THE SRY GENE: WHAT MAKES A MAN A MAN

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INTRODUCTION

Sexual differentiation is a sequential and ordered process beginning with the establishment of chromosomal sex at fertilization followed by the development of gonadal sex and culminating in the formation of the sexual phenotypes (Fig. 1). Each step in this process is dependent on the preceding one, and under normal circumstances chromosomal sex agrees with phenotypic sex. Occasionally, however, chromosomal sex and phenotypic sex do not agree, or the sexual phenotype is ambiguous. Such abnormalities of sexual development are rarely life-threatening, and the analysis of these disorders in humans has been especially informative in defining the molecular and genetic determinants of sexual development. Consequently, the overall process of sexual differentiation is understood in greater detail than any other embryonic system. What I propose to do today is to review the current understanding as to how the Y chromosome promotes testicular differentiation and to describe how this information has both depended on the study of and provided insight into the pathogenesis of several human disorders, including 46,XY women with the syndrome of pure gonadal dysgenesis and 46,XX men with true hermaphroditism or the sex reversal syndrome.

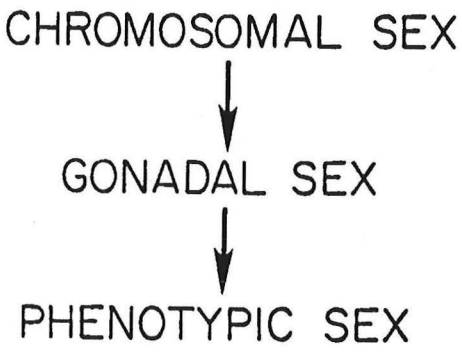


Fig. 1. The Jost Paradigm for Sexual Differentiation

CHROMOSOMAL SEX

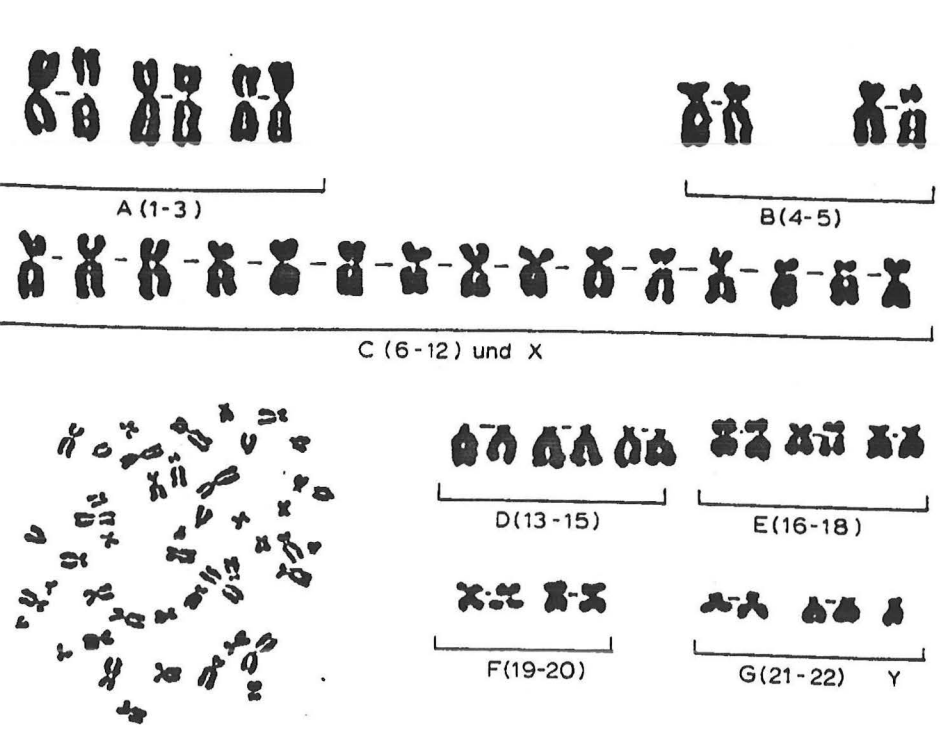
In the 1920's it was established by T.S. Painter at the University of Texas at Austin that in all mammalian species, including the human, females have two X chromosomes whereas males have one X and a chromosome not present in the female termed Y (1). It had previously been shown by T.H. Morgan and his colleagues that sex in the fruit fly Drosophila melanogaster is also determined by a similar chromosomal mechanism, the XX individual being female and the XY being male. These investigators further established that the critical feature for sex determination in the fruit fly is the number of X chromosomes; since XX is female and XO is male (albeit infertile) the Y chromosome can play no role in the process except to promote male fertility (reviewed in Ref. 2). It was generally assumed (and I was taught) that this system applied to all species; namely that in the human, as well, gonadal differentiation is a function of the X chromosomes, two X chromosomes causing female development and one X causing male development. In this model the Y chromosome was thought to carry no information essential for sex determination.

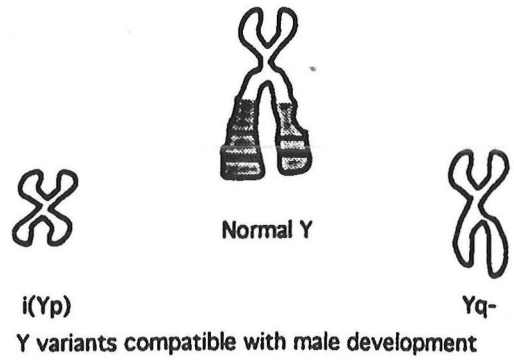
This formulation was thought to apply until the 1960's when it was established that the human Y chromosome specifies the development of the testes (subsequently shown to be true in all mammals). Namely, the 45,XO human is female, and no matter how many X chromosomes are present, a testis will develop as long as a Y chromosome is present (as in 47,XXY, 48,XXYY, 48,XXXY, 49,XXXXY, etc.) (reviewed in Ref. 2). Furthermore, the X chromosome does not appear to play an essential role in testicular development; although the 45,Y karyotype is not viable in the human, the X chromosome in the creeping vole is eliminated in the testicular germ line of cells, implying that the Y chromosome together with the autosomes carry all the necessary information both for testicular differentiation and spermatogenesis (3).

THE Y CHROMOSOME

The Y chromosome is one of the smallest of the human chromosomes (Fig. 2A), although the size can vary considerably in length due to variation in the length of the long arm (4). (Fig. 2B). At least three chemical features of the Y are thought not to be related to its role in sexual differentiation:

1.) The Y of virtually all species including the human contains satellites of DNA that are visible with special stains; because the Y-associated satellite DNA of one species does not cross-hybridize with that of another species, these satellites are not thought to play a role in the function of the chromosome (5).





A B

Fig. 2. A. The Y chromosome is one of the smallest of the human chromosomes, usually included in group G. B. The fluorescent part of the long arm of the Y chromosome is indicated by the stippled area.

2.) About 70 percent of the DNA of the human Y consists of repeated sequences on the long arm (6). These repetitive fragments are present in such diverse species as Drosophila, mouse, and the human and are composed of two repeating base quadruplets (GALA and GALA). These sequences are responsible for fluorescence of the Y, and variations in the number of repeats among normal men are thought to be responsible for the variability in the length of the long arm of the chromosome (Fig. 2B). These sequences are homologous to sequences on the human X chromosome, but there is no perceptible phenotypic effect when the long arm of the Y is either completely [i(Yp)] or partially (Yq-) deleted (Fig. 2B) (7). In brief, the long arm of the Y chromosome is thought to consist largely of "junk" DNA. In contrast, deletion of the short arm of the Y[i(Yp)] causes female development, indicating that the short arm carries the critical determination.

3.) A region on the distal tip of the short arm of the Y chromosome (Yp) is highly homologous to a similar region on the short arm of the X chromosome (8). These regions (the pseudoautosomal regions) are responsible for the pairing of the X and Y chromosomes during meiosis (see below). Although these sequences of DNA are specific to the sex chromosomes, they differ widely among species and are not thought to play a role in sex determination (9).

The critical portion of the Y chromosome for sex determination (and the focus of today's discussion) is the region of the short arm between the pseudoautosomal boundary and the centromere (10) (Fig. 3). A genetic map of the human Y chromosome has been constructed by assembling recombinant DNA clones, each containing a segment of the chromosome, into a single overlapping array (11). This map reveals that the Y-encoded genes are present partially in Y-specific sequences and partially in X-homologous sequences.

