

**BATTLE OF THE SEXES: NEW INSIGHTS INTO GENETIC PATHWAYS OF  
GONADAL DEVELOPMENT**

J. Larry Jameson

and by invitation, John C. Achermann, Gokhan Ozisik, and Joshua J. Meeks

Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL  
60611

Keywords: DAX1, SF1, sex determination, testis, gonadotropins

Corresponding Author:

J. Larry Jameson, MD, PhD  
Chairman, Department of Medicine  
Feinberg School of Medicine/Northwestern Memorial Hospital  
Galter Pavillon, Suite 3-150  
251 E. Huron St.  
Chicago, IL 60611-2908, USA  
Phone: (312)926-9436 Fax: (312)926-7260  
E-mail: [ljameson@northwestern.edu](mailto:ljameson@northwestern.edu)

Running Head: Mechanisms of Sex Determination

## Abstract

Sex determination is governed by a series of genetic switches that influence cell fate and differentiation during critical periods of gonadal development. Remarkably, the primordial fetal gonad is bipotential. Therefore, gonadal development provides an excellent opportunity to identify genes involved in differential organogenesis. The identification of the testis-determining gene, *SRY* (Sex-reversed on the Y), was a pivotal first step towards unraveling this genetic pathway. It is now clear that numerous other genes, in addition to *SRY*, are necessary for normal testis development. For example, human mutations in a variety of genes (*SOX9*, *WT1*, *SF1*) impair testis development. Murine models provide evidence for additional genes (*Lhx9*, *Emx2*, *M33*, *Dmrt*, *Fgf9*). This lecture will highlight insights gleaned from human mutations in the nuclear receptors, SF1 (Steroidogenic Factor1) (NR5A1) and DAX1 (Dosage-sensitive sex reversal, Adrenal hypoplasia congenita, X chromosome) (NR0B1). These studies reveal the exquisite sensitivity of SF1-dependent developmental pathways to gene dosage and function in humans.

## Introduction

During early gonadal development, a “battle of the sexes” is played out, as the bipotential gonad differentiates into either testis or ovary. Gene dosage plays a critical role in gonadal determination. Remarkably, however, the mechanism for sex determination varies widely among species, and is not highly conserved across evolution (Table 1). For example, in *Drosophila*, the ratio of X chromosomes to autosomes determines sex, emphasizing the importance of gene dosage. In many reptiles, temperature of the environment is the major determinant of the ratio of males to females. In birds, males are the homogametic (ZZ) sex and females are heterogametic (ZW) for sex chromosomes. Mammals, in contrast, reverse this phenomenon, as females are XX and males are XY.

Mammalian sex determination can be divided into 3 major stages: 1) genetic sex; 2) gonadal sex; and 3) phenotypic sex (Fig. 1). These designations are largely conceptual, as genetic and developmental pathways link each of these phases along a continuum that ultimately leads to a male or female capable of reproduction. Based on these stages, clinical disorders of sexual differentiation can be logically grouped into chromosomal disorders (genetic sex), dysgenetic gonads (gonadal sex), or abnormalities of hormone production or action (phenotypic sex).

Sex chromosomal composition (XX vs XY) is a critical determinant of gonadal sex. Most simplistically, this observation implies that Y-chromosomal genes are necessary for testis determination and/or the presence of two X-chromosomes favors ovarian development. Beginning with the discovery of *SRY* (Sex-reversed on the Y), much has been learned about the genes that lead to testis development; less is known about the genetic pathways that direct ovary development. In many respects, ovary development appears to be constitutive. That is, in the absence of testis-determining genes, an ovary develops, likely due to the inherent ability of germ

cells to enter meiosis if an inhibitory signal is not produced by the supporting Sertoli cells. Once an ovary has formed, a number of genes are necessary to ensure oocyte survival and normal follicle maturation (e.g., *GDF9*, *FOXL2*, *FSHR*). This review will focus primarily on testis differentiation and new insights gained from naturally occurring human mutations in the nuclear receptors, SF1 (steroidogenic factor1) and DAX1 (Dosage-sensitive sex reversal, Adrenal hypoplasia congenita, X chromosome, gene 1).

