

SF1 in the Development of the Adrenal Gland and Gonads

Gokhan Ozisik^a John C. Achermann^b Joshua J. Meeks^a J. Larry Jameson^a^aDivision of Endocrinology, Metabolism and Molecular Medicine, Northwestern University, The Feinberg School of Medicine, Chicago, Ill., USA; and ^bCentre for Human Growth and Maturation, Department of Medicine and Institute of Child Health, University College London, London, UK

Key Words

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Abstract

SF1 (steroidogenic factor-1; NR5A1) is an orphan nuclear receptor that is expressed in the adrenal gland, gonads, spleen, ventromedial hypothalamus and pituitary gonadotroph cells. Combined approaches of targeted mutagenesis in mice and examination of the effects of naturally occurring mutations in humans have clarified the role of SF1 in steroidogenesis and development. Targeted disruption of *Sf1* (*Ftzf1*) in mice prevents gonadal and adrenal development and causes male-to-female sex reversal. A heterozygous loss-of-function human *SF1* mutation (G35E) was described in a patient with adrenal failure and complete 46,XY sex reversal, indicating that haploinsufficiency of this transcription factor is sufficient to cause a severe clinical phenotype. In an infant with a similar clinical phenotype, a homozygous *SF1* mutation (R92Q) was identified. In functional assays, this mutant SF1 protein exhibited partial loss of DNA binding and transcriptional activity when compared with the more severe G35E P-box mutant. These patients reveal the exquisite sensitivity of SF1-dependent developmental pathways to gene dosage and function in humans.

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Discovery and Structure of SF1

The existence of a common ‘steroidogenic factor’ had been proposed based on the identification of similar regulatory elements in the proximal promoter regions of the cytochrome P-450 steroid hydroxylase gene family [1]. Steroidogenic factor-1 (SF1) (also known as Ad4BP) was cloned from adrenal cDNA libraries in 1992 (fig. 1) [2]. The mouse gene encoding this protein was mapped to chromosome 2 and named *Ftzf1*, as it is structurally homologous to the *Drosophila* gene, *fushi tarazu* factor 1 (FTZF1) [3, 4]. The human homologue, *FTZF1/NR5A1* contains seven exons and has been mapped to chromosome 9q33 [5, 6].

SF1 (*FTZF1/NR5A1*) encodes a 461 amino acid protein that is structurally similar to other members of the nuclear receptor superfamily (fig. 2). Functional domains of SF1 include a zinc finger DNA binding domain, an A-box (or FTZF1 box), a hinge region and a transactivation (AF2) domain. The first zinc finger of SF1 contains a proximal box (P-box) which confers DNA sequence specificity [7, 8]. The A-box stabilizes DNA binding [9, 10] and probably accounts for the ability of SF1 to bind to DNA as a monomer, in contrast to many other nuclear receptors, which bind to DNA as homo- or heterodimers. The AF2 domain of SF1 is involved in transcriptional activation, reflecting the recruitment of various co-activator proteins [11].

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www.karger.com/hreDr. J. Larry Jameson
Department of Medicine, Northwestern Memorial Hospital
Galter Pavilion, Suite 3-150, 251 E. Huron Street
Chicago, IL 60611-2908 (USA)
Tel. +1 312 926 9436, Fax +1 312 926 7260, E-Mail ljameson@northwestern.edu

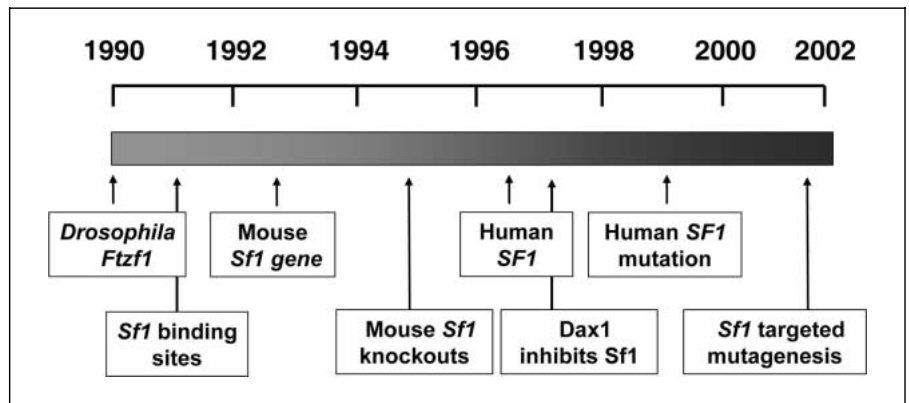


Fig. 1. Summary of selected key events that have helped to unravel the function of SF1.

