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Inactivation System of the Mammalian X Chromosome

(Lyon hypothesis/heterochromatin/genetic regulation/sex chromatin)

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ABSTRACT **In female mammals, one of the two X chromosomes present is inactivated during early development. In marsupials, the paternal X is inactivated; in eutherians, one of the two X chromosomes is inactivated at random. A mechanism is proposed to explain the cytogenetic data on inactivation and the derivation of the eutherian system from the marsupial system. In the marsupial system, a site on the X chromosome is sensitive to paternal origin: when the X chromosome is of maternal origin, this sensitive site is responsible for influencing an adjacent site, the receptor, to maintain the X in an active state; the paternal X becomes inactive. Transposition of the sensitive site to an autosome in eutherians would have two consequences. Since the receptor site of the X chromosome is no longer adjacent, the autosomal sensitive site of maternal origin would activate an X at random. The number of active X chromosomes would conform to the number of maternal sensitive sites and thus, generally, to the number of maternal sets of autosomes. The response of the sensitive site to its passage through the male may be designated as imprinting, a term used by Crouse to indicate that the behavior of *Sciara* chromosomes is determined by parental origin.**

The essential premise of the Lyon hypothesis (1), that in somatic cells of mammalian females one of the two X chromosomes normally present is genetically inactive, has been amply verified by a large body of biochemical, genetic, and cytological evidence (2). The focus of attention has, therefore, now shifted to the mechanism responsible for this remarkable situation in which an entire chromosome appears to be inactive and its homologue active or potentially active.

In cells of female eutherian mammals the inactive X can be either paternal or maternal in origin; the determination occurs independently in the several cells of the young embryo and, once made, is adhered to in all descendants of each cell. On the other hand, it has recently been found that among certain marsupial mammals, the kangaroos, it is always the paternal X that is inactive (3, 4). This somewhat unexpected finding, which may be a general characteristic of marsupials, has led Cooper (5) to propose that the random X inactivation of eutherian mammals has evolved from an ancestral condition still retained among the marsupials. He has further suggested that inactivation is brought about by the following mechanism: (*a*) among male marsupials, during meiosis or earlier, a controlling element is introduced into the X, probably by the Y, making the paternal X inactive in the next generation; (*b*) in eutherian mammals, the element is excised during early development and reinserted at random into one of the two X chromosomes, resulting in random inactivation. Cooper realized that, without further assumptions, his hypothesis could not account for several well-known facts of X chromosome behavior in man, mouse, and other mammals. Lyon (2, 6) has critically examined Cooper's model and those of others, and has pointed out (6) that it is advisable to start with the fewest assumptions and to develop a model that is both testable and consistent with currently available data. It may be added that the proposed mechanism for eutherian mammals should be capable of easy derivation from that of the marsupials, in which it is the paternal X that is always inactivated. A model satisfying these criteria is described here.

Abbreviations: Superscripts *m* and *p* indicate maternal and paternal origin, respectively; e.g., A^m is a set of autosomes derived from the mother; X^p, an X chromosome from the father.

PROPOSED CONTROL SYSTEM

On the basis of evidence to be discussed below, it is suggested that: (*a*) a locus (sensitive site) responsive to parental origin is located on an autosome in eutherians; (*b*) the paternal autosomal locus is presumed to be affected or influenced in a manner similar to that known for marsupials (3, 4), *Sciara* (7), and the coccids (8), while the maternal autosomal locus remains unaffected; the influence the paternal autosome undergoes during its passage through the male parent (see below, *Some General Considerations*) will be referred to as imprinting (9); (*c*) during early embryogeny, the unaffected sensitive site of the maternal autosome produces a single informational entity that attaches to a receptor site (10, 11) of one of the X chromosomes encountered at random; this X chromosome remains or becomes active; (*d*) at some later stage in development all other X chromosomes become inactive nonspecifically; that is, no special controlling elements are necessary to bring about the inactivation.

