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Steroidogenic Factor 1: A Key Determinant of Endocrine Development and Function

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I. Introduction

THE nuclear hormone receptor family represents a group of gene-specific transcription factors that mediate the actions of diverse ligands including steroid hormones, thyroid hormone, vitamin D, and retinoids (1). This family of transcription factors also includes orphan members for which activating ligands have not been identified (2). One of these orphan nuclear receptors, steroidogenic factor 1 (SF-1), has emerged as a key regulator of endocrine function within the hypothalamic-pituitary-gonadal axis and adrenal cortex and as an essential factor in sex differentiation. SF-1 was first identified as a transcription factor with limited tissue distribution that recognized a conserved regulatory motif in the proximal promoter regions of genes encoding the cytochrome P450 steroid hydroxylases. These studies established that SF-1 was responsible, at least in part, for the tissue-specific expression of genes involved in steroid hormone biosynthesis. Broader roles for SF-1 emerged from genetic studies in mice, where SF-1 was inactivated by targeted gene disruption strategies. These SF-1 knockout mice exhibited adrenal and gonadal agenesis, male-to-female sex reversal of the internal and external genitalia, impaired gonadotrope function, and ablation of a specific region of the hypothalamus. These latter studies delineated essential roles for SF-1 in the regulation of the hypothalamic-pituitary-steroidogenic organ axis at various levels, as well as in the complex processes of endocrine differentiation.

Despite these insights into the various roles of SF-1, the precise mechanisms through which SF-1 exerts its multiple effects remain to be determined. This review highlights the critical experiments that have established SF-1 as a pivotal determinant of endocrine function and differentiation and proposes additional studies that are needed to enhance our understanding of SF-1 action.

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II. The Initial Identification of SF-1 as a Key Determinant of Steroid Hormone Biosynthesis

A. Overview of steroidogenesis

All endogenous steroids are derived from cholesterol by the sequential action of a number of enzymes that are members of the cytochrome P450 family of mixed-function oxidases or hydroxysteroid dehydrogenases (reviewed in Ref. 3). The genes encoding the steroid hydroxylases display tissue-specific expression and are also regulated by trophic hormones. As shown in Fig. 1, the steroid hydroxylases exhibit overlapping, but distinct, profiles of tissue-specific expression. The cholesterol side-chain cleavage enzyme [P450scc, also designated CYP11A according to the standardized nomenclature for the cytochrome P450 genes (4)], which carries out the initial and rate-limiting reaction in the production of all physiological steroids, is expressed by all of the primary steroidogenic tissues and also is expressed in certain nonclassic steroidogenic tissues such as the brain. Similarly, steroid 17α-hydroxylase (P45017α/CYP17), which is also required for the biosynthesis of multiple classes of steroid hormones, is expressed throughout the primary steroidogenic tissues. In contrast, two related isozymes of steroid 11β-hydroxylase (P45011β/CYP11B1 and P450 aldo/P45011B2) and steroid 21-hydroxylase (P45021/CYP21), which carry out terminal reactions in adrenal corticosteroid biosynthesis, are uniquely expressed in the adrenal cortex. Finally, aromatase (CYP19), the most widely distributed steroidogenic P450 enzyme, is found in the gonads, placenta, and a range of tissues that do not carry out *de novo* steroid biosynthesis. These overlapping but distinct profiles of steroid hydroxylase expression suggested that shared mechanisms contribute to the regulated expression of the steroidogenic enzymes in primary steroidogenic tissues, while distinct mechanisms also allow P450scc and aromatase to be expressed at additional sites.

The second major form of regulation of the steroidogenic enzymes is their induction by trophic hormones. Treatment of steroidogenic cells with trophic hormones coordinately increases the transcription of most of the steroid hydroxylases (reviewed in Ref. 5). This effect can be mimicked by cAMP analogs, suggesting that hormonal regulation is via the cAMP-dependent pathway. The induction of the steroid hydroxylases by cAMP is delayed and appears to require ongoing protein synthesis, whereas most other cAMP-responsive genes in both steroidogenic and nonsteroidogenic cells are rapidly induced through a mechanism that does not require *de novo* protein synthesis. These latter observations raised the possibility that the steroid hydroxylase genes are regulated by trophic hormones via pathways distinct from those involving classic cAMP-responsive elements and their cognate binding proteins (6).

B. SF-1 and the regulation of steroidogenesis

To understand the molecular mechanisms underlying the regulated expression of the steroid hydroxylases, the 5′-flanking regions of the various genes were isolated, placed

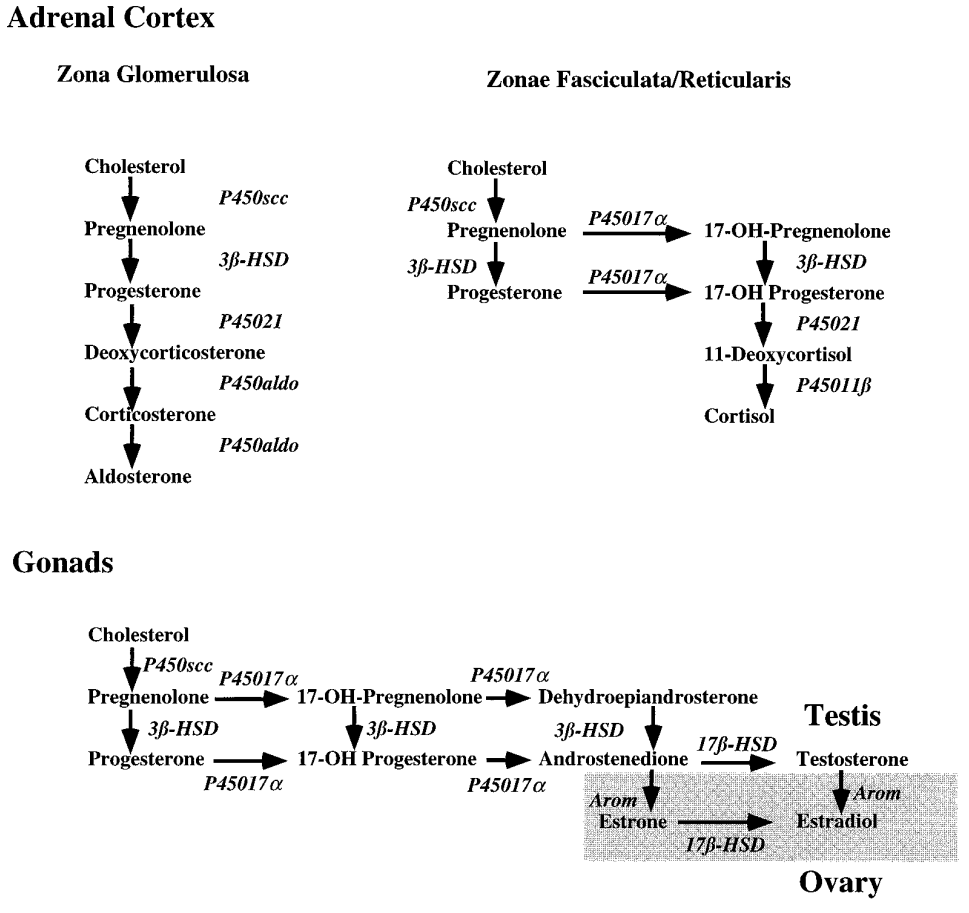

FIG. 1. Schematic overview of the biosynthetic pathways for steroid hormones. *Top*, The pathways by which adrenal corticosteroids are derived from cholesterol in the human zona glomerulosa and zonae fasciculata/reticularis. *Bottom*, The pathways by which sex steroids are produced in the gonads. Within the gonads, P45017α carries out two separate reactions: 17α-hydroxylation and C17–20 cleavage. Abbreviations: $P450_{scc}$, cholesterol side chain cleavage enzyme; 3B-HSD, 3β-hydroxysteroid dehydrogenase; P45017α, steroid 17α-hydroxylase; $P450_{21}$, steroid 21-hydroxylase; $P450_{aldo}$, aldosterone synthase; $P450_{11\beta}$, steroid 11β-hydroxylase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; Arom, Aromatase.

upstream of reporter genes, and transfected into steroidogenic or nonsteroidogenic cell lines to assess promoter activity (reviewed in Ref. 7). In these experiments, the 5′-flanking regions of the various steroid hydroxylases directed both tissue-specific and hormone-induced gene expression. In parallel, proteins that interacted with these 5′-flanking regions were studied by DNase I footprinting and gel mobility shift assays. From these studies, a number of regulatory elements were identified that contained variations of an AGGTCA motif, either PyCAAGGPyC or PuPuAGGTCA, and that interacted with a protein found only in steroidogenic cells, raising the possibility that a shared regulatory protein interacted with the steroid hydroxylase genes to regulate their coordinate expression (8, 9). Because of its apparent key role in regulating the steroid hydroxylases, this protein was designated steroidogenic factor 1 (SF-1) or adrenal 4-binding protein (Ad4BP).

C. Cloning and structural characterization of SF-1

Motivated by the belief that SF-1 was a key determinant of the expression of the cytochrome P450 steroid hydroxylases, two different laboratories independently set out to clone cDNAs encoding SF-1 (10, 11). Because the DNA-binding site for SF-1 was strikingly similar to the binding sites of other members of the nuclear hormone receptor family (12), Lala *et al.* (10) used a probe comprising the DNA-binding domain of the retinoid X receptor RXRβ to screen a mouse adrenal cDNA library for potential SF-1 clones. Independently, starting with extracts from bovine adrenal glands, Honda *et al.* (11) purified the corresponding protein by oligonucleotide affinity chromatography, determined its partial peptide sequence, and then used oligonucleotide probes designed from this peptide sequence to screen a bovine adrenal cDNA library. In each case, cDNA clones were isolated that, when expressed in transient transfection assays, activated promoter activity of the steroid hydroxylases, establishing that they represented authentic SF-1 clones (13, 14).

