Sex Determination and the Y Chromosome

KEN McELREAVEY* AND MARC FELLOUS

Although SRY was first identified 10 years ago, we still know remarkably little about its mode of action or downstream target genes. Recently, potential protein partners have been identified and there has been considerable activity to understand the roles of WT1, SF-1, DAX-1 and SOX9 in gonadogenesis. The emerging picture is one of complex interactions, involving both positive and negative regulatory signals that, depending on the cellular and promoter context, drive the expression of male-specific genes. Despite recent advances, however, we are still unable to explain the genetic cause of most cases of 46,XY gonadal dysgenesis or even a single case of Y-chromosome-negative 46,XX maleness. Am. J. Med. Genet. (Semin. Med. Genet.) 89:176–185, 1999. © 2000 Wiley-Liss, Inc.

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THE Y CHROMOSOME AND TESTIS DETERMINATION

Human Sex-reversed Conditions

Completely sex-reversed individuals are classified as either 46,XX males or 46,XY females. Incomplete or partial sex-reversed syndromes affect 46,XY and 46,XX true hermaphrodites, and 46,XY gonadal dysgenesis is characterized by a failure of testis determination [reviewed by Berkovitz, 1992]. These cases can be subdivided into 46,XY complete gonadal dysgenesis and 46,XY partial gonadal dysgenesis. The former (“swyer syndrome”) is characterized by completely female external genitalia, well-developed müllerian structures, and a gonad composed of a streak of fibrous tissue. The other form of dysgenesis is characterized by partial testis formation, usually a mixture of Wolffian and müllerian ducts, and varying degrees of masculinization of the external genitalia, which broadly correlates with the extent of testicular differentiation. All 46,XX males have testes and a normal male habitus, whereas 46,XX true hermaphrodites have both ovarian and testicular tissue, usually as ovotestes but less commonly as separate gonads. Both 46,XY females and 46,XX males are sterile. Pseudohermaphroditism occurs when the phenotype of the external genitalia is inconsistent with gonadal sex. This condition reflects dysfunction at the hormonal level of sexual differentiation after testis or ovary formation [Smith and Sinclair, 1999]. The analysis of these cases has been and remains essential for our understanding of the molecular basis of sex determination and the early development of the gonad.

SRY is the Y-located Testis Determinant

Although most of the human Y does not normally recombine with the X chromosome, there are two limited regions of sequence identity with the X that permit pairing and recombination during male meiosis [for review see Rappold, 1993]. These are the pseudoautosomal regions (PAR) located at the distal portions of the short and long arms of the Y chromosome. Absence of the Yp PAR is associated with short stature and male infertility. During meiotic prophase, germ cells require the Y chromosome as a pairing partner for the X. In the absence of pairing, caused by deletions of the pseudoautosomal region, germ cells undergo meiotic arrest resulting in azoospermia. Abnormal X-Y interchange during meiosis can lead to the transfer of Y material, including the sex-determining gene, to the short arm of the X chromosome [Anderson et al., 1986]. Transmission of this chromosome to a 46,XX individual usually confers a 46,XX male phenotype, though some cases of 46,XX true hermaphrodites have been reported [Berkovitz et al., 1992; McElreavey et al., 1992]. These pathologic conditions were used to map and finally identify the testis-determining gene SRY, which is situated approximately 5 kb centromeric to the pseudoautosomal region [Sinclair et al., 1990]. The human SRY gene comprises a single exon and encodes a protein of 204 amino acids, including a 79-residue conserved DNA-bending and DNA-binding domain, the HMG box (high mobility group) [Nasrin et al., 1991; Clépet et al., 1993]. This suggests that the SRY gene product regulates gene expression in a cell autonomous manner. In male mouse embryos, Sry is expressed briefly within the somatic cells of gonads at the onset of testis differentiation (between 10.5 and 12.5 dpc) [Koopman et al., 1990; Hacker et al., 1995]. In contrast...
to the expression profile in mice, human SRY can be detected by in situ hybridization and immunohistochemistry, from the moment of sex determination until the adult stage [Salas-Cortes et al., 1999, and our unpublished data]. The human SRY protein is present in both adult Sertoli cells and germ cells [Salas-Cortes et al., 1999]. It is formally possible that SRY plays a role in germ cell development or maintenance. SRY loss-of-function mutations are associated with a streak gonad lacking germ cells. The role of SRY in germ cell development, at least in the human, is unclear.

