

MAMMALIAN SEX CHROMOSOMES

H. SHARAT CHANDRA

Microbiology and Cell Biology Laboratory and ICMR Centre for Genetics and Cell Biology,
Indian Institute of Science, Bangalore 560 012, India.

CURRENT interest in the molecular biology of mammalian sex chromosomes originates, to a large extent, from two sets of observations made over twenty years ago. The first of these was the surprising finding that, unlike in *Drosophila*, XO individuals among mammals are female and XXY, male. This observation gave rise to the dogma that the mammalian Y chromosome carries one or more powerful male-determining genes. At about the same time, it was shown that "sex chromatin" or the "Barr body" characteristic of female mammals had its origin in one X chromosome retaining, during interphase, a level of condensation characteristic of metaphase chromosomes. The single X chromosome carried by the male does not show such condensation and hence no sex chromatin is present in males. Lyon¹ then proposed that it is possible to understand the unusual genetic behaviour of mammalian X-linked genes on the basis of certain assumptions based on the condensation of one of the two X chromosomes in female mammals. This hypothesis predicted that in somatic cells of female mammals only one of the two X chromosomes is genetically active whereas the other is inactive and that this inactivation, which occurs during early embryonic development, is random. It is this inactive X chromosome which appears as sex chromatin in interphase nuclei. Research during the past 20 years has amply confirmed the predictions set out by Lyon.

A variety of cytological, genetic and biochemical data have confirmed that in somatic cells of female mammals only one X is transcriptionally active while the other is inactive except for a few genes on a segment of the short arm². This inactivation of one of two homologous chromosomes occurs in the early embryo and, once established, is inherited clonally. In other words, inactivation can affect, apparently with equal probability, either of the two X chromosomes present in XX cells, but after the event, the same X continues to be inactive in all the descendants of that cell. In Marsupial mammals, inactivation is not random³. There is preferential inactivation of the paternal X chromosome, reminiscent of the inactivation of paternal chromosomes in certain coccids and elimination of paternal chromosomes in coccids as well as certain other insects⁴. The mechanism by which chromosomes in these organisms "remember" their parental origin, in contradiction of the rules of Mendelism, is not understood.

Even among Eutherian mammals, random inactivation is characteristic only of the cells of the embryo proper. In several extra-embryonic tissues the paternal X chromosome is preferentially inactive⁵. The significance of non-random inactivation in extra-embryonic tissues is not clear other than that it could possibly confer greater compatibility between mother and foetus.

In female germ cells both X chromosomes are active and evidence suggests that hitherto inactive X chromosomes are reactivated in the oocytes⁶.

One important genetical consequence of X inactivation is that it leads to dosage compensation. In this respect, the X chromosome of mammals resembles the X chromosome of *Drosophila*⁷. The two systems differ, however, in the mechanism by which dosage compensation is achieved. In *D. melanogaster* dosage compensation is brought about by transcribing the single X chromosome of the male at a rate twice that of either of the two X chromosomes in the female. According to a recent hypothesis, sex determination and dosage compensation in *D. melanogaster* are controlled by the same gene product^{8,9}. Increasing amounts of this product lead to increasing femaleness; if this product also act as a repressor specific to X-linked genes, then it would result in a corresponding decrease in the rate of transcription of those genes. In *D. melanogaster* it thus appears that sex determination and dosage compensation are linked functions. Is it possible that a relationship exists between sex determination and dosage compensation in mammals? Lyon¹⁰ has suggested that this may indeed be so because the gene *Tfm*⁺ is carried on the X chromosome of mammals. XY embryos carrying a mutant form of this gene develop as phenotypic females in spite of carrying a normal Y chromosome. This gene is considered to be a major determinant of the male somatic (or non-gonadal) phenotype. Lyon has reasoned that because differentiation of the male somatic phenotype depends on the product of the *Tfm* gene as well as on testosterone, it would be advantageous to the organism to polarize the two sexes with respect to the amount of *Tfm*⁺ product synthesized. Such polarization would appear to be especially desirable because females also produce small amounts of testosterone. Lyon has therefore suggested that dosage compensation, by equalizing the number of active copies of the *Tfm* gene in the two sexes, may have aided the evolu-

tionary polarization of the two sexes with respect to the level of *Tfm* product. Recent observations suggest that the primary consequence of mutations in the *Tfm* gene in mouse as well as man is androgen insensitivity. A receptor protein capable of binding the androgen dihydrotestosterone appears to be affected in these mutants. At least three quantitative types of heritable defects of this androgen receptor system have been discovered among humans showing androgen insensitivity¹¹. However, in normal males and females, the levels of this protein are not very dissimilar, suggesting that X-inactivation is not polarizing the two sexes with respect to the amount of *Tfm* gene product in the two sexes. Evidence of such polarization may therefore have to be sought among the products of other sex-determining genes on the X chromosome.

