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THE ORPHAN NUCLEAR RECEPTORS STEROIDOGENIC FACTOR-1 AND LIVER RECEPTOR HOMOLOG-1: STRUCTURE, REGULATION, AND ESSENTIAL ROLES IN MAMMALIAN REPRODUCTION

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Meinsohn M-C, Smith OE, Bertolin K, Murphy BD. The Orphan Nuclear Receptors Steroidogenic Factor-1 and Liver Receptor Homolog-1: Structure, Regulation, and Essential Roles in Mammalian Reproduction. *Physiol Rev* 99: 1249–1279, 2019. Published February 27, 2019; doi:10.1152/physrev.00019.2018.—Nuclear receptors are intracellular proteins that act as transcription factors. Proteins with classic nuclear receptor domain structure lacking identified signaling ligands are designated orphan nuclear receptors. Two of these, steroidogenic factor-1 (NR5A1, also known as SF-1) and liver receptor homolog-1 (NR5A2, also known as LRH-1), bind to the same DNA sequences, with different and nonoverlapping effects on targets. Endogenous regulation of both is achieved predominantly by cofactor interactions. SF-1 is expressed primarily in steroidogenic tissues, LRH-1 in tissues of endodermal origin and the gonads. Both receptors modulate cholesterol homeostasis, steroidogenesis, tissue-specific cell proliferation, and stem cell pluripotency. LRH-1 is essential for development beyond gastrulation and SF-1 for genesis of the adrenal, sexual differentiation, and Leydig cell function. Ovary-specific depletion of SF-1 disrupts follicle development, while LRH-1 depletion prevents ovulation, cumulus expansion, and luteinization. Uterine depletion of LRH-1 compromises decidualization and pregnancy. In humans, SF-1 is present in endometriotic tissue, where it regulates estrogen synthesis. SF-1 is underexpressed in ovarian cancer cells and overexpressed in Leydig cell tumors. In breast cancer cells, proliferation, migration and invasion, and chemotherapy resistance are regulated by LRH-1. In conclusion, the NR5A orphan nuclear receptors are nonredundant factors that are crucial regulators of a panoply of biological processes, across multiple reproductive tissues.

I. CLINICAL RELEVANCE

The literature relevant to two orphan nuclear receptors, liver receptor homolog-1 (LRH-1) and steroidogenic factor-1 (SF-1), has been reviewed with focus on the effects of these two factors on reproductive processes in mammals. The absence of LRH-1 results in early embryo death, while the absence of SF-1 causes adrenal agenesis. SF-1 mutations in male humans result in adrenal dysgenesis and phenotypic female genitalia due to regression of the Wolffian ducts and persistence of the Müllerian anlage. SF-1 expression is found in endometriotic tissue, where it plays a role in ectopic estrogen synthesis. Misregulation of both SF-1 and LRH-1 occurs in ovarian cancers, and LRH-1 is implicated in human pancreatic cancers. LRH-1 also promotes proliferation, metastasis, and invasion of breast cancer cells. LRH-1 antagonists may therefore serve as therapeutic modalities for cancer treatment.

II. INTRODUCTION

A fascinating narrative of the last century describes discovery of the many mechanisms of cell signaling. From these investigations, we have learned much about how extra- and intracellular messages are received, transduced, and translated within their target cells. A commonality of cell signaling is the presence of protein receptors that can be found integrated into the plasma membrane, in the cytoplasm, or within the nucleus. The signals that provoke cellular re-

sponses have been shown to display a wide diversity in structure, but most transduction systems fall into a few broadly defined categories. Protein signals usually interact with membrane receptors to induce cascades of intracellular modifications that drive cell functions. In contrast, lipid signaling differs from protein messaging, in that the messenger is believed to diffuse freely through membranes. Among lipid signals are the steroids, a class of derived lipids, that have evolved with signaling pathways that employ a category of proteins displaying structural similarity, known as the nuclear receptor superfamily **(FIGURE 1)**. In mammals, this extensive superfamily is composed of ~50 functional members, with 48 genes identified in the human genome, 49 in mice, and 47 in rats (322). In addition to steroids, members of the superfamily transduce signals as diverse as the thyroid hormones, retinoic acid, vitamin D, and bile acids. The unique characteristic of nuclear receptors, relative to other signaling modalities, is their capacity to bind directly to DNA, and thereby regulate transcriptional events, evoking a wide diversity of physiological actions. For this reason, nuclear receptors have been defined as a group of gene-specific transcription factors. The superfamily is subdivided into seven subfamilies (N0–N6), with three classes (I–III) based on the multiple similarities and differences that exist in their structure and DNA-binding characteristics (76).

Historically, the discovery of new hormones was achieved by analysis of their effects on physiological or developmental processes, and the purified hormone was subsequently used to identify its cognate receptor (140). By this means, the steroid receptor family (class I) was discovered, including the progesterone, estrogen, glucocorticoid, androgen, and mineralocorticoid receptors; as was thyroid/retinoid family (class II), including the thyroid receptor and vitamin D receptor. Proteins that recapitulated the nuclear receptor domain structure, but for which no ligand was known, were designated as orphan nuclear receptors, and they compose

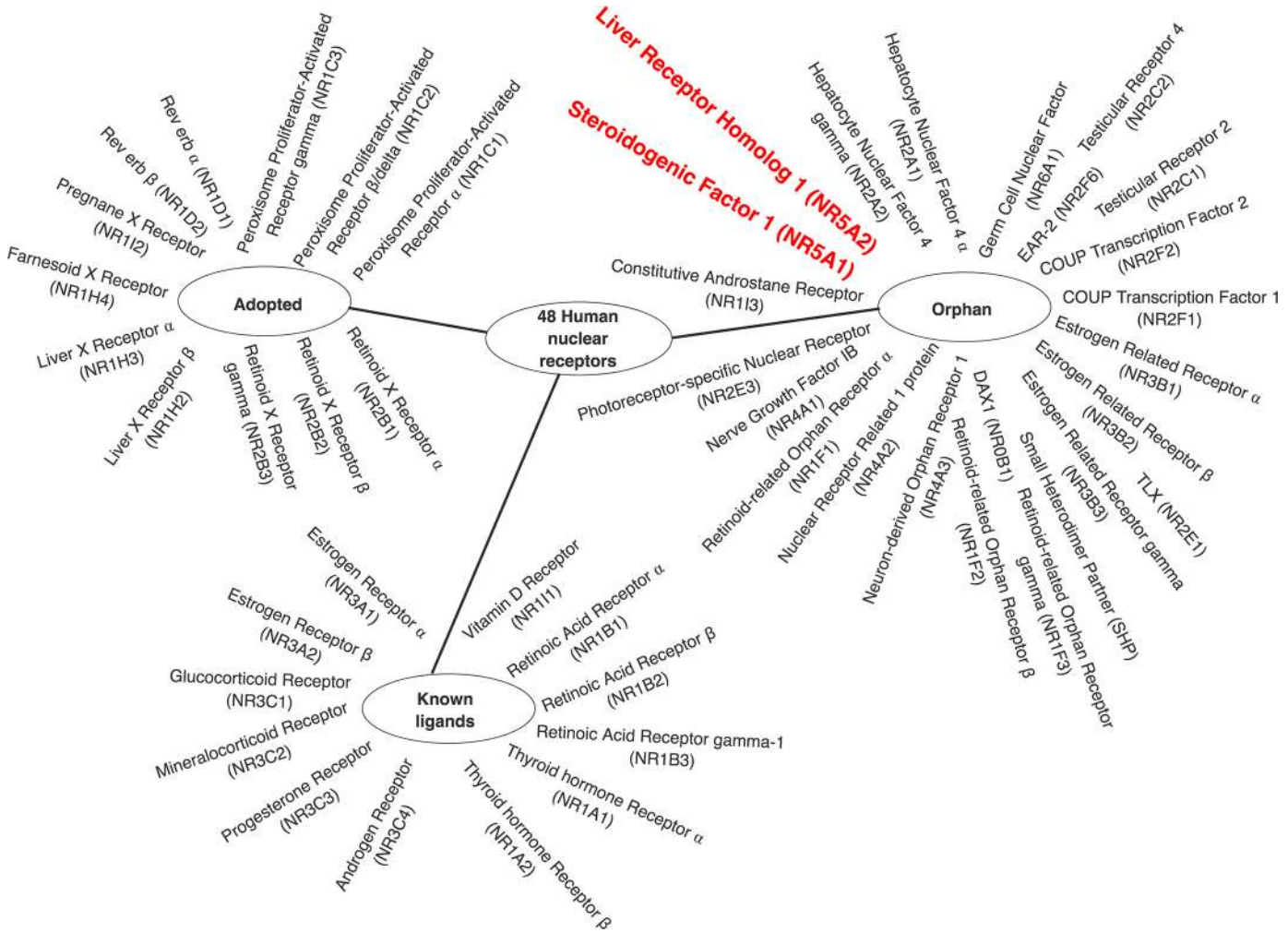

FIGURE 1. Classification of the 48 human nuclear receptors based on their discovery due to a known ligand, orphan receptors for which a ligand has been identified (adopted receptors), and true orphan receptors for which the ligand, if present, remains unknown. Steroidogenic factor-1 (SF-1) and liver receptor homolog-1 (LRH-1), the subject of this review, are highlighted in red. (Compiled from Refs. 12, 14, 64, 74, 84, 104, 105, 140, 193, 237, 288.)

the third family of nuclear receptors (class III). Recognition of their existence introduced a new era in endocrinology, in which the process of ligand-receptor discovery was inverted (71), where the orphan receptors were cloned and were then used to search for previously unknown ligands (140). By this means the ligand for the orphan RXR receptor, 9-*cis*-retinoic acid, was discovered, thereby allowing its new classification as an adopted nuclear receptor (71) **(FIGURE 1)**.

Many of the members of the nuclear receptor superfamily, when liganded, exert regulatory effects on mammalian reproductive processes. Some are clearly direct, best exemplified by the estrogen and progesterone receptors, while the effects of others, such as the thyroid (63) and retinoic acid receptors (92) on reproduction are both direct (91) and indirect (8).

The two orphan receptors treated herein are found in the NR5A family: NR5A1 (common name: steroidogenic factor-1 or SF-1) and NR5A2 (common name: liver receptor homolog-1 or LRH-1). They have been shown to be essential and significant regulators of reproductive processes. In spite of their closely related structures, these two nuclear receptors display differing and often nonoverlapping effects, in particular, on reproductive target tissues. This is remarkable, in that they bind to the same or highly similar response element in the genes they regulate **(FIGURE 2)** (52), and both are often expressed in the same cells and tissues **(FIGURE 3)**.

This review is an attempt to shed light on the multiple similarities and differences between these two receptors, in structure, in signaling, and in their roles in the mammalian reproductive system. We refer to these two receptors as SF-1 and LRH-1 throughout this treatise.

III. DISCOVERY

During the last decade of the 20th century, the initial evidence of a gene responsible for the transcriptional activation necessary for the proper expression of the fushi tarazu (*ftz*) gene was described in *Drosophila* (278). *Ftz* is a member of the pair-rule class of genes governing *Drosophila* embryo segmentation, and the purified sequence-specific DNA-binding factor was then called Ftz-f1 (269a). The embryonic long terminal repeat-binding protein in the mouse, now known as SF-1, proved to be a mammalian homolog of Ftz-f1 (151).

Some 20 years ago, the late Keith Parker reviewed the literature relative to SF-1, therein identifying it as a key determinant of the endocrine function at various levels within the hypothalamic-pituitary-gonadal axis (211). He noted that this transcription factor was detected in tissues known to express the cytochrome *P*-450 steroid hydroxylases, genes regulating steroid hormone biosynthesis, hence its name. New in vivo data for SF-1 were becoming available at that time, showing that it is highly expressed in steroidogenic tissues and that it plays an essential role in activating the expression of various steroidogenic enzymes, thereby regulating adrenal and gonadal formation, as well as sex determination and differentiation (176, 211, 234). Early studies also showed that SF-1 functioned as a factor in the development of hypothalamic control of pituitary function (116). Due to its constitutive activity, and the in silico predictions of secondary structure suggesting ligand-independent active conformation, SF-1 was initially identified as an orphan nuclear factor (62).

The homology of Ftz-f1 with LRH-1 was recognized when the sequence of the latter was determined (234, 252). LRH-1 plays a major role in multiple processes, and was identified early as a regulator of intracellular cholesterol homeostasis (reviewed in Ref. 76). Its roles in steroidogenesis, embryogenesis, and reproductive function are amplified in subsequent sections of this review.