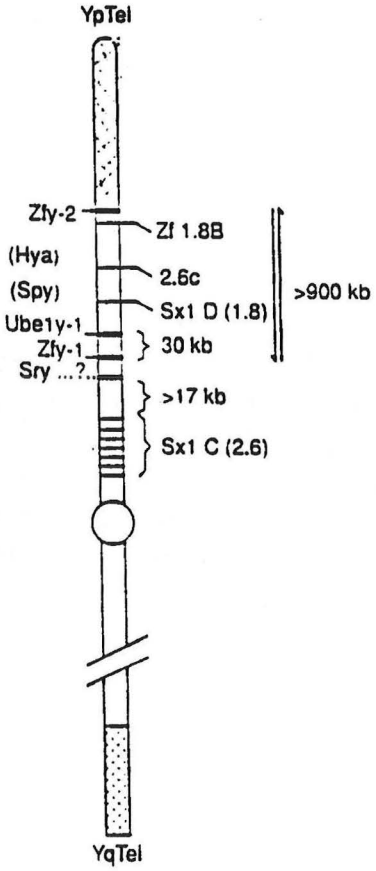


Fig. 3. Schematic diagram of the short arm of the mouse Y chromosome. The pseudoautosomal region is designated by the cross hatching.

PAIRING OF THE X AND Y CHROMOSOMES: PSEUDOAUTOSOMAL INHERITANCE

The two X chromosomes in females pair at the centromere and segregate during the first meiotic division of oogenesis by a mechanism analogous to the pairing of the autosomes to assure segregation of the chromosomes at cell division. The X and Y chromosomes also pair during spermatogenesis to assure appropriate segregation. However, the pairing of X and Y does not occur at the centromere but rather at the region of homology on the distal ends of the two chromosomes (12) (Fig. 4). In this way, the chromosomes duplicate and partition properly on the spindle, and two types of spermatozoa are produced at the second meiotic division, those containing a single X and those containing a single Y. The pairing of the two chromosomes causes the formation of the XY body that is identifiable in nucleoli between zygotene and mid pachytene (13).

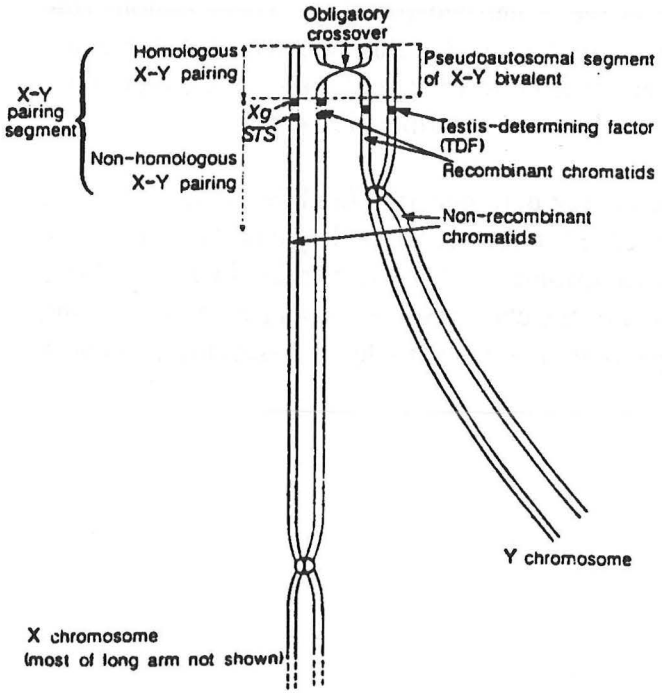


Fig. 4. Crossing over between the human X and Y chromosomes. The pseudoautosomal region is that part of the X-Y where crossing over occurs.

The genetic importance of this pairing first became apparent when it was recognized that crossing over is a consistent feature of meiosis and that this provides an explanation for the phenomenon termed pseudoautosomal inheritance. Garttler and his colleagues demonstrated that the enzyme steroid sulfatase in the mouse, which appeared to be inherited autosomally, is in fact encoded in the homologous region on the short arm of either the X or Y and that obligatory crossover proximal to the locus for this enzyme accounts for the fact that the gene appears to be inherited in an autosomal manner (14). Similar pseudoautosomal loci have been described in the human (15-17). It is noteworthy, as will be noted later, that the sex determining Y region (here labelled TDF) is located just proximal (within 20 kb) to the crossover site.

THE SEX-DETERMINING REGION Y (SRY) GENE

Cytological analysis of structurally abnormal Y chromosomes in humans has been extraordinarily useful in localizing the critical regions of the chromosome, beginning with the pioneering studies of Jacobs and Ross in the 1960's (18, also see Ref. 2 for review). Loss of the fluorescent segment of the long arm or formation of an isochromosome with a duplicated short arm and no long arm whatsoever are both compatible with formation of a normal testis (Fig. 2B). However, isochromosomes for the long arm of the Y in which there is loss of the short arm causes failure of testicular development and formation of a female phenotype (Fig. 2B). On the basis of such studies it was deduced that the testis determining region is located near the centromere on the short arm.

By studying patients with more and more subtle abnormalities it was eventually possible to map the human Y chromosome solely on the basis of naturally occurring deletions (19) (Fig. 5). In

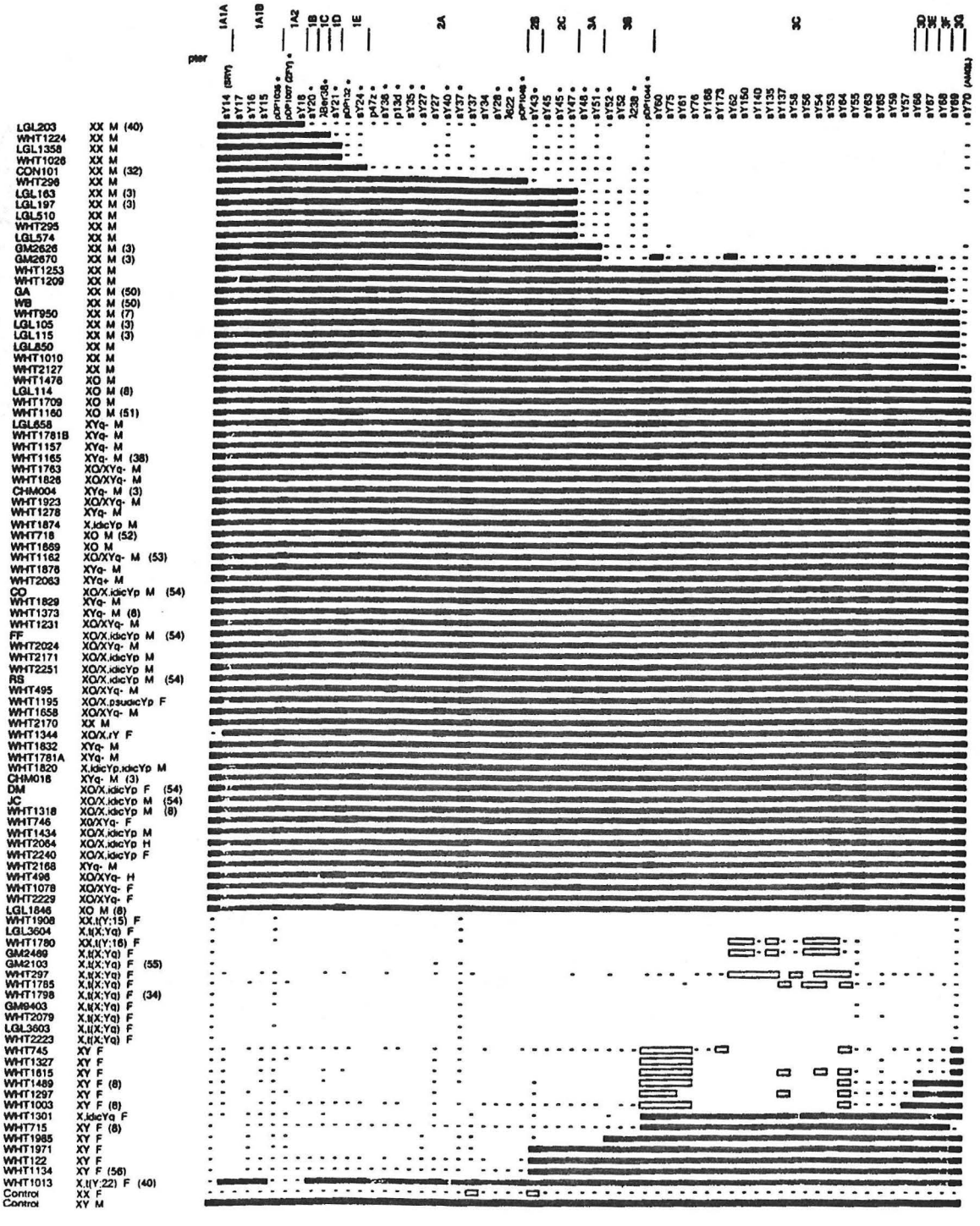


Fig. 5. Deletion map of the short arm of the human Y chromosome. Data from XX males are shown at the top, and data from XY females are shown at the bottom (Ref. 19).

this regard, the most informative such deletions were associated with the 46,XY form of pure gonadal dysgenesis. [Other maps of the Y have been constructed using restriction fragment and hybridization techniques (20,21)].