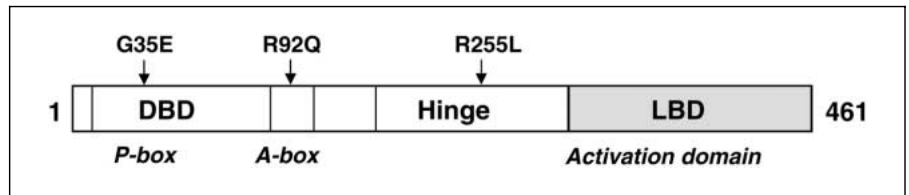


Fig. 2. Schematic representation of *SF1* depicting the locations of human mutations. DBD, DNA binding domain; LBD, ligand binding domain.

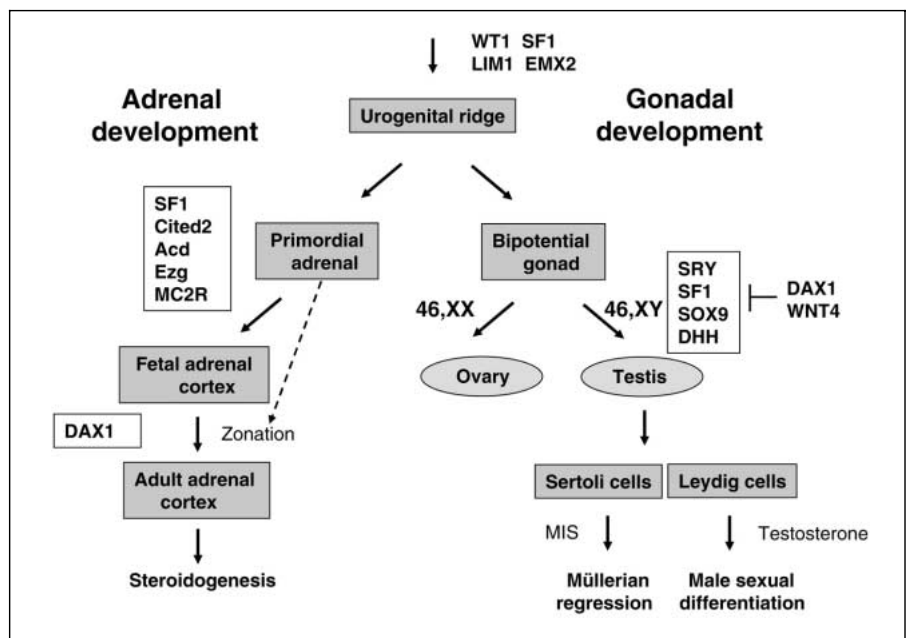


Fig. 3. Schematic illustration of adrenal and gonadal development from the urogenital ridge. Some of the genes involved in these processes are listed.

Pivotal Role of SF1 in Adrenal and Gonadal Development

The temporal and spatial expression of *SF1* is consistent with its critical role in adrenal development, steroidogenesis and gonadal differentiation (fig. 3). In the mouse, *Sf1* is first expressed in the urogenital ridge at embryonic day 9 (E9) [12]. It is subsequently localized to adrenal primordium (E11) and concentrated to adrenal cortical cells

(E13) [13]. A similar expression pattern is seen in humans [14, 15]. In the developing gonad, SF1 participates with several other transcription factors (WT1, DAX1, SRY and SOX9) to initiate patterning of the gonad as well as differentiation of the testis. In Sertoli cells, Sf1 regulates the expression of anti-Müllerian hormone, which leads to regression of the progenitors of the oviducts, uterus and upper vagina in males [16]. In Leydig cells, Sf1 regulates transcription of various enzymes involved in steroidogene-

Table 1. Examples of genes regulated by SF1

Adrenal gland and steroidogenesis
ACTH receptor
HDL receptor SR-B1
StAR
CYP11A1
3 β -hydroxysteroid dehydrogenase
Aromatase
Sexual differentiation
DAX1
AMH
AMH receptor
Insulin-like 3 gene product
Reproduction
GnRH receptor
Glycoprotein hormone α -subunit
LH β -subunit
Oxytocin
Inhibin α -subunit

sis and testosterone biosynthesis, allowing virilization of the male fetus. In the developing ovary, *Sf1* transcript levels fall during embryogenesis but are expressed in the granulosa and theca cells of the adult ovary at the onset of folliculogenesis [17]. Finally, Sf1 also plays an important role in the development of the ventromedial hypothalamus and pituitary gonadotrophs [18].

