Cold Spring Harbor Symposia on Quantitative Biology

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Cold Spring Harb Symp Quant Biol 1986 51: 229-235
Access the most recent version at doi:10.1101/SQB.1986.051.01.028

Sex Reversal: Deletion Mapping the Male-determining Function of the Human Y Chromosome

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The Developmental Genetics of Sex Differentiation

Mammalian molecular geneticists face the challenge of understanding how a fertilized egg develops into a mature organism, with all of its complex organ systems. Much has been learned about the molecular correlates of differentiation in, for example, hematopoietic cell lineages, but the molecular mechanisms of the development of mammalian organ systems remain virtually unexplored.

In mammals, the reproductive tract will undoubtedly prove to be among the organ systems most amenable to developmental genetic studies. Consider the invertebrates, where developmental genetics has been pursued with greatest success in *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (nematode). It is no mere coincidence that, in both fruit fly and nematode, the best understood developmental pathway is sex differentiation (Belote et al. 1985; Hodgkin et al. 1985; Maine et al. 1985; Nothiger and Steinmann-Zwicky 1985). The reasons are twofold and they apply to mammals as well as to invertebrates. First, although mutations in the development of most other organ systems (e.g., circulatory system, respiratory tract, liver) will often be lethal, the result of mutations in the development of the reproductive tract is generally sex reversal and infertility. Because they are not lethal, a great number and variety of mutations affecting sex differentiation can be observed. At least 19 distinct Mendelian mutations are known to perturb the pathway of sex differentiation in the human (Wilson and Goldstein 1975). A number of inherited disorders of sex differentiation are also known to occur in the mouse (e.g., Eicher 1982).

A second advantage in the analysis of sex differentiation is that the primary sex-determining signal can often be pinpointed. In both fruit fly and nematode, for instance, the sex-determining signal is the ratio of X chromosomes to autosomes. In most developmental pathways other than sex differentiation, one cannot make such inferences as to the initiating signal.

In mammals, the primary sex-determining signal is the Y chromosome. Positioned at the head of the sex differentiation pathway, the presence or absence of the Y chromosome determines the fate of the indifferent gonad in embryogenesis. XY, XXY, XXXY, and XXXXY embryos develop testes, while X, XX, XXX, and XXXX embryos develop ovaries. In turn, the embryonic testes or ovaries establish, respectively, a male or female hormonal environment. That embryonic hormonal environment determines the remainder of the sex phenotype, including the sex of the internal accessory organs and external genitalia (Jost 1970). The entire sex phenotype—male or female—hinges upon the function of a gene or genes on the Y chromosome.

This Y-borne gene or gene complex is referred to as the testis-determining factor, or *TDF*. Much debate in the field of mammalian sex determination has focused on the nature of this master regulatory gene. A model that has enjoyed widespread acceptance proposes that TDF and the H-Y antigen are synonymous (Wachtel et al. 1975). Another model assumes that the *TDF* gene will be found among a family of evolutionarily conserved, heterogametic-sex-specific DNA sequences (Epplen et al. 1983; Singh et al. 1984). Recent studies cast doubt on both of these hypotheses (McLaren et al. 1984; Kiel-Metzger et al. 1985).

In reality, we have no meaningful biochemical or cell biological insights into the nature or mode of action of TDF. It seems likely that the function of TDF will come to be understood only through cloning of the gene or gene complex. Despite our ignorance as to the biochemical and cell biological events set in motion by TDF, and despite the lack of a selection for TDF function in cell culture, it may well prove possible to clone the *TDF* gene purely on the basis of its genetic or chromosomal map position. In this sense, the search for the *TDF* gene is analogous to the searches for the genes underlying X-linked chronic granulomatous disease, Duchenne muscular dystrophy, Huntington's disease, and cystic fibrosis (all described elsewhere in this volume).

The remainder of this article focuses on two intertwined themes. The first of these is the chromosomal mapping of *TDF*. The second is the chromosomal basis of gonadal sex reversal (e.g., XX males and XY females).