Testis determination requires a series of developmental “switches” that direct the differentiation of Sertoli and Leydig cells from progenitor cells in the bipotential gonad (or urogenital ridge) (Fig. 2). These biological events are initiated by a transient wave of SRY (sex-related gene on the Y chromosome) expression that alters the fate of cells in the undifferentiated gonad to give rise to Sertoli instead of granulosa cells (1). Once Sertoli cells form, they coalesce with peritubular myoid cells that migrate in from the mesonephros to form testicular cords, the progenitor to the seminiferous tubules. Sertoli cells also produce MIS (Müllerian inhibiting substance, also known as Anti-Müllerian Hormone, AMH) and inhibin. MIS causes the regression of Müllerian structures, precluding formation of the fallopian tubes, uterus, and upper segment of the vagina. Inhibin selectively suppresses pituitary follicle-stimulating hormone (FSH). Leydig cells secrete testosterone, which is necessary for development of the Wolffian structures including the epididymides, vasa deferentia, and seminal vesicles. Testosterone is converted to dihydrotestosterone, a potent androgen that induces virilization of the external genitalia.

SRY is a member of the HMG (high mobility group)-box family of transcription factors, and it is likely that it interacts with other transcription factors to selectively alter the expression of target genes. However, the mechanism of SRY action remains enigmatic. A related protein, SOX9 (SRY-related HMG-box gene 9), is strikingly upregulated in the developing male gonad and is turned off in the female gonad. Targeted expression of *Sox9* is sufficient to initiate testis

formation in an XX genetic female, and mutations that disrupt *SOX9* impair testis development, indicating that it is a key gene in testis determination (2, 3). Whether *SOX9* is a downstream target of *SRY* or is regulated independently of *SRY* is currently unknown. *SOX9* binds to a specific site in the *Amh* (*Mis*) promoter, and synergizes with *SF1* to regulate tissue-specific expression of *MIS*. *SF1* is required for both adrenal and gonadal development, and it appears to function in conjunction with other transcription factors to regulate a large group of adrenal and gonadal genes. The expression pattern of *DAX1* largely parallels that of *SF1*. In contrast to *Sox9*, *Dax1* is down-regulated in the developing testis but not in the ovary. Overexpression of *DAX1* inhibits *Sry*-mediated testis determination, suggesting that *DAX1* may act as an “anti-testis” factor (4). The exquisite sensitivity of the male sex-determining pathways to gene dosage is apparent in humans, as haploinsufficiency of *WT1*, *SOX9*, *SF1*, and duplication of *WNT4* or *DAX1* are associated with impaired testis development in XY individuals (Table 2). In addition to those mentioned above, many other genes (e.g., *WT1*, *GATA4*, *DHH*) are also involved in gonadal differentiation and development as well as final positioning of the gonads (e.g., *INSL3*, *HOXA10*, *HOXA11*).

### **Mutations in *DAX1* Cause X-linked Adrenal Hypoplasia Congenita**

Adrenal hypoplasia congenita (AHC) is a rare disorder characterized by adrenal insufficiency and hypogonadotropic hypogonadism (HHG). Mutations or deletions of *DAX1* (AHC, NR0B1) cause the X-linked cytomegalic form of AHC (MIM 300200) (5). Boys with this condition typically present with primary adrenal failure in infancy or childhood. HHG is manifest as delayed or absent puberty, and is caused by decreased GnRH production and impaired gonadotropin responses to residual GnRH. Infertility is caused by gonadotropin deficiency in combination with a primary testicular defect in spermatogenesis (6, 7).

The *DAX1* gene encodes a 470 amino acid transcription factor with a carboxy-terminal region that resembles the ligand binding domain (LBD) of nuclear receptors (Fig. 3). The carboxyterminus of DAX1 confers potent transcriptional silencing activity (8, 9). In contrast to other nuclear receptors, the amino-terminal half of DAX1 consists of a repeated amino acid sequence that contains LXXLL-like motifs implicated in protein-protein interactions (10).

Although loss of DAX1 function is associated with adrenal failure and HHG in humans, transient gene expression studies suggest that DAX1 is a repressor of transcription mediated by SF1, a key factor involved in adrenal and gonadal development. DAX1 repression involves direct protein-protein interactions, accompanied by corepressor recruitment (10-12). The interaction between DAX1 and SF1 appears to be mediated by the amino-terminal half of the DAX1 protein (8), and likely involves the LXXLL repeat motifs. This paradox of DAX1 action—it is necessary for adrenal development, but inhibits SF1 action—is incompletely understood at present. It seems likely that DAX1 serves distinct developmental and regulatory roles by acting selectively on various target genes, or by changing its level of expression at different stages of development.