SF1 regulates the transcription of a vast array of genes involved in sex determination and differentiation, reproduction and steroidogenesis by binding to its cognate sites in their promoters (table 1). SF1 binds to DNA as a monomer and recognizes DNA binding sites that are variations on an extended estrogen receptor response element (PyCA AGGTCA). Once bound, transactivation of target genes by SF1 involves the recruitment of coactivators such as steroid receptor coactivator-1 (SRC1) [11], glucocorticoid receptor interacting protein (GRIP1) [19], CREB-binding protein (CBP)/p300 [20] or proline-rich nuclear receptor coregulatory protein (PNRC) [21]. It remains unclear whether a specific ligand modulates the activation of SF1. Although oxysterols were proposed to be SF1 ligands [22], subsequent experiments showed minimal effects on transcriptional activation [23]. Phosphorylation pathways have also been shown to modulate SF1-mediated transcription [19].

Several groups have performed targeted deletion of *Sf1* (*Ftzf1*) in mice [24–27]. Mice homozygous for the gene deletion ($-/-$) have complete adrenal and gonadal agene-

sis, male-to-female sex reversal and persistence of müllerian structures in males. Adrenal failure is apparent soon after birth. A virtual absence of the ventromedial hypothalamus occurs and there is decreased production of gonadotropins [27, 28]. However, these animals are able to respond to gonadotropin releasing hormone (GnRH) stimulation, suggesting that Sf1 deficiency does not result in an absolute loss of gonadotropin production from the anterior pituitary [28]. Furthermore, conditional knockout of *Sf1* in the pituitary has confirmed that the hypogonadotropic hypogonadism seen in these animals is also reversible with exogenous GnRH treatment [29].

Functional Interplay of SF1 and DAX1

SF1 is co-expressed with and stimulates the expression of another orphan nuclear receptor, DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia congenita, critical region on the X chromosome gene-1) [30–32]. In humans, mutations of *DAX1* cause adrenal hypoplasia, characterized by prolonged retention of the fetal adrenal and absent development of adult zone of the adrenal cortex [33, 34]. Targeted mutagenesis of *Dax1* (also known as *Ahch*) in mice causes a similar but less pronounced phenotype [35]. In mice, there is delayed regression of the X-zone, which may be homologous to the fetal zone, but the function of the mature adrenal cortex is apparently normal. *DAX1* mutations also cause reproductive defects that include hypogonadotropic hypogonadism and disordered formation of seminiferous tubules with ectopic Sertoli and Leydig cells, leading to impaired spermatogenesis [36, 37]. DAX1 inhibits SF1 transcription by interacting directly with SF1 and recruiting transcriptional co-repressors [38–40]. This observation, combined with the adrenal phenotype, led Babu et al. [41] to test whether the adrenal phenotype in Sf1 knockout heterozygotes [42] might be ameliorated on a background of *Dax1* deficiency. They observed that the absence of *Dax1* increased adrenal weight and function in *Sf1* heterozygous mice. These findings illustrate the interaction of these factors in vivo and underscore the fact that adrenal gland development is modulated by the interplay of multiple genes and pathways.

Phenotypic Effects of Human *SF1* Mutations

A human *SF1* mutation was first identified in a patient with primary adrenal failure, XY sex reversal and persistent Müllerian structures [43]. This phenotypically female

patient exhibited signs of primary adrenal insufficiency during the first 2 weeks of life. Laparotomy revealed normal Müllerian structures and streak-like gonads containing poorly differentiated seminiferous tubules and connective tissue. Mutation analysis revealed a de novo heterozygous G35E mutation within the P-box of the SF1 DNA binding domain (fig. 2). Functional studies showed that this mutation did not interfere with protein expression or nuclear localization. However, as predicted from the location of the mutation in the DNA binding domain, the mutant SF1 failed to bind or transactivate SF1 target genes (fig. 4). The mutant SF1 protein does not exhibit dominant negative activity and was able to partially activate several genes containing a 'perfect' SF1 binding site (e.g. *Cyp19*) [45]. Therefore, it is likely that SF1 acts in a dose-dependent manner. Since SF1 regulates so many genes involved in steroidogenesis, haploinsufficiency of SF1 could have a cumulative effect on multiple steps of steroid production and adrenal and gonadal development.