**RELATIONSHIP BETWEEN SRY AND HUMAN SEX-REVERSED CONDITIONS: XY FEMALES**

Since SRY is the primary sex determinant, one would predict that individuals with an absence of testis determination, 46,XY females with complete or partial gonadal dysgenesis, harbor mutations in the SRY gene. [Approximately 15% of 46,XY individuals with the complete form of gonadal dysgenesis carry mutations in the SRY gene, most of which are located in the HMG domain] [Cameron and Sinclair, 1997, and references therein]. These are summarized in Fig. 1. Some of these mutations are predicted to either abolish DNA-binding or alter the DNA-bending angle [Harley et al., 1992; Pontiggia et al., 1994; Schmitt-Ney et al., 1995; Werner et al., 1995]. The protein has two nuclear localization signals at either extremity of the HMG box. Thus, it is conceivable that some mutations interfere with the ability of SRY to enter the nucleus, though this has not been established [Sudbeck and Scherer, 1997]. Although the majority of deletions are de novo, a small minority are inherited from a normal fertile male [Vilain et al., 1992; Hawkins et al., 1992; Jager et al., 1992; Affara et al., 1993; Schmitt-Ney et al., 1995; Domenice et al., 1998]. These familial cases are, at first sight, difficult to explain. With one exception, a single base-pair change has resulted in an amino acid substitution in the HMG DNA-binding and -bending domains. These amino acid substitutions—S18N, V60L, I90M, F109S, and P125L—are associated with both 46,XY gonadal dysgenesis and an apparently normal fertile male phenotype.

Several hypotheses have been proposed that may explain incomplete penetrance in these families. The sequence variants may be polymorphisms unrelated to the phenotype. This hypothesis seems unlikely, since only one polymorphism has been described in the SRY gene in Japanese and Korean populations and it is not associated with sex reversal [Shinka et al., 1999]. A second hypothesis is interaction between the variant SRY protein and another genetic determinant of testis determination that is independently segregating in the family. The importance of the genetic background in mammalian sex determination is highlighted in the mouse, where Sry alleles from some mouse strains will cause sex reversal when placed in certain genetic backgrounds [Eicher and Washburn, 1986; Eicher et al., 1996]. A third hypothesis is that the in vivo activity of the variant SRY protein is altered such that it is around a critical threshold level at the moment of testis determination. This is a plausible explanation because there are many other examples of dosage effects resulting in sex reversal (see later discussion), but it has not yet been proved.

A smaller number of cases of 46,XY partial gonadal dysgenesis are associated with mutations involving the SRY gene. These include a de novo deletion of 3–7 kb that is located approximately 2–3 kb 3’ to the SRY polyadenylation site and a missense mutation (S18N) 5’ to the HMG box in a familial case of 46,XY partial gonadal dysgenesis [McElreavey et al., 1996; Domenice et al., 1998]. This sequence variant was present in the patient’s father and phenotypically normal brother, but not in 50 normal men. Other unusual phenotypes associated with SRY mutations include a de novo C to T transition resulting in a change from a glutamine to a stop codon at the second codon of the SRY open reading frame. This individual was a phenotypic female who had normal menarche, normal secondary characteristics, and at least partial ovarian function. The mutation was de novo, and experimental evidence indicated that the mutation was not leaky [Brown et al., 1998]. Two cases of true hermaphroditism have been described to be associated with a V60A amino acid change in the HMG box and a postzygotic mutation (L101H) within the HMG box respectively [Braun et al., 1993; Hiort et al., 1995].

**RELATIONSHIP BETWEEN SRY AND HUMAN SEX-REVERSED CONDITIONS: XX MALES**

Illegitimate recombination between the X and Y chromosome is not random but occurs between specific regions. The commonest form of Y-positive XX male results from a recombination between the homologous genes PRKX (on the X chromosome) and PRKY (on the Y chromosome).