1. Structural features of SF-1. Using these cDNAs as probes, SF-1 cDNAs from rat (15) and human (16, 17) were also isolated, and these sequences were compared with delineate conserved regions that might contribute to SF-1 function. As shown in Fig. 2, all four SF-1 cDNAs share conserved regions that correspond to known functional domains of other members of the nuclear receptor family (12). For example, SF-1 contains two zinc finger modules that mediate its binding to DNA. Both of these zinc finger modules, including the proximal (P) box in the first zinc finger and the distal (D) box within the second zinc finger, and the intervening linker region are conserved absolutely among the four species. In classic steroid hormone receptors, the P box determines the DNA sequence recognition for half-sites of the hormone-responsive elements, whereas the D box forms a dimerization interface that determines the appropriate spacing of these half-sites (18). Interestingly, each SF-1 sequence contains a hybrid P box that combines residues characteristic of glucocorticoid and estrogen receptor subclasses of nuclear receptors. A subset of nuclear receptors, including SF-1, NGFI-B, ROR, ERR1, and ERR2, interact as monomers with AGGTCA recognition motifs (1). Several of these receptors share an additional 30-amino acid carboxyl-terminal extension adjacent to the second zinc finger motif, designated the FTZ-F1 or A box, that recognizes additional bases 5′ to the AGGTCA hexamer (19, 20). Studies comparing the sequence requirements for SF-1 and the closely related orphan receptor NGFI-B have revealed differences in the preferred 5′-nucleotides (**PyCA**AGGTCA for SF-1 *vs.* **AA**AGGTCA for NGFI-B) and have identified amino acid residues within the A box that specify their differential binding (21). The 30 amino acids comprising the A box are conserved absolutely in all four mammalian SF-1 proteins.

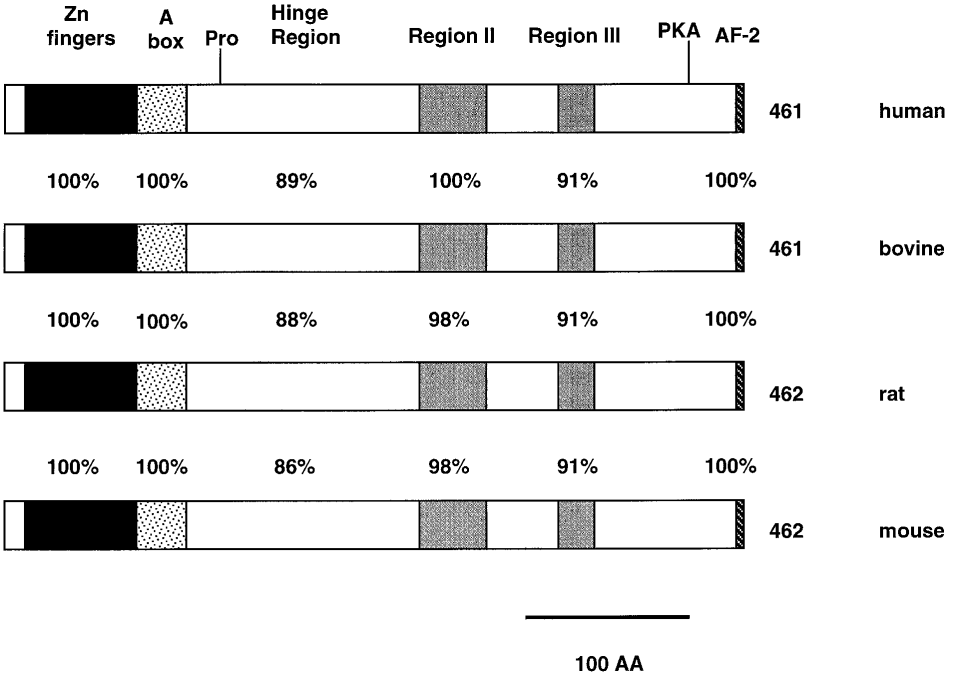

FIG. 2. Sequence conservation in different regions of SF-1. The functional domains of SF-1 in human, cow, rat, and mouse are drawn to scale. The *numbers above* each region indicate the percentage of identity in each region relative to the human sequence. The numbers at the carboxyl terminus indicate the total number of amino acids for each species. The positions of the zinc fingers (*black*), the A box (*stippled*), hinge region, Regions II and III (*gray*), and the AF-2 transactivation domain (*cross-hatched*) are indicated. Pro, The proline-rich sequence in the hinge region; PKA, the motif matching the consensus for phosphorylation by cAMP-dependent protein kinase. [Modified with permission from M. Wong *et al.*: *J Mol Endocrinol* 17:139–147, 1996 (17).]

In addition to the conserved regions that mediate DNA binding, SF-1 cDNAs from various mammalian sources also show homology in C-terminal regions that form the ligand-binding domain of ligand-activated nuclear receptors. In particular, the AF-2 transactivation domain is found at the carboxyl terminus of many ligand-inducible nuclear receptors, forming an amphipathic α-helix that apparently is essential for transcriptional activation (22–24). This region is conserved absolutely in all SF-1 proteins. To the extent that the AF-2 motif identifies ligand-activated receptors, the conservation of this domain raises the exciting possibility that a ligand may mediate SF-1-dependent transactivation.

Finally, there are other highly conserved regions of SF-1 for which functional roles have not yet been defined. Within the hinge region, there is a conserved stretch of seven (human) or eight (cow, rat, and mouse) consecutive prolines that lies within a proline-rich domain of approximately 100 amino acids (amino acids 124–226). This domain has been proposed to mediate transcriptional activation by SF-1 (11). Another conserved motif near the carboxy-terminal region of SF-1 (amino acids 427–430) is a potential consensus site for phosphorylation by cAMP-dependent protein kinase (11). As discussed below (*Section III.B*), the latter sequence may provide a mechanism for interactions between the cAMP-dependent signaling pathway and SF-1 transcriptional activation.

2. Multiple transcripts are encoded by the gene encoding SF-1. In addition to its general resemblance to other members of the nuclear receptor family, the mouse SF-1 cDNA strikingly resembled a cDNA isolated from mouse embryonal carcinoma cells, which was designated embryonal long terminal repeat-binding protein (ELP) because of its ability to bind a negative regulatory element in retroviral long terminal repeats (25). Isolation and characterization of the mouse gene encoding SF-1 revealed that the SF-1 and ELP transcripts arise from the same structural gene by alternative promoter usage and 3′-splicing (13). As shown in Fig. 3, subsequent characterization of various SF-1/ELP transcripts isolated