Recently, a second de novo heterozygous *SF1* mutation (R255L) was found in an XX female with adrenal insufficiency [46]. This mutation affects a conserved residue in the hinge region of SF1 (fig. 2). Although the mutation renders the molecule transcriptionally inactive, it does not appear to impair ovarian development.

A recessive (homozygous) *SF1* mutation has been identified in a baby born to consanguineous parents [44]. This autosomal recessive mutation affects the A-box region of SF1 that modulates monomeric DNA binding [45, 47]. In contrast to the P-box mutation, this A-box change (R92Q) is associated with a partial loss of function and

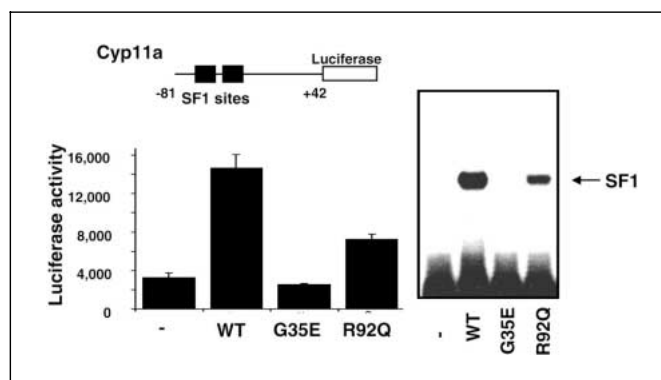


Fig. 4. DNA binding and transcriptional activity of human *SF1* mutations studied in vitro. A The R92Q A-box mutant shows impaired activation of a critical SF1 target gene, *Cyp11a* (P-450scc). B Reduced binding to a probe corresponding to the SF1 binding site (TCA AGGCTA) of this promoter. However, this loss of function was not as severe as that seen with the G35E P-box mutant. Reproduced with permission from Achermann et al. [46].

impaired binding to its response element (fig. 4). The surprising fact that the other family members are phenotypically normal despite having one mutant allele reveals the exquisite sensitivity of developmental pathways to gene dosage and residual function of SF1 in humans.

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References

- Rice DA, Mouw AR, Bogerd AM, Parker KL: A shared promoter element regulates the expression of three steroidogenic enzymes. *Mol Endocrinol* 1991;5:1552-1561.
- Lala DS, Rice DA, Parker KL: Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. *Mol Endocrinol* 1992;6:1249-1258.
- Swift S, Ashworth A: The mouse *Ftzf1* gene required for gonadal and adrenal development maps to mouse chromosome 2. *Genomics* 1995;28:609-610.
- Ueda H, Sonoda S, Brown JL, Scott MP, Wu C: A sequence-specific DNA-binding protein that activates fushi tarazu segmentation gene expression. *Genes Dev* 1990;4:624-635.
- Takeoto M, Parker KL, Howard TA, Tsukiyama T, Wong M, Niwa O, Morton CC, Miron PM, Seldin MF: Homologs of *Drosophila* fushi-tarazu factor 1 map to mouse chromosome 2 and human chromosome 9q33. *Genomics* 1995;25:565-567.
- Wong M, Ramayya MS, Chrousos GP, Driggers PH, Parker KL: Cloning and sequence analysis of the human gene encoding steroidogenic factor 1. *J Mol Endocrinol* 1996;17:139-147.
- Mader S, Kumar V, de Verneuil H, Chambon P: Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid-responsive element. *Nature* 1989;338:271-274.
- Umesono K, Evans RM: Determinants of target gene specificity for steroid/thyroid hormone receptors. *Cell* 1989;57:1139-1146.
- Ueda H, Sun GC, Murata T, Hirose S: A novel DNA-binding motif abuts the zinc finger domain of insect nuclear hormone receptor FTZ-F1 and mouse embryonal long terminal repeat-binding protein. *Mol Cell Biol* 1992;12:5667-5672.
- Wilson TE, Paulsen RE, Padgett KA, Milbrandt J: Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. *Science* 1992;256:107-110.
- Ito M, Yu RN, Jameson JL: Steroidogenic factor-1 contains a carboxy-terminal transcriptional activation domain that interacts with steroid receptor coactivator-1. *Mol Endocrinol* 1998;12:290-301.