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cous MSY1 displays an extremely high degree of structural diversity, and it is composed of 48–114 copies of a 25-bp repeat unit [Jobling et al., 1998a]. MSY1 is highly polymorphic in both repeat number and in the sequence of repeat units. This can be assessed by minisatellite variant repeat polymerase chain reaction (MVR–PCR) in which PCR products are generated between a fixed flanking primer and discriminator primers designed to anneal specifically to one kind of repeat variant. Of 81 chromosomes studied, 53 had an MSY1 code “MSY1 class 1” associated with DYS257A and 92R7T (class 1/A/T). The others (about one-third) were defined as MSY1 nonclass 1/G/C [Jobling et al., 1998b]. In XX males, only two of 24 had the class 1/A/T haplotype, however. This finding shows that one pathologic picture of sex determination, Y-positive XX males, is influenced by the Y chromosome haplotype. Approximately 80% of all XX males and only 10% of XX true hermaphrodites are SRY-positive. The source of the Y chromosome-negative cases is still unknown, though loci on the short arm of the X chromosome and chromosome 22 have been proposed [Tar et al., 1995; Aleck et al., 1999].

Figure 1. A: Schematic representation of the human Y chromosome showing the seven deletion intervals and subintervals and the position of genes in the non-recombining region. The two pseudoautosomal regions are indicated (PAR1 and PAR2). Recurrently deleted regions are associated with a failure of male spermatogenesis (AZFa, AZFb, and AZFc). The structure of the SRY gene is shown below. It should be noted that transcription of the SRY gene proceeds toward the telomere from the direction of the centromere. B: Mutations in the SRY open reading frame. This table is modified and updated from Cameron and Sinclair [1997]. Additional mutations included here have been reported by Dork et al. [1998], Scherer et al. [1998], Veitia et al. [1997], Domenic et al. [1998], and Brown et al. [1998].

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1CGD, 46,XY complete gonadal dysgenesis; PGD, 46,XY partial gonadal dysgenesis; TH, 46,XY true hermaphrodite; POF, 46,XY premature ovarian failure; NFM, normal fertile male. 2Father was mosaic for the mutation. 3Present in two sisters with CGD. 4Silent polymorphism in the same patient as L101H.
MECHANISM OF SRY FUNCTION

Current evidence indicates that SRY is expressed in pre-Sertoli cells within the presumptive testis and acts in a cell autonomous manner [Rossi et al., 1993]: Sertoli cells are predominantly XY in XXXY chimeric mouse gonads, and they are the first cell type to differentiate into the male gonad [Burgoyne et al., 1988; Magre and Jost, 1991; Palmer and Burgoyne, 1991; Patek et al., 1991]. The SRY HMG box is both necessary and sufficient to bind and bend DNA. SRY will bind to DNA in a sequence-specific manner, though there is a fairly high nonspecific binding affinity for linear DNA irrespective of the sequence [Nasrin et al., 1991; Harley et al., 1992; Harley et al., 1994]. Human SRY will recognize the sequence AACATG, whereas murine Sry will bind with somewhat higher affinity to the sequence CATTTG [Giese et al., 1992]. Differences in DNA-binding affinity may reflect distinct differences in the interactions of the two proteins with DNA. This view is supported by in vitro studies that indicate that human SRY interacts with DNA primarily through minor groove contacts, whereas murine Sry recognizes the major groove [Giese et al., 1992]. The DNA-bending activity of SRY is essential for the function of the protein. For example, the M64I amino acid change has a limited effect on DNA binding but significantly changes the angle of DNA bending and is associated with 46,XY complete gonadal dysgenesis [Pontiggia et al., 1994]. It is likely that the HMG box of SRY acts to juxtapose normally distant regions of DNA, thereby generating binding sites for other transcription factors [Giese et al., 1992]. SRY may therefore function by changing chromatin structure rather than acting as a specific transactivating molecule.

SRY PROTEIN PARTNERS

What factors may be interacting with SRY to regulate gene expression? One candidate is the gene SIP-1 (SRY interacting protein 1). This is a ubiquitously expressed nuclear protein with two PDZ domains that interact with the last seven C-terminal amino acids of the SRY protein [Poulat et al., 1997]. Other protein partner candidates include members of the POU domain family. The POU domain is a bipartite DNA binding domain consisting of two highly conserved regions tethered by a variable linker. The approximately 75-amino-acid N-terminal region is called a POU-specific domain, and the C-terminal 60-amino-acid region is the POU homeodomain. These proteins have transcriptional regulatory functions that are important for the terminal differentiation of several organs, including anterior pituitary, sensory neurons, and B lymphocytes [Ryan and Rosenfeld, 1997; Veenstra et al., 1997]. Direct functional interactions between SOX/HMG-containing proteins and POU domain proteins have been reported. The POU domain protein Tst-1/Oct-6 and the HMG protein HMG-1/Y act in concert to activate transcription of human papovavirus JC virus in transient transfections [Leger et al., 1995].