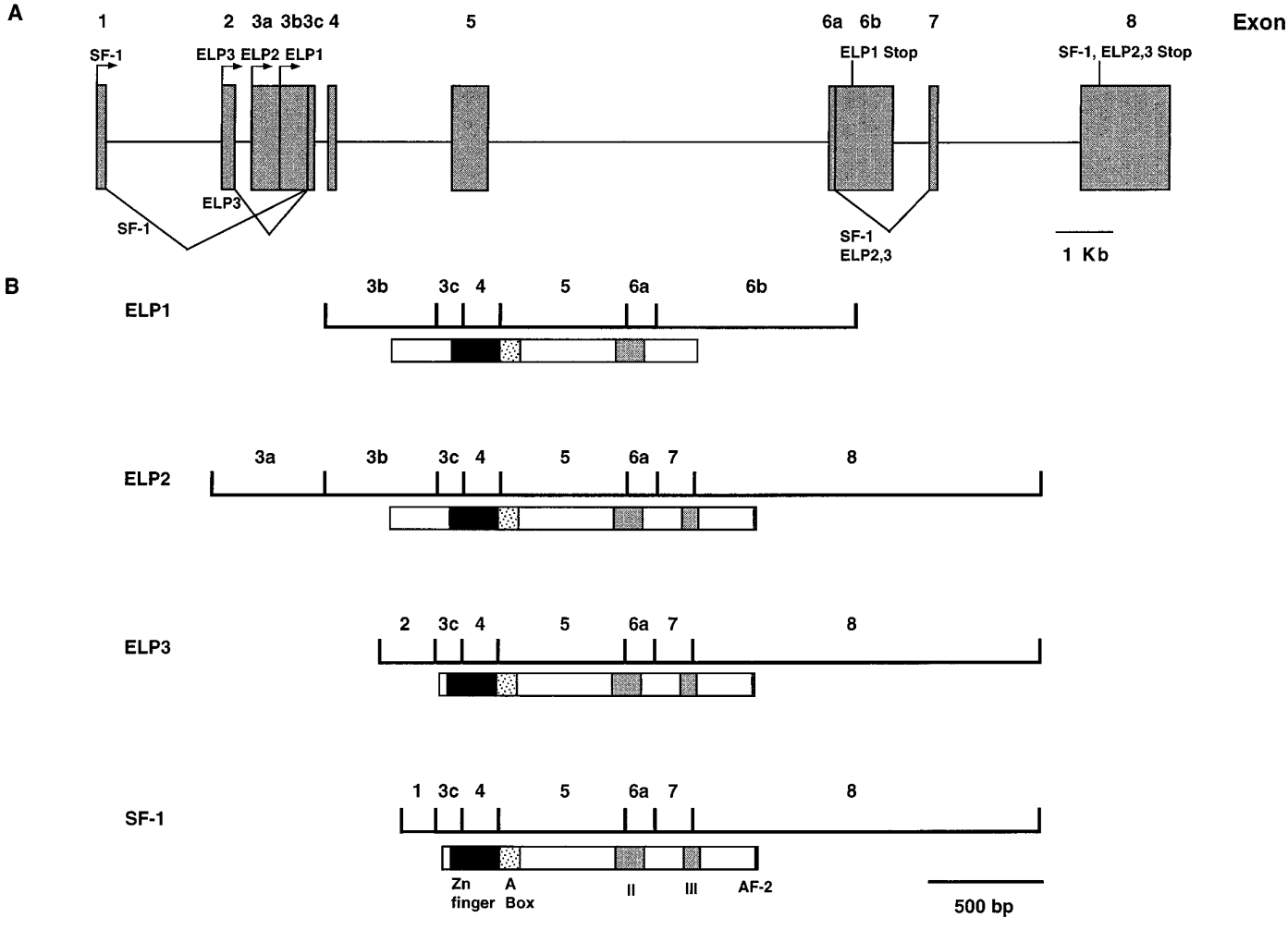

FIG. 3. A, Schematic organization of the mouse *Ftz-F1* gene. Shown are the relative positions of the transcription initiation sites (*horizontal arrows*), stop codons, and exons of the transcripts encoded by the mouse *Ftz-F1* gene, as described by Ninomiya *et al.* (26). Alternative splicing events that generate the SF-1, ELP2, and ELP3 transcripts are indicated *below* the diagram. B, Origins of the four transcripts encoded by the mouse *Ftz-F1* gene. The exons included in each transcript are shown above, using the same numbering system as in panel A. Shown *below* are diagrams of the proteins encoded by each transcript. *Highlighted* regions, using the nomenclature from Fig. 2, include the Zn finger modules (*black*), the A box (*stippled*), Regions II and III (*gray*), and the AF-2 domain.

from embryonal carcinoma cells and structural analysis of the mouse gene indicate that four distinct transcripts, designated ELP1, ELP2, ELP3, and SF-1, arise from different promoters and/or differ by their inclusion of alternative exons (26). As discussed in detail below, these transcripts differ in their profiles of expression and may serve distinct functional roles.

3. The gene encoding SF-1 is evolutionarily conserved in vertebrates and invertebrates. As initially noted with the ELP cDNA (25), SF-1/ELP closely resembled *Drosophila* fushi tarazu factor 1 (FTZ-F1), an orphan nuclear receptor proposed to regulate the expression of the *fushi tarazu* homeobox gene (27, 28). On this basis, the mouse gene was also named *Ftz-F1. FTZ-F1* homologs have now been identified in other invertebrates [*e.g.* the silkworm (29)] and vertebrates (10, 11, 15–17, 30). A subset of these genes have been mapped to specific chromosomal loci in *Drosophila* [cytological locus 75 CD (28)], mouse [proximal arm of chromosome 2 (31, 32)], and human [9q33 (31)]. Of considerable interest, all *FTZ-F1* genes characterized to date apparently encode at least two transcripts (33), suggesting that the different transcripts encode proteins that have different functions and thus are biologically significant.

In addition to these genes that presumably represent homologs of the mouse *Ftz-F1* gene, a very high level of sequence conservation was noted to an orphan receptor cloned from mouse liver, designated LRH1 (Genbank number M81385) and its human homolog, designated PHR-1 (34). Although LRH1 clearly derives from a separate gene and is expressed in tissues that do not express SF-1 (*e.g.* liver and pancreas), the LRH1 and SF-1 sequences are sufficiently similar, including the hybrid P box and the A box, to group them as members of the same subfamily of nuclear hormone receptors, designated NR5A (35). Moreover, based on the detailed analysis of DNA-binding specificity for SF-1, it is likely that LRH1 will also bind as a monomer to PyCAAGGTCA half-site motifs (21).

III. Characterization of Sites of SF-1 Expression and Identification of Its Target Genes

After the initial isolation and characterization of SF-1, a number of laboratories initiated studies to define its roles in endocrine function. Two complementary approaches were used to determine these roles: characterization of the tissues in which SF-1 is expressed and identification of target genes that are regulated by SF-1.

A. Profiles of SF-1 expression

Using reagents provided by the cloning of SF-1, several laboratories studied the tissue-specific expression of this orphan nuclear receptor, focusing first on the steroidogenic tissues of the adult and then extending their analyses to different developmental stages and to other tissues.

1. Adult steroidogenic tissues. Using *in situ* hybridization with a cRNA probe specific for SF-1, Ikeda *et al.* (13) showed that the expression pattern of SF-1 in adult tissues generally correlated with its proposed roles in regulating the steroid hydroxylases. Thus, SF-1 transcripts were detected in adrenocortical cells, testicular Leydig cells, and ovarian theca and granulosa cells. These sites corresponded to the tissues containing proteins that interacted with SF-1-responsive elements and correlated with the known profiles of expression of the cytochrome P450 steroid hydroxylases (3). In contrast, SF-1 transcripts were not detected in the placenta, which produces large amounts of progesterone by *de novo* synthesis from cholesterol and also converts adrenal androgens to estradiol, although more sensitive methods of RT-PCR revealed low levels of SF-1 expression at this site (11, 26). Complementary studies using a specific antibody against SF-1 confirmed that the expression profile of SF-1 protein in adult rats (14) and humans (36, 37) corresponded to sites where transcripts were detected.

2. Embryonic steroidogenic tissues. The acquisition of a sexually dimorphic phenotype is a critical event in embryonic development. In eutherian mammals (Fig. 4), the basic principle underlying these events is that genetic sex, determined at the time of fertilization by the presence or absence of the Y chromosome, leads to sexually dimorphic development of the embryonic gonads into either testes or ovaries (38). Recent studies have shown definitively that a gene on the Y chromosome, designated SRY for Sex-determining Region-Y chromosome, is sufficient to activate a cascade of events that ultimately leads to the formation of testes (39–42). Thereafter, hormones produced by the embryonic testes direct male sexual differentiation, whereas female sexual differentiation occurs in the presence of ovaries or in the complete absence of gonads. Two hormones produced by the testis are required for male sexual differentiation: Müllerian-inhibiting substance (MIS) and androgens (43, 44). The fetal Sertoli cells,

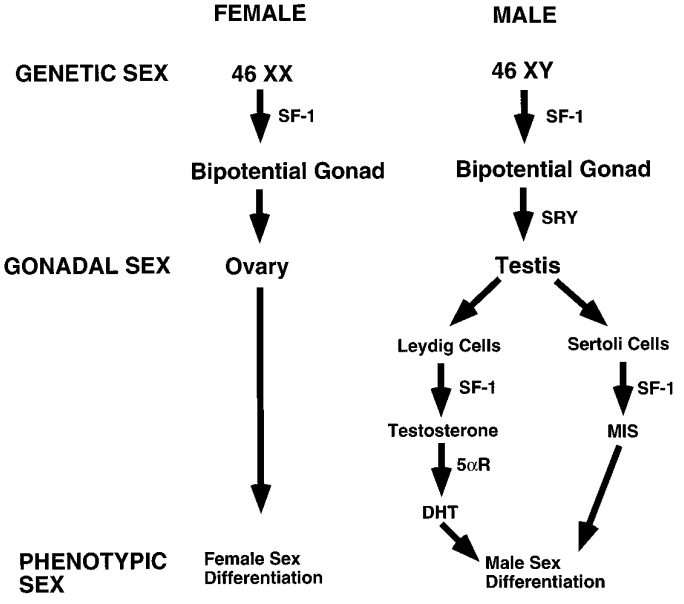

FIG. 4. Current concepts of mammalian sexual determination and differentiation. Diagrammed are the processes by which genetic sex is translated into gonadal sex, which then mediates phenotypic sexual differentiation. SRY, Sex-determining region Y chromosome; MIS, Müllerian-inhibiting substance; DHT, dihydrotestosterone; 5αR, steroid 5α-reductase; SF-1, steroidogenic factor 1.

contained within the testicular cords, secrete MIS, which causes regression of Müllerian duct-derived structures that would otherwise form the uterus, cervix, fallopian tubes, and the upper vagina. The fetal Leydig cells, within the interstitial region, produce testosterone, which leads to virilization of the male structures derived from the Wolffian duct, including the epididymis, vas deferens, and seminal vesicles. Virilization of the external genitalia further requires the conversion of testosterone to dihydrotestosterone, a reaction catalyzed by 5α-reductase (reviewed in Ref. 45).

Based on the known roles of steroid hormones in embryonic development and the apparent link between SF-1 and FTZ-F1, a key regulator of *Drosophila* development, the profiles of SF-1 expression during embryonic development were determined. Initial studies examined the spatial and temporal profiles of SF-1 transcripts in mouse embryos (46). As shown by *in situ* hybridization, SF-1 transcripts at embryonic day 9 (E9) localized to the urogenital ridge, which ultimately contributes cells to the adrenal cortex, gonads, and the mesonephros. At this early time, SF-1 was detected in a single population of cells. Later, the SF-1-expressing cells resolved into two discrete populations: a group of cells adjacent to the dorsal aorta that represent adrenocortical precursors and a larger group of cells adjacent to the coelomic epithelium that represent the gonadal precursors. These findings suggested that SF-1 was expressed from the very inception of the urogenital ridge, and that a common precursor ultimately gives rise to the steroidogenic compartments of both the adrenal gland and gonads. More recent studies have elegantly confirmed this model, showing that a common pool of rat cells expressing SF-1 protein subsequently resolve into distinct adrenocortical and gonadal precursors, with only the gonadal component supporting the migration of the primordial germ cells (47).