Targeted mutagenesis of *Dax1* was used to produce a murine model of X-linked AHC (13). A “Cre-loxP” targeting strategy was employed because mutations in *Dax1* cause infertility in males; this approach allowed Cre-mediated excision of *Dax1* in the progeny of females carrying the floxed *Dax1* allele. Male *Dax1* knockout (KO) mice are hypogonadal and infertile. Testis histology in the *Dax1* KO mice reveals progressive seminiferous tubule degeneration, loss of germ cells, impaired spermatogenesis, and Leydig cell hyperplasia. The efferent ductules and rete testis are blocked by aberrantly located Sertoli and Leydig cells, creating an obstructive pathology that leads to sperm necrosis within the seminiferous tubules (14). Testicular biopsy findings in patients with AHC are similar to those of the animal model (7).

Over 80 different human *DAX1* mutations have been described (15), most of which are nonsense or frameshift mutations that cause premature truncation of the protein (Fig. 3). Deletion of as few as the last nine amino acids of *DAX1*, which constitute a putative AF2 domain, is associated with a severe clinical phenotype (16). Relatively few missense mutations have been reported in *DAX1*. These mutations appear to cluster within restricted domains of the carboxy-terminus of the protein, potentially providing insight into important functional domains (17, 18). Most *DAX1* mutations result in similar loss of transcriptional repression in functional assays, despite somewhat variable clinical presentations. This observation suggests that modifier genes or environmental factors account for variability in clinical presentation of AHC. Rarely, patients have been identified with relatively mild *DAX1* missense mutations that result in partial loss of transcriptional repression (19, 20). These individuals first presented in adulthood (rather than childhood) with evidence of mild adrenal failure or partial HHG. However, the absence of *DAX1* mutations in a relatively large group of patients with familial and sporadic forms of HHG and delayed puberty indicates that mutations are infrequent in such patients unless there is associated adrenal failure (21).

### **Mutations in *SF1* (Steroidogenic factor-1) Cause Adrenal Insufficiency and XY Sex Reversal**

The *SF1* gene (also known as *FTZF1*) contains seven exons and has been mapped to human chromosome 9q33 (22, 23). The gene encodes a 461 amino acid protein that is structurally similar to other members of the nuclear receptor superfamily (Fig. 4). Identified functional domains of *SF1* include a two zinc finger DBD, an A-box (or *FTZF1* box), a hinge region, and an AF2 domain. The first zinc finger of *SF1* contains a proximal box (P-box), which confers specificity in the recognition of DNA binding sites (24, 25). The A-box appears to stabilize DNA binding (26, 27). The AF2 domain of *SF1* is involved in transcriptional activation. *SF1* binds to DNA as a monomer, and recognizes DNA binding sites containing variations on a

PyCA AGGTCA DNA sequence motif. Once bound to DNA, transactivation of target genes by SF1 involves the recruitment of coactivators such as steroid receptor coactivator-1 (SRC-1) (28), glucocorticoid receptor interacting protein (GRIP1) (29), CREB-binding protein (CBP)/p300 (30) or proline-rich nuclear receptor coregulatory protein (PNRC) (31).

The temporal and spatial pattern of SF1 expression is consistent with its critical role in adrenal development, steroidogenesis, and gonadal differentiation. In the mouse, Sf1 is first expressed in the urogenital ridge at embryonic day 9 (E9) (32), and subsequently in the adrenal primordium (E11) and adrenal cortical cells (E13) (33). A similar expression pattern is seen in humans (34, 35). In Sertoli cells, Sf1 regulates the expression of *Amh*, which leads to regression of Müllerian structures in males (36). In Leydig cells, Sf1 regulates various enzyme genes involved in steroidogenesis and testosterone biosynthesis, allowing virilization of the male fetus.

Targeted deletion of Sf1 (FtzF1) in mice results in complete adrenal and gonadal agenesis, male-to-female sex-reversal, and persistence of Müllerian structures in males (37-40). The ventromedial hypothalamus (VMH) is also absent and there is decreased production of GnRH and gonadotropins (40, 41).