Elucidation of the mechanism by which the Y dictates testicular development has not been so easy. Fisher originally proposed two alternative mechanisms for Y chromosome control of sex; either all the genes encoding testicular development are Y linked, or the Y chromosome encodes a single control gene that regulates expression of genes elsewhere in the genome (22). All the evidence is compatible with the second alternative, and there has been an enormous effort expended in the past two decades to identify this critical control gene. There were in fact two major false starts, namely claims for a primary role for genes encoding HY antigen and the so called zinc finger Y protein; these false starts, which have formally been disproved, were reviewed by Kittie Wyne for a recent Endocrine Grand Rounds (March 23, 1994) and will not further concern us today.

However, by 1987 the gene was known to be located within 200 kb of the pseudoautosomal boundary, and in 1990 Sinclair and his colleagues utilized gene walking techniques to identify a single copy gene termed the Sex determining Region Y (SRY) gene in the 1A1 region (Fig. 6) (23). The

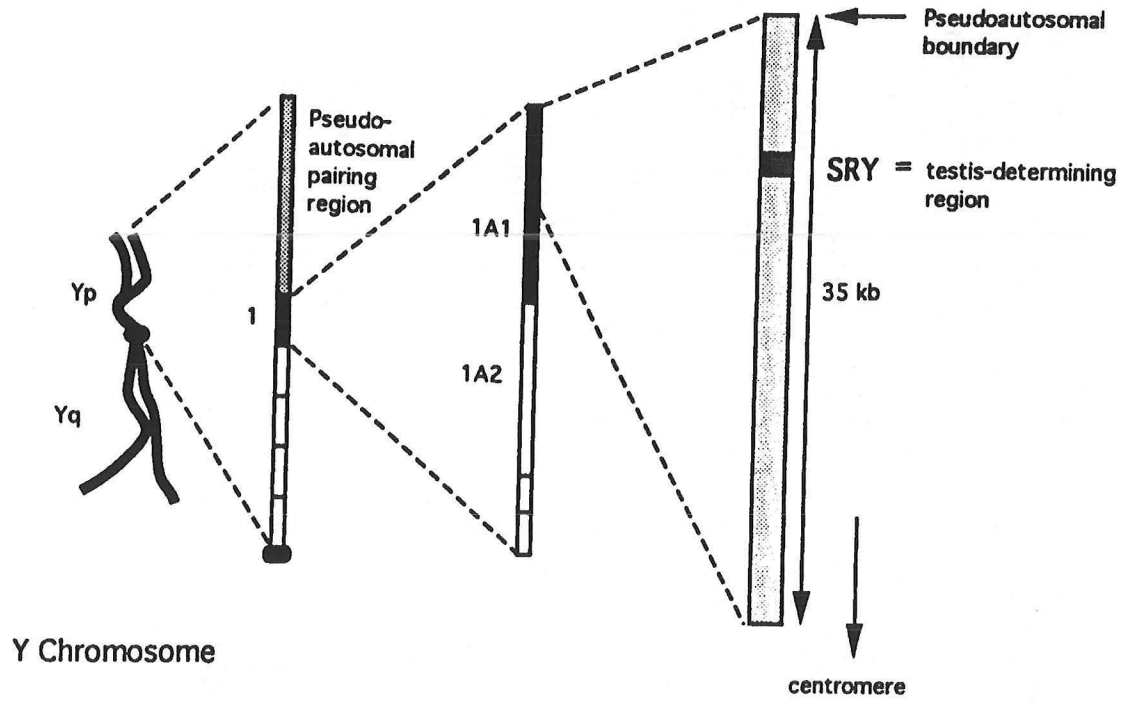


Fig. 6. Location of the Sex determining Y Region of the Y chromosome (Ref. 50).

SRY was shown to be conserved on the Y chromosome of all mammalian species tested including the mouse (24, 25), and direct evidence that SRY is the testis determinant was obtained when a 14 kb transgene containing the mouse SRY gene was introduced into mouse embryos and shown to cause XX mice to develop into male mice with testes, male secondary sex characteristics, and male sexual

behavior (26) (Fig. 7). The XXSRY transgenic males are infertile because of the presence of two X chromosomes which lead to an arrest in male meiosis (27) and/or because of the absence of other loci on the Y chromosome that are necessary for spermatogenesis (28).

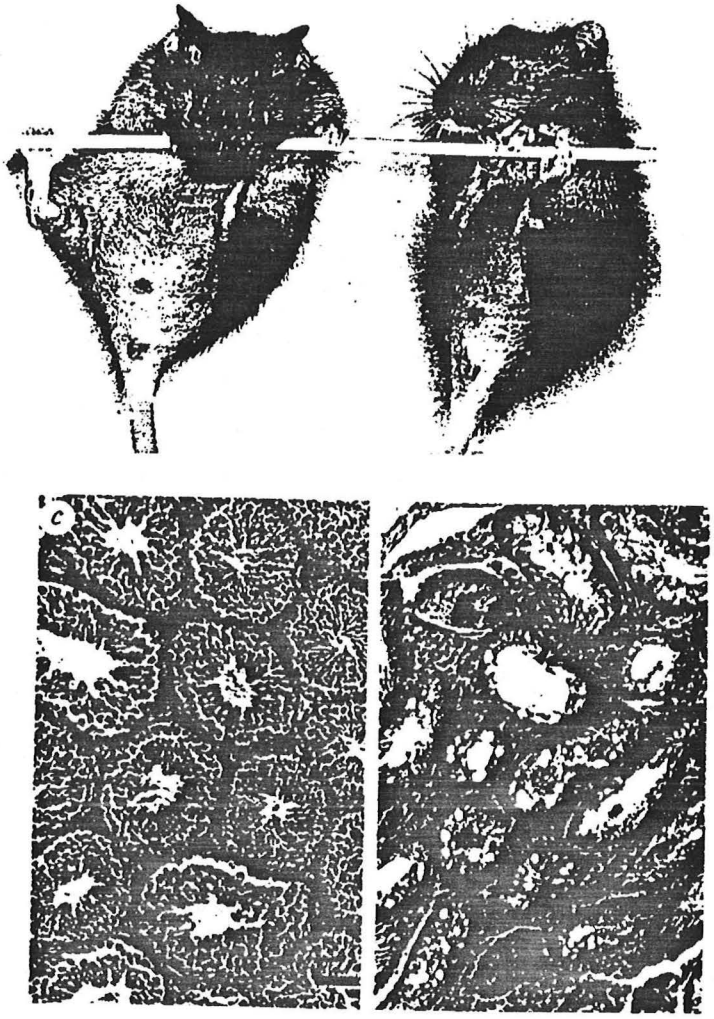


Fig. 7. Upper Panel: XY (left) and XXSRY transgenic male mice (right). Lower Panel: Histology of the testes of the male (left) and transgenic (right) mice (Ref. 26).

The SRY coding sequence, which contains no introns, has now been cloned from several species, including marsupials, the rat, the human, the mouse, and the rabbit (29) (Fig. 8). There are extensive sequence differences in the 3′ and 5′ regions of the gene and differences in the length of SRY among the species, and it is striking that the human gene, although expressed appropriately in transgenic animals, is not functional in the mouse. However, there is a high degree of homology in one region of the coding sequence among the various species, namely in the sequence between amino acids 60 and 140 (29). This similarity is illustrated by comparison of the mouse and human sequences which are 90% identical in this region (Fig. 9). Transcription of the human SRY involves multiple start sites, a principal site that results in a 900 nucleotide RNA transcript and at least one additional start site approximately 400 base pairs upstream that results in a much longer mRNA (30).