The evolution of the NR5A family was explored by Kuo et al. (147), who concluded that the mammalian versions of NR5A genes are orthologs of vertebrate forms that arose from a common ancestor, by means of a gene duplication

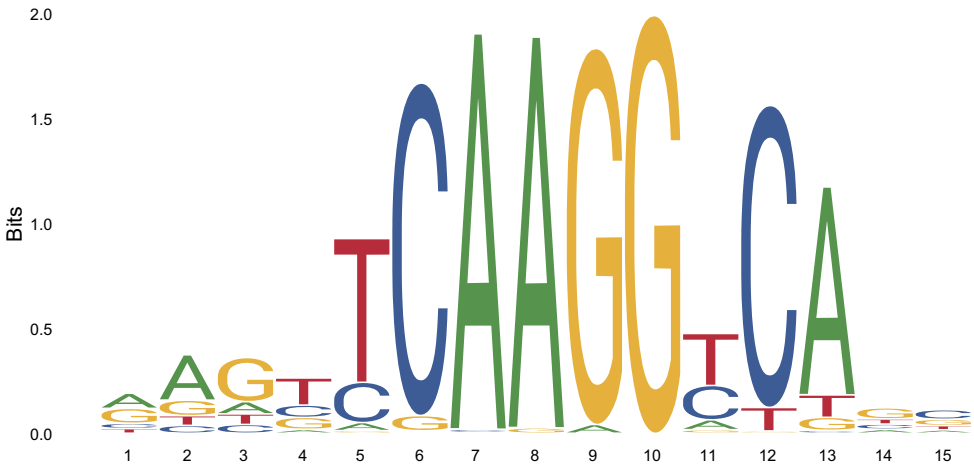

FIGURE 2. Consensus DNA sequence to which both steroidogenic factor-1 (SF-1) and liver receptor homolog-1 (LRH-1) have been shown to bind on target genes. The image is derived from the JASPAR open access database of nonredundant transcription factors, release 7 (jaspar.genereg.net).

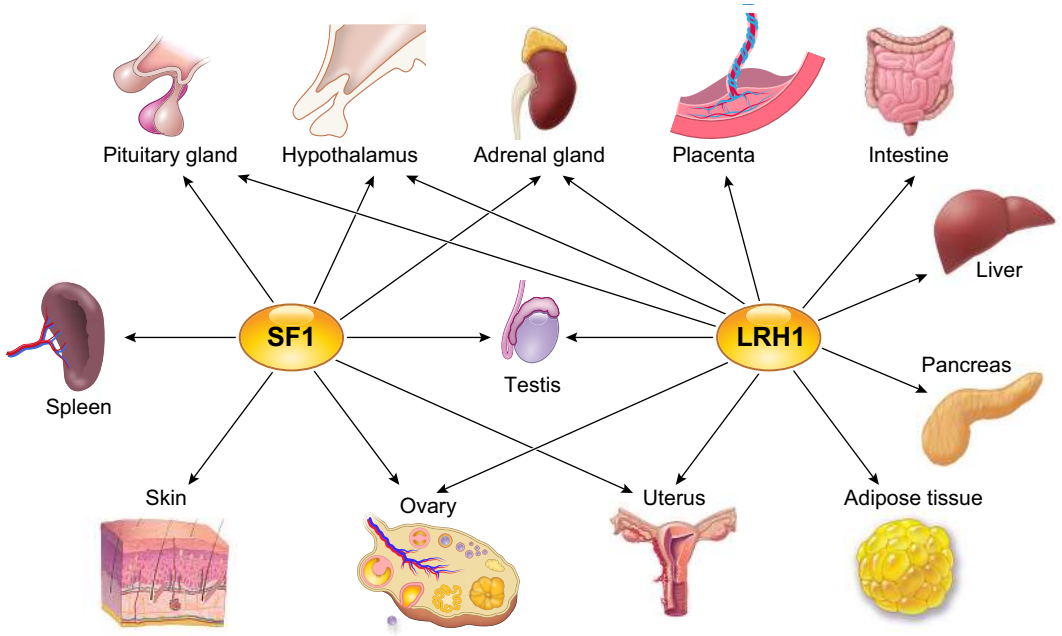

FIGURE 3. Tissue distribution of the NR5A receptors in mammals, showing liver receptor homolog-1 (LRH-1) expression exclusively in the digestive tract and glands, steroidogenic factor-1 (SF-1) expression specific to the spleen and skin, and overlap of the expression of the two receptors that occurs principally in endocrine and neural tissue.

event **(FIGURE 4)**. The ancestral form gave rise to a third NR5A gene, NR5A5, present in teleost fish, but that has been lost in higher forms (55, 147).

IV. STRUCTURE

LRH-1 is located on human and mouse chromosome 1 (83), whereas SF-1 is located on human chromosome 9 and mouse chromosome 2 (168). As noted above, both are classified as nuclear receptors because both display the typical structure of this family, albeit with some peculiarities. As nuclear receptors, they are imported from the cytoplasm via the nuclear pore complex, and therefore have two functional nuclear localization signals responsible for shepherding the receptors into the nucleus (162, 305). The domain structure of these two closely related nuclear receptors **(FIGURE 5)** comprises:

- a modulatory NH_2-terminal A/B domain; that is, in contrast to other nuclear receptors, devoid of the ligand-independent activation function-1 domain (AF-1) at their NH_2 terminals (76, 162);
- the highly conserved DNA-binding domain (DBD or C domain), responsible for targeting the receptor to specific DNA sequences, termed hormone response elements (213);
- the ligand-binding domain (LBD or E domain), which contains a conserved ligand-dependent activation function-2 (AF-2) motif that mediates co-activator interaction (213);
- the D domain serving as hinge between DBD and LBD (76, 211, 305), recently shown to be more than a flexible connector, as it is required to regulate the transcriptional activity of LRH-1 in humans (298) and has proven to be important for effective in vitro phosphorylation of LRH-1 (158).

Members of the NR5A subfamily further contain a hallmark feature, an additional 30-amino acid COOH-terminal extension, designated FTZ-F1 box or A box, located adjacent to the second zinc finger motif at the COOH terminus of the DBD (211, 270). The cooperativity between the P box in the first zinc finger motif of the DBD and the FTZ-F1 box allows for stable, high-affinity binding of NR5A factors to their target genes, thereby permitting their function as monomers (250, 269, 291). This was first shown in *Drosophila* Ftz-f1 by Ueda et al. (270), and subsequently confirmed in the mammalian homologs (reviewed in Ref. 174). As mentioned above, the NR5A receptors are constitutive, in that they have the capacity to adopt an active conformation without requiring a ligand or other modifications. The unliganded activity of the NR5A receptors evokes the two views of the evolution of nuclear receptors. First, parent forms were unliganded, and binding to specific ligands was a trait acquired independently by nuclear receptor lineages (70). Alternatively, the liganded forms were ancestral, a trait lost in the NR5A family (25). The presence of a ligand-binding domain and pocket in the NR5A genes argues for the latter scenario. In support of this view is the evidence that the active conformation is stabilized in SF-1 and LRH-1 when amino acid residues connect between helices, replacing connections usually found between the ligand and the receptor (25).

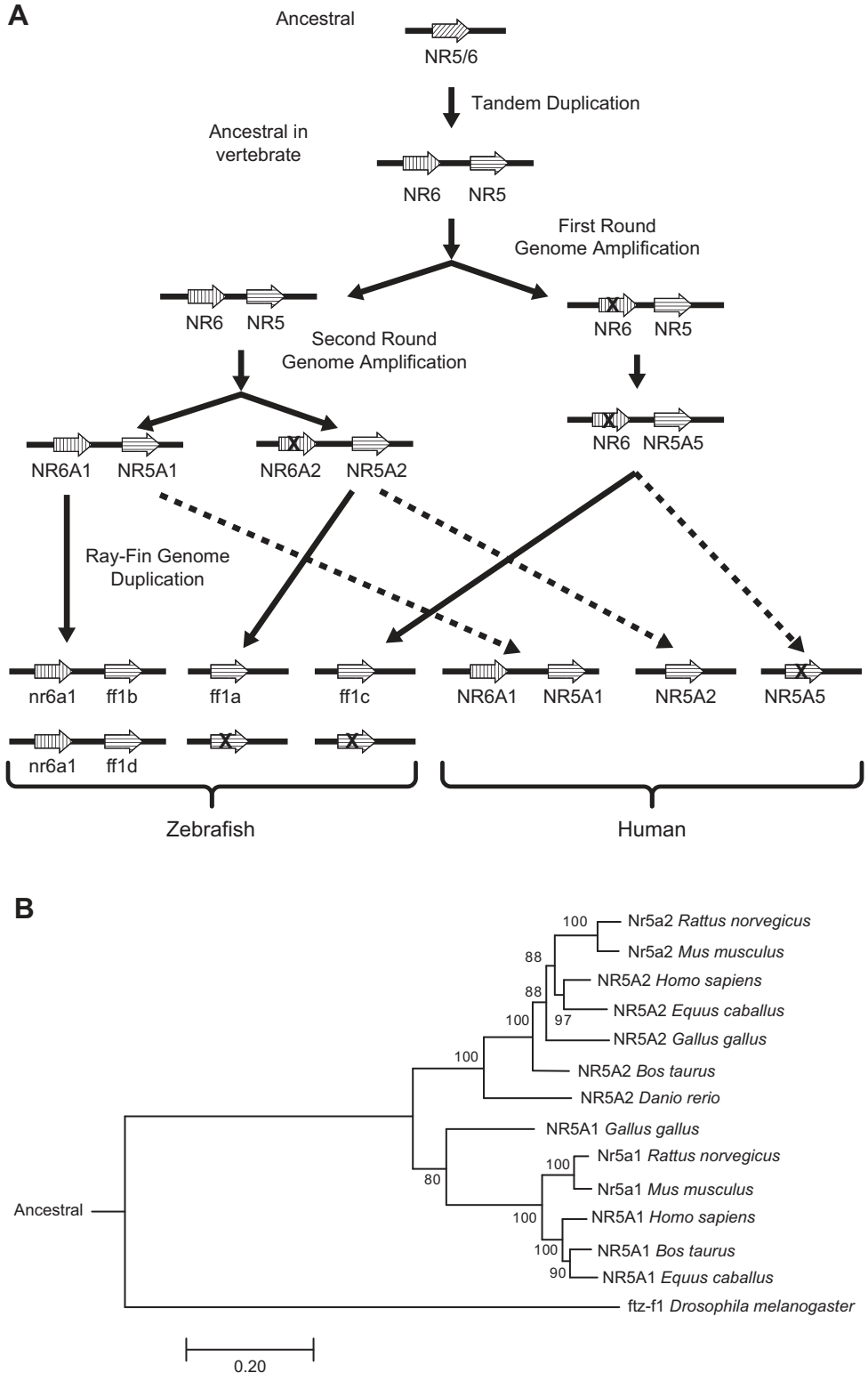

FIGURE 4. Evolution of the *NR5A* genes. *A*: schema to demonstrate the putative evolution of modern forms of the *NR5A* genes in vertebrates from an ancestral form containing one ancestral *NR5* gene that, after tandem duplication, became the precursor of two forms, *NR5A5* and *NR5A6*. After two rounds of genome amplification, the *NR5A1* and *NR5A2* genes emerged. Another product, *NR5A5*, persists in fish, but has been lost in mammals. [From Kuo et al. (147), with permission from the Biochemical Society of London.] *B*: phylogenetic tree demonstrating the proposed origin and evolutionary relationship between liver receptor homolog-1 (LRH-1) and steroidogenic factor-1 (SF-1) in vertebrate species and in *Drosophila*. The evolutionary sequence and history were derived with the Neighbor-Joining method, and evolutionary distances from the Maximum Composite Likelihood method. Figure compiled using Molecular Evolutionary Genetics Analysis, version 7.0 for NCBI. The marker 0.2 represents a 20% sequence difference.

V. TISSUE DISTRIBUTION

SF-1 expression is most pronounced in steroidogenic organs such as the adrenal cortex and the gonads. It is present in the urogenital ridge of the mouse at embryonic day (E) 9.5, in fetal and adult adrenocortical cells, in the Leydig and Sertoli cells of the developing and mature testes, as well as in granulosa and theca cells of the ovary (117, 118, 120). While early studies reported that there is no expression in luteal cells (76), more recent studies suggest that it is, in fact, present in corpora lutea of the cow (190) as well as in the rat (72) and mouse (106). Studies have also located SF-1 ex-

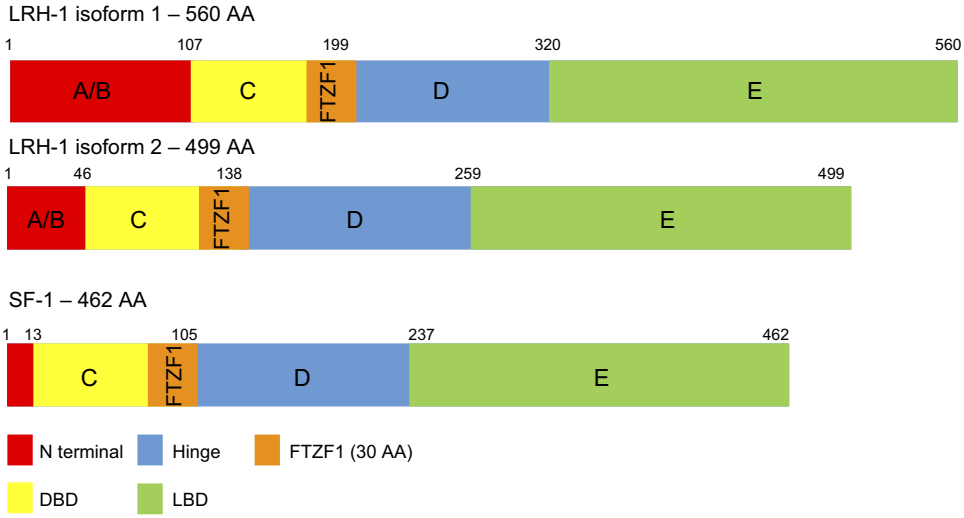

FIGURE 5. Representative domain structure of the NR5A proteins using the mouse, where two isoforms of NR5A2 (LRH-1) are present, one at 499 amino acids, that displays elevated homology with its mammalian homologs, and one with an extended A/B domain. The NR5A1 (SF-1) protein at 462 amino acids has similar domains, but the amino acid homology by BLAST is only 58%. DBD, DNA binding domain; LBD, ligand binding domain; FTZF1, FTZ-1 or A box specific to the *NR5A* genes.

pression in nonsteroidogenic tissues, more specifically the ventromedial hypothalamus (VMH), where it plays an important role in hypothalamic regulation of pituitary gonadotroph organization and function (245), not to mention in endothelial cells of the sinus and pulp vein of the spleen and in the skin (195, 212).