A Deletion Map of the Y Chromosome

How then does one go about determining the precise genetic map position of *TDF* on the Y chromosome? Most of the Y, the only haploid human chromosome, does not participate in meiotic recombination. (This is not true of the small portion of the Y that exhibits "pseudoautosomal" inheritance; Rouyer et al.; Cooke and Smith; Darling et al.; all this volume; D. Page,

unpubl.) It is therefore not possible to construct a genetic linkage map of the Y chromosome from recombination frequencies among markers. However, the natural occurrence of a wide variety of structural abnormalities of the Y chromosome suggests the possibility of constructing a deletion map. Indeed, attempts were made to infer the regional location of *TDF* on the human Y chromosome by karyotype-phenotype correlation (Buhler 1980; Davis 1981). Unfortunately, descriptions of structurally abnormal Y chromosomes from chromosome-banding studies are usually of limited precision and accuracy. Such studies left unresolved the debate as to whether *TDF* maps to the short arm (Yp), centromeric region, or long arm (Yq), or whether in fact multiple *TDF* genes might map to both Yp and Yq.

Hybridization with Y-DNA probes—in conjunction with chromosome-banding studies—is a superior method for characterizing Y-chromosome anomalies and hence for constructing a deletion map of the Y chromosome. We have tested more than 80 individuals for the presence of as many as 140 Y-DNA loci by hybridization to Southern (1975) transfers of restriction-digested genomic DNAs. The majority of the persons tested are XX males or XY females (to be described later) or have, as judged by cytogenetic analysis, a structurally abnormal Y chromosome. DNA studies showed that 50 of the individuals tested carry part but not all of the Y chromosome (Page et al. 1985; Disteche et al. 1986b; Vergnaud et al. 1986; D. Page, unpubl.). That is, each of these 50 individuals has some but not all of the Y-specific restriction fragments invariably present in normal (XY) males. Although only 19 of these 50 Y deletions had been detected by chromosome-banding studies, every Y deletion detected by chromosome banding was also revealed by DNA hybridization. The various patterns of Y-DNA loci present in these 50 individuals carrying part but not all of the Y chromosome are, as a group, most simply explained by the 8-interval deletion map shown in Figure 1. (The intervals are numbered according to the 7-interval map described by Vergnaud et al. [1986]. The subdivision of interval 4 into 4A and 4B is based on the additional findings of Disteche et al. [1986b]. Earlier ambiguities regarding the ordering of intervals on Yp [Vergnaud et al. 1986] have largely been resolved [D. Page, unpubl.].) With the exception of only one of these 50 individuals, this map accounts for each case on the basis of a single Y breakpoint; that is, this deletion map reconciles our hybridization data with the presence of a single, contiguous portion of the Y chromosome in all but 1 of these 50 individuals. The strength of the map lies in its internal consistency. There is little or no basis for ordering the 140 Y-DNA loci apart from this hybridization analysis of deleted-Y individuals. Though a few of these DNA loci have been regionally mapped on the Y chromosome by in situ hybridization—the results of which are consistent with the deletion map—the resolving power of deletion mapping is greater.

The 8 deletion intervals shown in Figure 1 have been ordered with respect to each other without reference to cytogenetic findings. However, by correlating the results of the DNA hybridization studies with cytogenetic findings, one can orient this otherwise abstract map with respect to the long and short arms of the Y chromosome. Several males with microscopically detectable deletions of distal portions of Yq and an apparently intact Yp (see XYq^- males in Fig. 1) are among the deleted-Y patients studied (Vergnaud et al. 1986; D. Page, unpubl.). Conversely, as judged by staining of extended (prometaphase) chromosomes, some of the XY females studied (Fig. 1) have deletions of minute portions of Yp and an apparently intact Yq (Disteche et al. 1986b). The DNA hybridization results obtained with these XYq^- males and XYp^- females allow us to assign intervals 1–4A to Yp and 5–7 to Yq. Evidence of two sorts indicates that interval 4B contains the centromere. First, 4B is the only interval present in all deleted but independently segregating Y chromosomes (as in XYq^- males and XYp^- females). Second, Wolfe et al. (1985) localized an alphoid repeated sequence to the Y centromere by in situ hybridization, and deletion analysis maps that repeated sequence to interval 4B (D. Page, unpubl.).