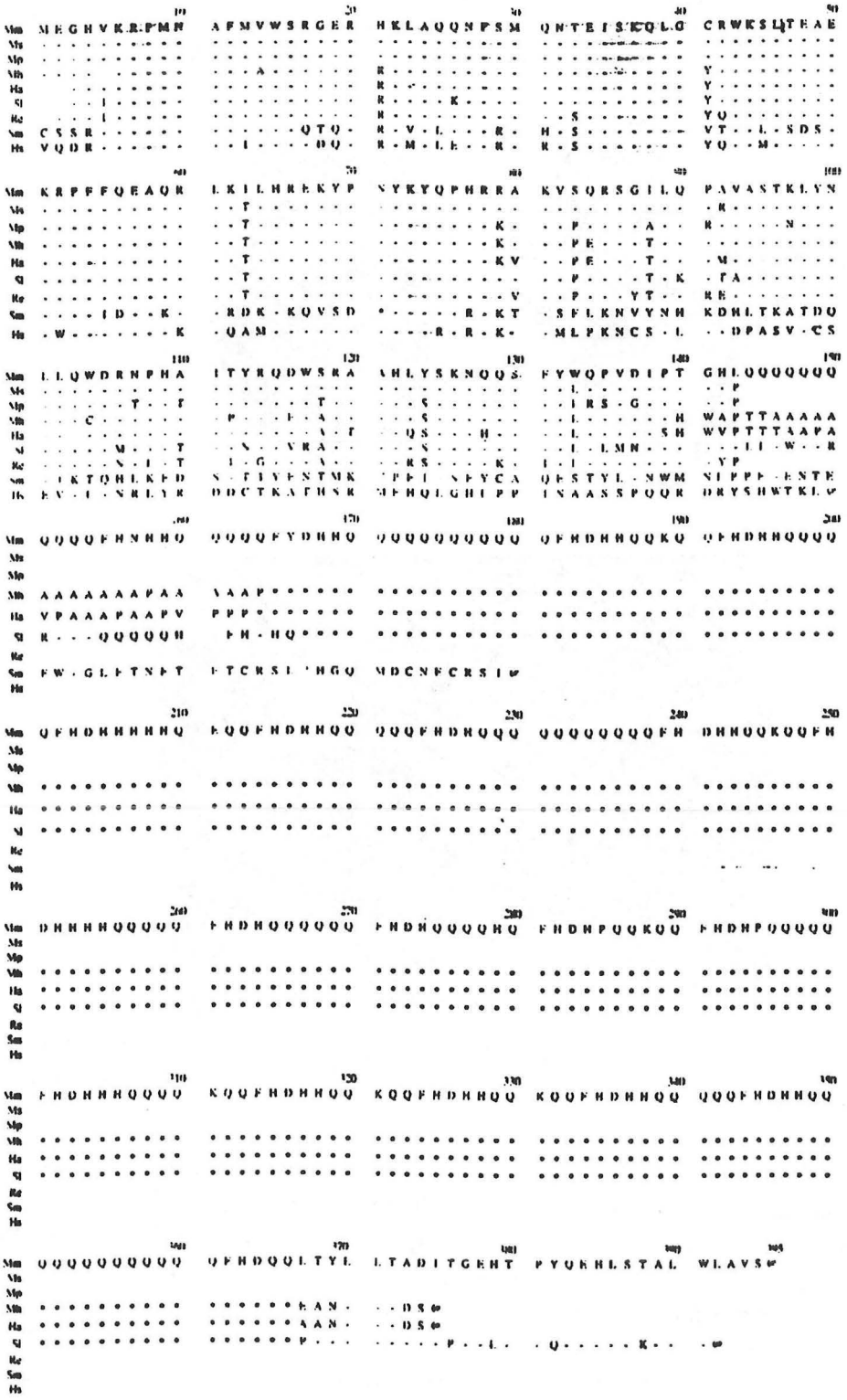


Fig. 8. Alignment of the SRY sequences from six species of mice, rat, marsupial, and human (Ref. 29).

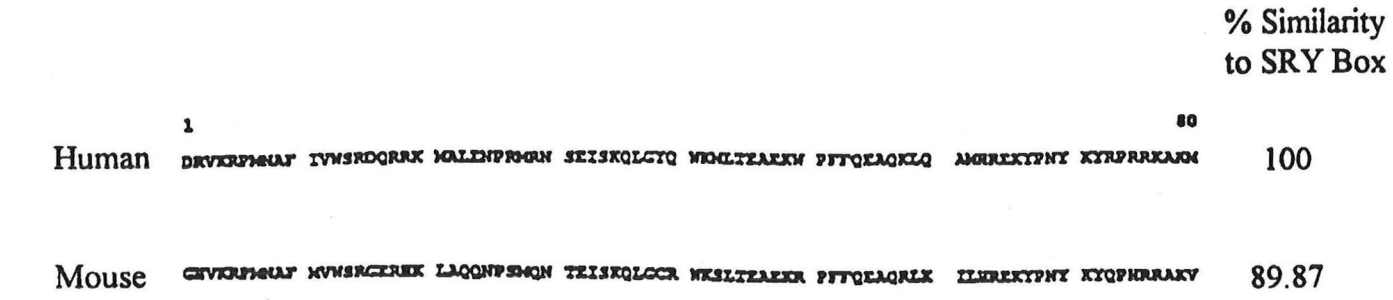


Fig. 9. Comparison of the sequences in the HMG box of Human and Mouse SRY.

Both of the mRNAs are believed to encode a protein predicted to contain 204 amino acids (30). Low level of expression of the RNA transcripts can be identified in several extragonadal tissues of the embryo, including brain and spleen, but these are thought to be nonfunctional (29).

In the adult mouse SRY is expressed in the testis, probably in round spermatids, but the testis transcript exists as a circular RNA, thought to be formed by the splicing of a longer primary transcript (Fig. 10). How this circular transcript functions and whether it is transcribed is unclear.

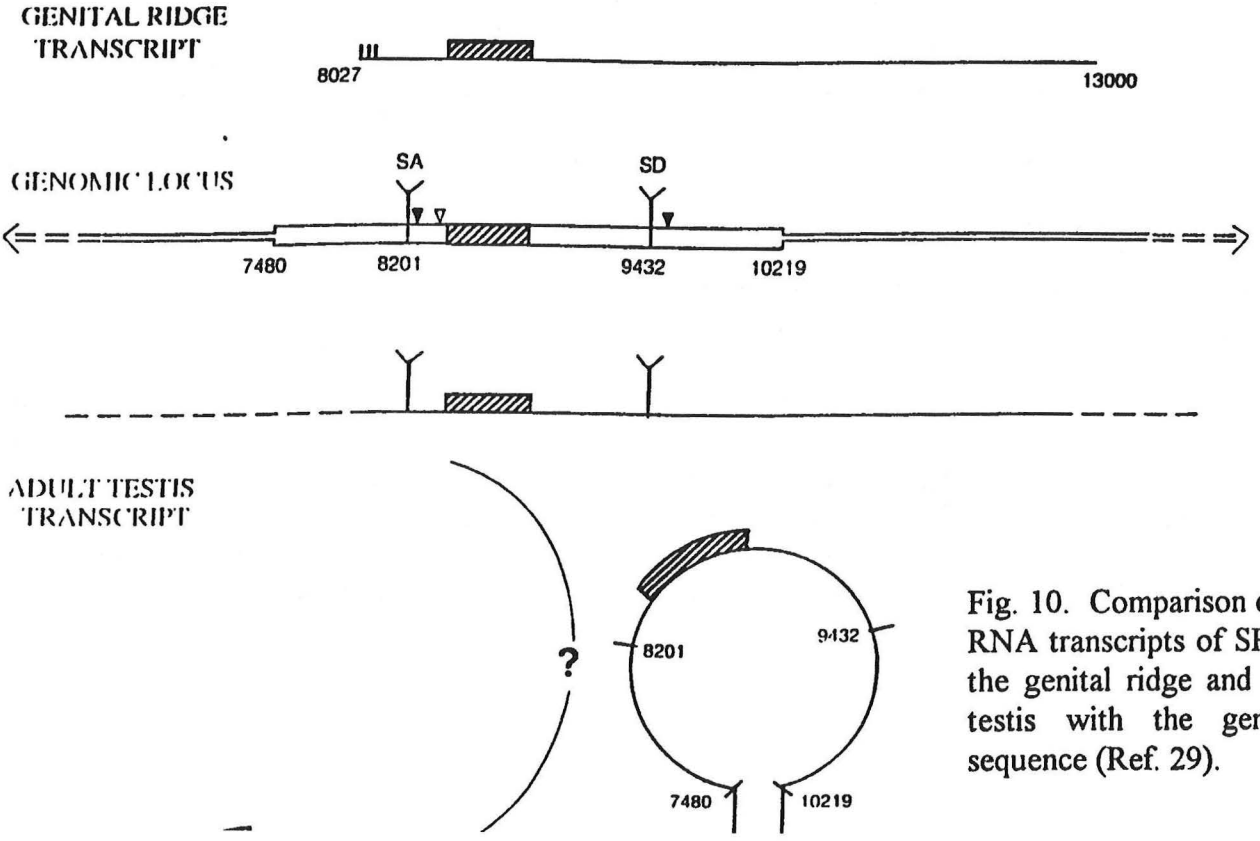


Fig. 10. Comparison of the RNA transcripts of SRY in the genital ridge and adult testis with the genomic sequence (Ref. 29).

