the specific functions of SF-1 at these sites.

What is the relationship of SF-1 to other closely related members of the NR5 nuclear receptor family? SF-1 most closely resembles another orphan nuclear receptor, NR5A2, particularly within the DNA-binding domain, suggesting that these two transcription factors may regulate overlapping target genes. Indeed, both SF-1 and NR5A2 can activate promoter activity of the small heterodimerization partner (SHP) nuclear receptor (Lee *et al.*, 1999), which is expressed in the adrenal cortex, liver, and other tissues. Interestingly, NR5A2 transcripts are expressed at high levels in the corpus luteum of the ovary (Boerboom *et al.*, 2000), where it may replace SF-1 as a critical regulator of the cytochrome P450 steroid hydroxylases at certain stages of the ovulatory cycle.

While many laboratories have identified a diverse group of SF-1 target genes, as summarized in Table I, these analyses largely have focused on transient transfection assays using relatively limited stretches of promoter/regulatory DNA. These studies may overemphasize the importance of SF-1 in gene regulation and verification of important roles *in vivo* ultimately is needed. Among the SF-1 target genes in Table I, evidence supporting such *in vivo* roles has been provided for MIS (Giuili *et al.*, 1997; Arango *et al.*, 1999) and LHβ (Keri and Nilson, 1996). Moreover, important questions about specific roles of SF-1 in development versus differentiated function may be too subtle to be addressed in either global or tissue-specific knockouts. Although it was anticipated that SF-1 knockout mice might provide *in vivo* evidence for the importance of SF-1 in gene expression, particularly for those genes involved in steroidogenesis, the failure of the knockout mice to develop the steroidogenic organs and the VMH precluded such analyses at these sites. Strong evidence for a developmental role of SF-1 came from studies in which forced expression of SF-1 in embryonic stem cells

TABLE I
Sites of Action and Target Genes for Steroidogenic Factor-1

Ventromedial hypothalamic nucleus	N-methyl-D-aspartate receptor
Gonadotropes	α subunit of glycoprotein hormones
	Luteinizing hormone (LH) β
	Follicle-stimulating hormone (FSH) β
	Gonadotropin-releasing hormone receptor
Adrenal cortex	Cytochrome P450 steroid hydroxylases
	3β-hydroxysteroid dehydrogenase
	Steroidogenic acute regulatory protein (StAR)
	Corticotropin receptor
	Scavenger receptor-B1
	Hydromethylglutaryl-CoA reductase
	DAX-1
	Aldose reductase-like protein
Gonads	
Leydig cells	Cytochrome P450 steroid hydroxylases
	StAR
	LH receptor
	Insulin-like polypeptide 3
	Prolactin receptor
	Mullerian inhibiting substance (MIS) receptor
Sertoli cells	MIS
	Inhibin
	FSH receptor
	Sex-determining region Y (SRY)
	SOX9 (SRY box)
Theca and granulosa cells	Cytochrome P450 steroid hydroxylases
	StAR
	Inhibin
	Oxytocin

induced the expression of the cholesterol side-chain cleavage enzyme (Crawford *et al.*, 1997b). In contrast, analyses of mutant Y1 cells with defects affecting SF-1 function provided evidence for multiple roles in differentiated function. The Schimmer laboratory showed that a SF-1 mutation was associated with decreased expression of the ACTH receptor, 11β-hydroxylase, cholesterol side-chain cleavage enzyme, and steroidogenic acute regulator protein (StAR) (Frigeri *et al.*, 2000). Interestingly, the SF-1 defect affected the expression of the ACTH receptor and 11β-hydroxylase to a much greater degree than cholesterol side-chain cleavage enzyme or StAR, suggesting further subtleties among the target genes in their regulation by SF-1.

The ability to disrupt SF-1 expression or function in a temporally specific manner after development of the steroidogenic tissues is completed would help to assess the importance of SF-1 in gene expression in a more physiological context. To this end, transgenic systems in which Cre recombinase is fused to ligand-inducible proteins (e.g., mutated versions of estrogen receptor or progesterone receptor) provide an opportunity to induce Cre pharmacologically by treatment with synthetic agonists. Such temporally regulated knockouts should provide a novel approach to examine the roles of SF-1 in this continuum of differentiated function. A clear delineation of SF-1 specific target genes, an increased understanding of the factors governing SF-1 function and expression, and the temporal staging of SF-1 action undoubtedly will provide important new insights into processes of endocrine differentiation and development.

ACKNOWLEDGMENTS

We thank the past and present members of the Parker and Schimmer laboratories, who have contributed to many of the studies reviewed here, and our many colleagues who communicated unpublished results. We especially acknowledge Professors Tsuneo Omura and Ken Morohashi, who independently provided many of the critical experiments that defined the multiple roles of SF-1 and who — even as competitors — were unfailingly generous in their sharing of reagents and unpublished data. Our studies were supported by the Howard Hughes Medical Institute and the National Institutes of Health (KLP) and the Medical Research Council of Canada and the Canadian Institutes of Health Research (BPS).

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