The map shown in Figure 1 provides a simple ac-

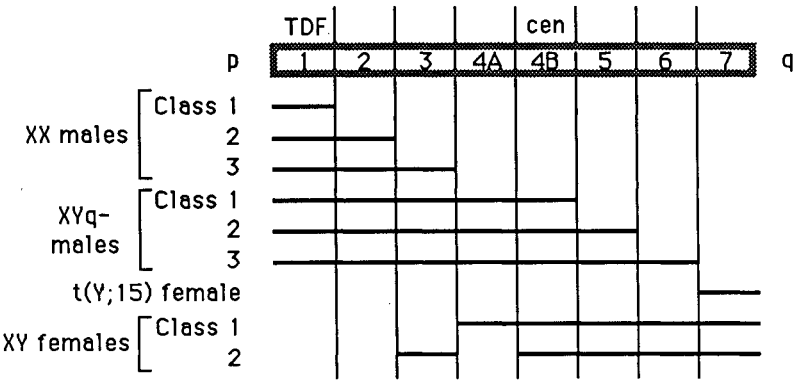

Figure 1. Eight intervals in a deletion map of the Y chromosome, based on DNA hybridization. This map is of the portion of the Y chromosome showing strictly sex-linked (as opposed to pseudoautosomal) inheritance. (p) Short arm; (cen) centromere; (q) long arm. (*TDF*) Testis-determining factor.

counting of all 50 Y deletions except that found in the class 2 XY female, who would appear to carry two noncontiguous portions of the Y chromosome. The class 2 XY female can be more easily accommodated if an implicit assumption underlying the map is relaxed. It has been assumed that the order of intervals on the Y chromosome is invariant among normal males, including the fathers of the patients studied. Suppose, however, that among the population, there exist two different Y chromosomes that differ by an inversion of intervals 3 and 4A. Perhaps such an inversion polymorphism would be easily tolerated on the Y, most of which does not participate in meiotic exchange. Then one might explain the class 3 XX males and class 1 XY females as arising from a Y chromosome of the 2-3-4A-4B form (as shown in Fig. 1) and the class 2 XY female as arising from a Y chromosome of the 2-4A-3-4B variety (with intervals 3 and 4A inverted). This would allow us to account for each of the cases via a single breakpoint on the Y. This dimorphism would also rationalize the finding that, on the 2-3-4A-4B chromosome, *DXYS1*-like sequences are found in two noncontiguous regions (Vergnaud et al. 1986); on the 2-4A-3-4B chromosome, the *DXYS1*-like sequences would occur in a single block (D. Page, unpubl.), consistent with their having transposed from the X as a unit (Page et al. 1984).

A working model of the Y chromosome is shown in Figure 2. Little is known about the physical size of the 8 deletion invervals, except that interval 7, composed largely of Y-specific tandemly repeated sequences (Cooke 1976; Kunkel et al. 1976), accounts for nearly half the chromosome. Intervals 1–4A are on the short arm, the centromere is in interval 4B, and intervals 5–7 are on the long arm. The pseudoautosomal domain is probably distal to all Y-specific sequences assigned to the short arm by deletion mapping (Rouyer et al.; Cooke and Smith; Darling et al.; all this volume; D. Page, unpubl.).

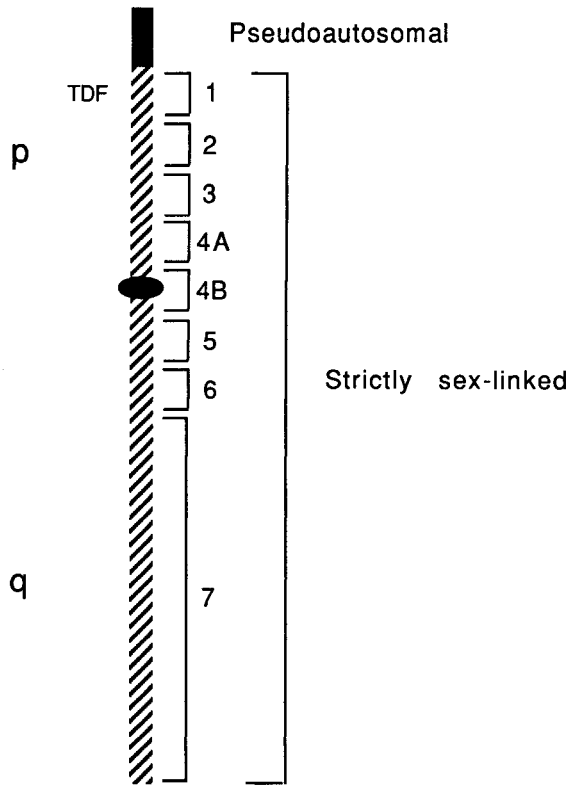

Figure 2. Cartoon of the Y chromosome.