SRY is expressed in the urogenital ridge of the male mouse embryo during the narrow window of time in development when the differentiation of the cords commences in the fetal testis (25) (Fig. 11). This correlation is striking in terms of its timing and its spatial and sexual restriction. It is particularly striking that expression of the gene is virtually undetectable in this tissue after day 12.5 pc, even using the PCR reaction for its detection.

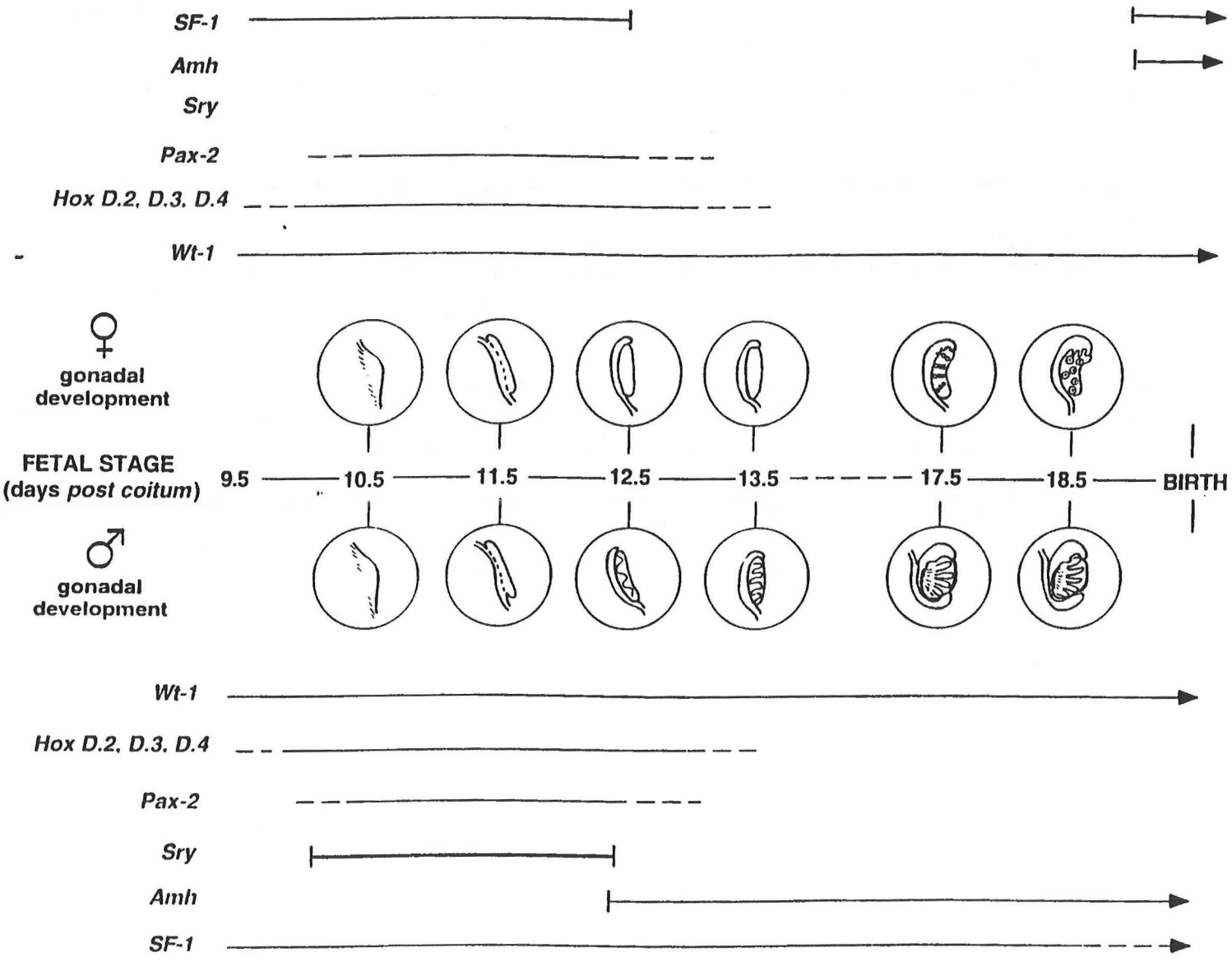


Fig. 11. Time course of expression of molecular markers in the urogenital ridge of the mouse embryo. Wt-1, Pax-2, and the Hox D genes are expressed identically in male and female gonads. SRY and antimullerian hormone (AMH) show sexually dimorphic expression, and Steroidogenic Factor-1 (SF-1) is expressed initially in both gonads and then shut off in the ovary (Ref. 29).

HOW DOES THE SRY GENE PRODUCT WORK?

The 80 amino acid sequence encoding the putative DNA-binding domain of the predicted protein product of the SRY gene shares a high degree of homology with a group of transcription regulatory proteins, the HMG box proteins (reviewed in 29). The HMG box was originally characterized in non-histone, high mobility group proteins present in chromatin. Some members of this family of proteins bind cruciform structural elements in DNA and show no sequence specificity. A second class of HMG-type proteins, to which SRY and its related genes (the so-called SOX genes) belong, exhibit both sequence-specific and structurally related binding to DNA (31). At least one of the SOX genes closely related to SRY is encoded on the X chromosome (32). Mutations that cause single amino acid substitutions in the HMG domain of SRY both impair binding of the protein to DNA and cause human disease (see below) so that it is believed that the DNA binding of the protein is essential to its function. The SRY HMG box intercalates in the minor groove of DNA to interrupt base stacking but not base pairing (33). However, the mechanism by which specificity is built into the system is unclear.

SRY protein can induce a 120 degree bend in DNA when bound, and the affinity of binding of the SRY is higher for bent than for linear DNA. However, since the amount of SRY protein in cells is orders of magnitude lower than the levels of the more ubiquitous members of this group of proteins that recognize similar motifs, it is not likely that SRY can perform such a function in vivo, although other transcription regulatory factors might lend specificity (34). Furthermore, the identified DNA motifs to which SRY and its related SOX proteins bind are so ubiquitous that it has proved difficult to utilize the techniques of modern molecular biology to identify the critical downstream genes that must be regulated by SRY. One group has suggested that SRY may function in part by activation of the AP-1 transcription family (35).

The findings with the XXSRY transgenic mice clearly indicate that most of the critical downstream genes must be encoded on chromosomes other than the Y itself. The most promising approach to identifying the downstream genes has arisen from studies on sexual differentiation in the mouse by Eva Eicher and her colleagues (36, 37). They made the observation that transfer of the Y chromosome from certain types of old world, wild mice (mus domesticus) into inbred laboratory strains derived ultimately from the house mouse (mus musculus) caused the production of hermaphroditic progeny; namely offspring carrying the domesticus Y (Y^{Pos}) had a high incidence of ovaries or ovotestes; when studied during embryogenesis all such animals start off as having ovotestes bilaterally, but in most the testicular element regress with time so that the adult animals tend to be sterile XY females. The most likely interpretation was that the SRY^{Pos} fails to interact with the critical downstream genes in common mouse strains to initiate normal testicular development (38). This possibility was made more likely when it was demonstrated that the SRY gene in various mus domesticus strains has a considerably different structure, including being shorter (Fig. 12). Indeed the suggestion has been made that the critical difference is in the number of CAG trinucleotide repeats (39). Eicher and her colleagues are now utilizing the techniques of recombinant inbred genetics in mice to attempt to identify the downstream genes. It may be that the true function of SRY is to repress a gene (termed Z) that functions in the absence of a Y chromosome to initiate ovarian development (40).

