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The mammalian Y chromosome: molecular search for the sex-determining gene – summary and perspectives

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Summary

Other presentations to this symposium have indicated that the search at the molecular level for the pivotal regulatory, or structural, gene responsible for determining the development of the undifferentiated gonad has been joined in earnest. It is also clear that genes on the Y chromosome are involved in processes other than primary determination of the testis. In this summary, we will review briefly 'the molecular search for the sex-determining gene' and consider the approaches that are available and the achievements that have been made in the areas relevant to an understanding of the roles and significance of other Y-located genes. The availability of molecular and physical mapping data also allow an examination of the evolutionary relationship of the mammalian X and Y chromosomes and a consideration of the possible homologies between the human and mouse Y chromosomes.

Key words: molecular organization, sex determination, human Y-linked genes, mouse, steroid sulphatase.

Molecular organization of the Y chromosome

The molecular dissection of the mammalian Y chromosome has been achieved through the application of a variety of approaches. In particular, both molecular and genetic analysis have progressed through the availability of rearranged Y chromosomes. These include both those significant in sexually dysgenic humans and also in the *Sxr* and *Sxr'* conditions of mice.

Observations on the obligatory pairing and exchange in the 'pseudoautosomal' region have been of considerable interest, not only in context of the proposed necessity for such an event in productive meiosis, but also in the ordering of loci with respect to a gradient of recombination within the region (see Burgoyne; Weissenbach *et al.*, this symposium).

The assignment of a locus for testis determination close to the region of active exchange on the human Y chromosome has been reviewed by several contributors. This location for an essential gene in sex determination could be considered to be unexpected and, in this context, it is significant that abnormal exchange between the X and Y chromosomes resulting in the transfer of *TDF* to the X chromosome occurs at the relatively high frequency of about 10^{-4} (see de la Chapelle, this symposium; Bengtsonn & Goodfellow, 1987). In mice, the testis-determining locus is distant from the pseudoautosomal region and, although transfer of *Tdy* to the X chromosome is observed, such an event is associated with the presence of a rearranged Y chromosome.

Some XX males and some XY females may arise from a similar type of event; that is, the inheritance of either of the reciprocal products of an aberrant paternal X–Y exchange. The comparatively lower frequency of XY females to XX males may reflect an ascertainment bias. Transfer of Y-chromosomal material to the paternal X chromosome of XX males is a frequent, but not an absolute, observation in the aetiology of these cases. However, where such exchanges do take place, there is evidence that they can result in the transfer of a contiguous terminal segment including *TDF* to the paternal X chromosome. This can occur without a perceptible alteration to the overall length of the recipient X chromosome. Other cases have been described in which unequal crossover events have taken place leading to duplication of terminal regions (Weissenbach *et al.*, this symposium) and one of the X chromosomes in at least some XX males is significantly larger than the other (Ferguson-Smith *et al.* this symposium).

A wide selection of Y-chromosome-derived sequences has been obtained by random cloning from Y-chromosome libraries. Many of these detect single- or low-copy repeat, Y-specific or Y/autosomal sequences; in addition, a proportion of the probes that have been localized to Yp also exhibits a high degree of homology with a region at Xq21. Pritchard & Goodfellow (this symposium) have described a more direct approach to obtaining Y-chromosomal sequences from specific regions of interest. This has been achieved through chromosome-mediated transfection using thymidine kinase (TK) or antibiotic resistance (neo) as selectable markers followed by fluorescence-activated cell sorting (FACS) for MIC2 (12E7 antigen). Transfectants retaining small Y-chromosomal fragments and which carry flanking markers to the *TDF* region have provided an enriched source of useful sequences.

Studies in a number of laboratories have allowed a deletion map for the human Y short arm to be deduced. From this map, notwithstanding the uncertainties introduced by the possibility of more complex rearrangements, or by the existence of inversion polymorphisms in the population, it can be estimated that the sequences involved in male determination are located between the proximal boundary of the pseudoautosomal region and the distal boundary of the X/Y homologous region (see Figs 1, 2). Indeed, it can be argued that the male-determining locus represents the proximal limit of the region that is normally exchanged (Weissenbach *et al.* this symposium; Bengtsonn & Goodfellow, 1987). Exchange between the X and Y chromosomes which include the *TDF* region results in sterile, sexually dysgenic, individuals and therefore the rearrangements will not persist in the population. This provides an effective barrier to the randomization of sequences on the sex chromosomes in the regions below *TDF*.