A human *SF1* mutation was first identified in a patient with primary adrenal failure, XY sex-reversal, and persistent Müllerian structures (42). This phenotypically female patient exhibited signs of primary adrenal insufficiency during the first two weeks of life. Laparotomy revealed normal Müllerian structures and streak-like gonads containing a few poorly differentiated seminiferous tubules surrounded by extensive connective tissue. Mutation analysis revealed a de novo heterozygous Gly to Glu (G35E) mutation within the P-box of the SF1 DNA-binding domain (Fig. 4). 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 and transactivate SF1 target

genes such as *Cyp11a* (P450<sub>scc</sub>), *Dax1*, or *LHβ*. The phenotype of this patient with a heterozygous point mutation in SF1 is less severe than the complete adrenal and gonadal agenesis seen in homozygous Sf1 (-/-) knockout mice. Recent evidence suggests that heterozygous Sf1 (-/+) knockout mice also exhibit impaired adrenal function, though not as severe as that seen in this patient (43). The mutant SF1 protein does not exhibit dominant negative activity (44). Therefore, it is likely that haploinsufficiency of SF1 precludes normal glandular development and function.

The identification of additional SF1 mutations has helped to clarify the functional requirements for this receptor. A second de novo heterozygous *SF1* Arg to Leu mutation (R255L) was found in a XX female with adrenal insufficiency (45). This mutation affects a conserved residue in the hinge region of SF1. Although the mutation renders the protein transcriptionally inactive, it does not appear to impair ovarian development. A homozygous SF1 mutation was recently identified in a baby born to consanguineous parents (Fig. 4) (46). This autosomal recessive mutation alters the A-box region of SF1 that modulates DNA binding by monomers (44, 47). In contrast to the P-box mutation, this A-box change (R92Q) is associated with a partial loss of function and impaired binding to its response element. The observation that heterozygous 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. Examples of the mouse and human mutations that exemplify the dose sensitivity of SF1 and DAX1 are summarized in Table 3. A striking feature of this table is that two-fold alterations in the gene doses of SF1 and DAX1 yield a broad spectrum of adrenal and gonadal phenotypes. Ongoing experiments in mouse models suggest that these phenotypes are readily altered by genetic background, indicating the presence of modifier genes.

In summary, naturally occurring mutations in humans, in combination with transgenic mouse models, have provided important new insights into the mechanisms that regulate sex determination. It is increasingly apparent that the timing and levels of gene expression are

critical determinants of gonadal development. Future studies should be able to further unravel this genetic pathway, providing an important paradigm for understanding cell fate determination and organogenesis.

#### Acknowledgments

This work was supported by NIH Grants U54-HD-29164 and PO1 HD-21921.

#### Figure Legends

**Fig. 1. Overview of Sex Determination.** T, testosterone; DHT, dihydrotestosterone, MIS, Müllerian inhibiting substance, E2, estradiol, P4, progesterone.

**Fig. 2. Genetic Pathways Leading to Gonadal Development.** The genes required for development of the urogenital ridge, bipotential and differentiated gonad are indicated, along with hormones produced by various cell lineages in the testis and ovary. A4, androstenedione.

**Fig. 3. Structure of DAX1 and Locations of Mutations.** (A) The DAX1 locus on the X chromosome is indicated. The carboxyterminus of DAX1 is structurally related to nuclear receptors and possesses potent repressor activity. The aminoterminal contains three and one-half repeats of a 66-67 amino acid repeat motif. (B) Locations of nonsense, frameshift, and missense mutations. Note that missense mutations cluster within the carboxyterminus.

**Fig. 4. Structure of SF1 and Locations of Mutations.** (A) The locations of human mutations are indicated above the structure of SF-1. The positions of the mutations G35E, R92Q, and R255L within the P-box, A-box, and Hinge region, respectively, are shown. DBD, DNA-binding domain; LBD, ligand-binding domain (which consists of an activation domain). (B) Expanded

view of the DNA binding domain (DBD) of SF-1, illustrating the location of the G35E mutation at the base of the first zinc finger. This mutation occurs at the position where the SF-1 DBD makes direct contact with DNA recognition sites.

