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Steroidogenic Factor-1 form and function: from phospholipids to physiology

Alexis N. Campbell[1,2,*], **Woong Jae Choi**[1,*], **Ethan S. Chi**[1,*], **Abigail R. Orun**[1,*], **James C. Poland**[1,*], **Elizabeth A. Stivison**[1,*], **Jakub N. Kubina**[1], **Kimora L. Hudson**[1], **Mong Na Claire Loi**[1], **Jay N. Bhatia**[1], **Joseph W. Gilligan**[1], **Adrian Quintana**[1], **Raymond D. Blind**[1,2,3,4]

[1]Department of Medicine, Division of Diabetes, Endocrinology and Metabolism, Vanderbilt University Medical Center, Nashville, TN 37232

[2]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232

[3]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232

Abstract

Steroidogenic Factor-1 (SF-1, *NR5A1*) is a member of the nuclear receptor superfamily of ligand-regulated transcription factors, consisting of a DNA-binding domain (DBD) connected to a transcriptional regulatory ligand binding domain (LBD) via an unstructured hinge domain. SF-1 is a master regulator of development and adult function along the hypothalamic pituitary adrenal and gonadal axes, with strong pathophysiological association with endometriosis and adrenocortical carcinoma. SF-1 was shown to bind and be regulated by phospholipids, one of the most interesting aspects of SF-1 regulation is the manner in which SF-1 interacts with phospholipids: SF-1 buries the phospholipid acyl chains deep in the hydrophobic core of the SF-1 protein, while the lipid headgroups remain solvent-exposed on the exterior of the SF-1 protein surface. Here, we have reviewed several aspects of SF-1 structure, function and physiology, touching on other transcription factors that help regulate SF-1 target genes, non-canonical functions of SF-1, the DNA-binding properties of SF-1, the use of mass spectrometry to identify lipids that associate with SF-1, how protein phosphorylation regulates SF-1 and the structural biology of the phospholipid-ligand binding domain. Together this review summarizes the form and function of Steroidogenic Factor-1 in physiology and in human disease, with particular emphasis on adrenal cancer.

Keywords

Ad4BP integrative structural biology of steroidogenesis and gene expression; R255L polymorphism; nuclear phosphoinositide function

[4]Correspondence: ray.blind@vanderbilt.edu.
[*]Equal Contributions

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1. A brief history of SF-1 physiology:

Steroidogenic factor 1 (SF-1) was first described in the early 1990s when researchers became aware that expression of many cytochrome P450s, which catalyze the addition of hydroxyl groups during the synthesis of steroids, were regulated by a single factor thought to be a master regulator of steroidogenesis[1–3]. This factor also was identified in cow, mouse, rat, fly, and human, and was shown to regulate expression of the cytochrome p450 genes[4–9] and act as a regulator of the hypothalamic-pituitary-gonadal axis[10–12]. SF-1 has since been shown to be expressed in a limited number of other organs including the pituitary, hypothalamus, adrenal gland, testis, ovaries, endometrium, skin and spleen, reviewed previously[13]. In mice, complete developmental genetic knockout of Sf-1 causes lethality by postnatal day 8 due to a lack of corticosteroids[14]. These animals lack adrenal glands and gonads in both male and female animals. Sf-1 knockout mice can be kept alive with perinatal corticosteroid injections and adrenal transplants, although Sf-1 knockout mice eventually develop an obese phenotype[15]. In male mice, loss of Sf-1 leads to persistent Mullerian structures and female external genitalia[16,17], reviewed by[18]. Conditional knockout models in mice have shown Sf-1 is required for ovulation and developing ovarian reserves[19,20]. So although SF-1 knockout leads to perinatal lethality, even complete loss of all alleles can be managed to permit a relatively low impact phenotype in mice, suggesting inhibitors of SF-1 would be well tolerated in human patients.