With respect to adrenocortical development, the profile of SF-1 expression was largely consistent with its presumed essential role in steroidogenesis. SF-1 transcripts were detected when the adrenal primordium first appears as a distinct structure at ~E10–10.5. This expression clearly preceded that of P450scc, which was not detected until ~E11, consistent with the model that SF-1 is necessary for expression of the steroid hydroxylases. As the chromaffin cell precursors subsequently migrated into the adrenal primordium at ~E12.5-E13.5, SF-1 expression localized to the steroidogenic cortical cells, where it was expressed throughout the remainder of gestation and postnatal life.

Analyses of SF-1 expression in the embryonic gonads not only supported a role for SF-1 in steroidogenesis, but also indicated that this transcription factor may play additional roles in development. As noted above, SF-1 transcripts were first detected in the urogenital ridge of both male and female embryos at ~E9, preceding the onset of Sry expression. At this time, testes and ovaries cannot be distinguished histologically and are termed indifferent or bipotential gonads. Thereafter, under the influence of Sry, the fetal testes become recognizable as they differentiate into two distinct compartments: the testicular cords, which contain the fetal Sertoli cells and the primordial germ cells, and the interstitial region, where the steroidogenic Leydig cells are found. At E12.5, coincident with formation of the testicular cords, SF-1 expression persists and is detected in both the steroidogenic Leydig cells and in the testicular cords. The expression of SF-1 within the testicular cords hinted that its role in gonadal development extended beyond regulating the expression of steroidogenic enzymes.

In the ovary, in contrast, there is an apparent decline in SF-1 transcripts (46) and protein (48) coincident with gonadal sexual differentiation, suggesting that normal female sexual differentiation is facilitated by a decrease in SF-1 expression. Thereafter, SF-1 levels in the ovary remain low or undetectable until the onset of follicular development (37). SF-1 expression is first detected in both theca and granulosa cells at the preantral stage, which precedes the expression of aromatase in granulosa cells, consistent with the model that SF-1 is an essential upstream regulator of aromatase expression. As follicular maturation proceeds, SF-1 is strongly expressed by both granulosa and theca cells of the antral follicles. Finally, levels of SF-1 expression decline considerably as follicles become atretic or convert to corpora lutea.

3. Other sites of SF-1 expression. Initial analyses of SF-1 activity in gel shift assays and *in situ* hybridization analyses of adult mice had indicated that SF-1 was restricted to the steroidogenic cells of the adrenal gland and gonads. Therefore, it was a surprise when *in situ* analyses of E11.5 mouse embryos revealed SF-1 transcripts within a discrete region of the developing diencephalon (46). Later in development, the SF-1 signal localized to regions that represent the hypothalamic precursor and then finally localized to neurons within a discrete hypothalamic nucleus, the ventromedial hypothalamic nucleus [VMH (49–51)]. Within the VMH, the expression of SF-1 was predominantly within the dorsomedial part, although some expression was also seen in the ventrolateral region. SF-1 transcripts also were detected in the developing anterior pituitary gland by E13.5, with subsequent restriction to cells of the gonadotrope lineage (50, 52–54). These findings raised the intriguing possibility that SF-1 played roles that extend beyond the maintenance of steroidogenic capacity within the primary steroidogenic tissues. A schematic summary of the sites and onset of SF-1 expression, both in primary steroidogenic tissues and in extrasteroidogenic sites, is given in Fig. 5.

More recent studies have used RT-PCR to analyze the profiles of expression of the various transcripts encoded by the mouse *Ftz-F1* gene (designated SF-1, ELP1, ELP2, and ELP3). Although SF-1 was expressed predominantly in the adrenal gland, ovary, and testis, SF-1 transcripts also were detected in the placenta and spleen (26, 55). Similar RT-PCR approaches also detected SF-1 transcripts in the human placenta (56), although studies in knockout mice lacking SF-1 suggest that SF-1 is not an essential regulator of placental steroid hydroxylase expression (see below).

Intriguingly, the pituitary contains transcripts corresponding to the ELP3 isoform. This *Ftz-F1* product encodes the same protein as SF-1, but its transcription is controlled by a different promoter (26). If confirmed, this finding raises the possibility that distinct promoters direct the expression of the same nuclear receptor protein—SF-1—in primary steroidogenic tissues *vs.* extrasteroidogenic sites such as pituitary gonadotropes and the VMH.

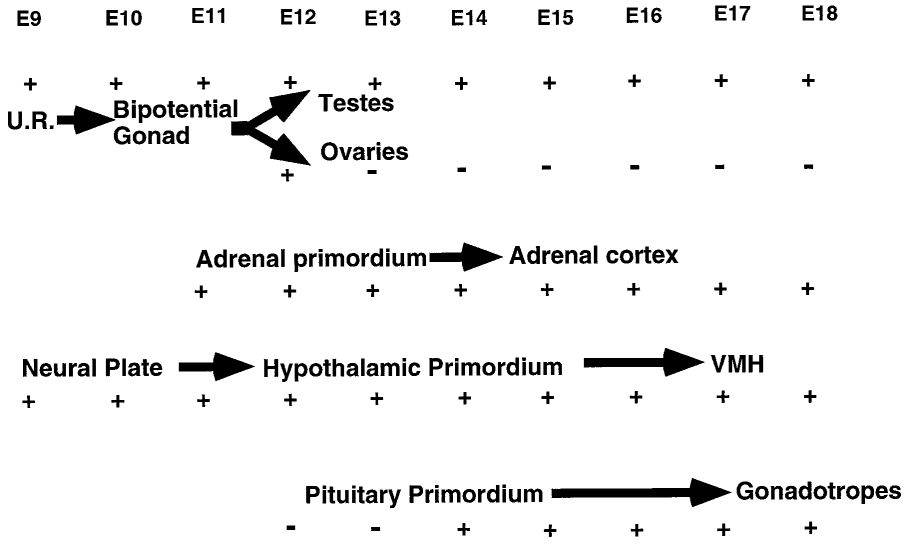

FIG. 5. Ontogeny of SF-1 expression in mouse embyros. The ontogeny of expression of SF-1 transcripts in developing mouse embryos from embryonic day 9 (E9) to E18 is summarized schematically. (+) Indicates that SF-1 mRNA was present, (−) indicates that transcripts were absent. The *arrows* depict the approximate transition times between the different stages of development. U.R., Urogenital ridge; VMH, ventromedial hypothalamic nucleus.

TABLE 1. Genes that are regulated by SF-1 in steroidogenic cells

Gene	Cells or Tissue	Reference(s)
mP450scc	Y1 adrenocortical cells	57
rP450scc	granulosa cells	58
hP450scc	Y1 adrenocortical	59
mP45021	Y1 adrenocortical cells	60
bP45011β	Y1 adrenocortical cells	61
mP450aldo	Y1 adrenocortical cells	62
bP45017α	Y1 adrenocortical cells	63
rP45017α	Y1 adrenocortical cells MA-10 Leydig cells	64
rAromatase	granulosa cells R2C Leydig cells	65, 66
hAromatase	granulosa cells	67
h3β-HSD 2	Y1 adrenocortical cells	68
hStAR	Y1 adrenocortical cells	69
mStAR	Y1 adrenocortical MA-10 Leydig	70
hACTH Receptor	adrenocortical cells	71
bOxytocin	ovary	72

Abbreviations: m, mouse; r, rat; h, human; b, bovine.

B. Target genes regulated by SF-1

1. Steroidogenic cells. Concurrent with studies to define the localization and temporal expression of SF-1, a number of laboratories have attempted to gain insight into the functions of SF-1 by identifying the target genes that it regulates. SF-1 was first identified and isolated as a critical regulator of the steroid hydroxylases within adrenocortical cells. This role for SF-1 was subsequently extended to other steroidogenic cells, as summarized in Table 1 (57–67), strongly suggesting that SF-1 truly acts as a global regulator of the cell-specific expression of the cytochrome P450 steroid hydroxylases.

In addition to these roles in cell-specific expression, some reports have linked SF-1 to the hormone-induced expression of the steroid hydroxylases. The mechanisms of cAMP-regulated expression of the steroid hydroxylase genes appear to be complex and may not involve a common underlying mechanism (reviewed in Refs. 5 and 7). For some of the steroid hydroxylases, *e.g.* P45011β and P450aldo, cAMP-induced expression apparently results from interactions of classic cAMP-responsive elements with cAMP response element binding protein. Other transcription factors, including Sp1, Pbx-1, and NGFI-B, also have been variously implicated in the hormonal regulation of the steroid hydroxylase genes. Of considerable interest, however, SF-1-responsive elements also have been linked to cAMP-induced expression. At least in certain settings, SF-1 sites only stimulate transcription when cAMP-dependent protein kinase is active (14, 73). Coupled with the conserved consensus motif for phosphorylation by cAMP-dependent protein kinase (Fig. 2) and the demonstration that the catalytic subunit of cAMP-dependent protein kinase can phosphorylate SF-1 (64), these findings raise the possibility that SF-1 may play pivotal roles in both the cell-specific and hormone-induced regulation of the cytochrome P450 steroid hydroxylases.

Studies have also implicated SF-1 as a key regulator of non-cytochrome P450 components of the steroidogenic complex within steroidogenic cells. As shown in Fig. 1, the non-cytochrome P450 enzyme, 3β-hydroxysteroid dehydrogenase (3β-HSD), is also required for the biosynthesis of all major classes of steroids, and SF-1 has been reported to regulate the Type II isozyme of 3β-HSD, which is expressed only in the adrenal cortex and gonads (68). Similarly, recent studies have indicated that SF-1 also regulates the expression of the Steroidogenic Acute Regulatory Protein (StAR), a mitochondrial protein that plays a critical role in cholesterol delivery to the mitochondria where the initial reactions in steroidogenesis are catalyzed by P450scc (69, 70). Finally, recent studies have suggested that SF-1 regulates the expression of the ACTH receptor within adrenocortical cells (71). Collectively, these studies point to a pivotal role in the maintenance of differentiated function and steroidogenic capacity in steroidogenic cells of the adrenal cortex and gonads.