Studies of XX males have enabled the identification of probes detecting low-copy Y-specific sequences that map to the interval embracing the testis-determining gene and which are frequently deleted in XY females. These are being investigated by a number of groups as possible starting points for walking, long-range restriction mapping and for the identification of sequence motifs characteristic of transcribed sequences (see Ferguson-Smith *et al.*, Muller, Page *et al.* and Pritchard & Goodfellow, this symposium).

Autosomal genes and sex determination

The presence or absence of a Y-linked gene (or genes) is not the only factor involved in testis determination. Developmental processes in complex organisms depend on the interaction of many genetic and possibly environmental components. Lack of Y-derived sequences in some human XX males and hermaphrodites, coupled with familial inheritance patterns, has led to the postulation of an autosomal testis-determining gene designated *TDFA* (de la Chapelle, this symposium), which may be homologous to one of the autosomal loci described for mice (see Erickson *et al.* this symposium). The appearance of Sertoli cells in the testicular cords is a basic event in testis formation; Burgoyne (this symposium) and Singh (this symposium) report that Sertoli cells in XX/XY chimaeras are exclusively XY and, since XX*Sxr* mice are seen to have such cells, it can be postulated that sequences necessary for their development are located in the *Sxr* region. There have been, however, several reports at this symposium of testis development in the absence of detectable Y sequences and the gene (or genes) necessary for Sertoli cell development could, like those for testis development, (see above) be autosomally located but normally regulated by Y-chromosomal sequences (Burgoyne personal communication).

Wiberg and Scherer (this symposium) have presented evidence indicating the presence of a serologically detected H-Y antigen (also known as SDM or Sxs) in true hermaphrodites who lacked detectable Y-genomic sequences. Sxs is normally confined to the heterogametic sex. There is evidence for the location of gene (or genes) responsible for expression of Sxs on human chromosome 6 (Lau, Chan, Kan & Goldberg, 1986) and it is suggested that this may be under the control of X- and Y-linked loci. It is of interest that both this autosomal locus and a testis-determining autosomal locus in mice (see Erickson *et al.*, this symposium) should be located on chromosomes carrying genes for major histocompatibility determinants (human chromosome 6 and mouse chromosome 17).

Identification of Y-linked genes

(A) *Humans*

The *MIC2* gene (see Goodfellow *et al.*, this symposium) represents the only Y-linked gene that has been characterised at the molecular level. It also represents the only known human pseudoautosomal gene. Recent sib testing of the inheritance of 12E7 antigen phenotype, not only confirmed the existence of a further Y-linked gene (previously referred to as *Yg*), which regulates the level of 12E7 antigen expression, but also provided evidence that this locus can be exchanged with a homologue on the X chromosome by recombination in the pseudoautosomal region. This regulatory sequence on the X chromosome