There is accumulating evidence that several members of the SOX gene family functionally interact with POU domain proteins. SOX2 will activate FGF-4 gene transcription in the presence of the OCT-3 protein but will interact antagonistically with OCT4 to repress osteopontin gene expression [Botquin et al., 1998]. In Drosophila the Sox-domain protein dichaete is required for the development of the central nervous system midline [Soriano and Russell, 1998]. The HMG box of Diachaete is 88% identical to vertebrate SOX2, and dichaete mutations can be rescued by expression of murine SOX2. dichaete also interacts with the POU domain gene ventral veinless, which suggests that SOX–POU interactions have been conserved during evolution. SOX10, a factor necessary for the development of neural crest derivatives, interacts synergistically with Tst-1/Oct-6 [Kuhlbrodt et al., 1998]. The interaction was mediated through the N-terminal regions of both molecules. Sox11 also interacts synergistically with the POU domain protein Bm1 in developing glial cells [Kuhlbrodt et al., 1998]. The Drosophila fishhook protein is a member of the SOX gene family that regulates the expression of the pair rule gene, even-skipped (eve) during embryonic segmentation, and may interact with the Drosophila POU domain proteins Pdm-1 and Pdm-2 to regulate eve transcription [Ma et al., 1998]. These data suggest that SRY may function to regulate gene expression by interaction with a POU domain protein.

Most mouse strains have an Sry gene that encodes a protein with a structure similar to that described in humans, but some mouse strains have a different Sry protein. Mus musculus molossinus-derived Y chromosomes contain an Sry gene that encodes an HMG box and a potential transactivating domain that has been mapped to a glutamine/histidine-rich C terminal. This region consists of repeating units of Q$_{5}$–13FHDH$_{1}$–5. There is no corresponding region in human SRY. Transgenic studies have shown that this domain is necessary for mouse Sry function [Bowles et al., 1999], perhaps through interaction with other proteins [Lau and Zhang, 1998; Zhang et al., 1999]. It is possible that in some mouse strains the biochemical mode of action of Sry is different from other strains or that proteins have been recruited that fulfill this role in other mouse strains.

NON-Y FACTORS INVOLVED IN SEX DETERMINATION

Several factors have been described that are necessary for gonadogenesis, but as noted by Swain and Lovell-Badge [1999], it is necessary to distinguish between a factor essential for early gonad development and one involved directly in sex determination (i.e., the decision to form either a testis or an ovary). The phenotype associated with mutations in genes encoding many of these factors is compatible with either or both func-
tions. It is becoming clear, however, that several factors play many roles during gonadogenesis, being essential to gonadal development and subsequent sex-specific differentiation.

The Wilms tumor gene WT1 consists of 10 exons spanning 50 kb. The last four exons encode C2-H2 zinc finger domains. Understanding the precise biological functions of WT1 is likely to be difficult because there are at least 32 different isoforms of the protein: it undergoes RNA editing; there are at least three alternative translation initiation sites; exon 5 is differentially spliced; and there is an alternative donor splice site at the exon 9 boundary that results in the differential splicing of three amino acids, “KTS” between the third and fourth zinc fingers [Scharnhorst et al., 1999]. WT1 proteins that contain the KTS insert preferentially associate with splicing factors and are incorporated into spliceosomes in vitro, whereas the KTS protein preferentially interacts with DNA [Davies et al., 1999]. During mammalian development, WT1 is expressed mainly in the developing kidney, uterus, and testis. WT1 transcripts are present in the bipotential genital ridge and subsequently in the Sertoli cells of the testis and the granulosa and epithelial cells of the ovary [Pelletier et al., 1991b]. The relative levels of WT1 + KTS and − KTS transcripts are maintained at all development stages of testis and ovary formation [Nachtalig et al., 1998].