In contrast **(FIGURE 3)**, LRH-1 distribution is more widespread, as it is found in multiple tissues of endodermal origin, including the liver, pancreas, and intestine (76). Elevated expression of LRH-1 was also found in the ovary, where it is restricted to the granulosa cell compartment (76). It is present at more modest levels in the hypothalamus and anterior pituitary gonadotrophs (80, 325), endometrium (318) and placenta, along with the adrenal gland and the testis (248).

VI. REGULATION

A. Potential Ligands

As noted above, a stable and active monomeric NR5A LBD exists in the absence of ligand, co-activator peptide or homo- or heterodimeric receptor partner, indicating that ligands are dispensable for SF-1 and LRH-1 basal activity (174, 291). Nevertheless, crystallography and mass spectrophotometry analyses revealed large and hydrophobic pockets in both SF-1 and LRH-1 LBD occupied by phospholipids, such as phosphatidylethanolamine and phosphatidylglycerol (79). Experimental addition of bulky side chains into LRH-1 empty hydrophobic ligand-binding pocket results in equal or greater activity of the nuclear receptor, suggesting that it can accommodate potential ligands (232). Other phospholipids, such as phosphatidylcholine or second messenger phosphatidylinositol phosphates, can modulate the two NR5A receptor interaction with co-activators (79, 144, 163, 205, 231). Variations in lipid environment and metabolism can modulate SF-1 function. For example, it has been shown that sphingosine can inhibit SF-1 and that cAMP can enhance SF-1 activity by inducing sphingosine catabolism (271). Introducing point mutations blocking phospholipid binding to these nuclear receptors generates mutant proteins, some of which are unable to be phosphorylated and then fail to recruit co-activators and induce transcription (161, 285).

While it is not clear whether there are active endogenous ligands for either of the NR5A receptors, a number of pharmacological ligands have been developed for SF-1 and LRH-1. Given that both receptors interact with the same gene sequences, the activity of these ligands is expected to be both gene target and cell context specific. Low-molecular-weight compounds with *cis*-bicyclo[3.3.p]oct-2-ene core structure selectively increase SF-1 activity, while 4-alkuloxy-phenol derivatives have an inverse agonist effect and suppress the constitutive activity of the receptor (60, 289, reviewed in Ref. 237). LRH-1 can be activated by dilauryol-phosphatidyl-choline (DLPC), a ligand agonist that, through its regulation of bile acids and glucose homeostasis, has been shown to decrease the quantity of glucose in diabetic mice (157).

Inverse agonists of LRH-1 that inhibit its constitutive activity, such as ML179 and ML180, have been synthesized (32). They function by inducing the translocation of LRH-1 from the nucleus (52) to the cytoplasm, thereby inactivating it. Another synthetic agonist molecule able to accommodate SF-1 and LRH-1 ligand binding pocket is GSK8470, developed by the GlaxoSmithKline company (289). This compound is described as capable of stimulating the transcription of downstream targets of LRH-1 in hepatic cells, as well as some downstream targets of the NR5A co-repressor short heterodimeric protein (SHP or NR0B2), an atypical orphan nuclear receptor that does not possess a DNA-binding domain (289).

The retinol-related molecule adamantyl hydroxyphenol chlorocinnamic acid (3Cl-AHPC) is another example of an anti-agonist molecule that represses LRH-1 by increasing its interaction with SHP (185). In addition to phosphatidylcholines, the signaling phospholipid phosphatidylinositol 3,4,5-trisphosphate (PIP_3) binds to LRH-1 (182), although downstream effects of this interaction have yet to be determined. It also binds to SF-1, inducing slight modifications in the LBD structure, indicating that a dynamic exchange of potential ligands may regulate the activity of this receptor (20).

While molecular dynamic simulations showed no overall conformational changes in SF-1 and LRH-1 when bound to phospholipids, the ligands affect the recruitment and affinity with cofactor peptides, with consequences on transcriptional capacity (30, 98, 231). It has been suggested that the phospholipid molecule is not an endogenous ligand for LRH-1, and even that there might be no endogenous ligand for this receptor (320). Furthermore, it has been postulated that the phospholipids that occupy the ligand pocket in LRH-1 and SF-1 serve not to stimulate, but rather to stabilize, the molecule and to reduce inhibitory cofactor binding (161). Although it is clear that ligands exist, information is yet fragmentary, and their role, mechanism of action, and function in cellular processes all require further investigation.

B. Co-regulators

Ligand-activated nuclear receptors are first used as adaptors between gene regulatory regions and the chromatin modifying enzyme complexes, and second as activators of ribonucleic acid (RNA) polymerase II, to suppress or enhance target gene expression (38). In turn, the nuclear receptors are modulated not only by ligand binding and posttranslational modifications, but also by recruitment of co-regulators. Co-activators and co-repressors are positive and negative co-regulatory proteins (201), and their actions may well be the most important mode of functional regulation of orphan nuclear receptors (153).

Several tissue-specific co-activators and co-repressors are known to regulate the transcriptional activity of SF-1 and LRH-1 in a context-specific manner **(TABLE 1)**. Importantly, these co-regulators bind to domains whose structure has been altered by binding of ligands and/or posttranslational modifications (100). Whether the posttranslational modifications are crucial to initiate, maintain, or simply facilitate the interaction is uncertain. It is, nonetheless, known that once the nuclear receptors are constitutively activated, they can interact with co-repressors that then further modulate their activity (76). This modification takes the form of alteration to the structure of the chromatin, leading either to a condensation that represses transcription or to a decondensation that facilitates the recruitment of the basal transcription machinery (24). In general, the structure that mediates the ligand-driven interaction with co-repressors and co-activators is the helix 12 (H12) of the receptor, which assumes an extended position in the absence of ligand, permitting the binding of co-repressors. Binding of agonist reorients H12 to a sequestered position that blocks the co-repressor binding site, while simultaneously forming a new docking surface for co-activators (222). The long H2 twists LRH-1 into an agonist-like conformation by affecting H12, even when LRH-1 ligand binding pocket is empty (232), while H1 and H12 of SF-1 are packed against the α-helical bundle, demonstrating its LBD ligand-independent active conformation (62).

Table 1. *The principal mechanism for regulation of the transcriptional activity of the NR5A receptors is interaction with cofactors*

Co-regulator	Regulation	Interaction Site/Mode	Nuclear Receptor	Reference Nos.
SHP (NROB2)	Negative	Attaches COOH-terminal domain and interacts with AF-2 domain	SF-1, LRH-1	88, 159
DAX1 (NROB1)	Negative	AF-2 domain	SF-1, LRH-1	6, 123, 262
NCOR1, NCOR2	Negative	Recruited by DAX1	SF-1, LRH-1	123, 252, 254
ALIEN	Negative	Recruited by DAX1	SF-1, LRH-1	123
FXR	Negative	Co-enriched nuclear receptor half site	LRH-1	48, 178, 286, 320
PROX1	Negative	Interact with LBD and DBD	LRH-1	224, 252
SMRT	Negative	No direct interaction	LRH-1	297
MBF1	Positive	Bridging factor	LRH-1	24
SRC1, SRC2, SRC3	Positive	Binding to the LBD	LRH-1, SF-1	159, 298
PGC1	Positive	AF-2 domain	LRH-1	311
β-Catenin	Positive	Distinct from the known interaction surfaces of LRH-1	SF-1, LRH-1	23, 127, 317

This regulation can be either positive (co-activators) to enhance constitutive activity or negative (co-repressors) to inhibit induction of transcription of target genes. The mechanisms vary, and some are unknown. LBD, ligand binding domain; DBD, DNA binding domain; SF-1, steroidogenic factor-1; LRH-1, liver receptor homolog-1.

The two best known SF-1 and LRH-1 cofactors are SHP, mentioned above, and dosage-sensitive sex reversal-adrenal hypoplasia congenital region gene on the X chromosome, gene-1 (DAX1; NR0B1), both of which act as repressors of these NR5A orphan receptors. SHP attaches to the nuclear receptor COOH-terminal domain and represses SF-1 and LRH-1 activity by interacting with its AF-2 transactivation domain (159, 163). There appears to be a negative feedback loop by which SF-1 and LRH-1 bind to the SHP promoter region to induce its transcription, which in turn reduces their activation and consequent transcriptional activity (88, 160). Structural and biochemical probe analysis has shown that SHP interaction with LRH-1 is significantly stronger than with SF-1, due to differential binding events occurring between the core LXXLL motif of SHP and the SF-1 coactivator binding site (164).

DAX1 functions as a ligand-independent nuclear receptor, and its repressive mechanism indicates that it is a competitive transcriptional co-repressor (233). Like SHP, DAX1 lacks a DNA-binding domain and additionally has neither a modulatory domain nor a hinge region (123, 204). SF-1 and LRH-1 also interact with DAX1 through its LBD with the NH_2-terminal LXXLL related motifs (262), binding with high affinity to the AF-2 domain, and repressing their transcriptional activity (6, 123, 262). There is also evidence that DAX1 recruits co-repressors, such as the nuclear receptor co-repressor (NCOR) and ALIEN, providing a further inhibitory mechanism of NR5A activity (53, 123). NCOR1 acts in the SUMOylation process of LRH-1, and one of the consequences of this association is the trans-repression of acute phase response proteins (252, 254). On the other hand, NCOR recruitment persists regardless of SF-1 SUMOylation state, as observed in un-SUMOylatable SF-1 knock-in mice (156). The homolog of NCOR1, NCOR2 (also called silencing mediator for retinoic acid and thyroid hormone receptor; SMRT), also represses the transcriptional activity of SF-1 and LRH-1 (99, 254). The mechanism of interaction between SF-1, LRH-1, and the NCOR2 is not yet well understood, as no direct regulation has yet been demonstrated. It could be, as is the case for NCOR1, that a supplementary protein is needed to link NCOR2 and the two nuclear receptors, indicating that it could be an indirect cofactor (297).

Another co-activator of LRH-1 related to SHP activity is the farnesoid X receptor (FXR; NR1H4), principally expressed in the liver, kidney, adrenals, and small intestine (178, 286). Studies with mice fed with FXR show that it is involved in cholesterol and bile acid metabolism, as well as in the regulation of glucose metabolism (178, 321). The interaction of FXR with LRH-1 allows for FXR-mediated activation of SHP, retinol dehydrogenase 9, pyruvate carboxylase, and phosphatidylethanolamine *N*-methyltransferase (48). The prospero-related homeobox transcription factor, PROX1, is another recognized co-repressor that acts similarly to DAX1 and SHP by directly interacting with both LBD and DBD of LRH-1 (224, 252). The interaction between the nuclear receptors and PROX1 leads to the repression of several target genes of LRH-1, including the steroidogenic gene *CYP17a1* (224).

Other cofactors act as bridging factors to regulate SF-1 and LRH-1, such as the multiprotein bridging factor (MBF1), which does not possess histone-modifying activities, but rather enables interactions of the nuclear receptors with the transcription machinery (24, 129). The three homologs of the p160 family, steroid receptor co-activators (SRC1, SRC2, and SRC3), act as strong regulators of the two NR5A nuclear receptor transcriptional activity by binding to their ligand binding domains (159, 298). SRC-1 interacts directly with the LRH-1 LBD in helix 1 and AF-2 (298), while SRC3 potentiates the interaction between CREB and LRH-1 (96). The phosphorylating kinase A (PKA) downstream of cAMP has been shown to stimulate SRC1 and SRC3 activation of SF-1-dependent transcription, but this signaling pathway also represses SRC2 co-activation of SF-1 by increasing ubiquitin-mediated degradation of SRC2 (22, 109, 111).

The co-activator peroxisome proliferator-activated receptor-γ (PPAR-γ)-coactivator-1 (PGC1) binds to the AF-2 domain of LRH-1 to promote differentiation of granulosa cells into progesterone-producing luteal cells (312). Interestingly, a novel isoform of LRH-1 in human granulosa cells was shown to be coordinately regulated by SF-1 and PGC1 (134). Blocking PGC1 is one of the strategies by which SHP and DAX1 repress LRH-1 activity (153), and LRH-1 is also inhibited following the recruitment of PGC1 by the sterol regulatory element-binding proteins (SREBP2) (130).

