The Role of X-Chromosome Inactivation during Spermatogenesis

(*Drosophila*/allocyely/chromosome evolution/male sterility/dosage compensation)

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ABSTRACT Inactivation of the single X chromosome in the primary spermatocytes of species with heterogametic males is postulated as a basic control mechanism on the chromosomal level that is required for normal spermatogenesis. This view is supported by (a) cytological observations of X-chromosome allocyely in the primary spermatocytes of all male-heterogametic organisms that were adequately examined, (b) autoradiographic evidence of early cessation of transcription by the X chromosome in the mouse and three species of grasshopper, and (c) the male sterility of animals with certain X-chromosome rearrangements that cannot be attributed to malfunction of specific genes. X-chromosome inactivation during spermatogenesis is proposed as the ideal system for studies of genetic control at the chromosomal level.

Sex chromosomes provide a striking example of differentiation of the chromosomal complement and inferentially of chromosomal function during evolution. It seems reasonable to suppose that natural selection has resulted in a particular distribution of genes between the sex chromosomes and the autosomes that is related to the role of these genes in the development and expression of sexuality. For example, male-fertility genes are found on the Y chromosome (Y) in *Drosophila*, whereas male-determining genes are found on mammalian Y chromosomes. Furthermore, sexual differentiation might depend on males differing in constitution from females (e.g. dosage) with respect to some genes but not others; Bridges' and Goldschmidt's genic balance theories of sex determination (1, 2) are based on such a requirement. If accompanying sex chromosome evolution there were the concomitant evolution of a chromosomal mechanism permitting coordinate control of sex-linked gene function, then the potential of functioning in coordination with other sex-linked genes might favor the location of certain loci on the sex chromosome. That such control could have developed is evident from other forms of control operating at the chromosomal level. For example, coordinate control of chromosomal activity is evident in all eukaryotic organisms at mitosis; furthermore, asynchronous control of different chromosomes or chromosome segments may be inferred from their allocyely (differences in chromosome condensation) during the cell cycle. Inactivation of one X chromosome (X) in somatic cells of female mammals (3) or of the entire paternally inherited chromosome set in certain coccids (4), as well as the physical elimination of chromosomal material from cell lines (5, 6), are other examples of gene inactivation at the chromosomal level. We conclude that the evolution of both genic and chromosomal processes

has culminated in heterogametic sexuality in a vast majority of animal species.

Two major developmental processes are involved in sexuality, sex determination and gametogenesis. These processes are not completely coupled, as gametogenesis is not a necessary concomitant of sex determination. It is the purpose of this communication to delineate the function of the X chromosome during spermatogenesis in heterogametic males. A wide range of seemingly unrelated cytological and genetic observations are assembled that support the unifying hypothesis that the single X chromosome in all male-heterogametic organisms is normally inactivated during a critical stage of spermatogenesis. If X chromosome inactivation is an essential control step, then factors interfering with it will upset the biochemical machinery of the cell; this may in turn lead to male sterility.

CYTOLOGICAL OBSERVATIONS

A large body of cytological literature, beginning with the discovery of the X chromosome in the hemipteran, *Pyrhocoris*, by Henking in 1891 (7), documents the generality of X-chromosome allocyely or heteropycnosis in the primary spermatocyte; this observation holds for all adequately-examined species in which males have a heteromorphic sex bivalent. During oogenesis, on the other hand, X-chromosome allocyely is not observed. Early observations are reviewed by Wilson (8). Precocious condensation of the sex chromosomes in *Drosophila melanogaster*, the species from which most of the genetic results reported here are derived, was tentatively described by Cooper (9) for X/Y males. We observed the same phenomenon in X/O males, eliminating the Y chromosome as the sole heteropycnotic element. In *Drosophila pseudoobscura*, the X chromosome is metacentric, one arm being homologous to the rod-shaped X and the other arm homologous to the right arm of the second chromosome of *Drosophila melanogaster* (10). In the spermatocytes of this species, we have observed that the arm of the X that pairs with the Y chromosome is heteropycnotic, whereas the other arm condenses in phase with the autosomes.

In mammals, early condensation of the sex-chromosome bivalent during first meiotic prophase is evident as the sex vesicle in all species studied. Several authors (11, 12) have shown that early condensation of the X in mouse spermatocytes is correlated with late replication; furthermore, Monesi (13) correlated early condensation with genetic inactivation, using cessation of uridine incorporation as the criterion. Similar autoradiographic studies of several species of grasshopper by Henderson (14) demonstrated that the X chromosome replicates later in S (synthetic phase of the cell cycle)

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and ceases transcription earlier in meiotic prophase than the autosomes. Clearly, the conclusion is that in the primary spermatocyte, the X chromosome is out of phase with the autosomes in a wide range of organisms.