2. Sertoli cells. The observation that SF-1 was expressed in nonsteroidogenic cells suggested that SF-1 might regulate target genes within these cells. One attractive candidate for an SF-1 target gene within Sertoli cells was the *MIS* gene,

whose expression closely parallels that of SF-1 (74). Sequence analyses of the 5′-flanking regions of MIS genes identified a conserved motif matching exactly the optimal sequence for SF-1 binding. Gel mobility shift experiments established that a protein in Sertoli cell extracts formed a complex with this element that migrated identically to known SF-1-dependent complexes. This complex was abrogated by the addition of a polyclonal antiserum specific for SF-1, proving that SF-1 was the protein in Sertoli cells that bound this element. Finally, transfection experiments in primary Sertoli cell cultures showed that this element was critical for MIS promoter activity. In contrast to these results, studies in immortalized cultures of cells derived from the embryonic gonad suggested that SF-1 does not activate, but rather represses, the MIS promoter (75). The basis for these divergent results in different transfection systems remains to be determined, and further studies are needed. Nonetheless, it is attractive to propose that SF-1 directly regulates the *MIS* gene, and thus is crucial for the production of both essential mediators of male sexual differentiation (74).

Another potential target gene for SF-1 in Sertoli cells is aromatase, which is expressed in prepubertal Sertoli cells (76). The role of SF-1-responsive elements in aromatase expression in other cell types (65–67) lends credence to this model, although studies analyzing aromatase promoter activity in Sertoli cells have not been reported.

3. Gonadotropes. The observation that SF-1 is expressed by pituitary gonadotropes has prompted a number of laboratories to seek target genes of SF-1 that might contribute to gonadotrope function. Previous analyses of the promoter of the gene encoding the α-subunit of glycoproteins (α-GSU) had identified an element (the gonadotrope-specific element, or GSE) that regulated α-GSU promoter activity by interacting with a protein that apparently was limited to gonadotropes (77). When it was recognized that the GSE resembled the SF-1-binding site, the possibility arose that SF-1 might bind the GSE; this model was confirmed by gel mobility shift and functional transfection assays, which demonstrated that SF-1 regulates α-GSU promoter activity via the GSE (52, 78). Subsequent studies have implicated SF-1 as an upstream regulator of the genes encoding the β-subunit of LH (79, 80) and the GnRH receptor (81). Thus, reminiscent of the findings in steroidogenic cells, it appears that SF-1 regulates a constellation of genes that are required for gonadotrope function.

4. VMH. As summarized below, knockout mice deficient in SF-1 have profound defects in the VMH, strongly suggesting the presence of SF-1 target genes at this site. To date, however, these target genes have not been identified, perhaps reflecting the complexities of studying gene expression within neuronal cells.

IV. The Roles of SF-1 *in Vivo*: Targeted Gene Disruption to Create SF-1 Knockout Mice

As summarized above, analyses of sites of SF-1 expression and identification of potential target genes provided intriguing hints into possible roles of SF-1 *in vivo*. To address directly these roles, several laboratories employed targeted gene disruption in embryonic stem cells to make SF-1 knockout mice. Two groups disrupted the *Ftz-F1* gene within exons encoding the zinc finger modules that are common to all *Ftz-F1*-derived isoforms (55, 82). The third group targeted a more 3′-exon, again encoding sequences shared by all *Ftz-F1* isoforms (50). Finally, an alternative approach was taken that mutated the initiator methionine that is shared by the products of the SF-1 and ELP3 transcripts; this approach was aimed at preventing selectively the expression of SF-1 and ELP3 without interfering with the ELP1 and ELP2 products (83). Despite these different targeting strategies, the effects of each SF-1 knockout on endocrine development and function are strikingly similar, suggesting that the observed phenotype results directly from the inactivation of *Ftz-F1*-derived transcripts. For convenience, these *Ftz-F1*-disrupted mice will be referred to below as "SF-1 knockout mice."

A. General features of the SF-1 knockout mice

Genotypic analysis of offspring of heterozygous +/− mice showed that SF-1 knockout mice were born at the expected frequency of 1:4, establishing that *Ftz-F1*-encoded transcripts are not required for survival *in utero*. At birth, the SF-1 knockout mice were indistinguishable from wild-type littermates, except that all SF-1 knockout mice had female external genitalia irrespective of genetic sex. Within 12 h after birth, the SF-1 knockout mice showed evidence of volume depletion and began to die. Most animals died within 3 days, and all were dead within 8 days. The SF-1 knockout mice had significantly diminished corticosterone levels and elevated ACTH levels and could be kept alive by injection of a glucocorticoid/mineralocorticoid cocktail, proving that the cause of their death was adrenocortical insufficiency (83).

B. Primary steroidogenic tissues in SF-1 knockout mice

The features of the SF-1 knockout mice—male-to-female sex reversal of external genitalia and adrenocortical insufficiency—were consistent with the proposed roles of SF-1 in androgen and corticosteroid biosynthesis. What was not anticipated, as shown in Fig. 6, was the complete absence of adrenal glands and gonads in the knockout mice—findings that revealed obligatory roles for SF-1 in the development of the primary steroidogenic tissues (55, 82). Both male and female SF-1 knockout mice had normal oviducts, uterus, and vagina, indicating that male internal genitalia were sex-reversed.

In SF-1 knockout embryos, some mesenchymal thickening was apparent in the region where the genital ridge normally develops, suggesting that gonadal development is initiated in the absence of SF-1. Shortly thereafter, however, cells in the genital ridge exhibited morphological features and DNA fragmentation consistent with apoptosis, and the structures regressed. These findings indicated that the lack of expression of SF-1 leads to the onset of programmed cell death within the developing gonads and adrenal gland at discrete developmental stages. Before gonadal regression, the primordial germ cells were detected in the genital ridge, indicating that SF-1 is not required for their migration into the

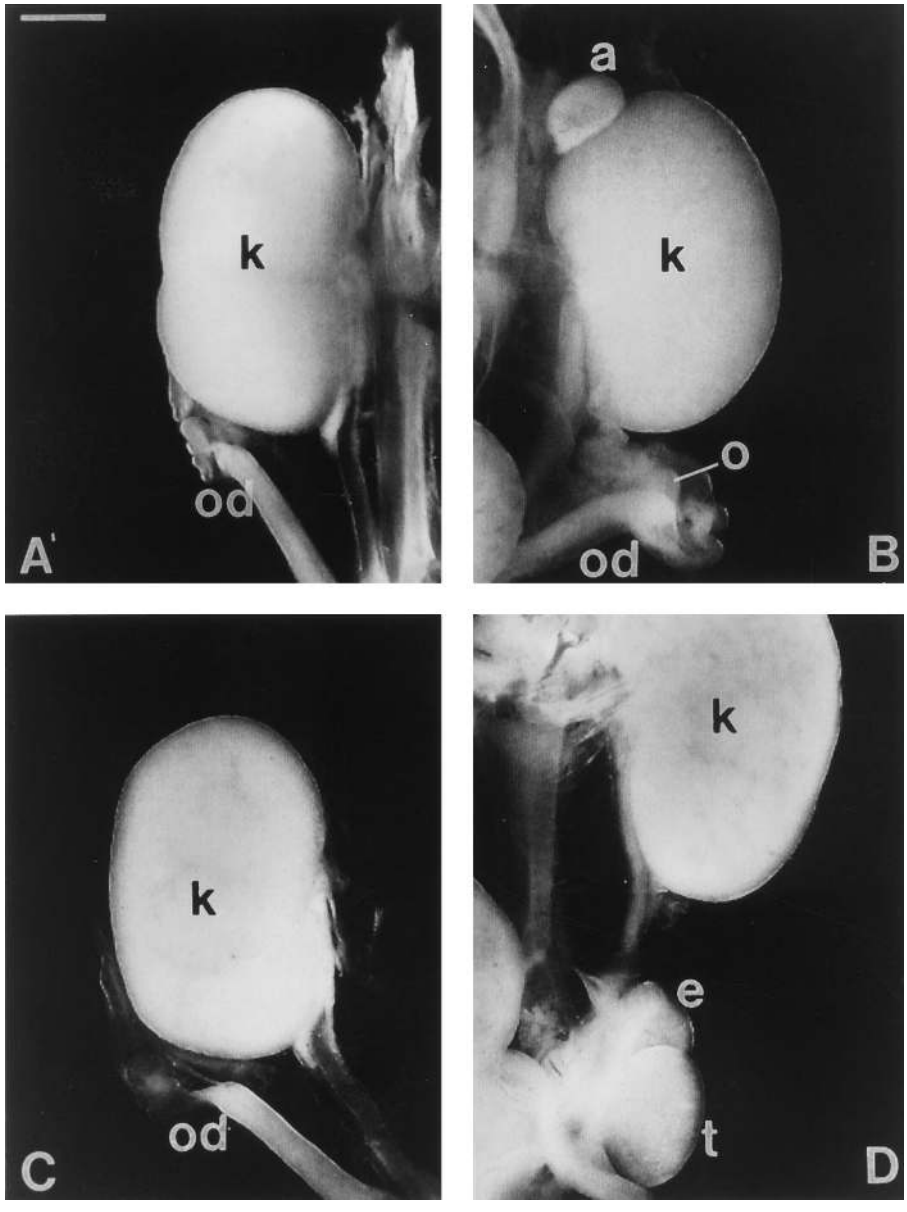

FIG. 6. Newborn SF-1 knockout mice lack adrenal glands and gonads and have female internal genitalia. SF-1 knockout mice (*left*) and wild-type littermates (*right*) were killed, and the genitourinary tracts were dissected. A, SF-1 knockout female. B, Wild-type female. C, SF-1 knockout male. D, Wild-type male. *Scale bar* = 1 mm. k, Kidney; a, adrenal; o, ovary; t, testis; e, epididymis; od, oviduct. [Reprinted with permission from X. Luo *et al.*: *Cell* 77:481–490, 1994 (82). © Cell Press.]

developing gonads. As the gonads disintegrated, however, there was a coincident loss of germ cells, which presumably is a secondary effect since these cells do not express SF-1. Presumably, the absence of the adrenal medulla in SF-1 knockout mice also reflects such secondary effects, since the chromaffin cell precursors also do not express SF-1.