Several human polymorphisms in SF-1 associate with infertility and a wide range of differences of sex development in humans[21–25]. The causative nature of some of these polymorphisms is unclear due to the presence of other polymorphisms in the affected patients[26]. In addition to classic loss-of-function polymorphisms which decrease SF-1 function, any improper expression of SF-1 that affects dosage also leads to physiological defects in mouse models and human patients. Indeed, SF-1 gene dosage is a critical aspect of SF-1 pathophysiology and normal function, with SF-1 appropriately coined a “Goldilocks” transcription factor in a recent review from Enzo Lalli’s lab[27]. Along those lines, mouse models in which SF-1 functions have been altered by mutation of post-translational modification sites cause an adrenal-like gene expression pattern in the gonads, and a gonad-like gene expression pattern in the adrenals of these knock-in mice[28]. The physiological data suggest SF-1 control of gene expression patterns under physiological conditions is fluid, and represents more of an equilibrium between states, rather than discreet on/off transcriptional regulation particularly sensitive to SF-1 dosage, in both human patients and mouse models.

Silencing of SF-1 in the endometrium is required for maintenance of pregnancy, and aberrant expression of SF-1 has been associated with endometriosis although the mechanism is less than clear[20,29–34]. Beyond reproduction, improper levels of SF-1 have been found in cancers, including SF-1 upregulation in adrenal cancer, specifically in adrenocortical carcinoma, where inhibition of SF-1 slowed the proliferation of these cancer cells[35,36]. In ovarian cancers there is some evidence that overexpressing SF-1 in ovarian cancer cell lines inhibits proliferation of the cells[37]. Together, the data suggest links between SF-1 and adrenocortical cancer[38] and endometriosis in adults[39], however full SF-1 function appears to

be somewhat dispensable in development, as suggested by mouse knockout studies and the identification of human polymorphisms with loss of SF-1 function.

2. The Brain and SF-1:

SF-1 is expressed in the ventromedial hypothalamus (VMH): a brain region integral for neuroendocrine homeostasis, notably modulating glucose sensitivity, metabolic regulation, appetite, and thermogenesis[40,41]. While the role of Sf-1 across VMH pathologies is not yet fully understood, Sf-1 expression is essential for differentiation of the VMH[42]. Knockout of Sf-1 in mice promoted development of metabolic disorders such as diabetes and obesity under high fat diet compared to wild type animals[43,44]. Many pathologies associated with Sf-1 deletion had been thought to derive from the loss of Sf-1 associated leptin receptors[45] and decreased glutaminergic excitation of POMC and AgRP neurons, which have been implicated in the regulation of satiety[46,47]. However, recent data suggests overeating may not be the only factor in metabolic imbalance associated with Sf-1 dysfunction. Instead, Sf-1 knockout models display increased insulin resistance, decreased blood glucose sensitivity, impairment in free fatty acid mobilization and decreased energy utilization leading to increased adiposity[42,48]. Further, Sf-1 expressing neurons in the VMH have recently been shown to control inflammation in fat depots associated with high fat diet-induced obesity[49].

Sf-1 knockout mice also show impairment in weight, muscle development, protein turnover and metabolite recovery post-exercise as compared to wild type animals[48]. Moreover, studies have shown that there is a correlation between Sf-1 neuronal activity and the leptin-PI3K-FoxO1 pathway in regulation of hypoglycemia in peripheral tissues[50,51]. Finally, optogenetic activation of Sf-1 neurons reduced food intake and increased energy usage, glucose regulation and insulin sensitivity[52,53]. Overall, SF-1 expression in the brain permits development of the VMH and allows the VMH to regulate metabolic processes. Further research is necessary to determine the molecular mechanisms of VMH and brain SF-1 activation by regulatory ligands and other brain-specific factors.