**Table 1. Mechanisms of sex determination across evolution**

<b>Species</b>	<b>Sex Determination Mechanism</b>
Hymenoptera	Genome copy number (2x female; 1x male)
Drosophila	Ratio of X chromosomes : Autosomes
Reptiles	Temperature
Turtles	Male: cool    Female: warm
Alligators	Male: warm    Female: cool
Fish	Social factors; Hormonal environment
Birds	Male (ZZ)    Female (ZW)
Mammals	Male (XY)    Female (XX)

**Table 2. Examples of gene dosage-sensitivity of gonadal determination in XY humans**

<b>Mutation</b>	<b>Clinical Disorder</b>
WT1 haploinsufficiency	Wilm's tumor; ambiguous genitalia
SOX9 haploinsufficiency	Campomelic dysplasia; ambiguous genitalia
DAX1 duplication	Ambiguous genitalia
WNT4 duplication	Ambiguous genitalia
SF1 haploinsufficiency	
Heterozygous severe mutation (G35E)	Male to female sex reversal
Homozygous mild mutation (R92Q)	Male to female sex reversal

**Table 3. Spectrum of SF1 and DAX1 Gene Dosage Effects on Gonadal Development**

<b>XY Gene Dose</b>		<b>Phenotype</b>
<b>SF1</b>	<b>DAX1</b>	
2x	1x	Normal
1x	1x	Adrenal insuff./ Dysgenetic testis
1x/0.5x	1x	Normal
0.5x/0.5x	1x	Adrenal insuff./ Dysgenetic testis
0x	1x	Adrenal & Gonadal Agenesis
2x	2x (dup)	Testis dysgenesis
2x	0.5x	Late onset AHC
2x	0x	Classic AHC/ Dysgenetic testis
1x	0x	Compensates for Dax1 knockout

## References

1. Albrecht KH, Eicher EM. Evidence that sry is expressed in pre-sertoli cells and sertoli and granulosa cells have a common precursor. *Dev Biol* 2001; 240:92-107.
2. Vidal VP, Chaboissier MC, de Rooij DG, Schedl A. Sox9 induces testis development in XX transgenic mice. *Nat Genet* 2001; 28:216-7.
3. Kwok C, Weller PA, Guioli S, et al. Mutations in SOX9, the gene responsible for Campomelic dysplasia and autosomal sex reversal. *Am J Hum Genet* 1995; 57:1028-36.
4. Swain A, Lovell-Badge R. Mammalian sex determination: a molecular drama. *Genes Dev* 1999; 13:755-67.
5. Muscatelli F, Strom TM, Walker AP, et al. Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 1994; 372:672-6.
6. Habiby RL, Boepple 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; 98:1055-62.
7. Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley WF, Jr. X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. *J Clin Endocrinol Metab* 1999; 84:4501-9.
8. 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; 17:1476-83.
9. Lalli E, Bardoni B, Zazopoulos E, et al. A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. *Mol Endocrinol* 1997; 11:1950-60.
10. Zhang H, Thomsen JS, Johansson L, Gustafsson JA, Treuter E. DAX-1 functions as an LXXLL-containing corepressor for activated estrogen receptors. *J Biol Chem* 2000; 275:39855-9.
11. 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; 18:2949-56.
12. Altincicek B, Tenbaum SP, Dressel U, Thormeyer D, Renkawitz R, Baniahmad A. Interaction of the corepressor Alien with DAX-1 is abrogated by mutations of DAX-1 involved in adrenal hypoplasia congenita. *J Biol Chem* 2000; 275:7662-7.
13. Yu RN, Ito M, Saunders TL, Camper SA, Jameson JL. Role of Ahch in gonadal development and gametogenesis. *Nat Genet* 1998; 20:353-7.
14. Jeffs B, Meeks JJ, Ito M, et al. Blockage of the rete testis and efferent ductules by ectopic Sertoli and Leydig cells causes infertility in Dax1-deficient male mice. *Endocrinology* 2001; 142:4486-95.
15. Phelan JK, McCabe ER. Mutations in NR0B1 (DAX1) and NR5A1 (SF1) responsible for adrenal hypoplasia congenita. *Hum Mutat* 2001; 18:472-87.
16. Nakae J, Tajima T, Kusuda S, et al. Truncation at the C-terminus of the DAX-1 protein impairs its biological actions in patients with X-linked adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 1996; 81:3680-5.