Table 1 Differences within the ORF of the *Sry*[129] and *Sry*[DOM] alleles[a]

Nucleotide position[b]	MUS	DOM	Consequence
8481	T	to C	Ile to Thr
8701	G	to T	Trp to Leu
8711	T	to C	No Change
8731	T	to C	Leu to Pro
8733–8738		to CAGCAG deleted	GlnGln deleted
8808–8813		to CACCAG deleted	HisGln deleted
8930	C	to G	His to Gln
8934	G	to C	Glu to Gln
9006	C	to T	Gln to Stop codon

[a] There are 15 changes not noted above that occur between position 9006 and 9489 (the stop codon for the 129 *Sry* gene). These include 7 base pair substitutions and 5 insertions.
[b] Numbers refer to the base pair position in GenBank entry X67204.

Fig. 12. Sequence differences between SRY from the house mouse Mus musculus (MUS) and Mus domesticus (DOM).

It is clear that we are still a long way from understanding how SRY plays its sex-determining role. What are its molecular targets? How is SRY itself regulated? What is the significance of its association with DNA bending? What cofactors are involved, and how is specificity achieved? What is the nature of the species specificity involved in the function of SRY? These and other questions are the subject of intense interest among workers in the field.

THE ROLE OF SRY IN HUMAN DISORDERS

Whatever the uncertainties about its mechanism of action elucidation of the structure and general function of SRY has provided insight into two puzzling disorders of human sexual development, namely those situations in which female development occurs despite the presence of a Y chromosome or in which testes develop in the absence of a Y.

46,XY Form of Pure Gonadal Dysgenesis

It has been recognized since the early 1960's that most instances of gonadal dysgenesis are due to either deletions or structural abnormalities of the X chromosome (33). The 46,XY form of pure gonadal dysgenesis encompasses women with bilateral streak gonads and sexual infantilism, as in the 45,X variety, but associated with a 46,XY karyotype. In this disorder the height is normal, and about 40% have some degree of feminization including a few with menses. Tumors may develop in the

streak gonads, particularly dysgerminoma or gonadoblastoma, and are frequently heralded by the appearance of virilizing signs.

Analysis of Y chromosomes from subjects with 46,XY gonadal dysgenesis associated with partial deletions of the short arm was central to the identification of the SRY gene (Fig. 5); such deletions totally prevent expression of the SRY gene, cause infertility in affected individuals, and usually arise as de novo mutations. However, the majority of 46,XY women do not have an abnormality of the Y that can be identified by light microscopy or by chromosomal banding techniques. Consequently, identification of mutations in the SRY in the genomes of two 46,XY women (42, 43) not only provided support for the critical role of SRY in male determination but was assumed to explain the pathogenesis of 46,XY gonadal dysgenesis. More than twenty such point mutations/small deletions in such patients have been described to date, all but one of these mutations have involved the HMG-box region of the gene (42-46) (Fig. 13). In some of these families more than one individual is involved (Fig. 14).

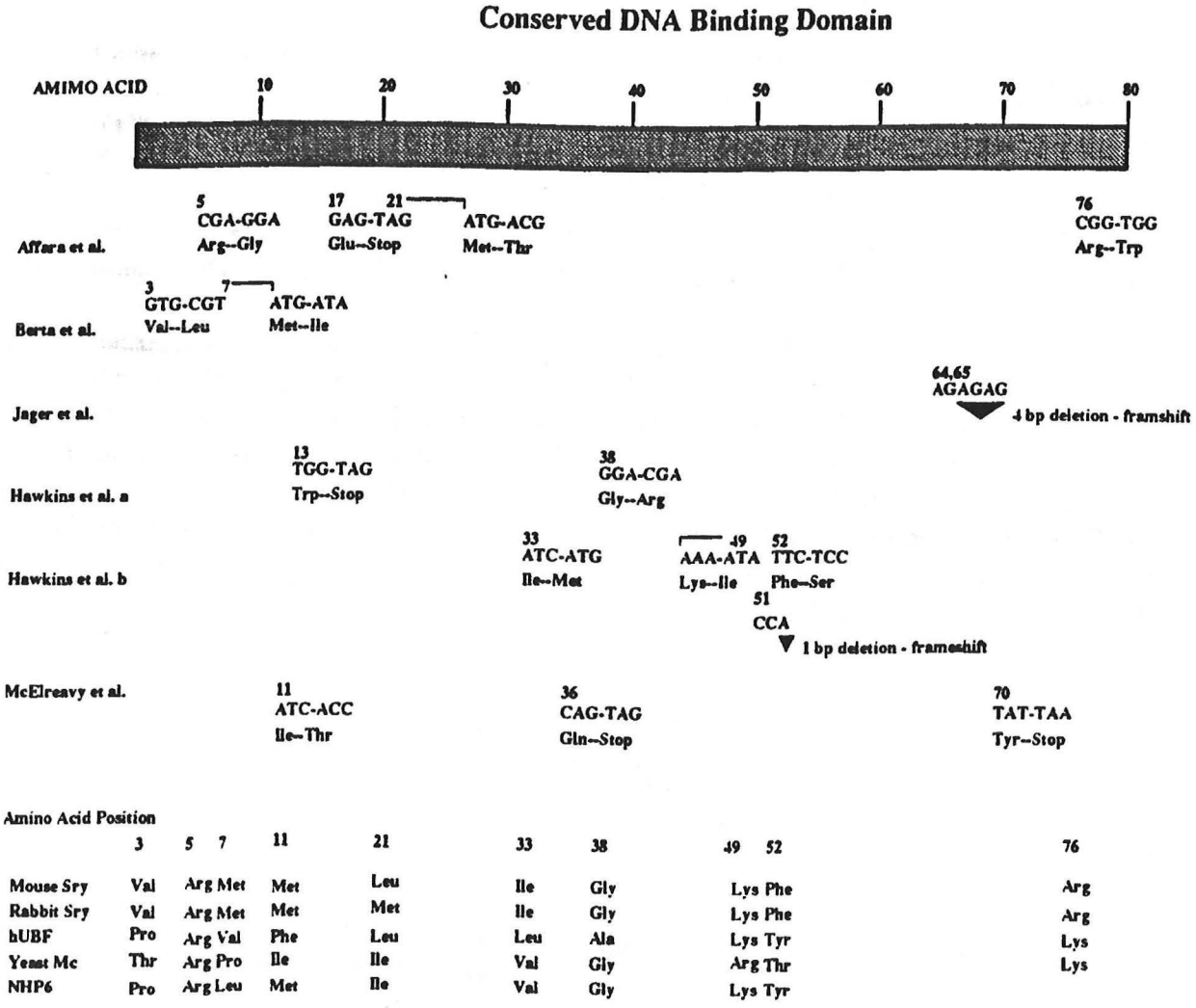


Fig. 13. Schematic representation of the HMG-box (DNA-binding domain) of the human SRY gene and the location of some of the mutations described in women with 46,XY gonadal dysgenesis (Ref. 44).

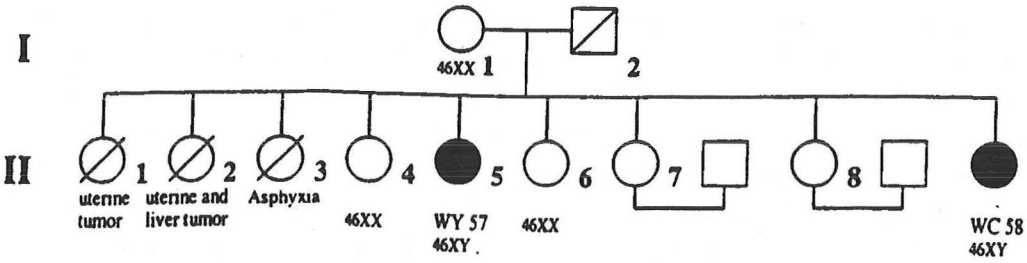


Fig. 14. Pedigree of a family in which two individuals with 46,XY gonadal dysgenesis have a premature stop codon at amino acid 17 of the HMG-box (Ref. 44).