3. Adrenal cancer and SF-1:

Outside the metabolic physiopathology in the brain, adrenocortical cancer has similarly been implicated in SF-1 function. The gene dosage of SF-1 is important in all aspects of SF-1 ability to regulate transcription and physiology, and regulation of the adrenal glands is no exception. SF-1 overexpression in mice increased development and proliferation of adult and pediatric adrenocortical tumors[54] and adrenocortical cancers have previously been linked to SF-1 overexpression[55,56]. Heterozygous SF-1 mice with a haploinsufficient decrease in SF-1 expression manifest with adrenal hypoplasia and decreased stress response from the adrenal glands[57,58]. These Sf-1 heterozygote +/− mice have smaller adrenals, but those smaller adrenals have increased steroid production per cell, suggesting a decoupling of SF-1 gene dosage affects in adult endocrine function of the adrenals *vs.* the developmental aspects of adrenal growth that are driven by SF-1 expression[59]. SF-1 knockdown and overexpression in a human adrenocortical carcinoma cell line (NCI-H295R) shows SF-1 dosage-dependent chromatin-binding site occupancy nearly triples during SF-1 overexpression cells[54]. SF-1 upregulation has been found in adrenal cancer, specifically adrenocortical carcinoma, and

further chemical inhibition of SF-1 slowed the proliferation of these cancer cells[35,36]. SF-1 has also been shown to control proper centrosome amplification in an adrenal cell line, a process that when dysregulated has well-established links to cancer[60,61].

SF-1 also associates with several factors that are known to regulate adrenal development and/or oncogenesis. In resected human adrenocortical tumors (both adenomas and carcinomas), expression of SF-1 was linked with expression of the GATA-6 transcription factor, and GATA6 expression correlated with expression of the classic SF-1 target gene *CYP17A1*, suggesting a functional link between GATA6 and SF-1[62]. Adrenocortical H295R cells show negative crosstalk between transforming growth factor beta (TGF-beta) and SF-1, as well as between Wnt/Beta-catenin and SF-1 in these human adrenal cancer cells[63], further linking SF-1 expression with activated adrenal cancer cell growth in this cell line. These same human adrenocortical H295R cells when overexpressing SF-1 can also change the cadre of transcriptional coregulators that associate with SF-1, specifically with the powerful transcriptional coregulator NRSF/REST coregulator[54], which has been previously shown to effect classic SF-1 target genes and steroidogenesis in these cells[64]. Together, the data suggest that SF-1 expression can drive growth adrenocortical carcinoma, with the clear implication that an SF-1 inhibitor could have therapeutic value in treating this rare form of cancer[65]. Further, the manageable side effects even from developmental loss of the entire Sf-1 gene in mice[14,42] suggests inhibitors of SF-1 in human adrenocortical carcinoma patients would be well tolerated[55].

3. Transcription Factors that collaborate with SF-1:

SF-1 collaborates with other transcription factors to regulate target gene expression, full regulation of SF-1 target genes usually requires a diversity of transcription factors, each with their own *cis* regulatory elements in the promoters of target loci. The final transcriptional output from these loci is the sum product of all these transcription factors, such as transcriptional regulation of SF-1 target gene *AMH*, which encodes Anti-Müllerian hormone (AMH; Müllerian inhibiting substance or MIS). The AMH gene product is necessary for normal male sex differentiation through Müllerian duct regression[66] and fully regulated expression of this locus is dependent on SF-1 collaboration with several other transcription factors known to regulate this locus, such as SOX-9[67], Wilm's Tumor protein (WT-1)[68], and GATA-4[69]. Although *AMH* is a well-validated target gene of SF-1[67], robust transcriptional activity is not achieved by SF-1 alone, but rather other transcription factors are required at the AMH locus for full transcriptional regulation. Another classic SF-1 target gene is *CYP11A1*, which encodes cytochrome P450 side-chain cleavage enzyme, a mitochondrial enzyme responsible for catalyzing the first and rate-limiting step in steroidogenesis[70]. The *CYP11A1* locus is regulated by several transcription factors including SF-1[3], c-JUN[71], SP1[72,73], and CBP/p300[74] in gonadal and adrenocortical cells. These transcription factors all collaborate to enhance gene regulation at this locus, causing changes of up to nearly ~20-fold[68]. Thus, the diversity of transcription factors at SF-1 target genes reflects a basic principle in metazoan transcriptional regulation, that many transcription factors often collaborate to regulate a target promoter.