SEX DETERMINATION

The central problem in mammalian sexual differentiation is the mechanism by which testis determination occurs. The mammalian embryo has a passive inclination towards the female phenotype. The testes suppress this passive inclination and induce the male phenotype. The indifferent gonads of the embryo develop into testes whenever the cells of the embryo, in particular the cells of the gonadal stroma, contain a Y chromosome. The mechanism by which the Y chromosome brings about testis differentiation is therefore a problem of considerable current interest and much of the remainder of this review will be devoted to a discussion of hypotheses about possible mechanisms.

Advances in the methods of somatic cell hybridization and genetic engineering have accelerated the rate at which human chromosomes can be mapped. A large number of assignments of genes to individual chromosomes, to specific arms, and to regions within an arm are now available¹². The X chromosome has more genes mapped on it than any other chromosome. To some extent, this is because the X is present hemizygotously in males. However, paradoxically, not even a single Mendelian gene has been assigned definitively to the Y chromosome although this chromosome, like the X, is present hemizygotously. At various times quantitative characters such as tooth size, height and total ridge count on fingers have been ascribed to genes on the human Y chromosome. The association of these characters with Y chromosomes (or additional Y chromosomes) is possibly attributable to the effects of additional amounts of the constitutive heterochromatin that the Y contributes rather than to specific genes on the Y chromosome. Such condensed chromatin could affect, for example, cell-cycle properties

such as the duration of the S phase and thereby influence quantitative characters such as those mentioned above¹³. The point of interest is that although current dogma requires the presence of Mendelian genes with male-determining properties on the Y chromosome of mammals, these genes have so far eluded us.

Genes determining the H-Y antigen and the serologically detectable male (SDM) antigen were until recently thought to be one and the same and that this gene played a role in testis differentiation. This gene is believed to be located on the Y chromosome. According to a recent critical assessment of the evidence¹⁴, the two antigens are probably specified by different genes and, it appears, neither gene is responsible for male differentiation. On the other hand, there exist several data which seem to suggest that the Y chromosome is not indispensable for male differentiation. The first of these is the occurrence of XX individuals among humans who are phenotypic males. In a few such males there is the possibility of translocation of presumptive Y material on to an X chromosome, but in the large majority of cases no translocation is demonstrable. A second example comes from the Scandinavian wood lemming, *Myopus schisticolor*, in which fertile females with an XY chromosome constitution have been found¹⁵. The Y chromosome in such animals is presumed normal because it was inherited from the normal fathers of the XY females. These and other lines of evidence suggest that the time is ripe for a reassessment of ideas on the role of the Y chromosome in mammalian sex determination. I wish to suggest, as a first step towards such reassessment, that the Y chromosome does not code for a product essential for the development of the male gonadal phenotype. A model for testis determination can be envisaged by postulating that a testis-determining gene (*Tdx*) is located on the X chromosome. The Y chromosome, according to this view, determines maleness not by synthesizing a product essential for the male gonadal phenotype but by preventing a repressor of autosomal origin from binding to the *Tdx* gene. When the Y chromosome is absent, the repressor binds to the *Tdx* gene and transcription of the *Tdx* product is thereby blocked, resulting in the development of the female phenotype. In other words, there is competition between a limited quantity of repressor synthesized by an autosome and RNA polymerase for binding to the *Tdx* gene as well as a set of postulated high-affinity sites on the Y chromosome. Because the repressor has higher affinity than polymerase for the Y chromosome, it preferentially binds to the Y chromosome in XY embryos and allows RNA polymerase to initiate transcription of the *Tdx* gene product. In embryos without a Y chromosome, such as those with an XX or XO constitution, there is no competition from the

Y-linked high-affinity sites and the repressor therefore binds to the single copy of the *Tdx* gene on the active X chromosome since one X is inactive in XX cells. The Y chromosome merely provides binding sites for the repressor and does not support synthesis of a product or products necessary for determining the male gonad.