How detailed a map of the Y can one derive from such DNA hybridization studies of naturally occurring deletions? Flow-sorted (Deaven, this volume) and other Y-enriched libraries provide an effectively endless supply of new Y-DNA hybridization probes. To be useful in deletion mapping, probes need not be absolutely Y-specific in their pattern of hybridization, so long as they detect one or more Y-specific restriction fragments on Southern transfers. Accordingly, one is ultimately limited by the distribution of endpoints among spontaneously deleted Y chromosomes—and by one's ability to screen for such deletions among the population. Many such deletions are ascertained because of resulting sterility or other abnormalities of sexual development. Deletions without marked phenotypic consequence will go unnoticed unless they occur at sufficient frequency to be detected in surveys of normal populations. Y deletions can also be produced in tissue culture (Darling et al., this volume), but then one does not have the opportunity to examine the organismal phenotype of the deletion. Recent studies suggest that characterization of an expanded set of deleted-Y patients with a growing number of Y-DNA probes will yield a substantially refined map. Intervals 1, 3, and 6, for example, have recently been subdivided (D. Page, unpubl.).

Mapping *TDF* and Other Genes

Since it is based on the results of hybridization with cloned Y-chromosomal DNA, this deletion map is quite revealing with respect to the organization of Y-specific repeated sequences and of X-Y homologous sequences within the Y chromosome (Vergnaud et al. 1986). Nonetheless, the principal reason for constructing such a map is to facilitate our understanding the biological roles of Y-borne genes. Foremost among these is the male-determining function of the Y.

Is the male-determining function of the Y chromosome the responsibility of a single gene or of several? If there are multiple male-determining genes on the Y, are they redundant, such that any one alone can initiate testis differentiation, or do they act in concert? Our results to date are consistent with there being a single *TDF* gene or gene complex, located on Yp (the short arm). The DNA hybridization results obtained with XX males (Fig. 1) suggest that interval 1 of the Y chromosome is *sufficient* to induce testis differentiation. Indeed, among the patients in whom we have detected part but not all of the Y chromosome, all those with testes carry interval 1 of the Y chromosome. If more than one Y-borne gene is required to initiate testis dif-

ferentiation, then those genes must all be located within interval 1. Conversely, the DNA hybridization results obtained with XY females (Fig. 1) suggest that interval 1 of the Y chromosome is *necessary* to induce testis differentiation. That is, among the patients in whom we have detected part but not all of the Y chromosome, all those lacking testicular tissue also lack interval 1. (Some XY females have recently been found to have deletions smaller than those shown in Fig. 1; D. Page, unpubl.) The male-determining function of the human Y chromosome, then, appears to reside entirely within a gene or gene complex found in interval 1, on Yp (Figs. 1 and 2).

This deletion map may also be of use in resolving other long-standing controversies regarding the Y chromosome. In particular, what is the function of the H-Y antigen? H-Y is a male-specific, minor histocompatibility antigen, first identified by graft rejection (Eichwald and Silmser 1955). Although it has been proposed that TDF and H-Y antigen are synonymous (Wachtel et al. 1975), H-Y does not appear to be required for testicular differentiation in mice (McLaren et al. 1984). A reliable method for H-Y typing of human B-cell lines using a cytotoxic-T-cell assay has been developed (Goulmy et al. 1983; Simpson 1986). H-Y typing of B-cell lines from patients with well-characterized Y deletions may allow one to unambiguously assign a gene responsible for H-Y antigen expression to a particular deletion interval. If H-Y antigen and TDF are one and the same, then this *H-Y* gene must map to interval 1 of the Y chromosome, where *TDF* resides (Fig. 2). If the *H-Y* gene maps outside interval 1, then it is not TDF. If H-Y antigen is required for spermatogenesis (Burgoyne et al. 1986), then one might expect it to map to one of the intervals on Yq, portions of which may be necessary for male fertility (Tiepolo and Zuffardi 1976).