- 12 Hatano O, Takayama K, Imai T, Waterman MR, Takakusu A, Omura T, Morohashi K: Sex-dependent expression of a transcription factor, Ad4BP, regulating steroidogenic P-450 genes in the gonads during prenatal and postnatal rat development. *Development* 1994;120:2787-2797.
- 13 Morohashi K, Iida H, Nomura M, Hatano O, Honda S, Tsukiyama T, Niwa O, Hara T, Takakusu A, Shibata Y et al: Functional difference between Ad4BP and ELP, and their distributions in steroidogenic tissues. *Mol Endocrinol* 1994;8:643-653.
- 14 Hanley NA, Ball SG, Clement-Jones M, Hagan DM, Strachan T, Lindsay S, Robson S, Ostrer H, Parker KL, Wilson DI: Expression of steroidogenic factor 1 and Wilms' tumour 1 during early human gonadal development and sex determination. *Mech Dev* 1999;87:175-180.
- 15 Ramayya MS, Zhou J, Kino T, Segars JH, Bondy CA, Chrousos GP: Steroidogenic factor 1 messenger ribonucleic acid expression in steroidogenic and nonsteroidogenic human tissues: Northern blot and in situ hybridization studies. *J Clin Endocrinol Metab* 1997;82:1799-1806.
- 16 Shen WH, Moore CC, Ikeda Y, Parker KL, Ingraham HA: Nuclear receptor steroidogenic factor 1 regulates the Müllerian inhibiting substance gene: a link to the sex determination cascade. *Cell* 1994;77:651-661.
- 17 Takayama K, Sasano H, Fukaya T, Morohashi K, Suzuki T, Tamura M, Costa MJ, Yajima A: Immunohistochemical localization of Ad4-binding protein with correlation to steroidogenic enzyme expression in cycling human ovaries and sex cord stromal tumors. *J Clin Endocrinol Metab* 1995;80:2815-2821.
- 18 Ingraham HA, Lala DS, Ikeda Y, Luo X, Shen WH, Nachtigal MW, Abbud R, Nilson JH, Parker KL: The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. *Genes Dev* 1994;8:2302-2312.
- 19 Hammer GD, Krylova I, Zhang Y, Darimont BD, Simpson K, Weigel NL, Ingraham HA: Phosphorylation of the nuclear receptor SF-1 modulates cofactor recruitment: integration of hormone signaling in reproduction and stress. *Mol Cell* 1999;3:521-526.
- 20 Monte D, DeWitte F, Hum DW: Regulation of the human P450sc gene by steroidogenic factor 1 is mediated by CBP/p300. *J Biol Chem* 1998;273:4585-4591.
- 21 Zhou D, Quach KM, Yang C, Lee SY, Pohajdak B, Chen S: PNRC: A proline-rich nuclear receptor coregulatory protein that modulates transcriptional activation of multiple nuclear receptors including orphan receptors SF1 (steroidogenic factor 1) and ERR α 1 (estrogen related receptor alpha-1). *Mol Endocrinol* 2000;14:986-998.
- 22 Lala DS, Syka PM, Lazarchik SB, Mangelsdorf DJ, Parker KL, Heyman RA: Activation of the orphan nuclear receptor steroidogenic factor 1 by oxysterols. *Proc Natl Acad Sci USA* 1997;94:4895-4900.
- 23 Mellon SH, Bair SR: 25-Hydroxycholesterol is not a ligand for the orphan nuclear receptor steroidogenic factor-1 (SF-1). *Endocrinology* 1998;139:3026-3029.
- 24 Luo X, Ikeda Y, Parker KL: A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 1994;77:481-490.
- 25 Luo X, Ikeda Y, Schlosser DA, Parker KL: Steroidogenic factor 1 is the essential transcript of the mouse Ftz-F1 gene. *Mol Endocrinol* 1995;9:1233-1239.
- 26 Sadovsky Y, Crawford PA, Woodson KG, Polish JA, Clements MA, Tourtellotte LM, Simburger K, Milbrandt J: Mice deficient in the orphan receptor steroidogenic factor 1 lack adrenal glands and gonads but express P450 side-chain-cleavage enzyme in the placenta and have normal embryonic serum levels of corticosteroids. *Proc Natl Acad Sci USA* 1995;92:10939-10943.
- 27 Shinoda K, Lei H, Yoshii H, Nomura M, Nagano M, Shiba H, Sasaki H, Osawa Y, Ninomiya Y, Niwa O et al: Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. *Dev Dyn* 1995;204:22-29.
- 28 Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL: The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. *Mol Endocrinol* 1995;9:478-486.