The control system is equally applicable to males. In normal XY and Klinefelter XXY males the presence of one maternal sensitive site would lead to one active X in each and one inactive X in Klinefelter males.

The eutherian system is presumed to have originated from the marsupial in an exceedingly simple fashion (Fig. 1). The marsupial X is sensitized by passage through the male parent, and becomes inactive during early female embryogenesis. All that is considered necessary is the transposition of the sensitive site, alone, to an autosome. The receptor site that controls activation versus inactivation remains on the X chromosome. The sensitive site in its new location on the maternal autosome is active and, since it is no longer coupled directly with an X chromosome, activates at random one of

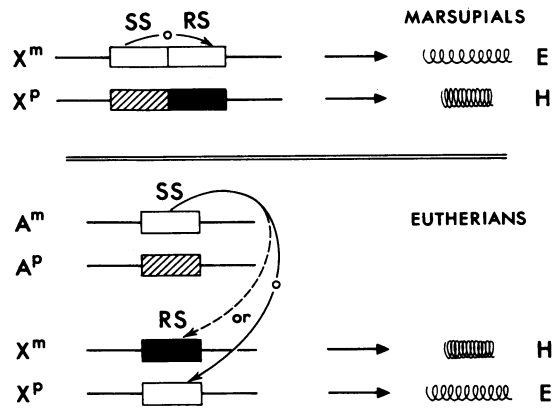

FIG. 1. *The marsupial system (above).* The sensitive site (*SS*) of the maternal X (X^m) produces an informational entity (*small circle*) that is transferred to the adjacent receptor site (*RS*). As a result, the maternal X remains euchromatic (*E*). The sensitive site of the paternal X (X^p) has been altered by its prior passage through the male phase of the life cycle: no informational entity is transferred to the adjacent receptor site. The paternal X becomes heterochromatic (*H*). (The paternal *RS* and *SS* regions have been marked for comparison with the eutherian system.) *The eutherian system (below).* Essentially the same as the marsupial system, except that the *SS* region has been inserted in an autosome. The *SS* of the paternally derived autosome (A^p) has been altered by prior passage through the male phase of the life cycle. The unaltered *SS* region of the maternal autosome (A^m) produces an informational entity that may attach to either X chromosome at random in each of the cells of the early embryo. The X chromosome receiving the entity remains euchromatic; the other becomes heterochromatic.

the X chromosomes present in the same nucleus. The sensitive site on the paternal autosome has been inactivated by its passage through the male and is, therefore, not capable of activating an X. This evolutionary step would be analogous to certain changes observed by McClintock (12) in gene-control systems in maize. In the Suppressor-mutator and Activator systems, one of the two essential components was not readily detected when both were close to the gene locus under control. A transposition of one component, the operator element, to a new location permitted identification of both it and the regulator element that had remained adjacent to the gene locus. Of special interest in the present context is McClintock's remark about one such case in which "A typical two-element system of control of gene action had evolved from an apparently one-element system."

The proposed evolutionary step would have the selective advantage of conferring on eutherian females a "mosaic heterozygosity" for the X, in contrast to the equivalent of a hemizygous X in marsupial females. For example, if the marsupial system were present in man, color blindness would be as common in females as in males; both would show the defect on receiving the mutant factor from the mother.

THE CYTOGENETIC EVIDENCE

Diploids

The expectation from the above scheme would be a strict concordance between the number of sets of maternal autosomes and the number of active X chromosomes. The parental origin of the X chromosomes themselves should have no bearing on their subsequent behavior. This is indeed what is observed (Table 1). In man, anomalous 46, X^mX^m males (13), who probably originate from XXY zygotes, show one sex chromatin body per diploid cell, and are in this respect indistinguishable from typical, diploid females (46, X^mX^p). Even in 49, XXXXY individuals, in whom all four X chromosomes were maternal in origin, all but one X appeared to be inactive (14, 15). It should be obvious that Cooper's hypothesis, as he himself was aware, cannot account for any of the observations cited in Table 1.