In addition to this wide and growing list of co-regulators for NR5A receptors, it has been shown that β-catenin is important for stable interactions with SF-1 and LRH-1, acting synergistically with both orphan receptors. In terms of downstream effects, LRH-1 interacts with β-catenin to promote cell cycle gene expression and cell proliferation in the intestinal crypt and granulosa cells (23, 183). Likewise, SF-1 and β-catenin interact in the signaling pathway that produces testosterone, a collaboration that can be interrupted by WNT4 overexpression (127).

As with other members of the nuclear receptor family, the regulation of the activity of NR5A receptors is a complex, context-specific process that depends heavily on cofactors. These interactions are the major, if not the principal, mechanisms of modulation of the multiple actions of SF-1 and LRH-1.

C. Transcriptional Regulation

The SF-1 gene contains seven exons and is closely situated downstream of the germ cell nuclear factor (GCNF;

NR6A1) gene, separated by ~13 kb (122, 292). An intervening insulator element is present, composed of sites that are target regions for the CCCTC-binding factor, histone tail acetylation regulation, and other nuclear matrix interaction to induce insulator activities, such as the adrenal specific DNase hypersensitive sites (adHS1–3) (122). Transient transfection experiments and protein/DNA binding assays suggest that the basal promoter of the SF-1 gene interacts with the ubiquitous transcription factors, such as nuclear transcription factor Y (NFY), stimulatory protein 1 and 3 (SP1/3), and upstream stimulatory helix loop helix (HLH) factors 1 and 2 (USF1/2), the latter of which enhances transcription through interactions with an E-box located on the basal promoter (293). Another HLH factor, Pod-1/capsulin, has been shown to repress SF-1 expression via regulation of the E-box, and while studies have failed to demonstrate direct interaction between this HLH factor and the basal promoter, targeted deletion of Pod-1/capsulin leads to an increased SF-1 expression in the developing testis and in adrenocortical tumor cells (56, 57, 264).

Chromobox homolog 2 (CBX2) is another factor shown to bind the SF-1 promoter (133), and mice carrying CBX2 null mutation display reduced SF-1 and sex determining region Y (*Sry*) expression, leading to a sexually dimorphic phenotype, small adrenal glands, and spleen malformation (132). Human CBX2 has been shown to bind directly to the SF-1 promoter, and mutations in this gene also show XY sex reversal (18). Other factors involved in gonadal development have been shown to interact with the SF-1 basal promoter, such as Wilm's tumor suppressor (WT1), Lim homeobox protein (LHX9), SOX9, and GATA4 (268, 290). Additionally, multiple intronic enhancers are specific to gonads, the adrenal gland, VMH, or pituitary gland, indicating, not unexpectedly, that regulation of SF-1 transcription is tissue specific (111).

DNA methylation also plays a role in the epigenetic regulation of the SF-1 gene. Studies in mouse tissues have shown that the SF-1 basal promoter is hypermethylated when SF-1 is not expressed, showing binding of DNA methyltransferase 3a (DNMT3A) and MECP2 factor to the SF-1 basal promoter (110, 302). In the hypo- or unmethylated state, SF-1 is expressed, with recruitment of transcription factors such as USF2 and RNA polymerase II (110, 302). Interestingly, analysis of the SF-1 promoter region in endometriotic stromal cells, which show aberrant increases in expression of SF-1, due to a high level of acetylation of associated histones, regulated by acetyltransferases (192).

From the above, it can be seen that SF-1 expression is modulated by a wide range of transcriptional regulatory processes, comprising transcription factors, intron enhancers, and epigenetic elements. This multiple array of factors is expected to be responsible for the highly variable, developmental stage, tissue and even species-dependent expression of SF-1.

Much less is known about the factors regulating the transcription of LRH-1. Studies of the 5′ upstream region of the gene have been summarized by Fayard et al. (76), indicating multiple transcriptional activators in regulation of LRH-1. In the mouse, these include GATA, HNF, and NKX motifs (76). Mouse *Pdx1* is co-expressed with LRH-1, and it has been shown that there are functional binding sites for this homeobox gene on the LRH-1 promoter (2). These findings have been interpreted to indicate that PDX1 exercises control over LRH-1 transcription during development.

D. Posttranslational Regulation

The expression of SF-1 and LRH-1 is also regulated by multiple modifications that occur following their translation. The intracellular second messenger cAMP, acting via protein kinase A (PKA), can induce p300 to acetylate SF-1 (47). This posttranslational change increases SF-1 DNA binding, induces its recruitment to nuclear clusters, and increases its dynamic interaction with regulatory cofactors (47). PKA has also been shown to promote dissociation of DAX1 from SF-1, thereby activating or amplifying transcriptional activity of the latter (73). LRH-1 possesses a large hinge domain on which the serine residues S238 and S243 can be phosphorylated, an action brought on by activation of the protein kinase C and the MAPK/ERK pathways (144, 158). The overall effect is an increase in the transcriptional activity of LRH-1. SF-1, on the other hand, has only one phosphorylation site in the AF-1 domain of the hinge region, S203, and it can be phosphorylated by the MAPK/ERK signaling cascade, as well as by cyclin-dependent kinase 7 (CDK7). The latter will form the CDK-activating kinase (CAK) complex with cyclin H (CYCH) and ménage à trois 1 (MAT1), which then anchors SF-1 to the basal transcriptional machinery of many of its target genes (161).

Another posttranslational modification of the NR5A receptors is the reversible covalent interaction engendered by conjugation to the small ubiquitin-like modifier (SUMO), at lysine residues (SUMOylation) (45, 306). Both NR5A receptors are direct substrates for the SUMO conjugation machinery, such that their activity is repressed by SUMOylation (40, 45, 308). The modification of SF-1 transcriptional activity by SUMOylation takes the form of reduced receptor binding to its cognate DNA sequences (35), or translocation of the transcription factor from the chromatin to inactive nuclear bodies (40, 45, 308). For example, the SUMO-E3 ligase RING finger protein 31 (RNF31) is involved in the ubiquitination (and stabilization) of DAX1, and present in the complex formed of DAX1, SMRT, and HDAC to induce SF-1 inactivation on target gene promoter regions (69). SUMOylation has been identified as a factor in

the interaction of corepressor PROX1 with LRH-1 to inhibit its role in cholesterol transport (253). Moreover, SUMO conjugation is implicated in the subnuclear localization of LRH-1, and the accumulation of LRH-1 in rat primary granulosa cell nuclear bodies can be suppressed by forskolin and cholera toxin treatment, suggesting the cAMP pathway is also involved in regulating LRH-1 activity (306). While in vitro studies showed that overexpression of un-SUMOylatable SF-1 and LRH-1 increased cell-based reporter activity (40, 161), the permanent elimination in vivo of SF-1 SUMOylation in mice did not lead to increased SF-1 activity; rather, it resulted in endocrine abnormality that reflected the inappropriate activation of specific SF-1 target genes (156). Indeed, loss of SF-1 SUMOylation elevates or induces ectopic activation of sonic hedgehog (*Shh*) in mouse testis and adrenal glands to amplify hedgehog signaling, promoting steroidogenesis and resulting in abnormal endocrine tissue development (156). In vivo studies using un-SUMOylatable LRH-1 mice lead to increased expression of genes involved in cholesterol transport and animals with diminished atherosclerosis development (253).

A further posttranslational regulation process that affects NR5A family members is ubiquitination, essential for many cell processes including signaling cascades, regulation of the cell cycle, DNA repair, and maintenance of protein integrity (44). It is generally inhibitory, and with respect to nuclear receptors, including SF-1, ubiquitination plays an important role in the reproductive and endocrine pathology. HDAC inhibitors such as trichostatin A and valproic acid are known to promote SF-1 ubiquitination, which then reduces steroidogenesis in adrenal tumor cells (46).

E. MicroRNAs

A wide range of microRNAs (miRNAs) is expressed in mammals, and they have been found to participate in physiological and developmental processes. They aid in modulation of gene expression by mediating RNA transcript degradation or regulating translation rate. In mouse embryonic stem cells, miR134 has been shown to promote differentiation due to its translational attenuation of LRH-1 and NANOG, both known as positive direct regulators of OCT4/POU5F1 and stem cell proliferation (265). miRNAs also play a role in several types of human malignancies such as cancer (250a). For example, the overexpression of miR-30d induces cell cycle arrest at G_0/G_1, decreases cell proliferation, migration, invasion, and tumor growth while increasing cell apoptosis in vitro (304). Bioinformatic analysis and dual-luciferase reporter assay revealed that LRH-1 is a direct target of miR-30d in colorectal cancer cells. The same study showed that LRH-1 overexpression could restore the inhibitory effect of miR-30d on these cells (304).

MicroRNAs are also involved in steroidogenic gene regulation and normal reproductive function. A recent report indicates that in the porcine ovary, miR-1275 is an endogenous regulator of LRH-1, reducing LRH-1 induction of CYP19A1 by means of effects on the 3′UTR of the LRH-1 gene (170). Studies have shown that various microRNAs interact with SF-1 to regulate its transcriptional activity. For example, miR-320 decelerates granulosa cell proliferation by decreasing SF-1 mRNA stability and impeding its transcriptional activity, while miR-764-3p has been shown to bind directly to SF-1 mRNA in mouse granulosa cells to inhibit aromatase transcription, resulting in reduced estrogen production (281, 314, 315). SF-1 has itself been shown to bind to the promoter region of miR-383 host gene, inducing its expression and allowing the miR-383-mediated estradiol release from granulosa cells (315).

F. Epigenetic Regulation

As noted above, SF-1 and LRH-1 both function as direct regulators of transcription and as transcriptional enhancers. They may also function as pioneer or licensing transcription factors, the elements that can program the epigenome during cell differentiation, by modifying chromatin accessibility to other transcriptional constituents (66). Nuclear receptors, including the glucocorticoid receptor (97) and the ecdysone receptor (246), have been demonstrated to act as pioneer factors in differentiating tissues. The evidence for LRH-1 as a possible pioneer factor has been derived from breast cancer cell lines where it regulates proliferation (16). In that study, it was shown by chromatin immunoprecipitation analysis that LRH-1 cooperates with the well-known pioneer factor, forkhead box protein A1 (FOXA1), to bring about expression of cell cycle genes (16). Furthermore, knockdown of LRH-1 altered FOXA1 binding and induced a second epigenetic effect, the depletion of histone deacetylase 2 from the regulatory region of cell cycle proteins. The concept of NR5A receptors as epigenetic modulators is novel and requires further investigation in the multiple tissues where these proteins are expressed.

VII. CELLULAR PROCESSES RELATED TO REPRODUCTION

A. Cholesterol Homeostasis

Cholesterol plays a central role in diverse biological processes, including in the formation of cellular membranes, and serves as the parent molecule for steroid hormone synthesis. Improper transformation, transport, or storage of cholesterol is the basis for a number of diseases, thus homeostatic regulation is essential, not only for normal reproductive function, but also, in some cases, for survival. Overall, appropriate intracellular concentrations of cholesterol are maintained by four complementary mechanisms: de novo synthesis; importation as elements of high-density lipoprotein (HDL) or low-density lipoproteins (LDL); re-

verse cholesterol transport, primarily as HDL; and cholesterol esterification and liberation (197). NR5A receptors play a significant part in cholesterol homeostasis by direct effects on some key regulators involved in these mechanisms.

The de novo synthesis of cholesterol, which relies on the rate-limiting enzyme HMG-CoA reductase (reviewed in Ref. 227), does not appear to be directly influenced by SF-1. One reporter assay study showed that LRH-1 does not drive transactivation of this gene (130), while Datta et al. (58) report binding of LRH-1 to the HMG-CoA promoter to induce specific activation of its transcription. Both of these studies are based solely on transient transfection assays; thus further investigation is required to determine the biological significance of these contradicting findings.

In many species, the main source of supply of cholesterol for steroid synthesis is via its importation from circulation by the HDL receptor, scavenger receptor class B type 1 (SCARB1) (197). It has been shown that SF-1 transactivates the SCARB1 gene (37) and mediates the uptake of cholesterol via this receptor (171). Similarly, chromatin immunoprecipitation (ChIP) analysis revealed that LRH-1 binds to its response element in the *SCARB1* promoter to induce its expression in both mouse and human tissue (150, 238).

Reverse cholesterol transport, another homeostatic process completed principally by HDL via SCARB1, is regulated by LRH-1 activity (76). Intermediates that shuttle cholesterol to HDL for efflux include members of the ATP binding cassette (ABC) family (reviewed in Ref. 219), and transient transfection assays have shown that LRH-1 activates transcription of isoforms ABCG5 and ABCG8 by binding to their promoter regions (82). In addition, hypomorphic expression of LRH-1 in the liver of heterozygote LRH-1 germline knockout mice leads to diminished expression of both *Abcg7* and *Abcg8* (300). One isoform of the ABC family, ABCG2, transports xenobiotics rather than cholesterol in reproductive tissues and has been shown to be upregulated by SF-1 in mouse Sertoli cells (295). The role of SF-1 in the expression of factors involved in reverse cholesterol transport has yet to be elucidated.