The creeping vole *Microtus oregoni* provides a particularly trenchant example of the elimination of X-chromosome activity during spermatogenesis. Ohno *et al.* (15) observed that females are XO and males are XY in constitution. In females, the X chromosome undergoes a regular mitotic nondisjunction in predefinitive gonial cells producing XX and nullo-X daughter cells; only the XX cells proliferate to produce oocytes, and subsequently ova that bear a single X chromosome. Nondisjunction of the X at a parallel stage in male germinal tissue produces XXY- and Y-bearing gonial cells; spermatocytes descend only from YO spermatogonia, with the consequence that equal numbers of Y-bearing and nullo-Y sperms are produced. Although this bizarre behavior serves to maintain the unusual sex-chromosome constitution found in this species, we see it as striking confirmation of the dispensability of the X chromosome in male gametogenesis; the early stage in germ-cell proliferation at which the contribution of the X chromosome ceases is surprising.

GENETIC ANALYSIS

If the phase of the X chromosome in relation to that of the autosomes plays an important role in spermatogenesis, then specific types of genetic alterations that upset this phase relation would be expected to lead to male sterility. Thus, analysis of genetic changes leading to male sterility is a logical method of obtaining genetic evidence on the role of the X chromosome in spermatogenesis. As stated above, the logic of the analysis requires that we distinguish between the role of the X chromosome in sex determination from that in spermatogenesis. Normal sex determination is a prerequisite of normal gametogenesis, but there is ample evidence that normal gametogenesis does not automatically follow normal sexual development. For example, XO individuals in *Drosophila* are morphologically and behaviorally normal males, but gametogenesis is incomplete; also XXY mammals (i.e. man and mouse) are normal-appearing but sterile males. In this communication, we are concerned with the role of sex chromosomes in gametogenesis and not in sex determination.

The genetic causes of male sterility in *Drosophila* fall into two major categories, genic and chromosomal, suggesting that the two levels of genetic organization discussed in the *Introduction* are important for normal gametogenesis. Genic sterility results from the mutation of specific genes, whose products are needed at one time or another for normal gametogenesis. Male-sterile mutations scattered throughout the chromosome complement identify the loci of such genes; the male-fertility factors on the Y are specific examples. Factors responsible for genic sterility are readily localized at particular chromosomal loci by standard mapping procedures and are for the most part recessive.

Chromosomal sterility on the other hand generally involves the X chromosome, is not readily attributable to the effects of particular gene loci, and is dominant. Understanding chromosomal sterility is necessary for ascertaining the basic role of the X chromosome in gametogenesis. We will discuss two types of chromosomal male sterility: X-autosome translocations and X-chromosome deficiencies.

Males carrying a reciprocal translocation between the X chromosome and an autosome T(X;A) are known to be sterile

in both the mouse (16) and *Drosophila* (17, 18). In the mouse, every recorded case of reciprocal T(X;A) is male sterile (16), and one established case in man appears to be subfertile (19). In contrast to *Drosophila* where translocations arrest spermatogenesis postmeiotically, the lesion in spermatogenesis in T(X;A)-bearing mice occurs before or during first meiotic prophase. This may explain the failure of Searle and his colleagues (20) to find a single T(X;A) in the mouse among 2000 translocations induced in primary spermatogonia and scored in primary spermatocytes.

Translocations have been intensively investigated in *Drosophila* and some generalizations regarding the correlation between translocations and male sterility are possible. Although X-2 translocations and X-3 translocations behave as dominant male sterilizers, other types of chromosome aberrations do not. Thus, translocations between autosomes are male fertile, as are X-4 translocations and about half the X-Y and Y-autosome translocations. Warters (21) has shown that more than 75% (85/110) of an unselected sample of translocations between the X chromosome and either chromosome 2 or 3 are male sterile. Salivary gland chromosome analysis (summarized in Fig. 1) reveals that with the exception of translocations that interchange chromosomal tips, all of which are male fertile, and translocations with one breakpoint in the heterochromatin adjacent to the centromere of the X chromosome, some of which are male fertile, all X-autosome translocations are male sterile. A second observation is that the sterility caused by X-autosome translocations is dominant; the addition to the T(X;A)/Y genotype of a segment of the X chromosome including the locus of the T(X;A) breakpoint and