In contrast to the profound consequences of the SF-1 knockout on the adrenal glands and gonads, the placenta was intact histologically and expressed normal levels of P450scc (55). Similarly, the expression of P450scc in the fetal intestine was preserved in SF-1 knockout mice (84). These findings strongly suggest that SF-1 does not regulate steroid hydroxylase expression beyond the classic, hormonally responsive steroidogenic tissues (*e.g.* the adrenal cortex and gonads). Steroid hydroxylase expression in these other sites may utilize distinct promoter elements or may reflect the use of alternative promoters, as has been shown to be the case for aromatase expression in the placenta (85).

C. Pituitary and hypothalamic defects in SF-1 knockout mice

The expression of SF-1 in the anterior pituitary and hypothalamus suggested that the SF-1 knockout mice might also exhibit abnormalities at these sites. Within the anterior pituitary, a number of cell lineages were apparently unaffected, as normal immunoreactivities for GH, PRL, TSH, and corticotropin were observed (52). In fact, mRNA levels for POMC, the precursor to ACTH, were elevated approximately 3-fold, consistent with the loss of negative feedback regulation by glucocorticoids. In contrast, the SF-1 knockout mice specifically lacked immunoreactivity for LH and FSH, two separate markers of gonadotropes—the pituitary cell

type that expresses SF-1 (50, 52). As measured by *in situ* hybridization, transcripts for α-GSU, LHβ, FSHβ, and the receptor for GnRH were all markedly decreased. These findings revealed important roles of SF-1 in gonadotrope function, suggesting that, as in the primary steroidogenic tissues, SF-1 regulates the expression of multiple genes that constitute the differentiated phenotype of gonadotropes.

Significant effects of the SF-1 knockout also were observed in the VMH, the hypothalamic region where SF-1 normally is expressed. As shown in Fig. 7, the VMH was virtually ablated in the SF-1 knockout mice (49, 50), demonstrating that SF-1 also plays essential roles within this hypothalamic nucleus. The defect was most pronounced in the dorsomedial part of the VMH, where SF-1 expression was strongest, and included a lack of the capsule delineating the VMH, which contains dendrites derived from VMH neurons (50). Subtle changes also were observed in the dorsomedial hypothalamic nucleus (DMH), a region that does not express SF-1 but has multiple connections with the VMH (50). Presumably, the changes in the DMH were secondary to the loss of connections from the VMH, although, alternatively, they may result from the absence of steroid hormones. Based on lineage analysis afforded by the expression of an SF-1/neo fusion transcript within cells that would normally express SF-1 in the knockout mice, it appears that the precursors migrated normally to the appropriate region of the hypothalamus in SF-1 knockout mice, but disappeared between E18 and postnatal day 1 (49). Thus, these results are reminiscent of the situation in the adrenal gland and gonads, which are relatively intact at early stages of development, but then regress at a discrete time point and ultimately disappear.

Most recent studies have sought to refine our understanding of the phenotype of SF-1 knockout mice. Given that hypothalamic abnormalities also are present, impaired gonadotrope function could reflect intrinsic defects in the gonadotropes or effects secondary to the ablation of the VMH. Although GnRH is present in GnRH neurons of the medial hypothalamus in apparently normal amounts, and is transported to the median eminence where its release normally occurs (S. Moenter and K. Parker, unpublished observation), treatment of the SF-1 knockout mice with GnRH restored pituitary expression of LH and FSH (49). These results suggest that gonadotropes, in contrast to the adrenals and gonads and the VMH, are not totally ablated in the absence of SF-1 and that SF-1 is not absolutely essential for gonadotropin production. These studies further suggest that the VMH, either directly or indirectly, interacts with the GnRH neurons to facilitate GnRH release. Although the VMH does not make major connections to the GnRH neurons, these effects could be indirect (*i.e.* transmitted via connections to intermediate neurons that then communicate with the GnRH

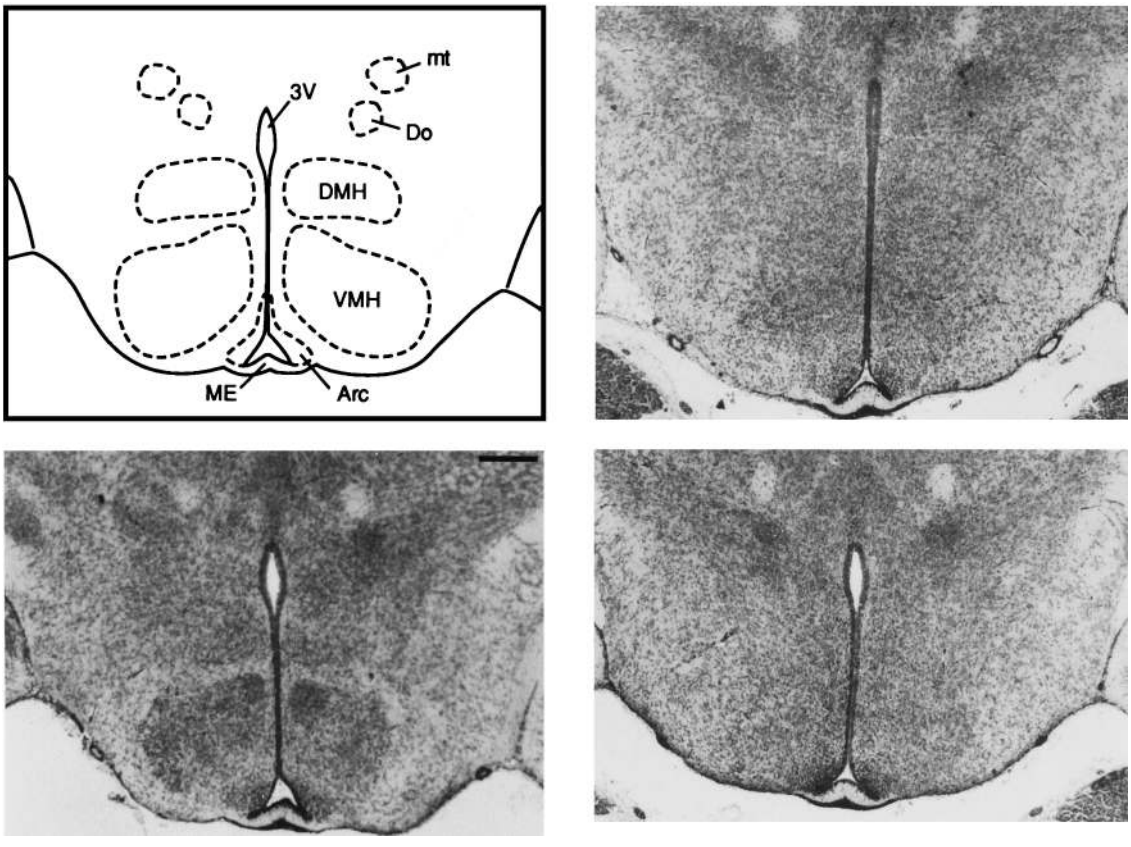

FIG. 7. Steroidogenic factor 1 knockout mice lack the ventromedial hypothalamic nucleus (VMH). Serial coronal sections from wild-type (*lower left*) and SF-1 knockout male (*upper right*) and female (*lower right*) mice were stained with cresyl violet and analyzed histologically. A schematic diagram of the anatomical regions found within these sections is shown (*upper left*). *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 Y. Ikeda *et al.: Mol Endocrinol* 9:478–486, 1995 (49). © The Endocrine Society.]

neurons). Alternatively, it is possible that the impaired GnRH release is secondary to the absence of gonadal steroids or other indirect effects of the SF-1 knockout. Further studies are needed, therefore, to define the relative roles of SF-1 at hypothalamic and pituitary levels in gonadotrope function, and to identify the mechanisms by which these effects are mediated.

V. Perspectives and Future Directions

The studies described in this review have defined essential, and sometimes unexpected, roles of SF-1 in endocrine differentiation and function, particularly within the reproductive axis. To date, SF-1 is the only transcriptional regulator demonstrated to play key roles at all levels of the hypothalamic-pituitary-steroidogenic organ axis. Despite this progress, as outlined below, a number of important questions remain to be addressed.

A. What are the roles of the various transcripts encoded by the Ftz-F1 gene?

As noted above, the generation of multiple transcripts is apparently a general feature of the *Ftz-F1* homologs in many species (33), raising the possibility that the different isoforms serve different functions. Intriguingly, studies in *Drosophila* have shown that the *FTZ-F1* gene encodes two distinct isoforms, which differ in their patterns of expression. The later-expressed isoform encodes a protein that may play key roles in metamorphosis (86), a process that is triggered by the *Drosophila* steroid hormone ecdysone. If different transcripts, derived from different promoters, direct the expression of SF-1 protein in the adrenal cortex and gonads or the gonadotropes and VMH (26), then it may be possible to use these promoters to effect tissue specific knockouts—*e.g.* by targeted expression of dominant negative SF-1 mutations, as has been done with other nuclear receptors such as NGFI-B (87)—thereby defining the roles of the different isoforms in endocrine function.