Cholesterol storage and its liberation are also important factors in sterol homeostasis, particularly in steroidogenic cells. Free cholesterol is esterified by the enzymes of the sterol *O*-acyltransferase (SOAT) family (228). At least one of the members of the family, SOAT1, was identified as a target of SF-1 in adrenocortical cells, and overexpression of SF-1 in an adrenal cell line upregulates its expression (77). In contrast, promoter assays have suggested that SF-1 also liberates cholesterol from the esterified state by activating hormone-sensitive lipase (LIPE), the principal lipase in steroidogenic tissues (112, 145). The latter concept is more in keeping of the role of SF-1 as a factor in steroid synthesis induction. No information appears available at this time on the role of LRH-1 in regulation of either SOAT1 or LIPE.

The sterol regulatory binding proteins (SREBPs) are transcription factors that have been shown to be the dominant regulators of components in cholesterol metabolism processes (241). Lopez and McLean (171) reported a synergistic interaction between SF-1 and SREBP1a in induction of transcription of SCARB1. Synergistic transactivation has also been demonstrated in the context of another lipogenic gene, the Niemann-Pick C1(NPC1)-like 1 (155). Given that LRH-1 also induces SCARB1 expression in both mouse and human tissue (76), it is somewhat surprising that at least two studies suggest reciprocal antagonism between LRH-1 and SREBPs. Lee et al. (157) reported that treatment with the LRH-1 agonist dilauroyl phosphotidylcholine (DLPC) reduced the expression of SREBP1c. Others presented evidence that LRH-1 directly inactivates SREBP1 transactivation in promoter assays, and they further showed that SREBP2 directly inhibits LRH-1 activity (130). It is proposed that this is achieved by binding of LRH-1 to SREBPs, which inhibits the recruitment of their co-activators CBP and PGC1β, potentiating SREBP transcriptional activity (167, 202). It is speculated that the inhibitory effects of SREBPs on LRH-1 activity have greater impact than the effects of LRH-1 on the SREBP functions because abundance of active SREBPs in the nucleus varies more strongly in response to metabolic alterations. The dissonance between multiple studies showing that LRH-1 stimulates SREBP-driven genes in vivo (13, 68) and in vitro (155) suggests that the antagonism between LRH-1 and SREBP may be gene specific.

Cholesterol transport within the cell is necessary for multiple biological processes, and since the aqueous intracellular milieu is hydrophobic, transport mechanisms are required. Among these are the cytosolic lipid transfer proteins including NPC1, sterol carrier protein-2 (SCP2), and the steroidogenic acute regulatory protein (STAR) (294). All of these factors are essential for reproductive function (reviewed in Ref. 256). Studies have shown that SF-1 regulates the transcription of the NPC1 gene in concert with cAMP (86). The promoter region of SCP2 contains an SF-1 (and presumably a LRH-1) response element, suggesting regulation by the NR5A receptors (172).

The STAR protein, first discovered by Clark and Stocco (50), transfers cholesterol from the outer mitochondrial membrane into the cristae, where it can initiate the enzymatic cascade that comprises steroid synthesis. There is a great deal of information implicating the NR5A receptors in modulating STAR expression, from early studies showing promoter transactivation by SF-1 in granulosa cells (229, 259) to more recent ChIP analysis of bovine thecal cells, demonstrating increased SF-1 binding to the bovine theca cell *STAR* promoter in response to luteinizing hor-

mone (LH) stimulation (196). SF-1 induces STAR synthesis, not only through classic promoter binding, but also by acting as an enhancer at sites >3 kb upstream of the *STAR* transcription start site (189). A nonexhaustive list of tissues where SF-1 regulates STAR includes adrenal cell lines (296), Leydig (273), theca (196), granulosa (210), and endometrial cells (67). LRH-1 also is an essential factor for expression of STAR in steroidogenic tissues. An early investigation showed that LRH-1 induces *STAR* transcription in human adrenal, testis, and ovarian tissues (217). Cre-loxP depletion of LRH-1 in the mouse ovary from primordial follicles forward (13, 14, 68) results in dramatic reduction in *Star* expression. Moreover, ChIP analysis by Duggavathi et al. (68) demonstrates that LRH-1 binds directly to the *STAR* promoter.

In summary, it is clear that there is remarkable scope in the regulation of cholesterol homeostatic and transfer mechanisms by the NR5A receptors. Virtually all of these processes are significant to mammalian reproductive function, as cholesterol is the parent molecule of the steroids that orchestrate folliculogenesis, spermatogenesis, and gestation.

B. Steroidogenesis

As noted above, cholesterol is transported from the cytosol into the inner membrane of the mitochondrion of steroidogenic cells to allow steroid hormone biosynthesis (50). Cholesterol side chain cleavage enzyme (P450scc, CYP11A1), resident in the mitochondrion, converts cholesterol to pregnenolone, and the diverse steroid products can subsequently be synthesized following transformations of pregnenolone by a variety of cytochrome *P*-450 oxidases (CYPs) and steroid dehydrogenases (HSD) enzymes (86, 211; reviewed in Ref. 187). The promoter regions of several of these enzymes have been shown to contain the SF-1/LRH-1 consensus site (194, 226) **(TABLE 2)**. These discoveries led to identification of the presence of SF-1 in most mouse steroidogenic tissues, including the corpus luteum (CL) (106), and LRH-1 was later shown to be present in the ovary and testis (248, 311) **(TABLE 2)**. Under the control of gonadotropins, the NR5A receptors enhance the activity of steroidogenic genes, such as STAR, CYP11A1, CYP17A1, 3βHSD, and the steroid 11β-hydroxylase (CYP11B1) (150). Moreover, LRH-1 serves as a critical factor in the transcriptional regulation of the aromatase (*CYP19A1*) gene, the rate-limiting enzyme in ovarian estrogen biosynthesis (184). In addition to regulating enzymes directly involved in cholesterol transport and steroid biosynthesis, SF-1 and LRH-1 control the expression of ferrodoxin 1 (*FDX1*), an iron-sulfur protein which functions as the electron donor for the catalytic activity of P450scc in ovarian granulosa cells (119). Activation of the *FDX1* promoter has been shown to occur following stimulation by cAMP (119).

C. Cell Proliferation

We have recently shown that LRH-1 plays a key role in granulosa cell proliferation, as depletion of LRH-1 causes a significant decrease in the number of granulosa cells entering S phase of the cell cycle, and in the abundance of transcripts of key genes such as the cyclins D and E and their downstream targets E2F1 and E2F2 (183). This effect on proliferation has been shown to be mediated by LRH-1

Table 2. *Both LRH-1 and SF-1 regulate the process of steroid synthesis in reproductive target tissues, sometimes in the same cells*

Steroidogenic Gene	Principal Reproductive Tissue Localization	Reference Nos.
	SF-1	
STAR	Theca, granulosa, luteal, Leydig cells	39, 49, 102, 125, 210
CYP11A1	Theca, granulosa, luteal, Leydig cells	27, 51, 102, 125, 210, 243, 244, 279
CYP17A1	Theca, granulosa, luteal, Leydig cells	27, 209, 210
CYP19	Granulosa, Leydig, Sertoli cells	177, 188, 280
HSD17B1	Endometriotic tissue	4
HSD3B1	Leydig, theca cells	27, 188
HSD3B2	Luteal, Leydig cells	180, 272
	LRH-1	
STAR	Granulosa, luteal, Leydig cells	137, 181
CYP11A1	Granulosa, luteal, Leydig cells	136, 244
CYP17A1	Granulosa, luteal, Leydig cells	72, 181, 311
CYP19	Granulosa, Leydig	106, 218
HSD3B1	Granulosa cells	188
HSD3B2	Luteal cells, Leydig cells	180, 215

LRH-1, liver receptor homolog-1; SF-1, steroidogenic factor-1; STAR, steroidogenic acute regulatory protein; CYP11A1, P450 side-chain cleavage; CYP17A1, P450 family 17; CYP 19, P450 aromatase; HSD3B1, 3β-hydroxy-steroid dehydrogenase-1; HSD3B2, 3β-hydroxy-steroid dehydrogenase-2.

interaction with β-catenin and CDKN1A, a cell cycle inhibitor, all of which are direct targets of LRH-1, as revealed by ChIP (183).

While there seems to be little information about the role of SF-1 in gonadal cell proliferation, several studies of SF-1 in mouse adrenal function have demonstrated direct correlation between adrenal size and SF-1 gene expression, and SF-1 gene copy number has an impact on proliferative potential adrenocortical cells (15, 19, 133). SF-1 overexpression in human adrenocortical cells increases the proliferation rate in vitro, and transcript and microarray analysis showed increased expression of regulators of cell cycle progression and reduced expression of pro-apoptotic factors (65). An interesting study of the role of SF-1 on glycolytic gene transcription demonstrated that knocking down SF-1 in vitro led to reduced proliferation of adrenocortical Y-1 cells (5). In terms of the mechanism by which this effect occurs, it was postulated that the reduced expression of key glycolytic genes due to SF-1 absence leads to significantly lower levels of cellular ATP production, essential for the generation of daughter cells (274).

D. Stem Cell Differentiation

After discovery of SF-1 in the early 1990s, Milbrandt et al. (54) demonstrated that it directed mouse embryonic stem (ES) cells towards a steroidogenic lineage, by inducing endogenous *Cyp11a1* expression via cAMP and retinoic acid activation. These differentiated cells were nonetheless unable to biosynthesize steroids, due to the lack of cholesterol transport proteins. This initial discovery led other groups to explore the role of both SF-1 and LRH-1 in stem cell differentiation, showing binding to the promoter region of the *OCT4* gene and the consequent activation of the transcription of this pluripotency factor, in both murine and human ES lines (94, 307). These authors showed that, as ES cells began to differentiate, both SF-1 and OCT4 expression decreased, indicating SF-1 plays a role in maintaining pluripotency (307). More recently, it has been demonstrated that both LRH-1 and SF-1 regulate and can replace OCT4 in stem cells (282). In fact, LRH-1 can take the place of OCT4 in the derivation of induced pluripotent stem cells from mouse somatic cells, with enhanced reprogramming efficiency, relative to the classic four factors required for induction of pluripotency (OCT4, SOX2, FOXD3, and NANOG) (7).

Following exit from the pluripotent state, cells respond differently to the NR5A receptors. LRH-1 is capable of inducing mesenchymal stem cell differentiation without SF-1 expression (311). SF-1 is also capable of inducing the adipose tissue mesenchymal stem cells (MSC) to differentiate into steroidogenic cells, as confirmed by the secretion of corticosterone (87). In contrast, the steroidogenic cells derived from bone marrow MSC secreted gonadal rather than adrenal steroids (87). Recently, it was reported that mouse ES cells could be differentiated into Leydig-like cells via SF-1 overexpression in vitro and that these cells are able to rescue testosterone secretion when transplanted in the testes of rats where Leydig cells had previously been ablated (309). In terms of potential mechanisms, SF-1 directs stem cell differentiation by inducing chromatin alterations, and by modifying the chromosomal conformation of genomic regions, via histone eviction and chromatin loop formation (189, 207).

In overview, there is substantial evidence to implicate the orphan nuclear receptors of the NR5A family in two prominent events in reproductive function, the regulation of stem cell pluripotency and differentiation.

VIII. PHYSIOLOGICAL PROCESSES RELATED TO REPRODUCTION

A. Embryogenesis

1. Early embryonic development

Both SF-1 and LRH-1 are expressed at multiple stages in embryonic and fetal life, and both are essential for normal embryonic development, demonstrated by their deletion via targeted mutagenesis in the mouse. LRH-1 is abundantly expressed in the morula and inner cell mass of the early embryo (94). At embryonic day E7.5 in the mouse, LRH-1 is detected in foregut endoderm and is progressively expressed during the differentiation of the foregut into liver, intestine, and pancreas (76). At day E17.5, LRH-1 exhibits its adult expression profile in the liver, exocrine pancreas, intestinal crypts, and stomach epithelium (76). By day E11.5, bipotential gonads express LRH-1 and at E15.5, when testis and ovaries are anatomically distinct, and the signal for LRH-1 declines in gonads of both sexes (76). At day E17.5, LRH-1 exhibits its adult expression profile in the liver, exocrine pancreas, intestinal crypts, and gastric epithelium (76).