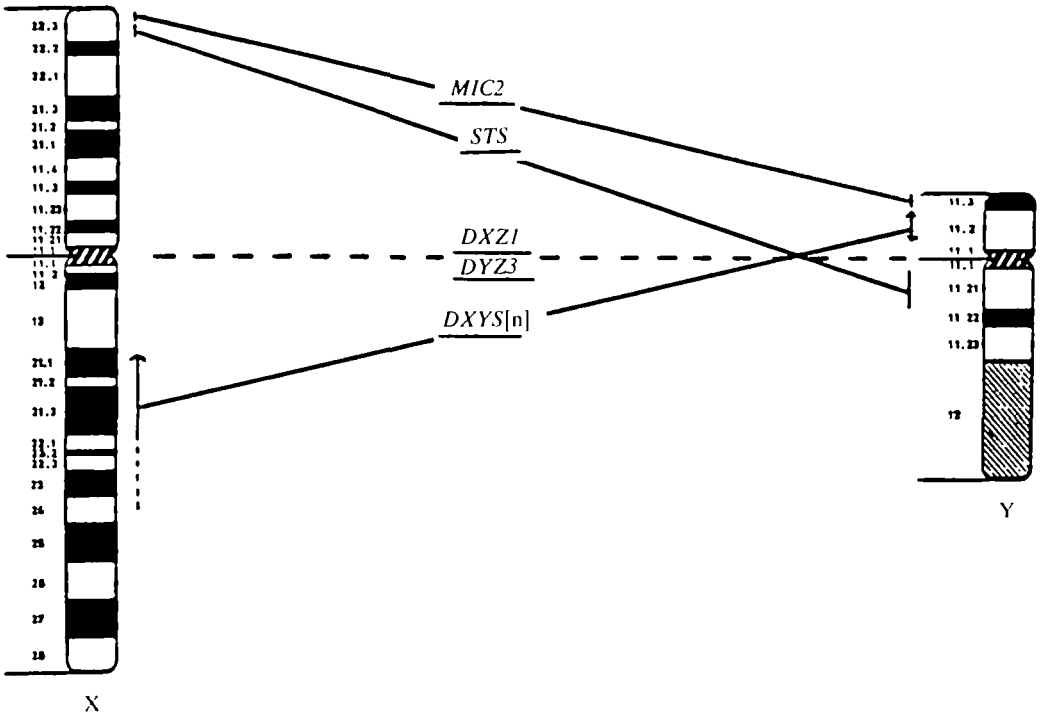

Fig. 1. Comparison of the organization of sequences on human X and Y. *MIC2* (*MXYS1*) coding sequence for the antigen 12E7. The Y-chromosomal location of STS sequences is proximal to that for *DYS20* (see Fraser *et al.*, this symposium and Buckle *et al.* 1987). The *in situ* hybridization grain distribution for *DYS20* is concentrated in Yp11.22–11.23. The low homology between the centromeric repeats *DXZ1* and *DYZ3* and the centromeric localization of DYZ3 has been described elsewhere (Wolfe *et al.* 1985). DXYS(n) designates a series of probes which hybridize to extensively homologous blocks of sequence located on Yp and Xq21. A single, recent duplication and transposition from the X chromosome to Yp is the most economic explanation (see Page, Harper, Love & Botstein, 1984) The X/Y homologous block on Yp is interrupted by Y-specific sequences (see also Fig. 2). Note: information concerning the structural organization of the telomeric sequences and of the alphoid repeat of the centromeric region of the Y chromosome (DYZ3) has been presented by Cooke and by Tyler-Smith (this symposium).

exerts *cis* control over Xg^- antigen and 12E7 antigen expression on red cells.

A variety of other genes in addition to *TDF* and *MIC2* has been attributed to the human Y chromosome, including those related to the control of growth and spermatogenesis (see Goodfellow, Darling & Wolfe, 1985). Extension of the H-Y transplantation antigen studies (described under mouse sex reversal below) to humans has been achieved through the use of T cell clones specific for H-Y. It now seems clear that the situation for humans is similar to that observed for mice; the locus for testis determination is distinct from that encoding H-Y. Moreover, in humans the two do not appear to be located close together. Whereas the detection of H-Y antigen by the T cell assay is dependent on a locus which is located between intervals 4B and 7 on the published deletion map (see Simpson *et al.*, this symposium), *TDF* maps to interval 1. Other data presented at this symposium suggest that the *H-Y* locus may be positioned on Yq. This observation raises the intriguing possibility that it may be identical to the spermatogenesis gene that has been suggested to reside in this region (Tiepolo & Zuffardi, 1976). Again interesting parallels with the mouse Y-chromosome functions are evident (see below). Page (this symposium) has proposed that a pleiotropic effect of the H-Y/spermatogenesis gene could explain the development of gonadoblastomas, which may occur in dysgenetic gonads. This effect has been attributed to a locus *GBY*.

There have been several preliminary reports of testis-specific transcripts that are encoded by Y-specific genomic sequences (e.g. see Arnemann *et al.*, this symposium); however their significance has yet to be fully evaluated. In this context, however, it is worth noting the remarkable detection of two pseudogene sequences (the original genes for which are autosomally located) which detect testis-specific transcripts (Leroy *et al.*, this symposium). This observation suggest that 'retroposition' to the Y chromosome may be more active in the testis. The Y

chromosome certainly represents a target area with few essential sequences and it is possibly more accessible to colonization by retroviral transposition in the testis.