17. Zhang YH, Guo W, Wagner RL, et al. DAX1 mutations map to putative structural domains in a deduced three-dimensional model. *Am J Hum Genet* 1998; 62:855-64.
18. Achermann JC, Ito M, Silverman BL, et al. Missense mutations cluster within the carboxy-terminal region of DAX-1 and impair transcriptional repression. *J Clin Endocrinol Metab* 2001; 86:3171-5.
19. Tabarin A, Achermann JC, Recan D, et al. A novel mutation in DAX1 causes delayed-onset adrenal insufficiency and incomplete hypogonadotropic hypogonadism. *J Clin Invest* 2000; 105:321-8.
20. Mantovani G, Ozisik G, Achermann JC, et al. Hypogonadotropic hypogonadism as a presenting feature of late-onset X-linked adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 2002; 87:44-8.
21. Achermann JC, Gu WX, Kotlar TJ, et al. Mutational analysis of DAX1 in patients with hypogonadotropic hypogonadism or pubertal delay. *J Clin Endocrinol Metab* 1999; 84:4497-500.
22. Taketo M, Parker KL, Howard TA, et al. Homologs of *Drosophila* Fushi-Tarazu factor 1 map to mouse chromosome 2 and human chromosome 9q33. *Genomics* 1995; 25:565-7.
23. 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; 17:139-47.
24. 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; 338:271-4.
25. Umesono K, Evans RM. Determinants of target gene specificity for steroid/thyroid hormone receptors. *Cell* 1989; 57:1139-46.
26. 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; 12:5667-72.
27. Wilson TE, Paulsen RE, Padgett KA, Milbrandt J. Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. *Science* 1992; 256:107-10.
28. 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.
29. Hammer GD, Krylova I, Zhang Y, et al. Phosphorylation of the nuclear receptor SF-1 modulates cofactor recruitment: integration of hormone signaling in reproduction and stress. *Mol Cell* 1999; 3:521-6.
30. Monte D, DeWitte F, Hum DW. Regulation of the human P450<sub>scc</sub> gene by steroidogenic factor 1 is mediated by CBP/p300. *J Biol Chem* 1998; 273:4585-91.
31. 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 $\alpha$ 1 (estrogen related receptor  $\alpha$ -1). *Mol Endocrinol* 2000; 14:986-98.
32. Hatano O, Takayama K, Imai T, et al. Sex-dependent expression of a transcription factor, Ad4BP, regulating steroidogenic P-450 genes in the gonads during prenatal and postnatal rat development. *Development* 1994; 120:2787-97.
33. Morohashi K, Iida H, Nomura M, et al. Functional difference between Ad4BP and ELP, and their distributions in steroidogenic tissues. *Mol Endocrinol* 1994; 8:643-53.

34. Hanley NA, Ball SG, Clement-Jones M, et al. Expression of steroidogenic factor 1 and Wilms' tumour 1 during early human gonadal development and sex determination. *Mech Dev* 1999; 87:175-80.
35. 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; 82:1799-806.
36. Shen WH, Moore CC, Ikeda Y, Parker KL, Ingraham HA. Nuclear receptor steroidogenic factor 1 regulates the mullerian inhibiting substance gene: a link to the sex determination cascade. *Cell* 1994; 77:651-61.
37. Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 1994; 77:481-90.
38. Luo X, Ikeda Y, Schlosser DA, Parker KL. Steroidogenic factor 1 is the essential transcript of the mouse Ftz-F1 gene. *Mol Endocrinol* 1995; 9:1233-9.
39. Sadovsky Y, Crawford PA, Woodson KG, et al. 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 U S A* 1995; 92:10939-43.
40. Shinoda K, Lei H, Yoshii H, et al. Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. *Dev Dyn* 1995; 204:22-9.
41. 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; 9:478-86.
42. 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; 22:125-6.
43. Bland ML, Jamieson CA, Akana SF, et al. Haploinsufficiency of steroidogenic factor-1 in mice disrupts adrenal development leading to an impaired stress response. *Proc Natl Acad Sci U S A* 2000; 97:14488-93.
44. 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; 275:31708-14.
45. 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; 67:1563-8.
46. Achermann JC, Ozisik G, Ito M, et al. 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; 87:1829-33.
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; 13:5794-804.

