REGULATION OF *X*-CHROMOSOME INACTIVATION IN MAMMALS

H. SHARAT CHANDRA

Institute for Genetic Studies, Bangalore 560027, India

SPENCER W. BROWN

Department of Genetics, University of California, Berkeley, California 94720

AS consistently demonstrated by confirmations of the Lyon hypothesis (LYON 1961, 1972), only one of the two *X* chromosomes is expressed genetically in females of eutherian mammals. Which of the two *X* chromosomes is to remain active is determined, apparently at random, during early embryogeny, and thereafter maintained in subsequent somatic cell lineages. In the germ line, however, both *X*'s appear to be active (EPSTEIN 1969).

The marsupial system appears to be simpler because inactivation does not occur at random (COOPER *et al.* 1971; SHARMAN 1971). In the species so far studied, the paternal *X* is inactivated in the female and the maternal *X* remains active. Thus, for the marsupial system it is possible to envisage that the different past history of the chromosomes brought in by the sperm is somehow responsible for the observed inactivation. One complexity has, however, appeared in the still quite limited number of marsupials which have been investigated. According to VANDEBERG, COOPER and SHARMAN (1973), there is some indication that the paternal *X* may also be active to a very limited extent in some marsupial tissues, but the basis for this limited activity is at present unknown. In addition, information on the activity of the paternal *X* in the germ line of the marsupial female is not yet available.

CONTROL SYSTEM

We have recently proposed a control system which has as one of its features the possibility for deriving the eutherian, random-*X* system from the marsupial, paternal-*X* system by a simple evolutionary step (BROWN and CHANDRA 1973). The proposed control system conforms precisely with all known eutherian cases of variation in number of sex chromosomes or genomes with the exception of two types of aberration in man. As will be explained later in this report, it seems likely that these aberrations provide significant evidence on the time in the life cycle when the chromosomes are pre-conditioned or "imprinted" for subsequent differential behavior. The term imprinting was originally used by CROUSE (1960) to indicate the process by which a chromosome is induced or "programmed" to behave differently from a genetically equivalent homolog during subsequent development, often many cell generations after the paternal and maternal homologs are combined in the same zygote.

In both the marsupials and the eutheria, a two-part control mechanism is envisaged in the proposed system. In marsupials, the two parts are pictured as lying close together or as being two components of a single controlling element. One of

Genetics 78: 343–349 September, 1974.

the two components, the "sensitive site," is somehow altered in the paternal-*X* chromosome prior to the completion of fertilization, either earlier, in the body of the male, or in the sperm *en route* to fertilization. The homologous sensitive site on the maternally derived chromosome is not so imprinted. At some stage in early embryogeny, this unimprinted sensitive site of maternal origin produces a single informational entity which attaches to or influences the adjacent receptor site and enables this *X* to remain active during subsequent development. The sensitive site of the paternal *X* does not transmit any information to the adjacent receptor site because of prior imprinting.

We have proposed that the evolutionary transition from the marsupial system to the eutherian system was accomplished by a transposition of the sensitive site, alone, to an autosome. This as yet unidentified autosome would, if maternal in origin, release, as in the marsupial case, a single informational entity and this entity would activate the receptor site of one of the two *X* chromosomes. This *X* would remain active. The other *X*, not receiving the entity, would remain or become inactive at some later stage of development. The well-known mosaicism for *X*-linked genes observed in eutherian females would reflect the time in develment when transfer of this informational entity occurred in any given cell line. This would mean that activation or inactivation is a two-step process and that there may be a considerable time lag between the two steps.

The proposed mechanism makes it more obvious than ever before that the question of mammalian *X*-chromosome inactivation really involves two different problems. The first of these is the mechanism by which the *X* chromosome or *X* chromosomes which are to remain active are chosen. Second, once chosen, how is the whole chromosome involved rather than just one or a few genes? We have referred to the latter phenomenon as the pervasive effect. There is very little evidence as to how this pervasive effect is brought about. More information about the mechanism behind the pervasive effect appears to be necessary in order to understand better many of the data on *X*-autosome translocations.

MOSAIC HETEROZYGOSITY

The eutherian system of random *X* inactivation is believed to have a selective advantage over the marsupial system because of its "mosaic heterozygosity" (BROWN and CHANDRA 1973). With only the maternal *X* active, marsupial females would be expected to be liable to sex-linked genetic defects in a manner similar to the eutherian male: a gene for color blindness or hemophilia in a marsupial female would be expressed if received from the mother even though a normal allele had been received from the father; the paternal allele would be on the inactive *X* and therefore not expressed. On the other hand, the mosaic heterozygosity enjoyed by the eutherian female would provide a type of flexibility analagous to fungal heterokaryons. It should be noted that the advantages of mosaic heterozygosity would accrue to the eutherian female irrespective of the mechanism by which random inactivation is achieved.