At least two major surprises have come from these studies. First, only about 15% of 46,XY women have either a deletion or a mutation of the coding sequence of the gene; the etiology of these other subjects is unknown. Some may result from mutations in the SRY gene but outside the coding sequence; some may be due to mutations in downstream genes that are normally controlled by SRY analogous to the sterile XY female mice described by Eicher; some may be mutations in other genes that influence the expression of SRY. An example of the latter type of disorder has been described in the mouse, namely deletion of sequences outside the SRY region prevents expression of SRY and causes development of XY females (47). Second, some of these mutations have not been studied very carefully; none has been shown to cause an abnormal phenotype in transgenic animals. In most families the father has been shown to have a normal Y chromosome so that the mutation is assumed to have arisen de novo. However, in one family studied by Berta et al (42) the mutant Y chromosome was shown to be present in two males and three females (Fig. 15). This mutation causes a G to C substitution at position 508 and does result in diminished DNA binding, but it is not intuitively obvious how it can be associated with either male or female development in different individuals; either this is a non functional polymorphism, or it is a mutation that can function normally in some but not all genetic backgrounds.

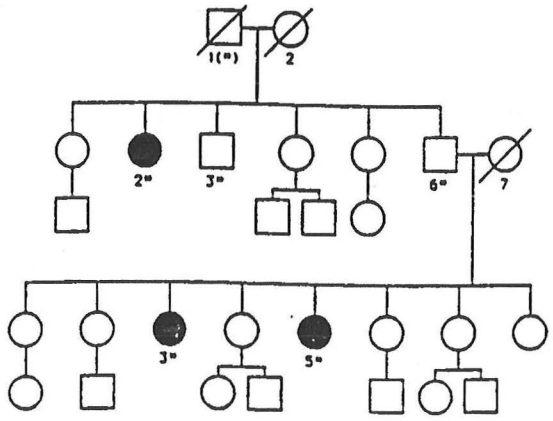


Fig. 15. Pedigree of a family in which 46,XY gonadal dysgenesis has passed through two generations. Individual II-6 has the abnormal Y chromosome but is a normal male. The mutation causes a single amino acid substitution in the HMB-box (Ref. 42).

The 46,XX Male and 46,XX True Hermaphrodite

Two other exceptions to the rule that the presence of a Y chromosome dictates development of a testis are the XX male and the XX true hermaphrodite. The phenotype of the XX male usually resembles that of the Klinefelter syndrome; the testes are small and firm (generally less than 2 ml in volume); gynecomastia is frequent; the penis is normal to small in size; azoospermia and hyalinization of the seminiferous tubules are usual. Such men differ from the typical Klinefelter subject in that the average height is less than in normal men and in that hypospadiasis more common (41). A subset of 46,XX men have ambiguity of the external genitalia at birth (see below).

True hermaphroditism is a condition in which both an ovary and a testis or a gonad with histological features of both (ovotestis) are present. The external phenotype displays all gradations of the female to male spectrum, but most have sexual ambiguity. There is no question but that this disorder, like the XX male, is genetically heterogeneous; approximately 70% have a 46,XX karyotype. Rare instances have been reported in which XX males and XX true hermaphrodites have existed in the same sibship (41). This feature suggests the possibility that in at least a subset these two disorders are variable expressions of the same fundamental defect.

Four theories have been proposed to explain how male development can occur in the absence of a Y chromosome: l) mosaicism in some cell lines for a Y containing cell line, 2) gain of function mutation of some autosomal gene, 3) deletion or inactivation of some gene or genes that normally suppress testicular development, and 4) interchange of a portion of the Y chromosome with the X chromosome.

In animal systems, examples of the latter two mechanisms have been clearly documented. For example, autosomal recessive mutations have been documented to cause XX sex reversal in the goat (48) and pig (49). Even more important has been the evidence from the XX male mouse. The sex reversal mutation in the mouse (Sxr) causes XX males to develop as phenotypic (but infertile) males (reviewed in Ref. 50). Y-specific DNA was detected on the distal end of an X chromosome in these mice in 1982 (51). The SRY gene on the distal end of the Y chromosome of mice carrying this mutation is believed to have duplicated. Translocation of this duplicated gene to the X chromosome during meiosis and transmission of the X chromosome carrying this sequence to XX offspring causes testicular differentiation (Fig. 16).

Application of similar techniques to the study of human disorders has been equally informative, and evidence has now been obtained for the operation of at least mechanisms l, 3, and 4 above in human XX sex reversal.

46,XX Males. Y chromosome sequences are detectable in approximately two thirds of 46,XX men and are found in the distal region of the X chromosome; this disorder is analogous to the situation in the XX sex reversal mouse (Fig. 5). The other third of 46,XX men are "Y-negative" and lack sequences for SRY. Clinically, the two groups differ in that the Y-negative group is more likely to have ambiguity of the external genitalia whereas the Y-positive group has the Klinefelter phenotype (52-54). The translocated region of the Y can be quite small and involve only the SRY gene itself;

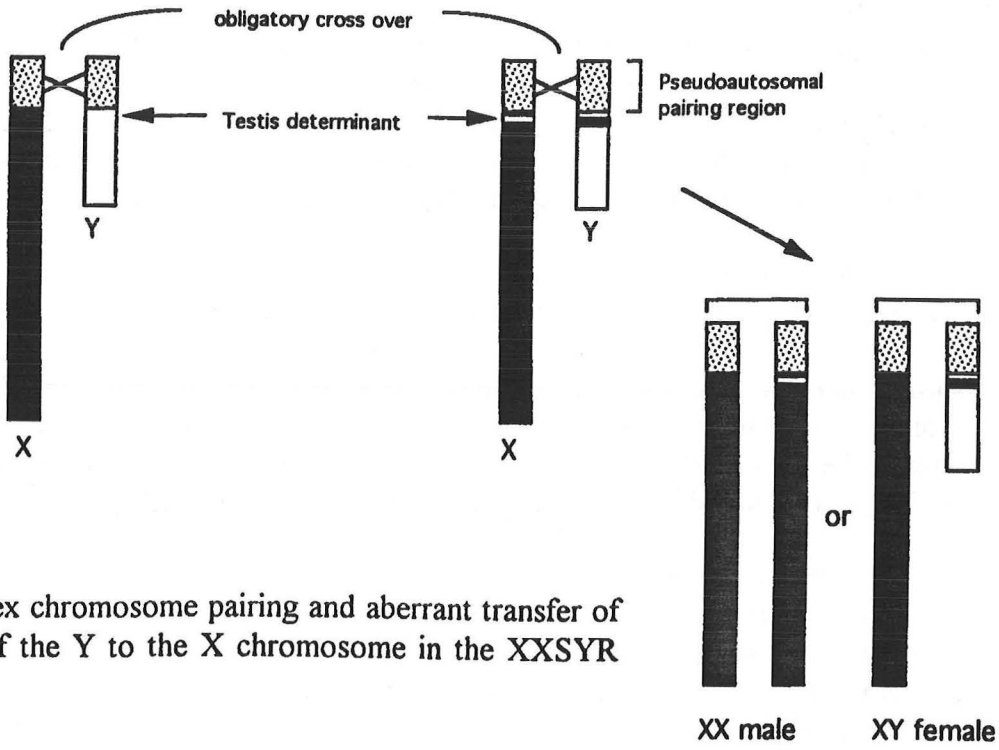


Fig. 16. Sex chromosome pairing and aberrant transfer of the SRY of the Y to the X chromosome in the XXSYR mouse.

such individuals are analogous to the situation in the transgenic mouse (54). In one study of ten 46,XX men Fechner et al found that six subjects had the SRY gene on the distal end of the X chromosome, one subject had mosaicism so that an intact Y was present in 1% of the cells, and three lacked Y sequences both by Southern blotting and by PCR analysis (55). Considered together, these various findings document the heterogeneity of the disorder, the majority having detectable Y chromosome sequences and the remainder of unknown etiology.

46,XX True Hermaphrodites. In contrast to the situation in the 46,XX males, the majority of 46,XX true hermaphrodites have no Y-specific sequences detectable (56-58). Indeed, in a study of 30 individuals with 46,XX true hermaphroditism, only three had detectable SRY, located as in 46,XX males to the Xp22 region of the X chromosome, suggesting that random X chromosome inactivation could lead to mosaicism of the testis determinant and result in development of both ovary and testis; mutations of other downstream genes were invoked to explain the 27 SRY-negative cases (59). A similar mechanism has been postulated to explain the development of testis and ovaries in other SRY-positive 46,XX true hermaphrodites (60). In 1990 it was suggested that SRY-negative true hermaphrodites and XX males are variable manifestations of the same mutation (Fig. 17); presumably if examined early enough all such individuals would have both ovarian and testicular tissue, but the ovarian tissue tends to regress with time, leaving the picture of XX males (56).