FIG. 1. The breakpoints of all two-break, male-viable translocations between the X chromosome and chromosome 2 or 3. The ordinate represents the X chromosome of the salivary-gland; the terminal division is at the top and the proximal-most division, which is heterochromatic and at the centromere, is shaded and at the origin. The abscissa represents all four autosomal arms with the proximal heterochromatic region of each represented by the shaded segment at the origin and with the distal-most division to the right. Solid circles, represent male-sterile and open circles, male-fertile translocations.

normal alleles of neighboring genes does not restore fertility. The association of dominant sterility, not with aberrations involving the X chromosome or autosomes *per se*, but specifically with translocations between the X and an autosome, as well as the special pattern of breakpoints found in male-sterile X-autosome translocations point to an effect at the chromosomal level. The apparent inability of X-chromosome duplications to rescue fertility leads us to suspect that translocations interfere with the normal inactivation, rather than any positive synthetic activity, of the X chromosome. If as a result of the translocation, the X chromosome were to remain active at a time when inactivation normally occurs, then the addition of more X-chromosome material would not be expected to rectify the abnormality. Approximately half the translocations involving the Y chromosome are also male sterile, but this sterility, in contrast to that caused by X-autosome translocations, is recessive. Unlike the X, the Y does engage in positive synthetic activity in primary spermatocytes (22, 23), and impaired Y function, whether caused by changing the state of the chromosome or deleting specific fertility factors, may be complemented by the presence of an additional normal Y in the genome (24, 25).

We think of the activity of a chromosome as being under the control of factors on the same chromosome, i.e. under cis control, such that translocation between two chromosomes under asynchronous control puts elements of each under dual and conflicting control. Conversely, we view sterility of T(X;A)-bearing males as evidence of asynchronous control of the X and the autosomes during spermatogenesis; thus, fertility of females that are either heterozygous or homozygous for a T(X;A) implies that there is no such asynchrony operative during oogenesis. Furthermore, fertility of X-Y translocations suggests that the X chromosome is more nearly in phase with the Y than it is with the autosomes; cytological observations confirm these suggestions. Similarly, the fertility X-4 translocations suggests relative synchrony of chromosomes X and 4 activity during spermatogenesis.

We presume that X-chromosome inactivation in primary spermatocytes, which is clearly reversible, and that occurring in somatic cells of female mammals, generally considered irreversible, are brought about by the same or similar mechanisms. In fact, recent evidence that the paternally inherited X chromosome is preferentially inactivated in female marsupials (26) provides an evolutionary connection between the two phenomena. On this presumption, we use observations on mammalian somatic cells to infer general properties of chromosomal control that apply to X inactivation in spermatocytes of many groups of animals. For example, studies of the somatic behavior of X-autosome translocations in female mice provide evidence that the phase or state of translocated segments is different from that of the same segments in their normal position; phenotypic observations reveal that autosomal genes, when near the breakpoint of an X-autosome translocation, may become inactivated in the same way as sex-linked genes in female cells (27, 28). Inactivation of translocated autosomal genes may place cells in which the translocated X is inactivated at such a disadvantage during proliferation that virtually all cells observed in the adult have the normal X inactivated and the translocated X active; X-autosome translocations that are preferentially active in cells of heterozygous females have been recorded in the mouse (29), man (19), and cattle (30). Evidence that translocations may affect

the control of normal chromosome activity is not confined to mammalian systems; in *Drosophila*, translocation of the Y chromosome with an autosome may alter the order of replication of Y-chromosome segments, as demonstrated by altered patterns of late labeling in cell culture (31).

Although we tend to think in terms of normally-inactivated sex-linked genes remaining active in X-autosome translocations, an alternative interpretation that ordinarily active autosomal genes become inactivated may also explain T(X;A) male sterility. Only the latter situation has been observed genetically in mouse somatic cells; however, Chu and L. B. Russell (32) have observed that only one segment of a translocated X chromosome is late-labeling, suggesting failure of X inactivation as well. There are three observations from *Drosophila*, that, although not individually compelling, taken together militate against inactivation of autosomal genes as causing T(X;A)-related disruption of gametogenesis: (a) A large fraction of small autosomal deficiencies are male fertile; (b) Two of three insertions of a segment of X into an autosome are male sterile, whereas five out of five insertions of autosomal segments into the X are male fertile; (c) The sterility of male-sterile Y-autosome translocation is Y-linked and not autosomal.