4. DNA binding properties of SF-1:

SF-1 binds DNA as a monomer to regulate the expression of many genes in tissues along the hypothalamic-pituitary adrenal/gonadal (HPA/HPG) axes in humans[75,76]. The SF-1 DNA-binding domain (DBD) has a bipartite structure containing a highly conserved nuclear receptor core DBD and a carboxy-terminal extension, but SF-1 also has an FTZ-F1-like box[77] that is unique to the NR5A subclass of nuclear receptors[76]. The SF-1 nuclear receptor DBD core (human amino acids 10 to 76) contains the typical and highly conserved nuclear receptor DBD structure containing two zinc atoms, each chelated by four cysteines[75,76,78]. The C-terminal extension is shared by the monomeric nuclear receptor proteins, where the C-terminal extension makes base-specific interactions in the DNA minor groove[76,79]. This C-terminal extension structure is shared with other nuclear receptors nerve growth factor-induced-B (*NR4A2*/NGFI-B)[80,81], estrogen-related receptor 2 (*NR3B2*/ERR2)[82], and the close SF-1 homolog liver receptor homolog-1 (*NR5A2*/LRH-1)[79,83]. Proximal to the SF-1 C-terminal extension is the 33-residue FTZ-F1 box (human amino acids 79 to 111), which is unique to the SF-1 sub-class of nuclear receptors[76]. The FTZ-F1 box is highly conserved across species within the NR5A nuclear receptor sub-class, with the drosophila FTZ-F1 box having 82% sequence identity to the human, and the mouse FTZ-F1 box having 100% sequence identity with the human SF-1 FTZ-F1 box[76].

As part of an NMR-based structural analysis of the SF-1 DNA-binding domain, Little and colleagues found that several single point mutations within the SF-1 FTZ-F1 box (R87A; R92A; Y99A and Y99F) and one double mutation (R101P/D102P) decreased SF-1 transcriptional on an SF-1 target promoter (Inhibin alpha) luciferase reporter, without affecting SF-1 expression levels[76]. However other single point mutations in the FTZ-F1 region had no effect on SF-1 transcriptional activity (L80K; R89A; M98A) in the same luciferase reporter assay[76]. Perhaps as expected, all tested mutations in the FTZ-F1 region (Y99F and Y99A single point mutations and the R101P/D102P double mutation) decreased DNA-binding to a canonical SF-1 DNA-binding site oligo by EMSA, and almost completely eliminated DNA-binding to an atypical SF-1 DNA-binding site oligo by EMSA[76]. This decrease in DNA-interaction induced by point mutations in the FTZ-F1 region of SF-1 is slightly different compared to the close homolog LRH-1, as Solomon et. al. found that a triple point mutant within the FTZ-F1 helix of LRH-1 (Y92A/F186A/Y178A) did *not* affect LRH-1 DBD binding to DNA by EMSA, yet dramatically decreased LRH-1 transcriptional activity to background levels[79]. This phenotype is consistent with the FTX-F1 region communicating with the LRH-1 ligand binding domain, as suggested by the proposed full-length model of LRH-1[84], however no full-length model of SF-1 (including the alpha-fold model) has been rigorously tested in the wet lab. Thus, the data suggest mutations in the FTZ-F1 region have non-identical effects on SF-1 *vs.* LRH-1, as well as different effects on SF-1 DNA-binding to higher-affinity *vs.* lower affinity DNA sequences. The critical FTZ-F1 region is a common feature to the NR5A subclass of nuclear receptors[76,79,83,85] and elicits specific DNA-binding at a 9-nt recognition element, 5'-YCAAGGYCR-3' [Y=T/C; R=G/A][86], yet mutation of the FTZ-F1 has different effects in different NR5A nuclear receptors.