A range of phenotypes are associated with mutations of the WT1 gene. In the human, heterozygous WT1 gene deletions are associated with mild genitourinary anomalies and a predisposition to Wilms tumor [Gessler et al., 1990]. Heterozygous missense mutations give rise to Denys-Drash syndrome (early-onset renal disease with diffuse mesangial sclerosis, 46,XY complete or partial gonadal dysgenesis, and Wilms’ tumor) [Pelletier et al., 1991a], and a change in the KTS+ /KTS− ratios (caused by donor splice-site mutations at the exon 9 boundary) are responsible for Frasier syndrome (late-onset renal disease characterized by focal glomerular sclerosis, 46,XY complete gonadal dysgenesis, and the absence of Wilms tumor) [Barbaux et al., 1997]. Since WT1 is expressed in the same cell lineage as SRY—before during, and after SRY expression—WT1 may be acting upstream to SRY during the development of the genital ridge, perhaps controlling SRY expression or interacting directly with SRY during sex determination or functioning immediately downstream of SRY.

There is now evidence that WT1 has many roles during gonadogenesis. WT1 null mutant mice of both sexes fail to develop kidneys and gonads [Kreidberg et al., 1993], indicating that WT1 acts upstream of the sex-determining decision. The molecular analysis of a familial case of Frasier syndrome has been described that suggests that WT1 has a male-specific role in sex determination. Two sisters in their late teens showed signs of renal disease, and each sister has a donor splice-site mutation that is predicted to result in an imbalance of the KTS+ /KTS− isoforms. One sister has 46,XY complete gonadal dysgenesis with a normal female phenotype, while the other sister has 46,XX chromosome complement with apparently normal ovarian development and function [Demmer et al., 1999]. This suggests that WT1 has either a male-specific function in sex determination or that testis formation is much more sensitive to dosage effects than ovarian formation.

DAX-1 (dosage-sensitive sex-reversal adrenal hypoplasia congenita critical region on the X chromosome, gene 1) and SF-1 (steroidogenic factor 1) are orphan nuclear hormone receptors that play critical roles in the development of the adrenal gland and reproductive systems [Parker, 1998; Goodfellow and Camerino, 1999]. Transcripts of both genes can be detected in specific endocrine tissues: the hypothalamus, anterior pituitary, adrenal glands, gonads, and placenta [Ikeda et al., 1996]. DAX-1 is an atypical orphan receptor. The carboxy-terminal region is homologous to the ligand-binding domain of nuclear receptors, but the DNA-binding region lacks the characteristic zinc finger motif and instead consists of three and a half repeats of a 65- to 67-amino-acid motif that is rich in glycine and alanine residues and has been proposed to have a DNA-binding function (Fig. 2). In contrast, the structure of the SF-1 protein has a more typical orphan receptor structure consisting of an N-terminal DNA-binding domain of tandem zinc finger motifs, a proline-rich region, and a ligand-binding domain (Fig. 2). To date,
In 46,XY males, loss of DAX-1 located at Xp21.3 [Zanaria et al., 1994]. Or DAX-1 have been identified. no high-affinity ligands for either SF-1 or DAX-1 have been identified. SF-1 will bind to DNA with high affinity and is a positive transcriptional regulator of a variety of steroidogenic enzyme genes in the adrenal glands and gonads [Lala et al., Morohashi et al., 1992; Wong et al., 1996; Parker and Schimmer, 1997]. The DAX-1 gene is located at Xp21.3 [Zanaria et al., 1994]. In 46,XY males, loss of DAX-1 function is associated with congenital adrenal hyperplasia and hypogonadotropic hypogonadism, but testis determination is normal (cryptorchidism is common, however) [Muscatelli et al., 1994]. Duplications of a 160-kb region that includes DAX-1 (the DSS locus) are associated with 46,XY gonadal dysgenesis and the consequent development of a female phenotype [Bardoni et al., 1994]. In mice, multiple copies of the Dax-1 gene cause sex reversal but only in the context of a weak Sry allele [Swain et al., 1998]. The absence of Dax-1 does not affect murine testis determination [Yu et al., 1998]. These data suggest that SRY and DAX-1 act antagonistically (see later discussion). Although DAX-1 was once considered to be a candidate ovarian determining gene, this hypothesis seems unlikely, since Dax-1 null mutant XX mice have normal ovarian development and function [Yu et al., 1998].