Although evidence from diploids effectively rules out the Cooper hypothesis, all examples have only one active X and can, therefore, be explained by the hypothesis of Grumbach, Morishima, and Taylor (10) that a single episome-like factor activates a single X.

Triploids

The concordance between the number of maternal sets of autosomes and the number of active X chromosomes is clearly better looked for in polyploid embryos, but the techniques that permit rapid identification of the chromosome sets in such embryos have only recently become available. However, even among those cases where the origin of the extra set is not known, a definite pattern emerges that is consistent with the scheme proposed here. Thus, in human 69,XXY triploids, one would expect two classes from the following gametic combinations: 2X2A ♀ + YA♂ and XA ♀ + XY2A♂, where each A denotes an autosomal set. On the basis of the control mechanism suggested here, the former class should be sex-chromatin negative and the latter class sex-chromatin positive, with a maximum of one sex-chromatin body per nucleus; XXY triploids of both classes have been observed (16, 17). Similarly, among 69,XXX triploids, two classes would be expected: some with only one sex chromatin body and others with a maximum of two bodies per cell; again both classes have been observed (16, 17). A minority of

TABLE 1. *Expected number of inactive X chromosomes (in parentheses), and, therefore, maximum number of sex chromatin bodies on a diploid autosomal background. All examples are human subjects reported in the literature**

Sex chromosome constitution (m = maternal, p = paternal)	Cooper model (5) No. of inactive X chromosomes = No. of paternal X chromosomes	Proposed model No. of inactive X chromosomes = No. of X chromosomes less No. of maternal sets of autosomes—here, one
X^mX^m (12, 36)	XX	X(X)
X^pO (33)	(X)O	XO
X^mX^mY (35)	XXY	X(X)Y
$X^mX^mX^p$ (36)†	XX(X)	X(XX)
$X^mX^mX^mY$ (36)	XXXY	X(XX)Y
$X^mX^mX^mX^m$ (34, 35)	XXXX	X(XXX)
$X^mX^mX^mX^mY$ (13, 14)	XXXXY	X(XXX)Y

* Only some of those genotypes that help differentiate between the two models and that are known to occur are listed here. See text for cases of typical diploidy and polyploidy.

† It is very likely that both $X^mX^mX^p$ and $X^mX^pX^p$ types occur, but it has not been possible to distinguish between them.

human XXX triploids have a maximum of two sex chromatin bodies per cell, whereas the majority have only one. According to the present interpretation, digyny would thus be the dominant mechanism in the origin of human triploidy. Edwards *et al.*, (17) have arrived at the same conclusion on the basis of other evidence.

Data on both the sex-chromatin status and parental origin of the extra set are available from one 69,XXY infant (17): his blood group data were more consistent with digyny than with diandry and the infant was sex-chromatin negative, as would be expected on the basis of the present hypothesis.

The most cogent evidence in favor of the hypothesis comes from the recent work of Bomsel–Helmreich (18), who induced triploidy in rabbit embryos by suppression of the second polar body. Triploidy was, therefore, always the result of digyny. XXX triploids had, like XX diploids, only one sex chromatin body, whereas XXY triploids were uniformly sex-chromatin negative. Assays of glucose-6-phosphate dehydrogenase, an enzyme believed to be X-linked in the rabbit (19), showed that enzymatic activity was consistently higher in triploid embryos as compared with diploid controls, indicating that more than one glucose-6-phosphate dehydrogenase locus was probably active among the triploid cells.

Although the triploid data do not permit a distinction to be made between the Cooper model and the one proposed here, they do rule out a single episome-like factor as an explanation of the number of active X chromosomes (10). An episome-like factor of extrachromosomal origin would remain a possible explanation only if the further assumption is made that the number of such factors somehow conformed to the number of sets of maternal autosomes. However, this added assumption is unattractive, if only because it would be difficult to picture the derivation of such an extrachromosomal mechanism from the marsupial system of paternal X inactivation.