Mice homozygous for a germline mutation in the gene encoding LRH-1 die between E6 and E7.5 **(TABLE 3)**, a time that corresponds to gastrulation, indicating that LRH-1 plays a crucial role in the formation of the early embryo (208). Interestingly, development of the embryo to a multicellular stage occurs (148), indicating that proliferation can occur in the absence of LRH-1. Embryos heterozygous for the mutation display growth retardation, epiblast disorganization, and impaired primitive streak morphogenesis (148). Failure of gastrulation is believed to be secondary to defective visceral endoderm development (148). The mechanism is postulated to be related to the maintenance of pluripotency of the embryonic cells. As noted above, LRH-1 colocalizes with the pluripotency factors, OCT4 in

Table 3. *Phenotypic characteristics of mouse models with germline deletion of LRH-1 or SF-1 or tissue-specific depletion of either gene in gonadal tissues showing the consequences of depletion on reproductive processes and consequent fertility*

			LRH-1	SF-1		Reference Nos.
Germline deletion	Homozygous		Embryo lethality at gastrulation period (E6–7.5)	Postnatal lethality ≤8 days after birth		176, 208
			Defective visceral endoderm development	Absence of adrenal gland and gonad regression at E11.5–12		148
			Impaired primitive streak morphogenesis	Adrenocortical insufficiency		
	Heterozygous		Reduced fertility in females due to impaired progesterone production	Fertile		149, 176
Cre-loxP conditional depletion	Cre recombinase	Male	Female	Male	Female	
	Amhr2	Fertile	Depletion in granulosa cells of all follicles including corpora lutea	Depletion in Leydig cells of testes	Depletion in granulosa cells of all follicles	68
			Infertile	Infertile	Infertile	214
			No ovulation	Arrested spermatogenesis	No ovulation	
			No luteinization	Delayed fetal testes organization	Absence of CL	
			Decrease in granulosa cells proliferation	Hypoplastic, undescended testes	Decrease in granulosa cells proliferation	
			No cumulus expansion	Decrease in testes somatic cell proliferation	Reduced follicle number	
	Cyp19a1	Fertile	Depletion in granulosa cells from antral follicles forward	Fertile	Fertile	14
			Infertile			
			Incomplete cumulus expansion			
			No ovulation			
			No luteinization			
	Pgr	Fertile	Depletion in corpus luteum	Fertile	Depletion in corpus luteum	318
			Infertile		Subfertile to infertile	249
			Corpus luteum unable to maintain pregnancy. Impaired decidualization		Impaired luteinization	

LRH-1, liver receptor homolog-1; SF-1, steroidogenic factor-1.

the inner cell mass and in the embryonic epiblast of the mouse, and LRH-1 is required to maintain OCT4 expression at the epiblast stage of embryonic development (94).

The ontology of SF-1 expression during development is less clear **(TABLE 3)**. Its first appearance is in the adrenal/gonadal primordium region of the mesoderm that condenses to become the urogenital ridge on E9 in the mouse, and by E11 it is found in the separated gonadal and adrenal anlagen (117). SF-1 expression then continues in the developing steroidogenic portion of the adrenal gland and then in the outer cortical region (E11–12) (117). It is not indispensable, as mice with a germline mutation of SF-1 survive in utero, but succumb by the eighth postnatal day due to adrenal agenesis and consequent adrenocortical insufficiency (176).

2. Neural development

Tissue-specific depletion strategies indicate that SF-1 expression is required at multiple sites in the hypothalamic-pituitary-gonadal axis (125, 138). In the adult mouse brain, SF-1 is expressed exclusively in the VMH (40). In the hypothalamus, SF-1 is found in mouse VMH precursor cells from E11.5 onward (61, 251). Its expression is essential for correct formation of this structure (116). In SF-1 knockout mice, the VMH is present at E17, but with decreased cellularity and abnormal organization that persists, at least until birth (116). Germline knockout studies are buttressed by results from a central nervous system (CNS)-specific SF-1 knockout mice model, where the animals show similar disruption of the VMH organization (139). Together these findings indicate that SF-1 is not involved in the early stages of VMH development, but that absence of this transcription factor results in incorrect architecture and premature regression of the structure.

SF-1 germline knockout animals can survive if supplemented with corticosteroids, and transplantation with wild-type adrenal glands restores hypothalamic-pituitary-adrenal function (179). These animals showed significant decreased locomotor activity and late-onset obesity (179). This suggests a role of SF-1 in energy homeostatic regulation, a concept supported by the occurrence of severe obesity in CNS specific SF-1 knockout mice (139).

3. Pituitary development

SF-1 is found in gonadotrophs of the developing pituitary at E13.5, following α-glycoprotein subunit appearance (αGSU), but before the expression of the β-subunits of LH and follicle-stimulating hormone (FSH) (120). DNA methylation analysis has shown that SF-1 is silenced in progenitor αT1 gonadotrophic cell lines, but active in both immature (αT3–1) and mature (LβT2) gonadotrophic cell lines (152). This indicates that SF-1 does not direct precursor cells to the gonadotrophic cell fate, rather, it regulates the final differentiation steps.

4. Gonadal development

SF-1 is strongly expressed in the genital ridge of both male and female mice, in the undifferentiated gonads from E9-E13, before the sex determining region Y (SRY) protein expression begins to induce sexual differentiation (117). Germline inactivation of SF-1 in mice does not interfere with normal early gonad development or with germ cell colonization, but differentiation is arrested around E11–11.5 and the cells degenerate via apoptosis (176). This suggests that SF-1 is not involved in the early stages of gonad identity specification, but required for differentiation, survival, and growth of the somatic cells already present in the early indifferent gonad. SF-1 has been shown to participate in the transcription of *Sox9* by binding to its gonad specific enhancer region together with SRY (240), and SOX9 in turn activates anti-Müllerian hormone (*Amh*) transcription together with SF-1 to induce male sexual development by inhibiting the formation of female gonads (59).

As gonad differentiation occurs, SF-1 continues to be expressed in a diffuse manner in the interstitial region, the testicular cords, and seminiferous tubules of the testis (E12.5–15). In contrast, it disappears from the developing ovary between E13.5 and 16.5, only to reappear after birth in this organ (117). Sex-specific regulation is achieved by repression of SF-1 by the forkhead box L2 (FOXL2) transcription factor during ovarian development (263). As chronicled above, one of the principal mechanisms by which SF-1 regulates gonadal function is the induction of steroidogenic enzymes (117). Furthermore, it has been shown that SF-1 (and LRH-1) can transform both pluripotent and mesenchymal stem cells into steroidogenic cells in vitro (310). The combination of SF-1 with WT1, DMRT1, GATA4, and SOX9 transformed mouse fibroblasts into Sertoli-like cells (28). Together these observations demonstrate the pivotal roles for both SF-1 and LRH-1 in gonadal and reproductive tract development.

B. Gonadotropin Synthesis and Release

Neurons that are positive for SF-1 in the VMH express estrogen receptor α (ESR1) as well as leptin receptors, both known to be essential for appropriate gonadotropin secretion (299). This regulation has been attributed to impingement of VMH projections onto gonadotropin releasing hormone (GnRH) neurons (89); thus it is to be expected that GnRH secretion would be impaired as the consequence of developmental disruption of the VMH in the absence of SF-1 (116). Support for this concept comes from CNS-specific SF-1 knockout mice that display diminished LH responses to exogenous GnRH relative to control animals (138). It was concluded that the absence of SF-1 in cells of the CNS and consequent abnormality has downstream effects in the form of reduced GnRH priming or synthetic capacity of pituitary gonadotrophs.

LRH-1 also plays a prominent role in the hypothalamus, particularly in the regulation of the female reproductive axis. LRH-1 expression in the CNS is localized to a limited area of the brain, the arcuate nucleus that includes kisspeptin (Kiss) neurons, a region well known for the regulation of GnRH secretion (3, 146). In mice, LRH-1 controls FSH levels, follicle maturation, and estrous cycle by binding directly to the *Kiss1* promoter and stimulating its transcription (3). The consequence of the depletion of LRH-1 is the reduction in the secretion of GnRH, which in turn reduces gonadotropin secretion (3).

The promoter regions of the common α-subunit and the subunits specific to the gonadotropic hormones, FSHβ and LHβ, display the SF-1/LRH-1 response element, indicating direct regulation by the NR5A receptors (81). Mutation of this site eliminated LHβ promoter activity (135). As noted elsewhere, germline deletion of SF-1 has severe consequences on prenatal reproductive development and postnatal reproductive function. The synthesis and secretion of both LH and FSH are impaired, as is the expression of the GnRH receptor on gonadotroph membranes (reviewed in Ref. 323). Pituitary-specific knockout, targeting floxed SF-1 by means of Cre recombinase driving the αGSU common subunit, depleted FSH and LH content in the pituitary to the point of near absence, while all other pituitary hormones were unaffected (323). The expected abrogation of fertility followed.

LRH-1 has recently been detected in the anterior pituitary gland, but its functional significance in vivo is only partially understood. Indeed, LRH-1 mRNA and protein expression were also found in both primary pituitary cells and gonadotroph-derived cell lines (325). LRH-1 has been shown to regulate gonadotropin gene expression, activating the FSH and LH secretion from the rat anterior pituitary gland and in gonadotropic cell lines, in vitro (325). This notwithstanding, a recent study using a mouse model with a gonadotroph-specific deletion of LRH-1 demonstrated that these mice had normal pituitary FSH and LH expression and intact fertility, indicating its expression in the pituitary is dispensable in vivo (80).

C. Gonadal Function

1. Testis

As noted above, it has been shown that SF-1 and LRH-1 are involved in the differentiation of mesenchymal stem cells into steroid hormone-producing cells, and in induction of the expression of the androgen-specific enzyme CYP17A1 (313). They therefore play a pivotal role in steroid hormone production in human Leydig cells (311).

Given the demonstrated importance of both SF-1 and LRH-1 in regulation of steroidogenic enzymes and factors (76), various groups have explored their roles in testicular function. Interestingly, LRH-1 was undetectable in the Sertoli cells that regulate the development of germ cells and where SF-1 expression is high (218, 248). On the other hand, LRH-1 is expressed at appreciable levels, quantitatively greater than SF-1, in Leydig cells as well as in pachytene spermatocytes and round spermatids (218, 248). Furthermore, LRH-1 is expressed in several rat and mouse testicular cell types where it regulates aromatase expression (218).

Additionally, as discussed above, SF-1 plays a role in the development of testis. It stimulates the expression of AMH, inducing the regression of the Müllerian ducts in the developing fetus and inhibiting female genitalia formation (242), and in the absence of SF-1 in male germline, a sex reversal phenotype is observed demonstrating the essential role SF-1 in testis formation and function (120, 235, 245). In mature testes, SF-1 is expressed in the Leydig cells, where it regulates progenitor cell formation and survival, and in the Sertoli cells of the seminiferous tubules, where, as it is the case in the developing testes, it plays a role in AMH transcription (113, 131). Leydig-specific SF-1 knockout mice have been produced via the inactivation of SF-1 in cells expressing the AMH type 2 receptor (Amhr2) **(TABLE 3)** (125). Male mice in this model were infertile and also showed undescended, hypoplastic testes with abnormal structure. The lumina of the seminiferous tubules were closed, and spermatogonia failed to develop into sperm. This effect was attributed to androgen deficiency, and indeed, CYP11A1 and STAR expression, essential upstream proteins in testosterone production, were significantly reduced.

Precocious expression of LRH-1 in mice leads to precocious induction of androgen synthesis and early puberty (68) most likely due to effects on the hypothalamic-pituitary mechanisms regulating the onset of reproductive function. In contrast, mice heterozygous for LRH-1 mutation, i.e., where only a single functional allele is present, have circulating testosterone levels that are less than half of what is observed in their wild-type littermates (277). Recent investigation showed by immunogold localization that LRH-1 is present in the head of human spermatozoa, with markedly reduced expression in the neck and across the tail, but also in different stages of testicular germ cell development (191). This study demonstrates that LRH-1 plays a role in sperm motility, survival, and cholesterol efflux and appears to serve as a downstream target of estrogenic signaling.

2. Ovary

In granulosa-specific SF-1 knockout mice, obtained via the depletion of SF-1 in the granulosa cells by means of Amhr2 Cre, females were infertile and showed hypoplastic ovaries with reduced numbers of oocytes and complete absence of luteal formation (214). This was an important indicator

that SF-1, like LRH-1, is crucial for normal ovarian function. Indeed, as noted in the section on steroidogenesis, SF-1 has been shown to directly regulate theca cell transcription of CYP19A1, and also to bind *CYP17* and *STAR* promoter regions in the ovary (196, 280). While studies have mainly reported low SF-1 expression in the mouse corpus luteum (106), bovine studies have shown that SF-1 inhibition leads to a significant decrease in progesterone production in luteal cells (190). Analysis of NR5A receptor expression in the rat ovary indicated that SF-1 was expressed in ovarian cell types, i.e., granulosa, thecal, and luteal cells at a higher level than LRH-1, which is restricted to the granulosa cells (72). In the macaque corpus luteum, SF-1 regulates the luteal secretion of inhibin-α (INHA), known to play a crucial role in suppression of FSH secretion (261). One of the important roles of SF-1 in granulosa cells, beyond biosynthesis of steroids, is its capacity to modulate the expression of AMH, a hormone involved in the ovarian reserve. In this context, SF-1 requires interaction with FOXL2 to bind the *AMH* promoter and induce its transcription (126). These interactions have not yet been well studied, but it has also been shown that FOXL2 is a suppressor of SF-1, leading to the inhibition of *CYP17A1* transcription and interrupted follicle development (209).

SF-1 has other target genes in ovarian tissue. One of these in human steroidogenic cells is 5-aminolevulinic acid synthase 1 (*ALAS1*), a rate-limiting enzyme for heme biosynthesis in mammals (128). This enzyme plays a role in progesterone production via the supply of heme as a prosthetic group of P450 steroid hormone-synthesizing enzymes (128). In another example, SF-1 combines with SMAD3 to activate transforming growth factor (TGF)-β3-induced CYP19A1 expression and subsequent estradiol synthesis and secretion in mouse granulosa cells (166). The expression of another SF-1 target gene, *CYP17A1*, is negatively regulated by the AP-1 family member FOS, a protooncogene that can reduce SF-1 activity by blocking both its transcription and binding to its LBD hinge region (247).