In mice that lack Sf-1, gonads and adrenal glands fail to develop, and these mice have hypogonadotropic hypogonadism, indicating hormone deficiencies at the hypothalamic and pituitary gland levels [Luo et al., 1994]. The gonads of mice lacking Sf-1 show developmental arrest between 11 and 11.5 dpc, which is after the first expression of Sry but immediately before the first morphologic signs of testis formation. In the human, a heterozygous mutation has been reported in the SF-1 DNA-binding domain (G35E) that abolishes DNA binding but not protein translation, stability, or nuclear localization [Achermann et al., 1999]. It is associated with 46,XY partial gonadal dysgenesis (immature tubules present in the testis), normal female external genitalia, and a uterus. This finding suggests that SF-1 functions as an important regulator of male sexual development after testis determination, which is consistent with the role of SF-1 as a positive transcriptional regulator of male-specific genes. These include the genes encoding enzymes necessary for testosterone biosynthesis, insulin-like 3 (INSL3) gene (recently shown to be necessary for correct testicular descent in mice) [Zimmermann et al., 1998; Nef and Parada, 1999], and the genes encoding AMH and its receptor [Shen et al., 1994; Giuili et al., 1997; Barbara et al., 1998]. In contrast to SF-1, DAX-1 functions as a transcription repressor. DAX-1 will repress the expression of StAR in cotransfection studies using a StAR promoter reporter construct [Sandhoff and McLean, 1999]. DAX-1 also has been implicated in the repression of p450scc and 3β-HSD [Lalli et al., 1998].

During mouse urogenital development, the expression profiles of both Sf-1 and Dax-1 are sexually dimorphic. Dax-1 is expressed in somatic cells of the genital ridge at 10.5–11 dpc and peaks at 12 dpc. In males the levels of Dax-1 transcripts decline as testis cords develop, whereas in females the levels of Dax-1 are unchanged, and the gene is expressed during ovarian development [Swain et al., 1996]. It should be noted, however, that in the rat the levels of Dax-1 actually increase in the male after testis determination and decline in the female [Nachitagl et al., 1998]. Sf-1 transcripts are first detected in the urogenital ridge of male and female mice at 10 dpc [Ikeda et al., 1994]. In male mice, expression is up-regulated following testicular determination, and expression can be detected in both Sertoli and Leydig cells. The sexually dimorphic expression of SF-1 and its role in male steroidogenesis suggest that it is located downstream of SRY in the cascade of gene activation necessary for male differentiation. The fact that ovaries fail to develop in XX female mice lacking Sf-1 suggests the critical role of Sf-1 in gonad development irrespective of the sex-determination process. The overlapping expression profiles and functional roles of SF-1 and DAX-1 indicate that they may be components of a common regulatory hierarchy.

An SRY-related protein, SOX9, also has been implicated in the mammalian sex determination process. SOX9 is necessary for cartilage formation and male sex determination, WY. Loss-of-function mutation is responsible for campomelic dysplasia; 60% of individuals with 46,XY, have sex reversal [Cooke et al., 1985; Foster et al., 1994; Wagner et al., 1994]. Like Sf-1, Sox9 exhibits sexually dimorphic expression profiles during gonadal development [Kent et al., 1996]. Sox9 is first expressed in the mouse urogenital ridge at 10.5 dpc in both sexes. At 11.5 dpc, SOX9 is expressed strongly in males and dramatically down-regulated in females. In males, expression is observed in the same cell lineage as SRY (Sertoli cells), which suggests that SOX9 may be a direct downstream target of SRY in the sex-determination cascade. This sexually dimorphic expression profile is conserved in both chicken and turtle and indicates that SOX9 may be a key...
regular of vertebrate male development [Moraes de Silva et al., 1996].