The structure of full-length SF-1 containing the DBD, hinge and LBD has yet to be elucidated, so any structural relationships between the DBD and LBD have yet to be

resolved. This is of particular interest as a polymorphism identified in a human patient within the LBD of SF-1 (R255L) was shown to dramatically decrease the ability of full-length SF-1 to interact with DNA oligos in electromobility shift assays[87], suggesting the R255L mutation in the LBD somehow affects the ability of SF-1 to bind DNA. Other studies have shown the R255L mutation can also alter the ability of SF-1 to bind phosphoinositide lipids while decreasing SF-1 transcriptional activity[88]. Mechanistically, it is therefore unclear if R255 in the LBD might directly mediate an interdomain interaction between the LBD and DBD to affect DNA-binding, or if R255 might participate in phospholipid ligand discrimination by SF-1, which in turn regulates SF-1 DNA-binding properties. Regardless of how R255 is translating structural information from the LBD to the DBD, the data indeed suggest that changes in LBD can be translated to the DBD within the context of the full-length SF-1. A structure of full-length SF-1 will be incredibly valuable in determining how the human R255L polymorphism in the LBD regulates SF-1 DNA-binding properties, and more generally how any potential DBD-LBD interactions might affect the overall activity of SF-1.

5. Structural biology of the SF-1 ligand binding domain:

Full-length SF-1 consists of the classic structural composition of a nuclear receptor: a DNA binding domain (DBD) with two zinc fingers, a flexible hinge region and a C-terminal activation function region (AF-2) that is part of the ligand-regulated transcriptional activation domain, called the ligand binding domain (LBD). There have been several crystal structures solved of the SF-1 LBD (see Table 1). The structure of the LBD is comprised of 12α helices distributed in 3 layers with an atypical 4th layer in the NR5A nuclear receptors SF-1 and LRH-1 formed by Helix 2[89]. The LBD also encompasses the AF-2 domain which is located at the c-terminal. Helix 12 largely accounts for AF-2 function as Helix 12 facilitates the formation of a surface which interacts with co-regulatory proteins by the nuclear receptor conserved Helix 12, α helical motif within the AF-2 region[90–93]. The final element of the LBD is the hydrophobic cavity known as the lipid-binding pocket (LBP). The SF-1 LBP has an elliptical shape and an opening at the bottom of the pocket with an area of around 100 Å^2 which is surrounded by the ends of helices H3, H6, H7, and H10.

While the ligand binding pocket cavity is largely hydrophobic, a hydrophilic patch exists near the entrance of the pocket[94]. Interestingly, the binding pocket of SF-1 is significantly larger than its close homolog Liver Receptor Homolog-1 (LRH-1, *NR5A2*), mouse SF-1 has a volume of 1640Å^3 compared to only 800Å^3 for the mouse LRH-1, and this trend was further seen in human SF-1 and human LRH-1[89,94]. The biological implications of the differing size of the ligand binding pockets in NR5A nuclear receptors have not been firmly established[95], however the simplest hypothesis is that SF-1 may bind endogenous ligands that are in some way larger than ligands for LRH-1. Crystallographic studies have shown that diverse bacterial phospholipids, aquired from the ectopic *E. coli* expression systems used to express SF-1, bind inside the canonical ligand binding pocket[89,96]. The data all suggest phospholipids are dynamically exchangeable regulatory ligands for SF-1, however it is unclear which of the many phospholipids that bind SF-1 is the most biologically relevant.

One class of SF-1 ligands that are of particularly high biological interest are the phosphoinositides since these lipids have been studied within the nuclear compartment for several decades[97–104] and have very potent signaling properties[105]. Direct binding analyses have suggested the phosphoinositides PI(4,5)P2 and PI(3,4,5)P3 bind to SF-1[89], confirmed later by co-crystal structures of several phosphoinositides with SF-1[106,107]. These structures suggest the phosphatidylinositol lipids take advantage of the amphipathic nature of the canonical ligand biding site, as the hydrophobic acyl chains of the ligand are sequestered from water deep within the lipid-binding pocket, and the hydrophilic headgroup is located at the water-exposed entrance to the ligand binding pocket[106]. There is evidence that PI(4,5)P2 associates with full-length SF-1 ectopically expressed in human HEK cells, as a PI(4,5)P2-specific kinase is able to incorporate radiolabel into immunoprecipitates of wild-type SF-1, but not a ligand-binding mutant of SF-1[108]. Further, displacement of all lipids from these SF-1 immunoprecipitates with a chemical competitor of PIP2 called RJW100[109,110] also prevented radiolabel incorporation by the PIP2-kinase[108]. These data suggest PIP2 associates with SF-1 at the canonical ligand binding site of SF-1, in a human cell line. More recent studies also suggest phosphoinositides functionally regulate the isolated ligand binding domain of SF-1, as changes to the acyl chain composition of PIP3 can alter the recruitment capabilities of SF-1 for coregulatory proteins[95,106,107]. It remains unclear if ligands might regulate the structure of full-length SF-1 in a way that could alter interactions that might occur between the ligand binding domain and other domains in SF-1, as has been suggested to occur with the close SF-1 homolog LRH-1[84]. More studies will be needed to evaluate how phospholipid ligands or other lipid molecules might affect regulation of SF-1 functions in transcription.