The presence of a Y chromosome in a gonadal female appears to strongly predispose her to gonadal neoplasia, particularly gonadoblastoma and dysgerminoma (Manuel et al. 1976). If this predisposition is the result of a single Y-borne gene or gene complex, then Y-DNA studies of females with these neoplasms and deleted Y chromosomes should allow one to map the locus. The presence of gonadoblastoma in a class 2 XY female (case 2 described by Disteche et al. 1986b; Fig. 1) suggests that this "gonadoblastoma locus," if it exists, is somewhere in intervals 3, 4B, 5, 6, or 7. It appears, then, that *TDF* itself is not the gonadoblastoma gene.

XX Males and XY Females: The X-Y Interchange Model

Two human "sex reversal" syndromes have been mentioned but not properly introduced. "XX males" are sterile but otherwise phenotypically male individuals whose karyotype is 46,XX (de la Chapelle 1981). They have testicular but no ovarian tissue. As judged by standard cytogenetic methods, they have the chromosomes of a normal female. "XY females" are sterile but otherwise phenotypically female individuals whose karyotype is 46,XY. They have "streak" ovaries devoid of follicles and no testicular tissue. The internal accessory structures are female. Secondary sexual characteristics are female but variably developed, and the somatic features of Turner syndrome (a phenotype classically associated with a 45,X karyotype) are present in some. As judged by routine cytogenetic analysis, XY females have the chromosomes of a normal male, with no evidence of mosaicism for a 45,X cell line. Thus, in both XX males and XY females, there is a discordancy between the gonadal sex and the chromosomal sex—as least as judged by chromosome banding. Given the Y-DNA hybridization findings already described, XX males and many XY females no longer represent exceptions to the rule that the Y chromosome is male-determining. Rather, they provide the basis for a stronger, refined rule: Interval 1 of the Y chromosome is male-determining.

Ferguson-Smith (1966) proposed that human XX males are the result of aberrant X-Y interchanges occuring during paternal meiosis, when terminal portions of the X and Y chromosomes pair (Fig. 3). According to this X-Y interchange hypothesis, an XX male inherits from his father an X chromosome whose terminus has been replaced by a *TDF*-bearing portion of the Y chromosome. Ferguson-Smith suggested that XY females might also result from aberrant X-Y interchanges. I will argue that this X-Y interchange hypothesis continues to provide a good working model on which to base investigations of XX males and XY fe-

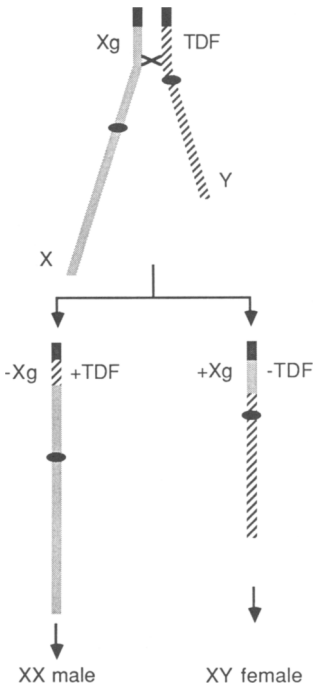

Figure 3. The X-Y interchange hypothesis: XX males and XY females receive reciprocal products of similar aberrant X;Y translocations occurring during or prior to paternal meiosis.

males. Specifically, our recent findings suggest that XX males and XY females may carry reciprocal products of similar X;Y translocations (Fig. 3). I will also outline additional predictions of the model that have not yet been tested.

The X-Y interchange hypothesis was motivated in large part by the anomalous inheritance in XX males of *Xg*, a dominant, X-linked antigenic marker. Many an XX male does not express his father's allele for *Xg* (de la Chapelle 1981), as if he has inherited two X chromosomes from his mother and none from his father. According to the X-Y interchange model, however, such an XX male has received a paternal X chromosome or, more precisely, a paternal X;Y translocation product that lacks *Xg* but carries *TDF* (Fig. 3).