IMPRINTING

Besides mammals there are two groups of organisms in which one or more paternal chromosomes function or behave differently from homologous maternal chromosomes. These groups are Sciara and its relatives among the Diptera (METZ 1938; CROUSE 1960), and mealybugs and related families among the coccid insects (Coccoidea:Homoptera) (BROWN and NUR 1964). A comparative study of the cytogenetics of these three groups should prove rewarding, but in this report we will confine ourselves to an analysis of some data from coccids which provide important evidence on the time in the life cycle when imprinting might occur.

Mealybugs: Several recent reviews of coccid cytogenetics are available (BROWN and NUR 1964; BROWN 1969; BROWN and WEIGMANN 1969) and they may be consulted for detailed accounts of these unusual genetic systems. Only those data especially relevant to chromosome imprinting are mentioned here. The chromosomes of the female mealybug are orthodox in behavior except for some characteristics they share with most other coccids. During early development of the male embryo, the paternal set of chromosomes becomes heterochromatic and remains so in most but not necessarily all somatic tissues (BROWN and NUR 1964; NUR 1967). Experimental evidence demonstrates a strict parallel between heterochromatization and genetic inactivity; in general, as shown by conventional genetic markers (BROWN and WEIGMANN 1969) and other evidence, the heterochromatic set is inactive in the male, but its genetic activity is restored in those few tissues in which the paternal chromosomes have undergone reversion to the euchromatic state (NUR 1967). The paternal set is always heterochromatic in the germ line. Meiosis is highly modified; the first division is equational for both eu- and heterochromatic chromosomes; at the second division, the two types of chromosomes are segregated into different nuclei. Therefore, of the four resultant products of meiosis, two are heterochromatic and two euchromatic. Only the euchromatic products form sperm, and only the maternal genes are transmitted (BBOWN and WEIGMANN 1969).

When mealybug sperm is irradiated, the diffuse centromere enables the variously rearranged chromosomes to perpetuate themselves without loss (CHANDRA 1963a). No matter how small or how large, the rearranged paternal chromosomes undergo heterochromatization during embryogeny, divide normally during mitosis, and are typically segregated at meiosis. Irradiation of both sperms and eggs or of young embryos sometimes results in combinations of eu- and heterochromatic segments (NUR 1970). The two types of chromatin maintain a fairly clear-cut distinction from each other in the mitotic chromosome; the aberrant meiotic manoeuvres of such a chromosome are those expected if both its segments are playing their allotted roles. Imprinting in the mealybug is thus the result of a generalized influence throughout the chromosome complement. This effect is not reversed nor more than slightly modified by translocation to an euchromatic chromosome.

In certain species of soft scale insects, a group of coccids related to the mealy-

bugs, both males and females are produced parthenogenetically by fusion of two haploid nuclei derived from a single division of the unfertilized egg (NUR 1971, 1972). There are basically two types of parthenogenetic development relevant to this discussion. In one kind, fusion of polar body II with the egg nucleus results in only females in the four known examples. A quite different mechanism occurs in three other species: the egg nucleus divides once and the resultant daughter nuclei unite to form a zygote substitute. Depending on the species, some or all the embryos formed in this way develop as males with typical heterochromatization of one complete haploid set. Both haploid sets in these males are presumably genetically identical derivatives of the single haploid set of the egg nucleus, but one set becomes heterochromatic during early development, the other does not. It is therefore clear that the factors responsible for inactivation of one set of chromosomes can be entirely maternal in origin. Furthermore, because it seems unlikely that an entirely new mechanism for inactivation would have evolved as a function of parthenogenesis, it seems reasonable to assume that in sexually reproducing species also the paternal set is imprinted within an egg destined to produce a male embryo.

The region within the egg which is responsible for imprinting appears to be restricted. The egg nucleus is presumed to lie outside this region because the maternal complement is not heterochromatized in sexually produced male embryos. Similarly the polar bodies also would lie outside the imprinting region; the evidence from parthenogenesis involving polar body II has just been cited and the evidence from two other sources leads to a similar conclusion. In mealybugs and their relatives, polar bodies do not degenerate, but participate in the formation of polyploid tissues which persist in the adult. Whether in males or females, embryos or adults, none of the chromosomes in these polyploid nuclei normally becomes heterochromatic. Furthermore, after prior heavy irradiation of mealybug sperm, development of the zygote is defective, and instead one polar body or both together may undertake normal embryogenesis (CHANDRA 1963a). The resultant embryos and eventual fertile adults may be diploid, triploid, or mosaic but they are always female, with only euchromatic chromosomes (CHANDRA 1962, 1963b).