6. Protein phosphorylation of SF-1:

Like many cellular proteins, SF-1 likely exists as a phosphorylated protein in cells (see Table 2). Phosphorylation can be detected when SF-1 is immunoprecipitated from adrenocortical cells[111]. More specifically, S203, a residue located in the hinge region of SF-1, has been shown to be phosphorylated by MAPK[112]. Mutating the serine residue to an alanine residue (S203A) leads to reduced ability to transactivate luciferase reporters. Structurally, phosphorylation of S203 has been shown to dramatically stabilize of SF-1 LBD[113], as demonstrated by chymotrypsin-based protease protection assay akin to other nuclear receptor LBDs undergoing similar protection from proteases upon binding activating ligand[114]. Mechanistically, SF-1 recruitment of GRIP-1, a coactivator of SF-1, is enhanced upon the phosphorylation of Serine 203[112]. The functional importance of phosphorylation of Serine 203 was suggested in studies where the S203A mutation leads to reduction of SF-1 dependent transcription of *StAR*, but not the high-density lipoprotein receptor (*HDLR*) gene, suggesting that S203 phosphorylation might selectively regulate SF-1 to induce context-dependent transcriptional regulation[115]. Serine 203 is phosphorylated by MAPK can also be phosphorylated by CDK7; an activity that has been confirmed *in vitro*[116]. The authors also report that a mutation within ligand binding pocket that prevents phospholipid ligand binding leads to decreased levels of Serine 203 phosphorylation by CDK7, suggesting a complex interplay between SF-1 phosphorylation and ligand binding.

In addition to the clear role of S203 phosphorylation in SF-1 function, there is evidence that other modes of phosphorylation may regulate SF-1. PKA has been shown to be able to phosphorylate the rat SF-1 DBD, which decreased SF-1 DBD DNA binding *in vitro*[117] however the exact residue that is phosphorylated has not yet been identified. Mutating a putative PKA phosphorylation site did not lead to a change in transcriptional activity of SF-1 relative to wild-type SF-1 in a luciferase reporter assay[115,118]. Together, the data suggest that SF-1 is a phosphoprotein, and that phosphorylation, particularly of S203, regulates SF-1 functions.

7. Non-canonical function of SF-1:

While nuclear receptors have traditionally been associated with regulation of transcription and gene expression, non-genomic functions have also been defined. These non-genomic actions usually can be detected within seconds to minutes, in contrast with transcriptional responses that often require dozens of minutes to hours for a detectable response[119,120]. Many non-genomic functions of nuclear receptors take place outside the nucleus, in the cytoplasm, at the plasma membrane, or within intracellular organelles[121]. SF-1 has been observed localized at the centrosome, where SF-1 was surprisingly shown to be required for maintaining centrosome homeostasis[122], which is a clear example of one non-canonical function of SF-1, independent of SF-1 gene regulatory activity.