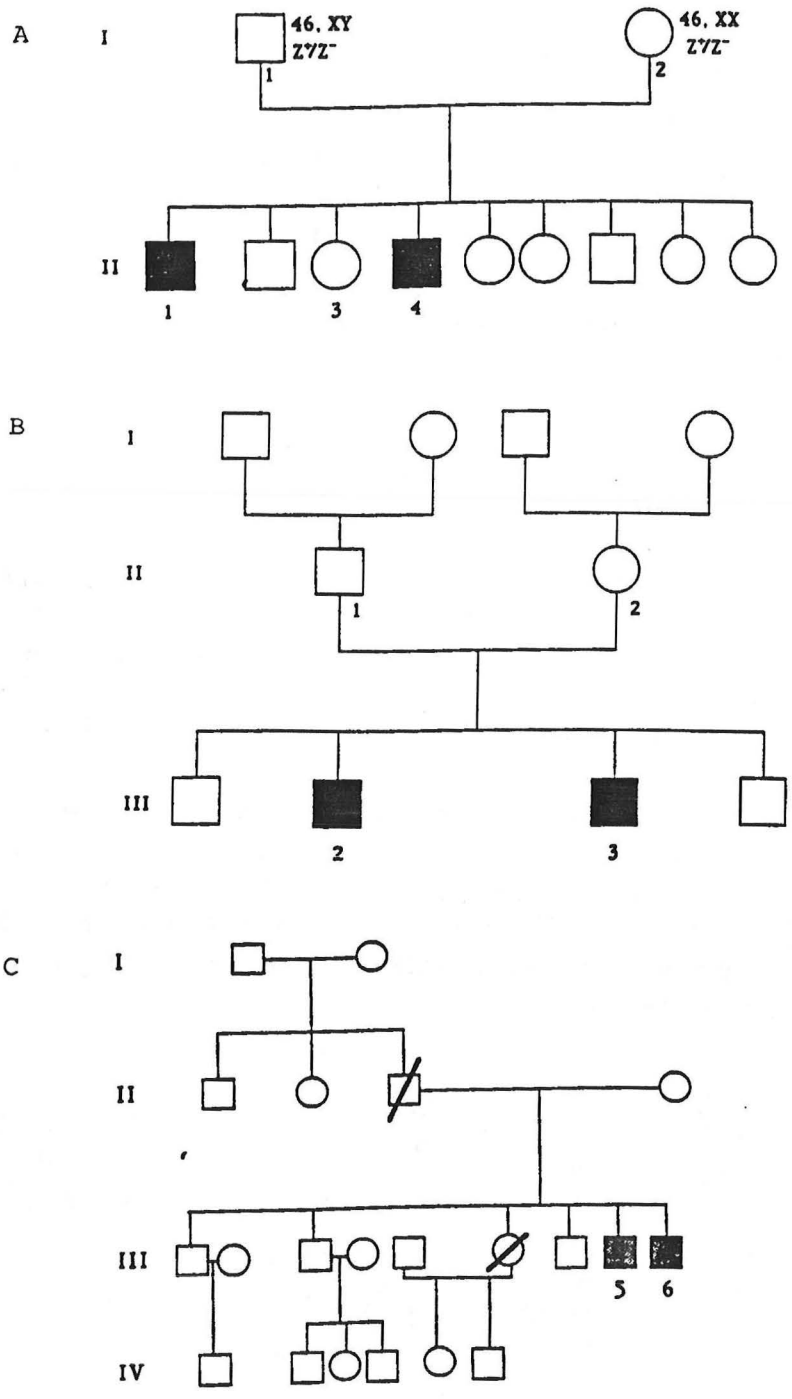


Fig. 17. Three SRY negative families in which 46,XX true hermaphroditism and/or 46,XX maleness is inherited in a fashion compatible with an autosomal recessive mechanism. In C.III.5 is a true hermaphrodite, and III.6 is a 46,XX male (Ref. 59).

In summary we have the following situation:

	46,XX Males	46,XX True Hermaphrodites
SRY Positive		
Translocations	70 %	10 %
Mosaics	1 %	
SRY Negative	30 %	90 %

In many instances it is difficult to separate 46,XX Males from 46,XX true hermaphrodites, as illustrated by the following patient who was evaluated at the Children's Hospital in the summer of 1993.

Case Report

M.A. was a 4 week old infant transferred from JPS Hospital for evaluation of ambiguous genitalia. Birth weight was 3317 g. The family history was uninformative, and the pregnancy was uneventful. Perineoscrotal hypospadias was present; the scrotum was bifid, and gonads were palpable in each hemiscrotum and were normally descended; the penis measured 2.5 cm. The chromosomal karyotype was 46, XX. Because "testes" were palpable bilaterally, the differential diagnosis was either 46,XX male or 46,XX true hermaphroditism.

On hCG stimulation, plasma testosterone rose appropriately. There was no evidence of a uterus on ultrasonography, and on urethography no wolffian derivatives were identified. Karyotyping on multiple skin fibroblasts excluded mosaicism and confirmed that the karyotype was 46,XX. No Y specific DNA including no SRY sequences were identified by fluorescent in-situ hybridization, Southern blot analysis using multiple probes, or PCR amplification. Surgery at age 10 months revealed the following anatomy (Fig. 18).

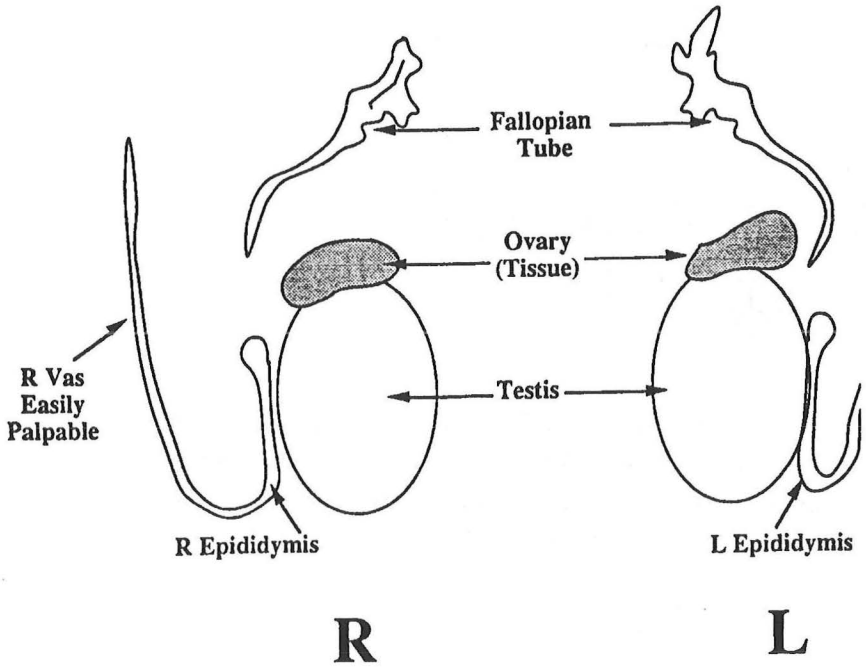


Fig. 18. Schematic diagram of the anatomical findings in M.A. at age 10 months.

The pathology revealed normal ovarian tissue containing numerous ovarian follicles at varying stages of development in the superior pole structures; the main gonadal structures contained testicular tissue with tubules composed of Sertoli cells. The child is thus a true hermaphrodite.

CONCLUSIONS

Demonstration that maleness is conveyed to the undifferentiated gonad by a single gene constitutes a signal advance in mammalian genetics and in understanding sexual differentiation and has provided insight into two types of human intersex - the 46,XY woman and the 46,XX male or true hermaphrodite. Elucidation of the mechanism by which this gene functions to cause differentiation of the testis and identification of the various genes involved in this regulatory cascade is now a major challenge and will almost certainly provide additional insight into these and other human disorders of human sexual differentiation. Insight into the genes that control ovarian development will follow, and in time we should be able to understand the entire program by which chromosomal sex is translated into phenotypic sex.

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