# Sex Determination

**Genetic Sex**

XY

XX

*Testis-determining genes*



*Ovary-determining genes*

**Gonadal Sex**

*Testis differentiation genes*



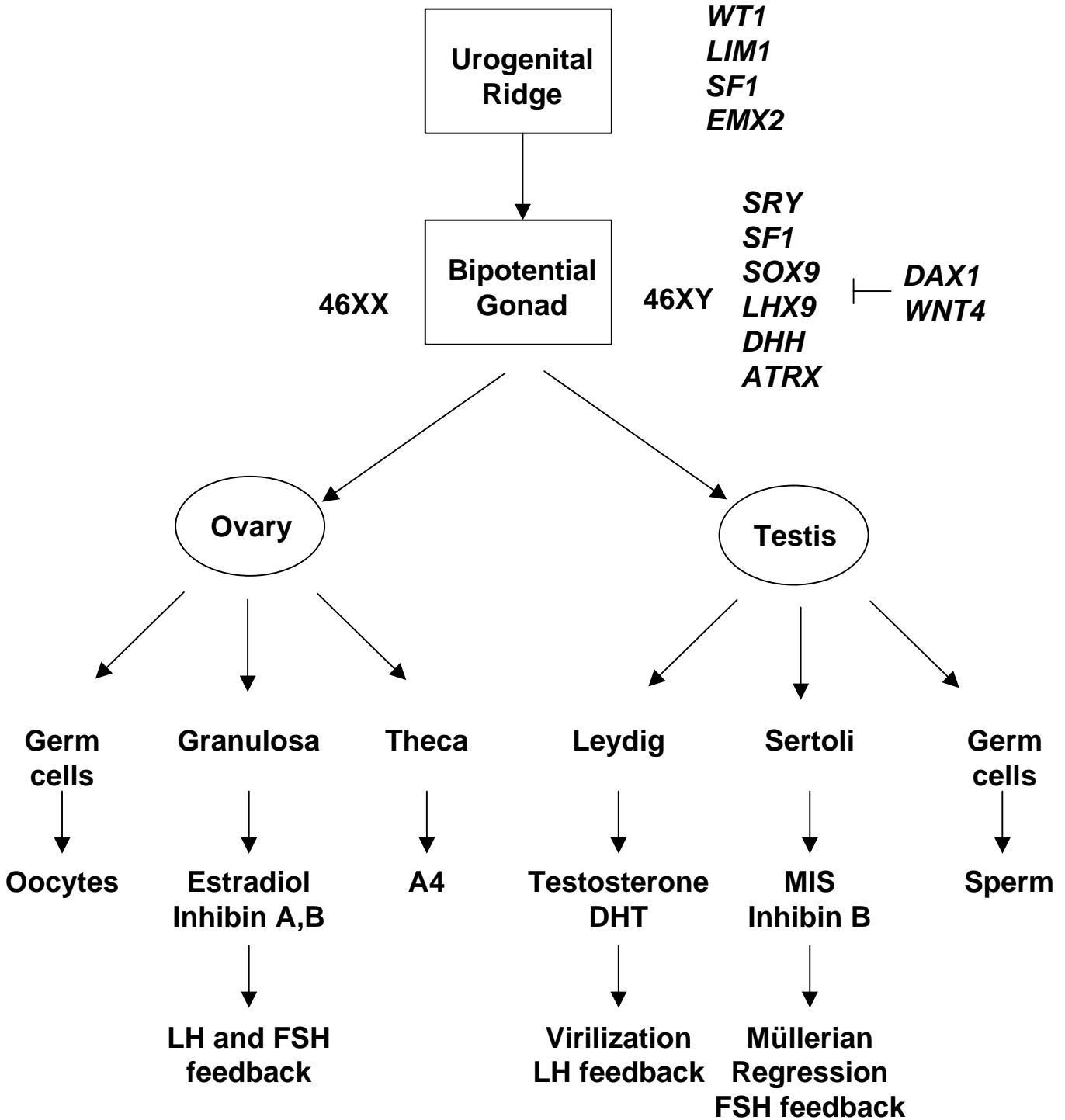
*Gonadal steroids (E2, P4)*

*Gonadal steroids & peptides (T, DHT, MIS)*