Tetraploids

Only two types of tetraploids are known, XXXX and XXYY. It seems very likely that these were derived from XX and XY zygotes in which early failure of cytokinesis led to a doubling of the chromosome complement (16). Two sex chromatin bodies have been observed in the XXXX tetraploids and none in the XXYY. These observations are comparable with those from tetraploid and higher endopolyploid cells occurring in normal XX and XY individuals (20) and offer no additional evidence bearing on our argument.

X-autosome translocations in mice

The mechanism responsible for inactivation of the X chromosome of mice has received much attention during the last decade. In all but one of the X-autosome translocations of mice, a "position-effect" type of variegation is induced for at least some of the genes of the autosome involved (2, 11, 21). The exceptional case is that of the Searle translocation (22); none of the genes on this autosome has been identified.

Considerable attention has been paid to the possibility that variegation patterns that have been observed may be the result of selection against cells in which inactivation has led to either (*a*) a modification of dosage, thus upsetting the normal dosage-compensation relationship, or (*b*) the equivalent of a homozygous deficiency. Inferences concerning the type of inactivation made from the observed variegation patterns thus become quite complex.

Nonetheless, there seems to be agreement among most mouse cytogeneticists that there is one major site on the X chromosome that is responsible for its inactivation (11, 21, 23). According to Russell and Montgomery (21), there is a single inactivation center on the X. Although Eicher (11) outlined a complex scheme that included two or more centers of inactivation, she believed that all were under the control of a single "empty" or unactivated receptor site on the X. The conclusions from analyses of inactivation in X-autosome translocations of mice are thus consistent with the main point made here, that there is a single, major controlling site on the X chromosome, regardless of secondary centers of inactivation.

Genetic and cytological evidence indicates that there are probably at least two inactivation centers on X chromosomes of mice, a primary center closely associated with the receptor site, and a secondary center elsewhere on the chromosome. The probability that the secondary center will induce inactivation is considerably lessened by complete physical separation from the primary center in typical, reciprocal translocations, and it may well be less effective in inducing inactivation in adjacent autosomal material. This concept reconciles some of the differences between the ideas of Russell (21) and those of Eicher (11); it is most like that of Eicher's defective memory system, even though objections may be raised in regard to the "memory" aspect of her system (2).

The Searle translocation is of special interest because the normal X is inactive in a large majority of cells and the translocation is correspondingly active. These data can be interpreted in several ways, including the obvious one of cell selection, but Lyon (2) has recognized that the autosome here may carry an activating center. In addition, it should be pointed out that the autosomal component of the Searle translocation is always of *maternal* origin, a requirement for activation in the system proposed here. Except for the Cattanach translocation, all other X-autosome translocations in mice induce male sterility; the chromosomes involved can be transmitted only by females. The autosome involved in the Searle translocation has recently been identified cytologically (24), and recognition of genetic markers on this chromosome would be of great value.

Deficient X chromosomes in man

Three different types of deficient X chromosome have been observed. The long arm may be missing (Xq-), and the short arm present singly or duplicated in an isochromosome (Xpi). Likewise, simple deficiencies of the short arm (Xp-) and isochromosomes of the long arm (Xqi) are also known. These two types are invariably inactive (25, 26). Because an inactivation of the normal chromosome would be equivalent to a gross homozygous deficiency in these cases, cell lineages of this sort would not be expected to survive. In the third type, that of the ring X, the positions of the breaks determine the size of the deficiency; the closer both breaks are to the ends of the chromosome, the smaller the deficiency. It is not surprising, therefore, that nearly all ring-X chromosomes are invariably inactive (25).

Since Xq-, Xpi, Xp-, and Xqi all become inactive (25, 26), it is obvious that there cannot be, as in mice, a single center of inactivation in human X chromosomes. The simplest

scheme, and one in agreement with that suggested for mice, is that there is a primary inactivation site—coupled with or acting as the receptor site—on one arm, and a secondary site on the other.