(B) *Mice*

Burgoyne (this symposium) has reviewed the wide range of phenotypic characteristics that have been attributable to strain-specific variants at Y-chromosomal loci. A minimum of four loci have been postulated (Stewart, 1983; Jutley & Stewart, 1985; Stewart & Jutley, 1987). Arguably the most important recent contribution to arise from studies on sex reversal in mice (*Sxr*) is the clear distinction between the locus for testis determination (*Tdy*) and that for H-Y as determined by cytotoxic and proliferative T cell clones (Simpson, 1985; Simpson *et al.* 1986 & this symposium). A comparison between XO*Sxr* and XO*Sxr'* mice convincingly demonstrated that, whereas the *Sxr* region bears both *Tdy* and H-Y, the *Sxr'* region carries only *Tdy*. Spermatogenesis studies on such individuals and on the testes of XO/XY mosaic mice (Burgoyne, this symposium) have provided evidence for a spermatogenesis gene (*Spy*) on the *Sxr* Y-chromosomal fragment, which is excluded in the *Sxr'* variant and is expressed in a cell autonomous fashion. Given the paucity of genes on the Y chromosome and the correlation between the loss of spermatogenesis and the absence of H-Y antigen expression, it is tempting to conclude that H-Y antigen is a product of the spermatogenesis gene. It is also plausible that the two functions are encoded by separate but tightly linked genes.

The functional importance of the *Sxr* region is beyond doubt; attempts to identify genes within this region (Bishop *et al.*, this symposium) have yielded sequences of potential significance. One of these, pCRY8/B, detects a single transcript of approx. 3·5 kb in testis, but does not hybridize to RNA from liver; its presence/absence in *Sxr'* will be of considerable interest.

Other Y-genomic sequences have been obtained that detect testis-specific transcripts (e.g. see Bishop *et al.*, this symposium): PY353/B is one such which, although not obviously of retroviral origin, is represented about 250 times and distributed at various positions along the length of the Y chromosome.

Steroid sulphatase: a second X and Y homologous gene?

Genetic analysis of steroid-sulphatase activity has indicated that the gene necessary for expression of the enzyme is X linked, but only partially inactivated in humans, with no evidence for an expressed Y-chromosomal copy. In contrast, evidence has been presented for X- and Y-localization in mouse with regular exchange between the alleles on the two chromosomes (Keitges, Rivest, Siniscalco & Gartler, 1985). Further support for an pseudoautosomal localization is suggested by its linkage to the most distal X-chromosome marker cream, *Crm*, (Cattenach & Crocker, 1986) and by its cosegregation with *Sxr* in an appropriate backcross (Bishop *et al.*, this symposium). Its pattern of linkage to the *Crm* locus through female meiosis is of interest, as it suggests that recombination in the pseudoautosomal region of female mice is significantly lower than that in males – a similar situation to that observed for the two sexes in humans (Rouyer *et al.* 1986; Weissenbach, this symposium).

The availability of a cloned probe for the STS structural gene (Ballabio *et al.* 1987) has allowed us to localize the coding sequences to Xp22.3 (Fraser *et al.*, this symposium). Two additional, Y-chromosomal-specific restriction fragments have been assigned provisionally to Yq11.2. It therefore seems reasonable to speculate that the distribution of the STS sequences in humans reflects a rearrangement from an ancestral sex-chromosome organization in which both X and Y alleles were functional and located in the pseudoautosomal region, as is apparently the situation in mice. However, as no extensive cross-hybridization between human STS coding sequences and rodent DNA has been detected, the nature of the relationship between human and mouse steroid sulphatase genes and their products remains to be established.

The data now available concerning the organization of the mouse and human sex chromosomes can be used to construct a model for the types of rearrangement that would be necessary to explain the different distribution of sequences in the human and mouse Y chromosomes (see Fig. 2). It should be stressed that current evidence indicates a very rapid evolution of the bulk of the Y chromosome. Viral retroposition and adventitious colonization by members of repetitive sequence families is a significant feature. Alterations which may result from these and other Y-chromosome rearrangements may modify the expression of the critical gene functions in a temporal or quantitative manner and may, therefore, be significant in speciation.