B. Does a ligand mediate SF-1 transcriptional activation?

As diagrammed in Fig. 2, the C-terminal regions of mammalian SF-1s are highly conserved within their putative ligand-binding domains. In particular, the sequences are conserved absolutely within the AF-2 domain, which forms an amphipathic α-helix proposed to participate in ligand-induced transcriptional activation (22). This raises the possibility that SF-1 also is activated by a ligand. To date, none of the obvious candidates, *e.g.* steroid hormones or biosynthetic intermediates such as pregnenolone, have been shown to act as ligands for SF-1. Even if SF-1 is ligand-regulated, several problems may explain the failure to detect this ligand. First, SF-1 may be activated by a ligand that is widely expressed in multiple cell types, thus hindering efforts to identify it. Of considerable interest in this regard, a recent report showed that the oxysterol 22-hydroxycholesterol can activate the transcriptional activity of the orphan nuclear receptor LXRα, raising the possibility that oxysterols or their metabolites act as ligands for a subset of nuclear receptors (88). Based on the position of oxysterols as potential intermediates in the biosynthetic pathways for steroid hormones, one can envision a positive feedback loop in which oxysterol intermediates, perhaps produced by the action of P450scc, would amplify the transcription of SF-1 target genes, thereby increasing the expression of the steroid hydroxylases and "locking in" the steroidogenic phenotype. Alternatively, SF-1 ligand(s) may have eluded detection because their action is indirect and mediated upon a heterodimerization partner for SF-1. Precedence for this model stems from the demonstration that nuclear receptors such as NGFI-B, previously believed to activate transcription as monomers in a ligand-independent manner, can interact with the heterodimerization partner RXR to activate transcription in a retinoid-dependent manner (89, 90). This finding raises the possibility that SF-1 may also regulate a subset of responsive elements as a heterodimer with RXR, perhaps in a retinoid-dependent fashion, or with a yet-to-be-detected heterodimerization partner. Obviously, the identification and characterization of SF-1 ligands would provide important insights into SF-1 action and might provide potential targets for pharmacological alteration of SF-1 function.

Even if SF-1 is not directly or indirectly activated by a ligand, it still may be activated by posttranslational modification. The conserved phosphorylation motif for cAMP-dependent protein kinase (11) provides one candidate site at which such ligand-independent modulation might be effected. It is hoped that further structure-function studies of SF-1 will provide insights into the roles of this, and other, phosphorylation sites in modulating SF-1 activity.

C. Where does SF-1 fit within hierarchical cascades of endocrine development?

1. Which other genes also contribute to tissue-selective expression of SF-1 target genes? Although SF-1 plays a pivotal role in determining the tissue specificity of its target genes, other transcriptional factors must also regulate their expression. For example, the enzymes that catalyze terminal reactions in adrenal corticosteroid biosynthesis—the 11β-hydroxylase isozymes and P45021—are only expressed in the adrenal cortex (see Fig. 1). In contrast, P450scc and P45017α also are expressed in the gonads. These different profiles of expression obviously cannot be explained solely by SF-1, as it is found in both the adrenal cortex and gonads. Similarly, it is apparent that different subsets of SF-1-responsive genes are expressed in the primary steroidogenic tissues (*e.g.* the adrenal cortex and gonads) and the extrasteroidogenic sites (*e.g.* the pituitary gonadotropes and VMH). Finally, there are genes whose expression is regulated by SF-1 in certain tissues that also are expressed in sites where SF-1 is not (*e.g.* aromatase in germ cells, P450scc in oligodendrocytes and fetal intestine). Thus, an important task for future studies is to identify other transcriptional regulators that act in concert with SF-1 to restrict the expression of different SF-1-responsive genes to appropriate cell types or that permit the expression of SF-1-responsive genes in sites where SF-1 is not expressed.

2. Which target genes of SF-1 mediate its key roles in maintaining the adrenal glands, gonads, and VMH? Although SF-1 has been

shown to regulate endocrine function at many levels, there remain key gaps in our understanding of the target genes that mediate these effects. For example, none of the known SF-1-responsive genes explain important features of the SF-1 knockout mice, such as adrenal and gonadal regression and absence of the VMH. In particular, natural or induced mutations in many SF-1-responsive genes have been characterized in detail, including: MIS (91), the various cytochrome P450 steroid hydroxylases (reviewed in Ref. 92), the α-GSU (93), StAR (94), and the ACTH receptor (95). None of these loss-of-function mutations causes adrenal or gonadal aplasia. It remains possible that the phenotype in the SF-1 knockout mice is so severe because multiple genes have been affected in concert, but these results may also indicate that additional genes mediate the adrenal and gonadal regression in the knockout mice. Based on the finding that the regressing gonads and adrenal glands exhibit changes typical of programmed cell death, it is likely that SF-1 regulates genes that modulate the apoptotic pathway (96, 97). Direct roles of SF-1 in growth regulation and differentiation are further suggested by studies in which treatment with SF-1 antisense oligonucleotides inhibited differentiation and facilitated mitosis of primary cultures of rat granulosa cells (98). Identifying the genes through which SF-1 influences cell division and programmed cell death is another important goal to understand fully how SF-1 works in endocrine development.

3. What mechanisms regulate the expression of SF-1? Given that the identical SF-1 protein within different cell types may result from distinct promoters that direct the transcription of the SF-1 and ELP3 transcripts, the mechanisms of cell-specific regulation may be highly complex. Additionally, published reports suggest a sexually dimorphic expression in the embryonic gonads, with SF-1 levels increasing in the testis but decreasing in the ovaries coincident with sexual differentiation (46, 48, 74). An important task, therefore, is to define the mechanisms that determine the cell-selective and sexually differentiated regulation of the *Ftz-F1* gene. To date, relatively little is known about the genes that activate the expression of SF-1 at critical periods of endocrine development, or that maintain SF-1 expression later in life. The lack of cultured cell lines that recapitulate the phenotype of gonadal and adrenal cortical precursors at these critical periods of development has hindered efforts to delineate these mechanisms, forcing investigators to substitute cells such as Y1 adrenocortical cells. Morohashi and colleagues (15) identified a conserved E box motif, located in the 5′-flanking region of the rat *Ftz-F1* gene, that played an important role in SF-1 promoter activity in transfected Y1 adrenocortical cells (15). They further showed in gel shift assays that this E box motif interacted with a protein whose abundance in gonadal extracts varied in a sexually dimorphic pattern: extracts from E12 testes expressed the E box-binding protein, whereas those from ovaries at comparable time points lacked this protein. No such sex-dependent differences were observed with adrenal extracts. Basic helix-loop-helix proteins, which bind E box motifs, have been linked to tissue-specific development in many species and to sex determination in *Drosophila* (99). It thus is plausible that a member of this family of transcriptional regulators may play a key role in SF-1 expression. In very similar 5′-deletion and mutational analyses of the mouse *Ftz-F1* gene, however, the E box motif did not play a major role in SF-1 promoter activity, and an adjacent motif that recognizes a yet-to-be-identified protein predominantly regulates promoter activity (D. Lala and K. Parker, unpublished observation). Thus, further studies are needed to reconcile these findings. Ultimately, transgenic analyses of reporter gene expression driven by SF-1 5′-flanking sequences—either wild-type or carrying mutations in the E box or other elements—may be necessary to define the roles of different promoter regions *in vivo*.

Morohashi and colleagues (100) also identified an element within the first intron of the rat *Ftz-F1* gene that was essential for full promoter activity in transfected Y1 adrenocortical cells. This element bound SF-1, raising the intriguing possibility that an autoregulatory loop determines the tissue-specific expression of SF-1 in adrenocortical cells. Similar autoregulatory loops have been implicated in the regulation of a number of other genes—both in *Drosophila* (*e.g. fushi tarazu*, *Ultrabithorax*, *even-skipped*, *deformed*, and *sex-lethal*) and in mammals (*e.g.* several Hox genes, *MyoD1*, and Pit-1)—that play important roles in development (100). Further analyses of the roles of this SF-1 autoregulatory loop may provide important insights into the mechanisms by which differentiated function of adrenocortical cells becomes "fixed" during development.

Little is currently known with respect to hormonal regulation of SF-1 expression. Northern blotting analyses in mouse Y1 adrenocortical cells suggested that ACTH treatment did not alter the levels of SF-1 mRNA (D. Rice and K. Parker, unpublished observation). A recent study, however, showed that GnRH treatment increased the levels of SF-1 mRNA in rat pituitaries by ~50% (101). Although the physiological significance of this induction remains to be defined, this finding raises the possibility that SF-1 transcription in pituitary gonadotropes is induced by GnRH.