Eicher¹⁶ has interpreted data on hybrids between *Mus. musculus* and *M. poschiavinus* as evidence for the presence of a male-determining gene, *Tdy*, on the Y chromosome. It is possible, however, to understand the transformation of XY hybrids with a *M. poschiavinus* Y chromosome into phenotypic females on the basis of the above model if we assume that the *M. musculus* repressor does not have sufficient affinity for the high-affinity sites of the *M. poschiavinus* Y chromosome. As a result, it interferes with the binding of polymerase to the *Tdx* gene and brings about a reduction in the amount of *Tdx* product synthesized. A detailed account of this hypothesis, including interpretation, within this framework, of anomalous conditions of sexual differentiation in man and other mammals, will be published separately¹⁷.

EVOLUTION OF X AND Y CHROMOSOMES

Do the X and Y chromosomes of mammals share some genetic homology and, therefore, a common evolutionary origin? It has been known for some time that in the male, the X and Y chromosomes pair and form a chiasma, which is usually considered as evidence of genetic recombination. More recently, electron microscopic evidence has demonstrated a distinct pairing segment between the two chromosomes and a synaptonemal complex¹⁸. Since synaptonemal complexes are associated with recombination, it is assumed that recombination must occur between segments of the short arms of the X and Y chromosomes. Based on these and other observations Burgoyne¹⁹ has recently developed an interesting model on genetic homology and recombination between the X and Y chromosomes. He has postulated that there is an obligatory cross-over between the short arms of the X and Y chromosomes and that the genes distal to the point of crossover will behave as autosomal genes. These genes, referred to as "pseudoautosomal genes", escape inactivation and include, among them, genes which are necessary in two doses for normal development to occur. According to this hypothesis, a primary reason for the developmental anomalies in XO Turner women is the presence of only one set of these pseudoautosomal genes. The model also provides a

possible explanation for several other curious observations, in particular, the inheritance of *Sxr* and *Xg* phenotypes. The model implies that the X and Y chromosomes may have evolved from a single pair of homologous chromosomes which differentiated subsequently into morphologically and functionally distinct elements.

The author's research reported here was supported by the Indian Council of Medical Research and by a grant from the Department of Science and Technology, Government of India.

5 March 1983; Revised 18 March 1983

1. Lyon, M. F., *Nature, (London)*, 1961, **227**, 372.
2. Mohandas, T., Shapiro, L. J., Sparkes, R. S. and Sparkes, M. C., *Proc. Natl. Acad. Sci. U.S.A.* 1979, **76**, 5579.
3. Cooper, D. W., VandeBerg, J. L., Sharman, G. B. and Poole, W. E., *Nature New Biol.*, 1971, **230**, 155.
4. Chandra, H. S. and Brown, S. W., *Nature, (London)* 1975, **253**, 165.
5. Takagi, N., Sugawara, O. and Sasaki, M., *Chromosoma*, 1982, **85**, 275.
6. Gartler, S. M., Andina, R. and Gant, N., *Exp. Cell Res.*, 1975, **91**, 454.
7. Chandra, H. S., in *Scientific Culture in the Contemporary World*, eds. V. Mathieu. and P. Rossi-Monti (Milan and Paris:Scientia/UNESCO), 1979.
8. Gadagkar, R., Nanjundiah, V., Joshi, N. V. and Chandra, H. S., *J. Biosciences*, 1982, **4**, 377.
9. Cline, T. W., *Dev. Biol.*, 1979, **91**, 522.
10. Lyon, M. F., *Helv. Paediat. Acta Suppl.*, 1974, **34**, 7.
11. Kaufman, M., Pinsky, L. and Feder-Hollander, R., *Nature (London)* 1981, **293**, 735.
12. McKusick, V., *Cytogenetics and Cell Genetics*, 1982, **32**, 7.
13. Mittwoch, U., *Nature, (London)* 1969, **221**, 466.
14. Silvers, W. K., Gasser, D. L. and Eicher, E. M., *Cell*, 1982, **28**, 439.
15. Winking, H., Gropp, A. and Fredga, K., *Hum. Genet.*, 1981, **58**, 98.
16. Eicher, E. M., in *Prospects for Sexing Mammalian Sperm* (eds. R. P. Amann and G. F. Seidel, Jr.) Colorado Assoc. Univ. Press, Boulder, Colorado, 1982.
17. Chandra, H. S., (Submitted for publication.)
18. Solari, A. J., *Chromosoma* 1980, **81**, 315.
19. Burgoyne, P., *Hum. Genet.*, 1982, **61**, 85.