The sex ratio of the mealybug has long been known to be highly variable and modifiable by influences affecting the mother. In the light of NUR's work (1971, 1972) with soft scales, it is tempting to believe that the sex of mealybug embryos is determined by the functioning or nonfunctioning of imprinting mechanisms within the egg, presumably in that part of the egg through which the sperm passes on its way to the egg pronucleus.

Mammals: The only evidence now available on the possible stage in the life cycle of mammals when imprinting might occur comes from a class of tumors of the human ovary (LINDER 1969) and from a human diploid/digynic-triploid mosaic girl (ELLIS *et al.* 1963). The evidence is meagre, but the fact that both these exceptional situations appear to have originated as the result of postmeiotic episodes appears significant to us.

1) Ovarian teratomas are bizarre tumorous growths, mostly benign, which

originate from the ovary. They are often highly differentiated, sometimes with teeth, hair and other tissue systems. In such teratomas, no genetic markers are present that are not present in the host female, but some of the maternal markers are missing (LINDER 1969; LINDER and POWER 1970). The chromosome complements of the teratomas are normal, 46*XX*, and one sex chromatin body is present in the nuclei. The most likely interpretation of these data is that the teratomas are postmeiotic products of strictly maternal origin (LINDER 1969). If so, this is contrary to the expectations based on our hypothesis, since the presence of two sets of maternal autosomes should result in two active *X* chromosomes and not one, as observed.

2) A particularly relevant case is that of a human diploid/triploid (46,*XX*/69,*XXX*) mosaic female (ELLIS *et al.* 1963). The extra haploid set in triploid cells was apparently of maternal origin but it was not possible to determine whether it was genetically the same as the maternal set in the diploid tissue. Union of one of the early cleavage products of the zygote (diploid) with polar body II (haploid) is the simplest way of accounting for the origin of this mosaic individual. One sex chromatin body was found in the diploid cells, two in the triploid (MITTWOCH, ATKINS and ELLIS 1963), and not one as would be expected from the proposed eutherian model.

We feel that the coccid data summarized earlier provide important clues towards understanding these exceptional cases. In the ovarian teratomas, a detailed consideration of the results led to the assumption that in the host female, the second meiotic division had been suppressed or the equivalent obtained through the "re-entry of the second polar body" (LINDER and POWER 1970). The idea of re-entry of polar body II is attractive because such a union might provide a stimulus for further, albeit abnormal, development. In other words, a decided possibility exists, that if the "zygote" is formed by two maternal sets arriving by two *different* routes, one may have undergone imprinting, but if both sets are derived by the same route, such as suppression of second meiotic division by colchicine (BOMSEL-HELMREICH 1971), then neither would normally undergo imprinting. According to this interpretation, the presence of two sex chromatin bodies in the triploid cells of the diploid/triploid mosaic girl is the result of imprinting of polar body II prior to its fusion with one of the cleavage nuclei.

The above interpretation of the mammalian data conforms generally with the evidence from the coccids that the immediate past history of the pronuclei combining to form the zygote or zygote substitute is of paramount importance. A specific difference with regard to polar bodies needs further comment: these appear to be subject to imprinting in the mammals but not in coccids. The apparent absence of imprinting of polar bodies in mealybugs and other coccids may be related to the fact that in these insects the polar bodies do not degenerate but are involved in the formation of large polyploid tissues which persist in the adult.

To recapitulate, the evidence from parthenogenetic coccids and human ovarian teratomas leaves no doubt that the factors responsible for inactivation can be strictly maternal in origin. These results and the data from the diploid/triploid mosaic girl further indicate that imprinting occurs in the egg, and that there may

be differences between coccids and mammals in the pattern of localization of imprinting factors.

In the control system suggested for eutherians, a single sensitive site is presumed to occur in one of the autosomes. Because of the ambiguities mentioned above regarding the origin of maternal chromosome sets in some instances, it seems likely that attempts to identify the responsible autosome will provide a more appropriate test of the hypothesis than would correlations between the number of active *X* chromosomes and the parental origins of extra chromosomal sets in polyploid embryos. Monosomic embryos, lacking the maternal homolog of the specific autosome, would be expected to have no active *X* chromosomes and succumb shortly after the normal time for *X* inactivation. Trisomic embryos having two maternally derived homologs of this autosome would be expected to have two active *X* chromosomes; genetic imbalance would be less extreme than in the monosomic cases and embryos might survive long enough to permit accurate assessment of the number of active and inactive *X* chromosomes.