During mitosis, centrosomes function as the microtubule organizing centers and form the bipolar spindles that facilitate the equal division of chromosomes[123]. Like chromosomes, centrosome division only occurs once in each cell cycle[124]. Correct division and amounts of centrosomes are required for proper cell growth and genetic stability, while centrosome amplification leads to aberrant mitosis[60,125,126]. SF-1 maintains correct centrosome counts in mouse adrenocortical Y1 cells by regulating the activity of DNA-dependent protein kinase (DNA-PK) in the centrosome[127]. The data suggest SF-1 interacts with DNA repair proteins Ku70 and Ku80, sequestering them from the catalytic subunit of DNA-PK (DNA-PKcs)[127]. Loss of SF-1 induces activation of DNA-PK, causing overactivation of Akt and CDK2/cyclin[127]. CDK2 phosphorylates proteins involved in the initiation of centrosome replication[128–130] and is associated with centrosome overduplication. Activation of DNA-PK also leads to the phosphorylation and inactivation of GSK3β, leading to accumulation of β-catenin and centriole splitting[131], itself an important step in centrosome amplification[132]. Overexpression of SF-1, however, does not appear to inhibit the duplication of centrosomes. Instead, SF-1 appears to block centrosome overduplication under challenged or stressed conditions[127]. This observation suggests SF-1 can prevent abnormal duplication of centrosomes without obviously regulating normal duplication, consistent with the function of other centrosomal proteins[133,134].

Depletion of SF-1 does associate with centrosome over-duplication, genomic instability, and decreased cell counts[122], and these defects were not only rescued by wild-type SF-1, but also by a transcriptionally inactive point mutant of SF-1 (G35E), was well as a truncated SF-1 lacking the DNA-binding domain[122,131]. These important rescue experiments suggest the activity of SF-1 in the centrosome is independent of SF-1 transcriptional regulation, and the ability of SF-1 to bind DNA directly. A centrosomal localization signal was also

identified and shown to be dependent on SF-1 amino acids 348–367[122]. Overall, these non-transcriptional actions of SF-1 describe a mechanism of centrosome amplification through DNA-PK that is independent from previously described mechanisms involving the DNA damage checkpoint. Although adrenocortical cancers have previously been linked to SF-1 overexpression[55,56], centrosome amplification has also been linked to cancers[60,61]. Thus, it remains possible that the link between SF-1 and adrenocortical cancers could also involve defects in centrosome biology.

8. Mass spectrometry to identify SF-1 ligands:

Mass spectrometry (MS) is an analytical tool used to detect and measure a large array of small molecules, including most species of lipids. It was thus mass spectrometry-based approaches that have been used to unequivocally chemically identify the electron density seen in crystal structures of SF-1[101,135]. Some studies have used lipidomic mass spectrometry to confirm the presence of particular phospholipids bound to the SF-1 ligand binding domain[88,89,94,96] while other studies have used mass spectrometry in *de novo*, discovery-based ligand identification. It was reported that 25-, 26-, or 27-hydroxycholesterol regulate SF-1 activity in living cells, and that deletion mutants of the SF-1 ligand binding domain did not respond to these ligands[136], in line with a feedback-regulatory role for oxysterol-based steroid metabolism controlled by SF-1. A great deal of work has now made it clear that oxysterols do not likely bind SF-1 within the canonical ligand binding pocket[137], although the cholesterol-based detergent CHAPS is required for efficient purification of the SF-1 ligand binding domain from bacterial cell lysates[88,106].