- 29 Zhao L, Bakke M, Krimkevich Y, Cushman LJ, Parlow AF, Camper SA, Parker KL: Steroidogenic factor 1 (SF1) is essential for pituitary gonadotrope function. *Development* 2001;128:147-154.
- 30 Ikeda Y, Swain A, Weber TJ, Hentges KE, Zalaria E, Lalli E, Tamai KT, Sassone-Corsi P, Lovell-Badge R, Camerino G, Parker KL: Steroidogenic factor 1 and Dax-1 colocalize in multiple cell lineages: potential links in endocrine development. *Mol Endocrinol* 1996;10:1261-1272.
- 31 Ikeda Y, Takeda Y, Shikayama T, Mukai T, Hisano S, Morohashi KI: Comparative localization of Dax-1 and Ad4BP/SF-1 during development of the hypothalamic-pituitary-gonadal axis suggests their closely related and distinct functions. *Dev Dyn* 2001;220:363-376.
- 32 Yu RN, Ito M, Jameson JL: The murine Dax-1 promoter is stimulated by SF-1 (steroidogenic factor-1) and inhibited by COUP-TF (chicken ovalbumin upstream promoter-transcription factor) via a composite nuclear receptor-regulatory element. *Mol Endocrinol* 1998;12:1010-1022.
- 33 Zalaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER et al: An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature* 1994;372:635-641.
- 34 Achermann JC, Meeks JJ, Jameson JL: Phenotypic spectrum of mutations in DAX-1 and SF-1. *Mol Cell Endocrinol* 2001;185:17-25.
- 35 Yu RN, Ito M, Saunders TL, Camper SA, Jameson JL: Role of Ahch in gonadal development and gametogenesis. *Nat Genet* 1998;20:353-357.
- 36 Habiby RL, Boeppel P, Nachtigall L, Sluss PM, Crowley WF Jr, Jameson JL: Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalamic and pituitary defects in gonadotropin production. *J Clin Invest* 1996;98:1055-1062.
- 37 Jeffs B, Meeks JJ, Ito M, Martinson FA, Matzuk MM, Jameson JL, Russell LD: Blockage of the rete testis and efferent ductules by ectopic Sertoli and Leydig cells causes infertility in Dax1-deficient male mice. *Endocrinology* 2001;142:4486-4495.
- 38 Ito M, Yu R, Jameson JL: DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol Cell Biol* 1997;17:1476-1483.
- 39 Lalli E, Bardoni B, Zazopoulos E, Wurtz JM, Strom TM, Moras D, Sassone-Corsi P: A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. *Mol Endocrinol* 1997;11:1950-1960.
- 40 Crawford PA, Dorn C, Sadovsky Y, Milbrandt J: Nuclear receptor DAX-1 recruits nuclear receptor corepressor N-CoR to steroidogenic factor 1. *Mol Cell Biol* 1998;18:2949-2956.
- 41 Babu PS, Bavers DL, Beuschlein F, Shah S, Jeffs B, Jameson JL, Hammer GD: Interaction between Dax-1 and steroidogenic factor-1 in vivo: increased adrenal responsiveness to ACTH in the absence of Dax-1. *Endocrinology* 2002;143:665-673.
- 42 Bland ML, Jamieson CA, Akana SF, Bornstein SR, Eisenhofer G, Dallman MF, Ingraham HA: Haploinsufficiency of steroidogenic factor-1 in mice disrupts adrenal development leading to an impaired stress response. *Proc Natl Acad Sci USA* 2000;97:14488-14493.
- 43 Achermann JC, Ito M, Hindmarsh PC, Jameson JL: A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet* 1999;22:125-126.
- 44 Achermann JC, Ozisik G, Ito M, Orun UA, Harmanci K, Gurakan B, Jameson JL: Gonadal determination and adrenal development are regulated by the orphan nuclear receptor, steroidogenic factor-1, in a dose-dependent manner. *J Clin Endocrinol Metab* 2002;87:1829-1833.
- 45 Ito M, Achermann JC, Jameson JL: A naturally occurring steroidogenic factor-1 mutation exhibits differential binding and activation of target genes. *J Biol Chem* 2000;275:31708-31714.
- 46 Biason-Lauber A, Schoenle EJ: Apparently normal ovarian differentiation in a prepubertal girl with transcriptionally inactive steroidogenic factor 1 (NR5A1/SF-1) and adrenocortical insufficiency. *Am J Hum Genet* 2000;67:1563-1568.
- 47 Wilson TE, Fahrner TJ, Milbrandt J: The orphan receptors NGFI-B and steroidogenic factor 1 establish monomer binding as a third paradigm of nuclear receptor-DNA interaction. *Mol Cell Biol* 1993;13:5794-5804.