On the other hand, it may be possible to investigate experimentally the question of imprinting in the mammalian polar body provided that re-entry of polar body II could be induced and the resultant zygote substitute enabled to develop further, perhaps by the techniques used to develop chimeras.

These investigations were supported by grants from the Indian National Science Academy and the World Health Organization to H.S.C. and by a grant to S.W.B. from the National Science Foundation (NSF GB–8196:2). We thank Dr. Stanley M. Gartler for drawing our attention to the ovarian teratoma data.

LITERATURE CITED

Bomsel-Helmreich, O., 1971 Fate of heteroploid embryos. Advan. Biosciences **6:** 381–403.

Brown, S. W., 1969 Developmental control of heterochromatization in coccids. Genetics **61:** 191–198.

Brown, S. W. and U. Nur, 1964 Heterochromatic chromosomes in the coccoids. Science **145:** 130–136.

Brown, S. W. and L. Weigmann, 1969 Cytogenetics of the mealybug *Planococcus citri* (Risso) (Homoptera, Coccoidea): Genetic markers, lethals and chromosome rearrangements. Chromosoma **28:** 255–279.

Brown, S. W. and H. S. Chandra, 1973 Inactivation system of the mammalian X chromosome. Proc. Natl. Acad. Sci. U.S. **70:** 195–199.

Chandra, H. S., 1962 Inverse meiosis in triploid females of the mealy bug, *Planococcus citri*. Genetics **47:** 1441–1454. ——, 1963a Cytogenetic studies following high dosage paternal irradiation in the mealy bug, *Planococcus citri* I. Cytology of X_1 embryos Chromosoma **14:** 310–329. ——, 1963b Cytogenetic studies following high dosage paternal irradiation in the mealy bug, *Planococcus citri* II. Cytology of X_1 females and the problem of lecanoid sex determination. Chromosoma **14:** 330–346.

Cooper, D. W., J. L. VandeBerg, G. B. Sharman and W. E. Poole, 1971 Phosphoglycerate kinase polymorphism provides further evidence for paternal X inactivation. Nature New Biol. **230:** 155–157.

Crouse, H. V., 1960 The Controlling element in sex chromosome behavior in *Sciara*. Genetics **45:** 1429–1443.

ELLIS, J. R., R. MARSHALL, I. C. S. NORMAND and L. S. PENROSE, 1963 A girl with triploid cells. Nature **198:** 411.

EPSTEIN, J., 1969 Mammalian oocytes: X chromosome activity. Science **163:** 1078–1079.

LINDER, D., 1969 Gene loss in human teratomas. Proc. Natl. Acad. Sci. U.S. **63:** 699–704.

LINDER, D. and J. POWER, 1970 Further evidence of post-meiotic orgin of teratomas in the human female. Ann. Human Genet. **34:** 21–31.

LYON, M. F., 1961 Gene action in the X-chromosome of the mouse. Nature **190:** 372–373. ——, 1972 X-chromosome inactivation and developmental patterns in mammals. Biol. Rev. **47:** 1–35.

METZ, C., 1938 Chromosome behavior, inheritance and sex determination in *Sciara*. Am. Naturalist **72:** 485–520.

MITTWOCH, U., N. B. ATKIN and J. R. ELLIS, 1963 Barr bodies in triploid cells. Cytogenetics **2:** 323–330.

NUR, U., 1967 Reversal of heterochromatization and the activity of the paternal chromosome set in the male mealybug. Genetics **56:** 375–385. ——, 1970 Translocations between eu- and heterochromatic chromosomes, and spermatocytes lacking a heterochromatic set in male mealybugs. Chromosoma **29:** 42–61. ——, 1971 Parthenogenesis in Coccids (Homoptera). Am. Zoologist **11:** 301–308. ——, 1972 Diploid arrhenotoky and automictic thelytoky in soft scale insects (Lecaniidae:Coccoidea:Homoptera). Chromosoma **39:** 381–401.

SHARMAN, G. B., 1971 Late DNA replication in the paternally derived X chromosome of female kangaroos. Nature **230:** 231–232.

VANDEBERG, J. L., D. W. COOPER and G. B. SHARMAN, 1973 Phosphoglycerate kinase *A* polymorphism in the wallaby *Macropus parryi*: Activity of both X chromosomes in muscle. Nature New Biol. **243:** 47–48.