The first evidence that phospholipids serve as regulatory ligands for SF-1 came when bacterial phospholipids from the recombinant protein expression system used to express SF-1, fortuitously co-crystalized with SF-1, with 1:1 stoichiometry and clearly bound within the canonical nuclear receptor ligand binding pocket[89,94,96]. Again, mass spectrometry-based lipidomic analyses then unequivocally confirmed the chemical nature of the electron density observed in the crystal structures as bacterial phospholipids from the *E. coli* system used to express and purify SF-1[88,89,94,96]. In one of these studies, the mass spectrometry data suggested phosphatidylethanolamine (PE) with shorter C12:0 acyl chains associates with SF-1, however the phospholipid ligand in the co-crystal structure in that same study was identified as a palmitic ester of PE diC16:1[96], suggesting PEs with longer acyl chain lengths (14–18 carbons) and with various degrees of saturation would also fit into the SF-1 ligand binding pocket[96]. Krylova et al., demonstrated phosphatidic acid (PA) and phosphatidylinositol (3,4,5) triphosphate (PIP3) also bind SF-1, suggesting phosphatidylinositols as potential SF-1 ligands[89], later confirmed by studies in human cells (Blind et al., 2012) and in several x-ray co-crystal structures[106,107]. In another study, Yong et al., used electrospray ionization (ESI) and tandem MS/MS to identify PE C34:2 or C32:1 lipids associated with SF-1[94]. Later work from Marion Sewer and Al Merrill on full-length SF-1 purified from human adrenocortical cells suggested SF-1 binds to sphingosines and sphingomyelin using a combination of LC-ESI-MS/MS techniques and multiple reaction monitoring (MRM)[138,139], and further interaction with some of these sphingolipids was altered by cAMP signaling. Another study used lipid mass spectrometry to suggest PE species with 16:0/16:1, 16:1/16:1, and 18:1/16:1 were associated with the isolated SF-1

ligand binding domain expressed and purified from bacteria[107], with both structural and functional changes induced by PIP3 with 18:1/18:1 dioleoyl acyl chains[107], observations also in line with the C34:2 chain length reported by other groups for phospholipids bound to SF-1[94]. Although these studies show the extensive mass spectrometry used to identify lipid ligands that associate with SF-1, there have been no mass spectrometry studies identifying phosphoinositides in particular associated with SF-1, which require highly specialized protocols[140,141].

9. Conclusions and Future perspectives:

SF-1 has high potential therapeutic value in adrenocortical carcinoma, however without understanding the molecular details of how SF-1 is regulated by endogenous phospholipids, the development of any compounds that inhibit SF-1 becomes a far more difficult task. Thus, more high-resolution structural studies of SF-1 are needed to unlock the full therapeutic potential of SF-1, and to develop new therapies for adrenal cancer, a disease currently with no FDA approved targeted therapies. As reviewed here, the role of SF-1 in adrenal physiology is well established in both humans and mice, and mouse knockout models suggest that even developmental loss of SF-1 function does not lead to physiological consequences that cannot be successfully managed. The genetic studies therefore suggest that chemical inhibitors of SF-1 will be well tolerated, although that remains to be tested. Together, the connections between SF-1 and adrenal cancer are too important to ignore, and deserve further investigation.

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Table 1,

Crystal structures of the SF-1 ligand binding domain in the protein data bank:

PDB	Organism	Co-crystalized ligand	Co-crystalized LXXLLPeptide	Reference
4QJR	Homo Sapiens	PIP3 (PIZ)	PGC-1 alpha	106
4QK4	Homo Sapiens	PIP2 (PIK)	PGC-1 alpha	106
8DAF	Homo Sapiens	6N-10CA, Bacterial Phospholipid (PEF)	NCOA2	95
1YP0	Mus Musculus	Phosphatidylethanolamine (PEF)	SHP	94
1YOW	Homo Sapiens	Phosphatidylethanol (P0E)	TIF2	89
7KHT	Homo Sapiens	PIP3 (WES)	PGC-1 alpha	107
1ZDT	Homo Sapiens	Phosphatidylethanolamine (PEF)	NCOA2	96
1YMT	Mus Musculus	Phosphatidylglycerol (DR8)	NR0B2	89
3F7D	Mus Musculus	Phosphatidylcholine (P42)	PGC-1 alpha	88

Table 2,

SF-1 phosphorylation:

Description of SF-1 Phosphorylation Event	Comments	References
PKA phosphorylation of SF-1	PKA phosphorylates rat SF-1 DBD and decreased DNA binding[117], phosphorylation site not definitively identified.	117
Ser 203 by MAPK	Genetic evidence (S203A) that S203 phosphorylation is necessary for full SF-1 transcriptional activity.	112,113,115
Ser 203 by CDK7	*In vitro* phosphorylation of S203 by CDK, SF-1 ligand binding pocket mutant phosphorylated less efficiently by CDK7.	116