Conclusion

Finally, with the molecular pincer movement closing in on the presumptive location of the testis-determining gene on the human Y chromosome and with the rapid dissection of the *Sxr* region in mice, it is worth speculating what might be at the centre of all this

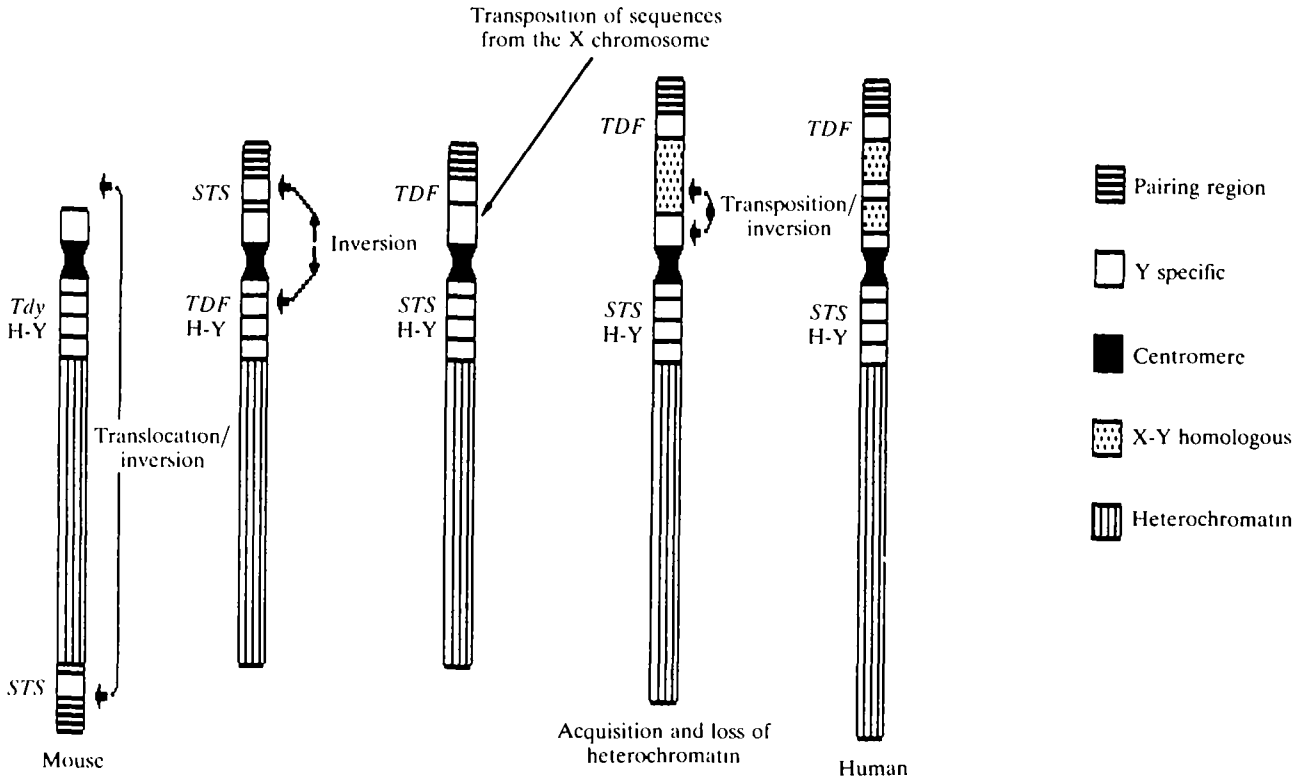

Fig. 2. Possible rearrangements that have occurred in the divergence of mouse and human Y chromosomes. The suggested rearrangements represent the minimum necessary – see text. The order of events indicated is one of several possible, but it is probable that the separation of the X/Y homologous sequences on human Yp with Y-chromosome-specific sequences has followed a recent dispersal from the X chromosome.

attention. The elegant genetic analysis of sex determination in *Drosophila* and in nematodes (Nothinger, this symposium; Hodgkin, this symposium) have emphasized the primary signal recognition by a KEY gene which regulates in turn a cascade of subordinate control genes. It appears inescapable that in some systems, at least, sex determination and inactivation mechanisms are inextricably linked, and it would be surprising if a similar situation did not obtain in mammals. The mutation of an autosomal regulatory gene in *C. elegans* (*tra-1*) and its potential to give rise to ZZ male/WZ female type of chromosomal sex-determining mechanism when segregating in an appropriate backcross, is a provocative observation. In the case of mammals, two roles can be proposed for the sex-determining sequence(s) on the Y chromosome. It may either be part of a counting system for sequences or regulatory molecules which, on reaching a threshold value, trip a KEY gene into the ON or OFF position; or it may be a gene analogous to one of those in the regulatory cascade seen in simpler organisms. While the exponents of mammalian sex determination still have to consider the various possible functions for *TDF* (or *Tdy*), those working on the worm and fly may have the appropriate sequences cloned and available for comparison before the mammalian chromosome walkers arrive at their destination.

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