4. How is SF-1 related to other genes whose disruptions lead to phenotypes that mimic aspects of the SF-1 knockout mice? Another important task is to define the pathways by which SF-1 interacts with other genes to regulate the development and function of the primary steroidogenic tissues, the pituitary gonadotropes, and the VMH. One approach to this problem is to identify genes that, when mutated or inactivated by targeted disruption, lead to phenotypes that resemble the SF-1 knockout mice.

a. Gonads. Studies using knockout mouse models have identified two other genes encoding transcription factors whose disruption is associated with gonadal agenesis: the Wilm's tumor-related tumor suppressor gene WT1 (102) and the homeodomain protein LIM1 (103). Both of these genes are expressed at approximately the same stage of gonadogenesis as SF-1 (*i.e.* ~E9.0). The effects on gonadogenesis in the WT1 knockout mice are remarkably similar to those seen in SF-1 knockout mice: the gonads appear normal at early stages but regress via apoptosis as they differentiate through a critical point in development. We have carried out preliminary studies to examine possible interactions between SF-1 and WT1. Analyses of the genital ridges of SF-1 and WT1 knockout mice indicate that SF-1 expression persists in the WT1 knock-

out mice, and WT1 expression persists in the SF-1 knockout mice (Y. Ikeda and J. Kreidberg, unpublished observation). These findings suggest that the simplest model, *i.e.* that one of these two essential genes is required to activate the other's expression, is not tenable, although more complex interactions may still be possible. Less is known about the precise effects of the LIM1 knockout on gonadogenesis, as the embryos die relatively early in their development, apparently because of forebrain abnormalities unrelated to their gonadal defects.

b. Gonadotropes. Female mice that are deficient in the zinc finger transcription factor NGFI-A (also called Egr1, zif-268, or Krox-24) are infertile secondary to hypogonadotrophic hypogonadism (104). Analyses of pituitary gene expression showed that there was a selective deficiency in the expression of LHβ, but not in other gonadotrope markers. Moreover, a conserved motif in the 5′-flanking region of the LHβ gene, which bound NGFI-A and through which NGFI-A increased promoter activity, was identified. Finally, cotransfection of NGFI-A and SF-1 led to synergistic activation of the LHβ promoter. These results suggest that gonadotrope defects in SF-1 and NGFI-A knockout mice result from their action in concert to regulate a common downstream gene essential for gonadotrope function. Similar conclusions of synergistic effects on a common target gene were drawn from studies of the salmon gonadotropin II gene, where interactions between SF-1 and estrogen receptor were found (105). Whether these synergistic interactions require direct physical interactions between SF-1 and other transcription factors will require further study.

c. Complex endocrine phenotypes. Like SF-1, mutations of the orphan nuclear receptor *DAX-1* also cause a complex endocrine phenotype that includes impaired adrenal development and hypogonadotrophic hypogonadism. *DAX-1* was isolated by positional cloning of the gene causing X-linked adrenal hypoplasia congenita (106). Patients with this disorder present in childhood with adrenocortical insufficiency; a subset later exhibit hypogonadotrophic hypogonadism. The gene responsible for this disorder maps to Xp21, a region associated with dosage-sensitive sex reversal wherein males with an extra copy of Xp21 have impaired testicular development and consequent sex reversal (107), prompting the designation *DAX-1* (Dosage-sensitive sex reversal-Adrenal hypoplasia congenita critical region on the X chromosome). The nucleotide sequence of *DAX-1* showed that it is a novel and highly unusual member of the nuclear receptor superfamily, with sequences homologous to the ligand-binding domain of nuclear hormone receptors but without the characteristic zinc finger DNA-binding motifs. Analyses of affected patients established that *DAX-1* mutations cause both X-linked adrenal hypoplasia and hypogonadotropic hypogonadism (108, 109), thereby proving that alterations in a single human gene lead to a compound endocrine phenotype with adrenal hypoplasia and impaired gonadotropin release.

The phenotypic similarities that accompany disruption of the *Ftz-F1* gene encoding SF-1 and natural mutations of *DAX-1* suggest that both genes act in the same developmental pathway, determining adrenal development and modulating reproductive function at hypothalamic/pituitary levels. The similar phenotypes associated with mutations in SF-1 and *DAX-1* raise two possibilities: that SF-1 and Dax-1 act sequentially within a hierarchical pathway or that the two proteins interact directly to regulate target genes that are critical for endocrine development. The phenotype of the SF-1 knockout mice, which includes complete absence of the adrenal glands and gonads, is more severe than that of patients with *DAX-1* mutations, suggesting that SF-1 should act upstream of *DAX-1* if the sequential model is correct.

Both models predict that SF-1 and Dax-1 expression should colocalize in the relevant endocrine cell lineages during embryonic development and in adult tissues, and initial reports using Northern blotting analysis (106, 110) or RT-PCR (110, 111) showed very similar profiles of expression. Although one report suggested that SF-1 and Dax-1 immunoreactivities do not colocalize in all cells of the developing rat testes (112), recent studies using *in situ* hybridization of serial sections have demonstrated a striking colocalization of SF-1 and Dax-1 expression in sites that include the adrenal cortex, both compartments of the embryonic testis, the VMH, and pituitary gonadotropes (113, 114). The colocalization data and the generation of similar complex endocrine phenotypes with SF-1 and *DAX-1* mutations point strongly to a functional interaction between SF-1 and Dax-1. A sequence motif in the 5′-flanking region of the human *DAX-1* gene has been shown to bind SF-1 (115), raising the possibility that SF-1 acts upstream of *DAX-1* to regulate its expression. However, cell transfection studies, in which deletion of the SF-1-binding motif did not impair Dax-1 promoter activity, and analyses of SF-1-deficient knockout mice, which maintain Dax-1 expression, argue against a hierarchical pathway in which SF-1 regulates Dax-1 expression (114). Similarly, it is unlikely that Dax-1 regulates SF-1 expression, since SF-1 expression either precedes or coincides with that of Dax-1, and the SF-1 knockout phenotype is more severe than that seen in patients with *DAX-1* mutations. An alternative model for consideration is that SF-1 and Dax-1 interact directly as heterodimers. This model is particularly intriguing because Dax-1 lacks the zinc-finger DNA-binding domain conserved among the vast majority of nuclear receptors, while retaining the ligand-binding region that is implicated in dimerization of other superfamily members. Although SF-1 belongs to the monomer-binding group of nuclear receptors, recent studies have shown that other members of the monomer group, such as NGFI-B, can form heterodimers that are transcriptionally active (89, 90), as discussed above. These findings provide precedence for the model that SF-1 and Dax-1 may regulate endocrine development, at least partly, via heterodimerization.

If SF-1 and Dax-1 interact physically, then the functional consequences of their interaction may differ in a tissue-dependent manner. In certain sites (*e.g.* adrenocortical cells, gonadotropes, and the VMH), SF-1 and Dax-1 may activate target gene expression in a cooperative fashion. Consistent with this model, loss-of-function mutations of either gene impair adrenal development and lead to hypogonadotrophic hypogonadism. In gonadal cells during critical periods of sexual differentiation, the actions of SF-1 and Dax-1 may be functionally antagonistic. *DAX-1* deletions or mutations that completely inactivate it are compatible with normal testicular differentiation, whereas presumptive gain-of-function

of *DAX-1* due to an extra copy of the *DAX-1* gene in patients with dosage-sensitive sex reversal impairs testicular development. These findings suggest that *DAX-1* overexpression impairs testicular development. In contrast, the extinction of SF-1 expression in the ovary suggests that persistent expression of SF-1 in the female gonad may impair ovarian development and female sexual differentiation, perhaps by aberrantly activating target genes such as MIS. These findings thus suggest that the balance between SF-1 and *DAX-1* in the gonads is critical for appropriate sexual differentiation.

D. Do other transcription factors/nuclear receptors serve dual roles in development and maintenance of the differentiated phenotype?

It is apparent that SF-1 plays dual roles in endocrine function. SF-1 is essential during embryonic development for the emergence of discrete cell lineages that form the adrenal glands, gonads, and VMH. In addition, SF-1 regulates the expression of multiple genes that constitute the differentiated function of SF-1-expressing cells. In this respect, SF-1 resembles other transcription factors that also play dual roles in endocrine function. One of these proteins, a homeodomain protein designated Pit-1 (alternatively named GHF-1) was actually the first gene shown to serve dual roles in endocrine development and function. Pit-1 is essential for the development of a subset of pituitary cells, including the lactotrope, somatotrope, and thyrotrope lineages, and also regulates the expression within these cell types of a number of genes that are required for their differentiated function, including GH, PRL, and the β-subunit of TSH (reviewed in Ref. 116). A similar dual role was recently defined for another homeodomain protein—thyroid-specific enhancer binding protein (T/ebp), alternatively designated thyroid-specific transcription factor. T/ebp plays key developmental roles in the lung, thyroid gland, pituitary gland, and ventral forebrain and also regulates the expression within these tissues of a number of genes that comprise the differentiated phenotypes of these tissues (117, 118).

Given the known developmental roles of steroid hormones and retinoids and the fact that the nuclear receptor family comprises the largest known family of transcription factors, it is not surprising that the ligand-activated receptors play key roles in mammalian development (reviewed in Ref. 119). What was not anticipated, however, was that orphan nuclear receptors like SF-1 would play essential roles in the development of specific tissues or cell types, as well as in the regulation of multiple genes that constitute the differentiated phenotype. The compound roles of SF-1 and DAX-1 are summarized above. Hepatocyte nuclear factor-4 (HNF-4) is another nuclear receptor that is essential for embryonic development. HNF-4 knockout mice exhibit impaired embryonic growth and severely disrupted gastrulation; HNF-4 also regulates the expression of a number of genes that constitute the hepatocyte phenotype, including albumin, coagulation factors, hepatic cytochromes P450, apolipoproteins, erythropoietin, and enzymes involved in intermediary metabolism such as phosphoenolpyruvate carboxykinase (120, 121). Similarly, recent studies on the differentiation of adipocytes have implicated the peroxisome proliferator activated receptor γ (PPARγ) as a master determinant of adipocyte differentiation and as an essential regulator of many genes required for adipocyte function (122, 123). It is likely that other genes, including other members of the nuclear receptor family, will ultimately be shown to play similar dual roles in developing and maintaining the differentiated phenotype.

VI. Conclusion

From the above considerations, it is evident that SF-1 has received wide interest as a cell-selective orphan nuclear receptor that profoundly influences endocrine differentiation and function. Studies on SF-1 regulation and function, however, are still in their infancy, and further delineation of the sites and mechanisms of SF-1 action as outlined here may provide a model for understanding the roles of other transcription factors that influence endocrine differentiation and function at multiple sites. Conversely, studies of other transcription factors that play similar "master" roles in endocrine function and development may provide important and useful clues to the mechanisms of action of SF-1.

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