LRH-1 is highly expressed in the mouse ovary and is specific to the steroidogenic granulosa and luteal cells, and distinctly absent in theca cells and ovarian stroma (106). It has also been identified in equine (21), rat (72), rabbit (1), bovine (75), and human (248) ovaries. During folliculogenesis, LRH-1 is expressed in the pre-granulosa cells of primordial follicles, in the granulosa cells of primary follicles, and at all later stages of follicular development (76, 183). LRH-1 plays a major, but not indispensable, role in granulosa cell proliferation (68, 183) and is also induced significantly in the CL during pregnancy (107). In rodents, the expression of LRH-1 is increased by FSH in granulosa cells, and by prolactin in luteal cells (72).

The essential role of LRH-1 in reproductive function and steroidogenesis in vivo is evidenced in mice heterozygous for a null mutation of LRH-1, where females are infertile due to a dysregulated luteal function (149). Additionally, granulosa-specific LRH-1 knockout females are also infertile, due to the failure in both cumulus expansion and ovulation, effects that cannot be redressed by gonadotropin stimulation (68, 125). The normal expression of a number of genes is disrupted in this mouse model, including the steroidogenic genes *Cyp11a1, Cyp19a1*, and *Star*, as well as the rate-limiting gene in prostaglandin synthesis, the prostaglandin-endoperoxide synthase 2 (*Ptgs2*), and genes associated with cholesterol transfer such as *Scarb1* (68).

Another transgenic mouse model with the effect of depletion of LRH-1 in granulosa cells of antral follicles produced infertile females due to incomplete cumulus expansion, as well as a lack of ovulation (14). Key genes involved in the process of cumulus expansion and ovulation such as epiregulin (*Ereg*), amphiregulin (*Areg*), betacellulin (*Btc*), and tumor necrosis factor stimulated gene-6 (*Tnfaip6*) are significantly downregulated while connexin 43 (*Gja1*) is drastically upregulated in this knockout mouse model. Interestingly, the nonovulated oocytes can be fertilized by intracytoplasmic sperm injection (ICSI), indicating that their viability is not affected, thus confirming that the absence of LRH-1 in granulosa cells and failure of ovulation is the genesis of their infertile condition (14). To further study the role of LRH-1 in ovulation, a CL-specific LRH-1 knockout mouse model was created and produced females capable of breeding, where ovulation and fertilization occurred, but the animals were infertile. In this mouse model, luteal size is reduced, and luteal function compromised, as evidenced by reduced circulating levels of progesterone. Ovarian expression of steroidogenic factors, including STAR and CYP11A1, is dramatically reduced, demonstrating that LRH-1 is required for luteal function (318).

These ovarian results are largely recapitulated in another transgenic mouse model generated by means of an inducible shRNA under the influence of a TET promoter, where LRH-1 depletion is actuated at will in vivo (85). The TET-treated females present a consistent reproductive phenotype that mimics the LRH-1 granulosa-specific knockout. Interestingly, the infertility is fully reversible after the cessation of LRH-1 knockdown, with no signs of permanent changes due to the transient reduction of LRH-1 expression.

These various examples demonstrated that SF-1 and LRH-1 are essential modulators of ovarian function in mammals, and that they are promising targets for novel fertility and contraceptive treatments.

D. Gestation

Years of fundamental and applied research have shown that progesterone, produced principally by the corpus luteum during early gestation, is crucial for the initiation of preg-

nancy, for embryo implantation, for maintenance of pregnancy, and for suppression of the LH secretion that induces ovulation in mammals. Progesterone further drives decidualization, a process by which stromal cells differentiate into decidual cells. Decidualization of the maternal stroma is key to a successful implantation and appropriate placental formation, and it confers maternal immunotolerance to the fetus (reviewed in Ref. 221). Studies have reported that SF-1 expression is absent from the fetal components of the placenta, including trophoblast cells in humans and in rodents (258, 303). SF-1 expression does not appear to be a regulatory factor in the healthy uterine tissues, although its continued expression in the CL of pregnant bovine and porcine ovaries indicates that it plays an essential role in maintaining gestation (190, 223). The cofactors DAX1 and WT1 have been shown to inhibit the cAMP-dependent transcriptional activity of SF-1 on CYP19A1 expression in cultured human endometrial cells (95). In mouse models where SF-1 expression is depleted specifically in granulosa cells, uterine development is significantly reduced in the epithelial, myometrial, and stromal layers, resulting in absent or fewer complex endometrial glands (214). The effect was not interpreted to be direct; rather, absence of SF-1 in ovarian granulosa cells leading to impaired steroidogenic gene expression, and the consequent reduction in ovarian estradiol production, is insufficient to stimulate normal uterine differentiation. In a novel mouse model, induction of overexpression of SF-1 in cells expressing progesterone receptor (PGR) was characterized by abnormal uterine morphology, with enhanced endometrial gland and cyst development, and consequent infertility in females (275). Endometrial cells of the SF-1 overexpressing mice did not express PGR and were unable to decidualize in response to hormone stimulation, suggesting that SF-1 silencing is essential for normal uterine function.

Zhang et al. (318) demonstrated that LRH-1, expressed in the mouse and human endometrium, is necessary for endometrial decidualization, placenta formation, and ultimately successful pregnancy. This was shown in a CL-specific LRH-1 knockout mouse that displayed luteal insufficiency, and where gestational failure cannot be mitigated by progesterone supplementation (318). Progesterone treatment induces successful implantation, but embryo development fails due to defects in placentation. These effects transpire at different times among animals, some soon following implantation, some as late as day 16 of pregnancy, and some at varying intervals. Uterine genes essential for gestation that are deregulated when LRH-1 is depleted from mouse CL and endometrium are *Hoxa10*, *Wnt4, Wnt5, Bmp2, Ppard*, and *Hbegr*. In humans, siRNA reduction of LRH-1 impaired decidualization of the endometrium (318) and therefore the development of the placenta by affecting the invasion of extravillous trophoblastic cells into the uterine decidua (319). Thus LRH-1 is essential for appropriate establishment of the maternal-fetal connection, and insufficient expression may be a factor in human gestational pathology, including pre-eclampsia (319).

E. Reproductive Behavior

As mentioned above, SF-1 is strongly expressed in the VMH nucleus and is essential for the normal function of neurons of this region, with impact on mammalian behavior including physical activity, anxiety, and aggressiveness (90, 179, 324). The VMH also regulates reproductive behavior, and the female sex hormones estrogen and progesterone secreted at different stages of the ovarian cycle lead to modifications in VMH neuron morphology (90). CNS-specific SF-1 knockout mice show impaired female reproductive function, with abnormal sexual behavior, irregular estrous cycles, and subfertility (138). The marked reduction in lordosis and receptivity observed in females of this mouse model may be caused directly by altered VMH organization, preventing neuron projection to the medial central gray and peri-aqueductal gray regions where lordosis is controlled (36), or indirectly where ventromedial neurons of the VMH are unable to transmit excitatory signals to GnRH neurons and with effects on steroid synthesis and subsequent reproductive behavior (316). Although studies have shown that some components of male sexual behavior and copulatory performance are induced by androgen activation in the VMH, in addition to the essential androgen receptor activity in the medial preoptic area (MPOA), CNS-specific SF-1 knockout male mice do not show impaired reproductive behavior (101, 138). To our knowledge, no studies have been published on the effect of LRH-1 on reproductive behavior.

IX. PATHOLOGICAL PROCESSES RELATED TO REPRODUCTION

A. Genetic Abnormalities

Although no complete deletions of the SF-1 gene appear to have been observed in humans to date, non-silent single nucleotide polymorphisms, frameshifts, and partial deletions have been described as causal to disorders in the heterozygous state (reviewed in Ref. 260). Due to its essential role in steroidogenesis, the SF-1 mutations in humans were initially linked to adrenal insufficiency and gonadal dysgenesis, where 46XY patients presented external female genitalia, uterine and upper vagina structures, and primary adrenal failure (46,XY DSD) (77). Heterozygous mutations in SF-1 are the cause of up to 20% of 46,XY DSD cases, without affecting adrenal function, and reported to account for ~4% of infertile men with severe spermatogenic failure that do not have chromosomal anomalies (9, 141). Single nucleotide variations and missense mutations in the human SF-1 gene have been associated with low testosterone levels, elevated gonadotropin secretion, azoospermia, oligozoo-

spermia, and hypospadias (9, 142). Women that carry SF-1 heterozygous mutations show a wide range of clinical phenotypes, some with no impact whatsoever, while others show 46,XX gonadal dysgenesis or agenesis, accompanied by conditions such as primary or secondary amenorrhea, precocious depletion of the ovarian reserve, and consequent premature ovarian failure (34, 173). Recent clinical evaluations have demonstrated the presence of SF-1 mutations in women with primary ovarian insufficiency (POI) to be rare, excluding it as a prevalent genetic factor for this condition (124, 276). Most clinical accounts of SF-1 anomalies have been associated with loss of function, but there have been reports of SF-1 overexpression and overactivity as well. This has been attributed, in some instances, to copy number variation of SF-1 through genomic duplication at its chromosome locus or, alternatively, upregulation of SF-1 through decreased promoter methylation, leading to tumor development (78, 220). There appears to be no published information on human genetic abnormalities attributable to LRH-1.

B. Endometriosis

Endometriosis is characterized by presence of endometrial glands and stroma in ectopic locations, usually the peritoneal cavity, causing abnormal growth that can lead to persistent pelvic pain and infertility (31). In addition to some genetic predisposition, estrogen dependency, progesterone resistance, and inflammation are clear molecular indicators of the disease, mediated by growth factors, metalloproteinases, prostaglandins, and cytokines (26, 31, 230). Bulun et al. (200) showed that, while normal endometrial tissues do not express steroidogenic genes, uterine tissues from women with endometriosis showed detectable expression of CYP19A1 and estrogen production. Further studies showed that inflammation and estrogen production in endometriosis are connected by positive feedback that promotes expression of factors including STAR, CYP19A1, and PTGS2, all of which have been shown to be aberrantly overexpressed in endometriotic lesions (29, 206). The regulation of these steroidogenic genes strongly suggests that SF-1 and LRH-1 play a role in endometriosis. Indeed, studies have shown that while in normal endometrial cells, chicken-ovalbumin upstream-transcription factor (COUP-TF) and WT1 bind to the promoter region of *CYP19* to inhibit its expression, in endometriotic stromal cells, the presence of SF-1 expression competes with these two transcription factors to induce steroidogenic gene transcription (4, 95). Experiments in which prostaglandin E_2 (PGE_2) was overexpressed in vitro demonstrated that de novo steroidogenesis in endometriotic tissues is regulated by the PGE_2-cAMP-SF-1 pathway, with driving estradiol production (4). Moreover, Tian et al. (267) showed the abundance of SF-1 and its target gene STAR to be correlated with the severity of endometriosis.

In terms of mechanisms, it has been shown that the SF-1 gene is differentially methylated in the endometrium of women with endometriosis, compared with those not afflicted with the disease (114). The hypothesis that SF-1 is a determinant factor in endometriosis has been supported by the fact that, in nonpathological conditions, the SF-1 promoter and exon I region of endometrial stromal cells shows a dense methylation pattern, epigenetically silencing SF-1 expression (301). When aberrant demethylation of the SF-1 promoter occurs, as it is observed in endometriosis, expression of SF-1 is upregulated (301). In turn, this overexpression of SF-1 causes an increase in steroidogenesis, favoring inflammation and growth of ectopic endometrial tissue. The mouse model described above with conditional uterine overexpression of SF-1, where mice showed enlarged, aberrant endometrial glands and activated immune response, provides experimental support for a role of this transcription factor in endometriosis (275).

C. Cancer

Accumulating evidence indicates that LRH-1 participates in the pathogenesis of tumors of multiple sorts (reviewed in Ref. 198) including pancreatic (11, 216), breast (266), gastric (284), and colon cancer (239). Suppression of LRH-1 in colon cancer (10) or osteosarcoma cells (165) inhibits but does not eliminate proliferation. Recent studies showed that, in some pancreatic cancer cell lines, higher LRH-1 mRNA levels were present compared with normal pancreatic ductal epithelium cells (11). Overexpression of LRH-1 in these pancreatic cancer cell lines is characterized by a phenotype of increased cell proliferation, via upregulation of genes, including cyclins D and E that regulate cell cycle and c-Myc, a protooncogene that controls generation of self-renewing metastatic cancer cells (121). Also upregulated are the metalloproteinases MMP2 and MMP9, implicated in metastasis and known to facilitate tumor growth, cell migration, and tumor invasion (93). Moreover, LRH-1 can promote pancreatic cancer metastasis (11, 169) and promotes intestinal tumor proliferation in gastrointestinal tumors by activating the Wnt/β-catenin pathway (10, 284). LRH-1 also contributes to intestinal tumor formation via its interaction with β-catenin/TCF4, known to induce cyclins D_1/E_1, which in turn regulate the cell entry in the G_1/S phase of the cell cycle and their subsequent proliferation (23). LRH-1 has been shown to drive colon cancer cell growth by repressing the expression of CDKN1A in a p53-dependent manner (143), the same mechanism that is involved in LRH-1 regulation of breast cancer (see below). Overall, LRH-1 acts in the initiation of intestinal tumor formation through effects on the cell cycle and through impact on inflammatory pathways.