SOME GENERAL CONSIDERATIONS

If the proposed mechanism is basically valid, then the choice of which X becomes activated has to be made by a single entity in an embryonic diploid cell and, thus, it would appear that only one site on the chosen X can be "hit" to begin with; a subsequent, pervasive effect is therefore also necessary to involve the whole chromosome. The mechanism by which such a pervasive effect is obtained is much less clear in any of its aspects than the choice of the X to be activated. The overall problem of mammalian X chromosome inactivation can thus be approached at two separate levels: (*a*) the basic facultative determination of which X chromosome or chromosomes are to remain active, and (*b*) the mechanism by which the pervasive effect is achieved. We are concerned here primarily with the basic determination; more insight into the mechanism would increase our understanding of the translocation data.

Imprinting

Crouse (9) introduced the term imprinting to indicate a change of state of a chromosome that allowed it to be "recognized" as different from its homologue. In *Sciara*, the imprinting is believed to occur in maternal and paternal germ lines before fertilization, and to determine the later behavior of the X chromosomes and the autosomes. Once imprinted, the future behavior of the chromosome is apparently irrevocably determined. The heterochromatic L chromosomes, limited to the germ line, are not subject to imprinting (27). According to the scheme outlined here for mammals, the fate of the paternal X chromosome in marsupials would be irrevocably determined by an imprinting of the sensitive site in the male parent. In eutherian mammals, imprinting of the sensitive site of the paternal autosome would occur before the completion of fertilization. The same site on the maternal autosome, not so imprinted, would remain active and lead to a random determination of which X chromosome is to remain active; at this time the fates of the X chromosomes are irrevocably determined. Analysis of variegated phenotypes in adult eutherians would indicate the time in development when the fates of the X chromosomes are determined, not when inactivation occurs. Inactivation would be a realization of the prior determination and could occur either simultaneously or at a later stage of embryogenesis. A combined analysis of variegation in the adult and of heterochromatization and the action of specific genes during early development should eventually establish the correct chronology.

It should also be noted that the differentiation between maternal and paternal origin does not imply that the alteration that leads to subsequent differential behavior of homologues necessarily occurred in the body of the male parent. In a parthenogenetic scale insect, Nur (28) found that diploidy was restored by fusion of the first two cleavage nuclei; however, in some instances the chromosomes derived from one of these nuclei later became heterochromatic, and indistinguishable in this regard from the heterochromatization of the paternal chromosome set in early embryogeny of the males of related bisexual species. The observed differential behavior of the paternal X chromosome in marsupials and the proposed imprinting in an eutherian autosome could be the result of influences present after sperm emission, but before nuclear fusion completed fertilization. The term "male phase" may, therefore, be suggested as a comprehensive term to include, in addition to events occurring in the body of the male, the period between emission and completion of fertilization.

Activating entity

There is no information concerning the nature of the agent acting on the sensitive site, or that transfers information from there to the receptor site. The sensitive site might perhaps be sensitized to respond to minor influences in the male phase; there is no reason either to presume or to rule out specific informational macromolecules. On the other hand, the fact that only a single X-chromosome site is normally activated in a diploid indicates that a single specific macromolecule may well be involved in the transfer of information from the sensitive to the receptor site. It has been suggested that such specific macromolecules are episomes or episome-like entities (29, 30). In view of recent critical review of the use of the term episome (31), it seems best to use a noncommittal term such as informational entity to designate the agent responsible for the transfer of information by one or a few macromolecules.

Prediction of sex chromatin status

Published discussions of sex chromatin have often included a formula by Harnden (32) to predict the expected number of sex chromatin bodies under various genotypic conditions. It was soon discovered that the formula, $S = X - A/2$ (where S is the expected number of sex chromatin bodies, X is the number of X chromosomes, and A is the number of autosomal sets), could not account for the data from triploids. On the other hand, the control system proposed here, when reduced to $S = X - A^m$, where A^m is the number of maternal autosomal sets, is a satisfactory formulation for all situations thus far observed. Indeed, if exceptions to this rule should occur, such as among human abortuses trisomic or monosomic for autosomes, they could very well provide material for assigning the locus in question to a specific autosome.

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