To determine the parental origin of the X chromosomes in XX males, the inheritance of several X-linked restriction-fragment-length polymorphisms (RFLPs) was followed in 10 XX males and their immediate relatives. While *Xg* maps to distal Xp, the X-linked RFLPs used to establish the parental origin of the X chromosomes map to Xq or to more proximal portions of Xp. In each of these 10 families, the XX male was found to inherit a paternal and a maternal X-linked RFLP allele (Page and de la Chapelle 1984 and unpubl.). Among these XX males were 2 who were known not to express their father's alleles for *Xg*. Thus, most if not all XX males inherit a paternal X and a maternal X, as normal 46,XX females do.

If XY females received the reciprocal product of similar X;Y translocations, then many an XY female should express her father's allele for *Xg*—despite having a single, maternally derived X chromosome. The only informative matings will be those in which the XY female's father is phenotypically Xg(a^+) and the mother Xg(a^-). Given the frequencies of the *Xg* alleles, less than 10% of matings will be of this sort. Unfortunately but not surprisingly, then, the two XY females shown to have deletions of *TDF* were products of matings uninformative for *Xg* (Disteche et al. 1986b).

The X-Y interchange model predicts, of course, the presence of Y-chromosomal material in the genomes of XX males. DNA and antigenic marker studies provided direct evidence of such Y material in XX males. Guellaen et al. (1984) detected certain Y-specific DNA sequences in three XX males by Southern (1975) blotting. We described an XX male who expressed his father's allele for 12E7, a Y-linked antigenic marker; he did not express his father's allele for Xg (de la Chapelle et al. 1984).

It is the distal short arms of the X and Y chromosomes that pair in male meiosis (Fig. 3; Chandley et al. 1984). According to the X-Y interchange model, then, the Y material present in XX males should originate from distal Yp. As judged by DNA hybridization, XX males do appear to carry terminal portions of Yp, albeit of varying size (Fig. 1). Conversely, the model would predict the absence of terminal portions of Yp in XY females. Class 1 XY females do appear to lack terminal portions of Yp (Fig. 1). Indeed, the deletion in class 1 XY females corresponds to the portion of the Y present in class 3 XX males, suggesting that class 1 XY females and class 3 XX males carry reciprocal products of similar recombination events. If, as earlier postulated, the class 2 XY female's father has a Y chromosome in which intervals 3 and 4A are inverted, then she too lacks a terminal portion of Yp.

The X-Y interchange model predicts that the Yp DNA found in XX males would be transferred to the distal short arm of an X chromosome. This prediction was confirmed by the results of in situ hybridization with a probe detecting Y-specific repeated sequences present in class 3 XX males. In all three class 3 XX males tested, this Y-specific probe hybridized unambiguously to the most distal portion of Xp (Andersson et al. 1986).

According to the X-Y interchange model, XX males should be hemizygous for some strictly X-linked (as opposed to pseudoautosomal) DNA sequences on distal Xp (Fig. 3). At such a locus, the single copy present in an XX male should be of maternal origin. We have not found any X-specific sequences for which XX males are hemizygous, but these negative results do not disprove their existence. The simplest explanation for many XX males not expressing their fathers' alleles for *Xg* is hemizygosity for the *Xg* locus. However, in the absence of a DNA probe for *Xg*, it remains possible that, in many XX males, the paternal *Xg* locus is present but is not expressed because of the position effect of a nearby translocation breakpoint.

Conversely, the model predicts, that, despite having only one X chromosome, many an XY female should have two copies of some strictly X-linked DNA sequences from distal Xp. At such a locus, one copy should be of maternal origin and the other of paternal origin. Further, the paternally derived copy should be transferred to distal Yp.

If each XX male is the result of a single Xp-Yp crossover proximal to the pseudoautosomal region (Fig. 3), then XX males should carry pseudoautosomal sequences derived from their fathers' Y chromosomes but not from their fathers' X chromosomes. Conversely, XY females should carry pseudoautosomal sequences derived from their fathers' X chromosomes but not from their fathers' Y chromosomes. Highly informative pseudoautosomal RFLPs (Rouyer et al.; Cooke and Smith; Darling et al.; all this volume; D. Page, unpubl.) should allow these predictions of the model to be tested.