Estrogens have been shown to promote proliferation of malignant ovarian cancer cell lines, while progesterone inhibits proliferation and promotes apoptosis of these cells (175).

Ovarian cancer researchers have identified SF-1 as a potential repressor of cancer cell proliferation, due to its crucial role in progesterone biosynthesis. Studies have demonstrated that transient expression of SF-1 in certain human ovarian cancer cell lines inhibits estrogen-induced proliferation and promotes apoptosis (225). While SF-1 gene expression is observed in human epithelial and metastatic tumors of the ovary, these adenoma and carcinoma cells do not present SF-1 immunoreactivity (236). Conversely, clinical studies have suggested that ovarian tumors with functioning stroma secrete estradiol via the regulation of aromatase (CYP19), due to overexpression of SF-1 (103). A recent extensive meta-analysis of a wide range of ovarian cancer clinical studies was aimed at determining whether SF-1 is associated with ovarian tumor progression (115). The results demonstrated that SF-1 expression level is significantly lower in ovarian cancer than in normal ovarian tissues, perhaps due to epigenetic silencing via increased methylation of the SF-1 gene (186). A further conclusion was that SF-1 expression pattern could serve as a marker to differentiate ovarian sex cord stromal tumors, where SF-1 is higher, from ovarian cancer, where it is markedly lower. A similar observation was made in granulosa cell tumors, where both SF-1 and LRH-1 expression was increased when compared with normal ovarian tissues as well as, in the case of SF-1 only, cells from other types of ovarian cancer (mucinous and serous) (42). This study also demonstrated that in the granulosa cell tumor-like in vitro cell line (KGN), SF-1 binds preferentially to the aromatase promoter II region relative to LRH-1, indicating that SF-1 is driving aromatase expression in this type of ovarian cancer (42). LRH-1 action in this context remains unknown, possibly functioning to activate proliferation. Similar results were observed in both human and rat testicular Leydig cell tumors, where elevated levels of aromatase mRNA were linked to increased SF-1 expression (257).

These clinical and cellular cancer studies demonstrate that both SF-1 and LRH-1 play different roles in ovarian cancer, depending on the type of tumor, or even the cell type of origin of the tumor.

Finally, there is considerable evidence implicating LRH-1 involvement in breast cancer (84). In the human breast, LRH-1 is expressed in the stromal compartment and in undifferentiated adipose tissue where it regulates aromatase expression and promotes estrogen biosynthesis (84). Its expression promotes proliferation, migration, and invasion of breast cancer cells in vitro (154). LRH-1 expression is regulated by estrogen, and its mRNA transcript levels have been proven to be higher in ESR1-positive but more stable in ERα-negative cells (154). In ERα-positive breast cancer cells, LRH-1 promotes cell proliferation by increasing estrogen biosynthesis by regulating aromatase expression (266) and by ESR1-mediated transcription of target genes such as *GREB1* (43). Estrogen-dependent breast cancer is often treated with aromatase inhibitors or estrogen receptor agonists such as tamoxifen. Altered expression and functions of microRNAs have been reportedly associated with tamoxifen resistance (326). The microRNA miR-027b-3p directly targets and inhibits the expression of LRH-1 and CREB1, and its levels were found to be significantly negatively correlated with LRH-1 and CREB1 levels in breast cancer tissues (326). As further evidence of a role for LRH-1 in mammary carcinogenesis, LRH-1 levels have been shown to be particularly elevated in chemoresistant breast cancer tissues from patients after recurrent chemotherapy (283). LRH-1 promotes breast cancer cell resistance to chemotherapy by upregulating the checkpoint protein MDC1 to enhance DNA damage repair (283). Furthermore, independent of its interactions with estrogen, LRH-1 overexpression was observed to promote remodeling of the actin cytoskeleton and E-cadherin cleavage, contributing to increase migration and invasion of the cancerous cells (41). Analysis of breast cancer samples by Bianco et al. (17) also revealed that LRH-1 regulates cell proliferation by inhibiting CDKN1A expression, thus removing an endogenous brake on proliferation. As above, this mechanism occurs independent of the proliferation involving ESR1 or p53. High levels of LRH-1 are also associated with poor breast cancer prognosis (16, 17). Given its important role in the progression of both estrogen-positive and -negative cancers, and its involvement in regulating hormone-independent pathways such as CDKN1A, it is clear that inhibition of LRH-1 could provide a powerful new approach for the treatment of endocrine-resistant breast cancer (16, 17). To our knowledge, no studies have shown SF-1 deregulation or involvement in breast cancer.

X. LRH-1/SF-1 INTERACTIONS

Given the structural similarities of SF-1 and LRH-1, along with their demonstrated ability to bind to the same DNA sequences, one would expect that they could reciprocally compensate for one another. In a number of tissues, particularly those in the digestive system, compensation does not occur because of the lack of significant overlapping expression (199). Other factors may be in play, due to differences in structure between the two nuclear receptors that have been shown to exist, including variation in helix length (144), which could result in differential responses to endogenous agonists. There are species differences as well, particularly in the size and primary structure of the ligand-binding pocket, such that the mouse appears to have undergone a radical reduction in the capacity to bind ligands by both SF-1 and LRH-1 (144). In addition, there is some evidence that the two nuclear receptors are differentially sensitive to the inhibitory effects of DAX1, and this cofactor is more potent in repressing the transactivation of steroid synthetic enzymes by LRH-1 than by SF-1 (33).

LRH-1 and SF-1 are coexpressed in the ovary at all stages of the estrous cycle (106). The ovary-specific models of deple-

tion targeting the follicular granulosa cells by means of the Amhr2 Cre driver argue that compensation of LRH-1 for SF-1 depletion, or conversely, SF-1 for LRH-1, does not occur (68). Indeed, there are only modest increases in mRNA for LRH-1 in the ovaries of the Amhr2/SF-1 granulosa specific knockout mouse, in both the gonadotropin stimulated and untreated conditions (214). As noted above, the Amhr2/SF-1 female mouse is infertile due to disruption of the follicle development trajectory (214), while in the Amhr2/LRH-1 ovary, large antral follicles that appear structurally normal are present, but these fail to ovulate (13, 68). These differences occur even though the two receptors regulate many of the same genes, particularly those associated with steroidogenesis, and in the same cells.

Both SF-1 and LRH-1 are present and active in the murine corpus luteum (106), but, as above, SF-1 cannot compensate for LRH-1 deficiency in this tissue (318). This may be due to the differential amounts that are recruited to promoters of the genes of luteal function in response to luteotropic stimuli, as reported by Weck et al. (287). By means of a chromatin immunoprecipitation technique, these authors showed that SF-1 binding to the *Inha* subunit promoter decreases and LRH-1 binding increases following activation of the cAMP signal that induces granulosa cell differentiation to luteal cells. One potential mechanism for this change is the relative expression (LRH-1 up, SF-1 down) of the two nuclear receptors following stimulation of the ovarian cells with gonadotropins (287). A further possibility is that stimulatory ligands may differentially regulate LRH-1 versus SF-1 transactivation by acting through different intracellular signaling mechanisms (287). The mechanisms of selective action clearly merit further investigation.

XI. SUMMARY, CONCLUSIONS, AND FUTURE PERSPECTIVES

The NR5A receptors are nuclear receptor proteins that act as transcription factors. They are evolutionarily conserved, as orthologs and paralogs are found in metazoans, from roundworms to mammals. The two mammalian forms, known as LRH-1 and SF-1, are common to reproductive tissues, but are also differentially expressed across a wide range of organs. While they interact with the same or highly similar DNA sequences, they have multiple, often nonoverlapping actions, and cannot compensate for each other. While a good deal is known about SF-1 and LRH-1, the interaction, synergy, or antagonism of the multiplex array of regulators, from cofactors to miRNA to epigenetic mechanisms, remains far from completely explained. Regulation by the two receptors appears to be cell and context specific, and the extent of commonality is unclear.

Much new information has emerged in recent years with respect to their differential roles in the regulation of fertility, derived primarily from conditional, cell-specific mutations in mice **(TABLE 3 AND FIGURE 6)**. Again, this information

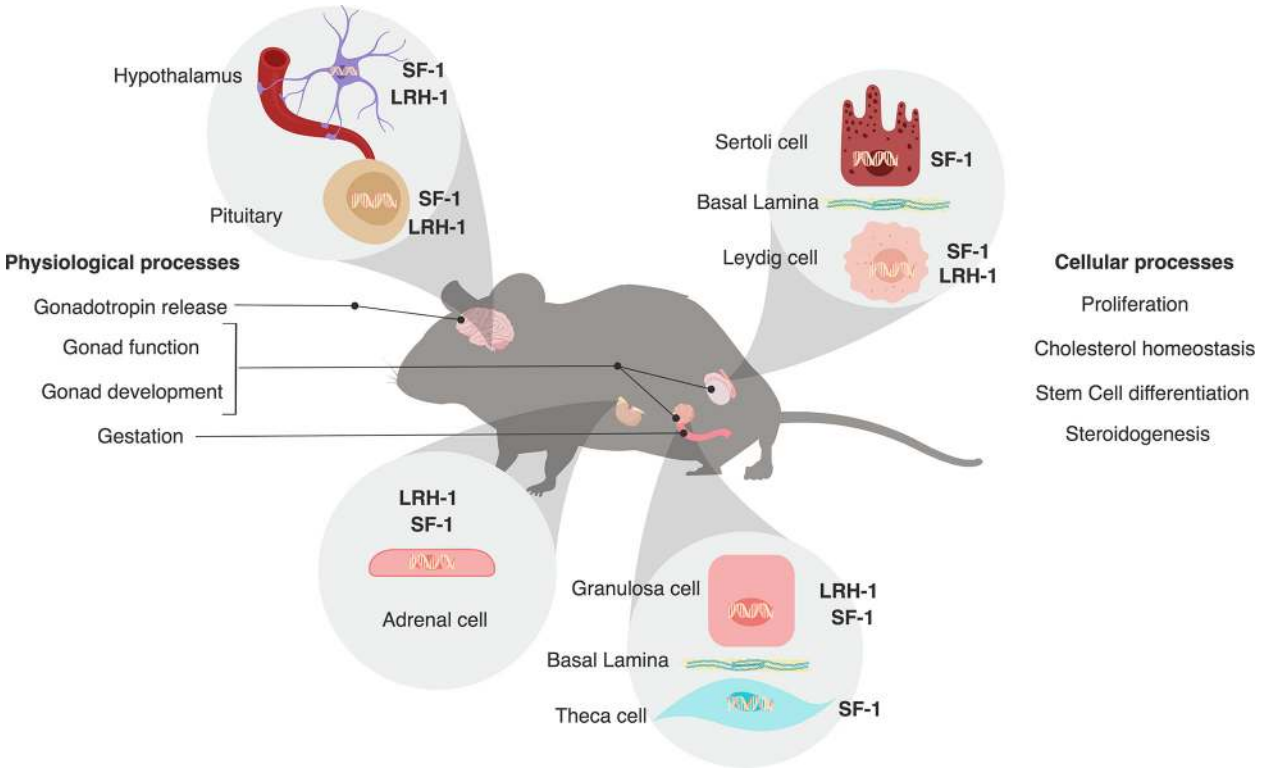

FIGURE 6. Summary of the essential cellular and physiological effects of the NR5A receptors liver receptor homolog-1 (LRH-1) and steroidogenic factor-1 (SF-1) in reproductive tissues using the mouse as a model.

demonstrates that SF-1 depletion in granulosa cells disrupts the follicle development process, while folliculogenesis proceeds to the large antral stage in LRH-1-depleted follicles. Most models of depletion of either of the nuclear receptors in granulosa cells are anovulatory, with the exception of the knockdown at the late peri-ovulatory state in the PgrCre/LRH-1 mouse. LRH-1 is an essential regulator of endometrial decidualization, while SF-1 appears to be present in endometriotic tissue. Both LRH-1 and SF-1 regulate steroid synthesis, but often in different tissues: LRH-1 in granulosa cells, SF-1 in theca and Leydig cells. In some tissues, such as the corpus luteum, LRH-1 and SF-1 are co-expressed and the extent of overlap in their targets is yet unknown.

It is enigmatic that two receptors that bind to the same DNA sequence in the same tissue can have disparate effects. Are there yet undiscovered endogenous ligands that selectively modulate their activity? Are the differential effects related to the dose of the receptors present in the cell? Both receptors are implicated in stem cell pluripotency, but in other circumstances, such as decidual conversion of endometrial stromal cells, LRH-1 is essential for terminal differentiation. LRH-1 appears to be a potent impetus for cell proliferation, but is not an absolute requirement, as embryos develop to a multicellular stage in germline deleted mice, and depletion in the ovary only partially compromises granulosa cell multiplication. These are among the many questions that need to be addressed in future research.

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