Several other factors are necessary for correct testis development. Mice that lack the homeobox gene Lim1 do not have head structures and die early [Miyamoto et al., 1997]. Surviving fetuses lack kidneys and gonads. Emx2 is the mammalian homologue of the Drosophila head gap gene empty spiracles, which is essential for the development of the dorsal telencephalon. Absence of murine Emx2 is associated with severe developmental anomalies of forebrain development as well as urogenital anomalies [Pellegrini et al., 1996; Yoshida et al., 1997]; absence of kidneys, ureters, gonads, and genital tracts. M33 is a member of the mammalian polycomb group family. Polycomb group proteins act in a multiprotein complex as negative regulators of hox gene expression. Targeted disruption of the M33 gene results in homeotic transformation as well as gonadal dysgenesis [Katoh-Fukui et al., 1998]. There are relatively few studies of these factors, and their role in gonadogenesis or function in sex determination is unclear.

**AN INTEGRATED MODEL OF MAMMALIAN SEX DETERMINATION**

Of course, caution is required in extrapolating the current data, but an integrated model of sex determination and differentiation is emerging. Based on pedigree analysis of sex-reversed conditions, we previously suggested that SRY may function in sex determination to repress an inhibitor of male-specific genes [McElreavey et al., 1993]. Now we can begin to speculate on the factors involved in this process.

There is increasing evidence for a close functional relationship among the SF-1, DAX-1, and WT1 proteins. WT1-KTS protein did not bind to the AMH-reporter sequence, a DNA component provided by the AMH-RE1 element was required for WT1 synergy with SF-1. The addition of a DAX-1 expression construct to this system resulted in a dramatic reduction of AMH activation achieved through heterodimerization with the SF-1 protein, though there was no evidence of DAX-1 DNA motif. In a yeast two-hybrid system, interaction was observed between Dax-1 and SF-1 proteins, but not between Dax-1 and WT1. The major sites of interaction on the SF-1 protein for DAX-1 and WT1 appear to be distinct. DAX-1 therefore antagonizes the functional interaction between SF-1 and WT1–KTS. In Frasier syndrome there is an imbalance between WT1 ~KTS and WT1 +KTS isoforms. The reduction in +KTS isoforms may result in more of the ~KTS being recruited into the spliceosome complex, thereby reducing the functional interaction of the ~KTS isoform with SF-1. An alternative hypothesis, proposed by Hastie and colleagues [Davies et al., 1999], states that the +KTS as part of the splicing apparatus is the key component of urogenital development mediated by WT1. Obviously a reduction in this isoform by aberrant splicing would have dramatic effects on sexual development.

Other cofactors are likely involved in this process. Co-activators and co-repressors play an important role in modulating ligand-activated nuclear receptors. Experiments by Crawford et al. [1998] have shown that Dax-1 recruits the nuclear receptor co-repressor N-CoR to SF-1. Nuclear receptor corepressor molecules transduce repressive transcriptional signals to orphan nuclear receptors by recruiting a complex of proteins, which ultimately results in the deactylating of histones. Furthermore, the interactions of SF-1, DAX-1, and N-CoR require a number of domains within each molecule. To interact with Dax-1, SF-1 requires a carboxyl-terminal repressive domain and a proximal interactive domain. The latter domain is also required for SF-1-mediated transactivation [Crawford et al., 1997]. To interact with SF-1, DAX-1 requires its DNA-binding domain, while its repressive function requires two regions of the ligand-binding domain—both of which are required to recruit N-CoR to DNA (transcriptional silencing domains). Other potential cofactors involved in AMH expression include SOX9 and GATA-4 [De Santa Barbara et al., 1998; Tremblay and Viger, 1999].

As mentioned earlier, both SF-1 and WT1 are essential for male development. Increased levels of DAX-1 protein, as in 46,XY individuals with Xp duplications, may result in inference with male-specific gene expression via the SF-1 protein. These data suggest that after SRY expression, both SF-1 and WT1 (~KTS) act downstream to control the expression of male-specific genes (perhaps including SOX9). Antagonism of this process, such as through interference by DAX-1 of SF-1/WT1 synergy, will lead ultimately to ovarian development. A function of SRY may be to repress DAX-1. Of
course, other cofactors are involved in this process, and in some cellular or promoter contexts the presence or absence of a particular ligand may convert a molecule from a repressor to an activator or vice versa. Some of these missing pieces of the jigsaw puzzle may be encoded by genes located at 9p24.3, 2q33, and 10q26 [Slavotinek et al., 1999; Wagggoner et al., 1999; Veitia et al., 1998], since deletions of these regions are associated with a failure of testis determination.

REFERENCES