The *Sxr* mutation in mice results in sex reversal, and it provides an interesting contrast to the phenomenon of human XX males. The *Sxr* strain is characterized by a high frequency of sterile XX males. The trait is transmitted by carrier males, in whom a duplicate of the testis-determining locus is found in the pseudoautosomal region of the Y chromosome. Meiotic recombination regularly transfers this pseudoautosomal copy of the testis-determining gene from the abnormal Y to the X, resulting in XX males (Burgoyne 1982; Evans et al. 1982; Singh and Jones 1982). Thus, sex reversal in *Sxr*

mice is due to frequent recombination between an aberrant Y chromosome and a normal X chromosome.

In contrast, human sex reversal appears to be due to aberrant recombination between normal X and Y chromosomes. We have studied the parents and siblings of many XX males by Southern hybridization using Y-DNA probes (Page et al. 1985; Vergnaud et al. 1986; D. Page, unpubl.). No Y DNA was detected in mothers or sisters, and the Y chromosomes of fathers and unaffected brothers appeared to be normal. In particular, fathers do not carry a duplication of the male-determining region that they transmit to their XX male sons. These results were particularly notable in the case of a family with three XX males (Page et al. 1985), in which one might have suspected a mechanism more like that seen in *Sxr* mice. Similarly, no Y DNA has been detected in the mothers of XY females with Y deletions, and their fathers appear to have normal Y chromosomes as judged by DNA hybridization (Disteche et al. 1986b; D. Page unpubl.). Thus, each such human XX male and XY female appears to be the result of a new mutation. This is particularly remarkable given that one in 20,000 males is an XX male (de la Chapelle 1981); this constitutional translocation is generated anew at a high frequency.

Unexplained Sex Reversal

XX males and XY females are not the only examples of apparent inconsistency between chromosomal sex and gonadal sex. X males have testes but are sterile, and they have a 45,X karyotype. As judged by Y-DNA studies, one X male is mosaic for a Y-bearing cell line (de la Chapelle et al. 1986), while another has a cytogenetically undetected translocation of a *TDF*-bearing portion of the Y to chromosome 15 (Disteche et al. 1986a). Not all cases of gonadal sex reversal, however, are due to anomalies of the Y chromosome. For example, several families have been described in which there are multiple XY females with streak ovaries. (The XY females in whom we detected Y deletions are all sporadic cases.) In most of these families, sex reversal is inherited in an X-linked or autosomal manner (e.g., see Espiner et al. 1970), suggesting mutations in genes that function downstream of *TDF* in the pathway of gonadal differentiation; the Y chromosome is probably intact. To be sure, autosomal mutation can cause gonadal sex reversal in XY mice (Washburn and Eicher 1983). In addition, there is at least one X male and several XX males and XX hermaphrodites (testicular and ovarian tissue) in whom no Y DNA has been detected (de la Chapelle et al. 1986; Vergnaud et al. 1986; D. Page, unpubl.). Some of these individuals may yet prove to have very small, *TDF*-bearing portions of the Y chromosome. On the other hand, perhaps some of these individuals do not carry *TDF* but have testicular tissue because of a gain-of-function mutation in a gene that acts downstream of *TDF*.

SUMMARY

An 8-interval deletion map of the human Y chromosome has been constructed using DNA hybridization to characterize naturally occurring structural abnormalities. The map is oriented with respect to the short and long arms, and the position of the centromere is known. The deletion map may be dimorphic; that is, within the normal population there may occur two Y chromosomes that differ by an inversion within the short arm.

Y-DNA studies have reconciled many cases of gonadal "sex reversal" with the rule that the human Y chromosome is male-determining. Gonadal sex is determined according to the presence or absence of interval 1 which is on the short arm of the Y chromosome. XX males are the result of X;Y translocations that occur during or prior to meiosis in the fathers. Some XY females may carry the reciprocal product of similar X;Y translocations. Not all cases of gonadal sex reversal can be explained on the basis of Y-chromosome aberrations, and these provide evidence of genes that function downstream of *TDF* in the pathway of gonadal differentiation.

ACKNOWLEDGMENTS

I am indebted to my co-workers, and in particular to David Botstein, Laura Brown, Albert de la Chapelle, Christine Disteche, and Jean Weissenbach. This work was supported in part by grant HD20059 from the National Institutes of Health.

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