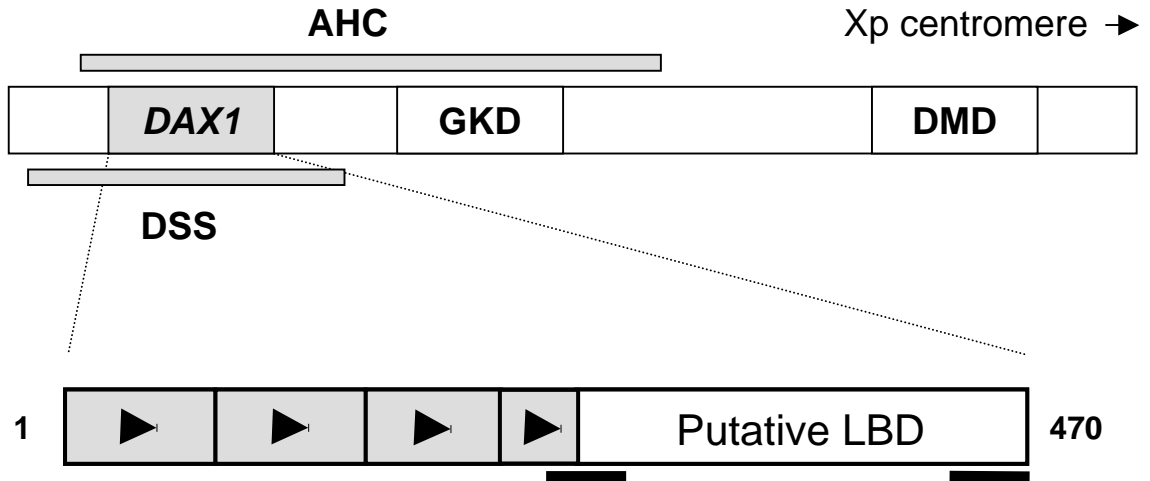
**Phenotypic Sex**

# Gonadal Development



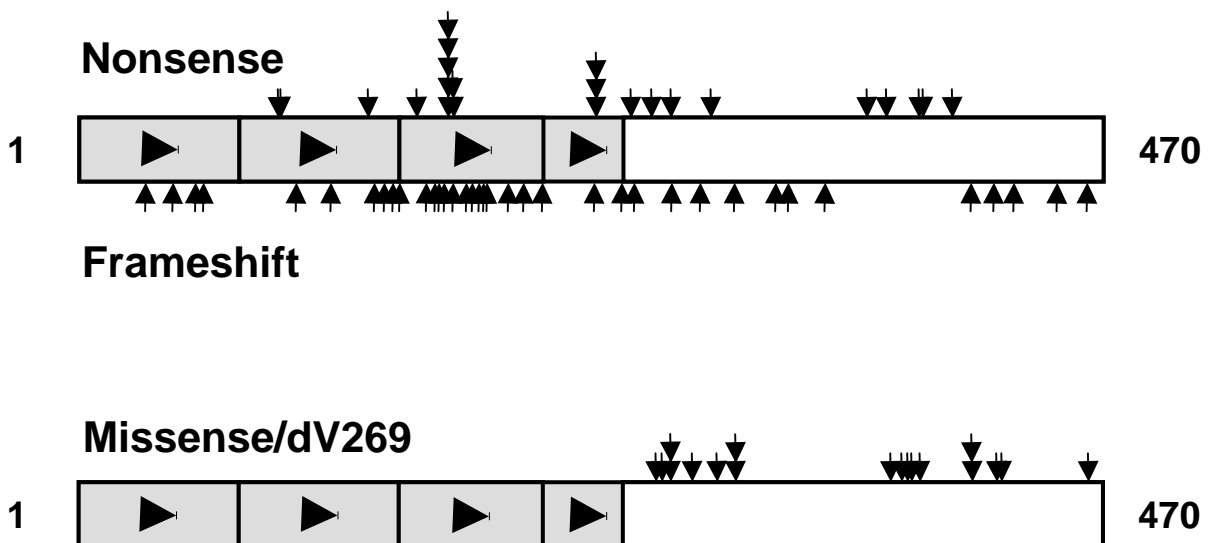
A

## DAX-1 Locus and Structure

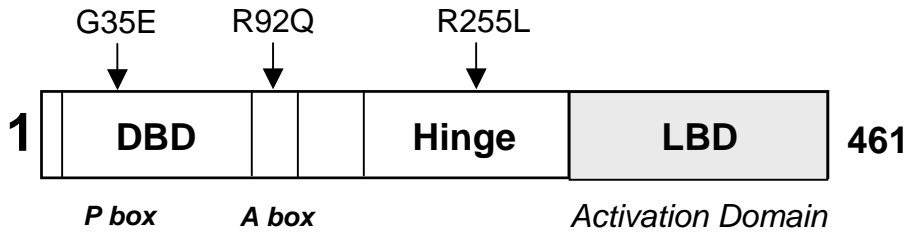


B

## DAX-1 Mutations



# A Steroidogenic Factor-1 (SF-1)



# B SF-1 DNA Binding Domain (DBD)