The second category of chromosomal sterility that we wish to discuss in the context of X-chromosome inactivation is deficiency for a specific region in the centromeric heterochromatin of the X chromosome of *Drosophila melanogaster*. We have observed that in a large collection of proximal deficiencies, those that are deficient for both *su(f)* at 67.3 on the genetic map and *bb* at 67.4 are invariably male sterile, while deficiencies for *su(f)* but not *bb* and *bb* but not *su(f)* are in every instance male fertile. These results show that sterility is caused by deficiency for a region and cannot be attributed to the absence of a particular gene locus. The male-sterile deficiencies are all lethal in the absence of a compensating duplication for the proximal segment of the X chromosome (provided by *mal⁺Y*). The fact that males carrying the deficiency in combination with the duplication survive but are sterile demonstrates the recessive nature of the lethality as well as the cis dominance of the sterility associated with the deficiency. We interpret these observations as indicating that the distal segment of the proximal X heterochromatin plays an important role in regulating X-chromosome activity during spermatogenesis. This region may be analogous to the inactivation centers postulated by Cattanaach (33) and L. B. Russell (34) to control X-chromosome inactivation in mammals. Other examples of a heterochromatic role in chromosomal control is found in controlling elements in maize (35) and the phenomenon of variegated-type position effect in *Drosophila* (see review by Baker, ref. 36).

It should be stressed that we view inactivation in relative rather than absolute terms; the mechanism should be understood as a gross modulation of the state of the chromosome with different regions of the chromosome under more or less stringent control. Such differential response to chromosomal control is exemplified for X-chromosome inactivation in mammals by the apparent failure of the sex-linked gene specifying the Xg antigen in man to be inactivated (37) and by the spreading inactivation of autosomal genes in X-autosome translocations in mice (27). In *Drosophila melanogaster* it seems likely that even though the major part of the X chromosome is inactivated in the primary spermatocyte, the genes in

the proximal heterochromatin that code for rRNA continue to transcribe; this argument supposes ribosomal RNA synthesis during spermatocyte growth in XO males that would necessarily involve X-chromosome cistrons. Additional arguments favoring activity of ribosomal cistrons in otherwise inactivated chromosomes can be made with regard to the Y chromosome. This chromosome is dispensable to the normal function of, and inferentially inoperative in, somatic cells of males and all cells of females; yet in genotypes in which there are no other ribosomal cistrons available (NO^-/Y males and $\text{NO}^-/\text{NO}^-/\text{Y}$ females, where NO^- symbolizes a deficiency for the Nucleolus Organizer) the Y-linked cistrons are able to transcribe rRNA. The Y is even capable of supporting the highly active ribosome synthesis required during oocyte growth.

GENERAL CONSIDERATIONS

Three observations on sex-linked genes support the view that a nonrandom distribution of genes between the sex chromosomes and the autosomes is a fundamental consequence of evolution: (a) Genes with special and related functions are located on the Y chromosome both in *Drosophila* (male-fertility factors) and in mammals (male-determining factors); (b) Insofar as it may be ascertained, the genetic content of the X chromosome has been conserved during mammalian evolution (39); (c) Coordinate control of sex-linked-gene action is observed in the phenomenon of dosage compensation and in spermatogenesis. We postulate that selection favored the location on the sex chromosomes of only those genes that are able to function effectively when constrained by the mechanisms of chromosomal control. It is reasonable to suppose that some genes are sex linked because they are related directly or indirectly to sexuality itself, whereas others are simply at a selective advantage when subject to control by the elements regulating sex-chromosome activity. Genes that could not operate to advantage under these restrictions were gradually eliminated from the sex chromosomes and accumulated by the autosomes as coordinate control of the sex chromosomes became more stringent.

Whatever the evolutionary sequence, the relation between sex-linked and autosomal genes is a crucial feature of genome organization in heterogametic species. Genetic alterations that affect this relation lead to reduced fertility. The extreme sensitivity of the male germline to alterations in X-autosome relations provides a powerful selective force for stabilizing this relation. We have seen how X-autosome translocations and proximal X-chromosome deficiencies lead to male sterility. Other candidates for this type of male sterility are X-chromosome duplications that have much more severe effects on male than female fertility, and which are more severe in their effect on male fertility than autosomal duplications of comparable size. A final indication of the extreme sensitivity of the male germline to genetic constitution is seen in species hybrids where, as recognized by Haldane in 1922 (40), fertility of the heterogametic sex seems to be the most fragile attribute of the progeny of interspecies crosses. Hybrids between closely related species may be normal in every respect, except that males are sterile; the sex chromosomes are implicated in that the fertility of progeny from reciprocal crosses may differ.

If the hypothesis proposed in this communication is correct and sex-linked genes are subject to collective control, then a reconsideration of Muller's (41) version of dosage compensation is in order. Although dosage compensation in *Drosophila*

is often considered a counterpart of X inactivation, the basic control mechanism postulated by Muller in his original definition of the phenomenon is entirely different from X inactivation. Muller proposed that the activity of sex-linked genes is increased in males relative to females in order to compensate for the fact that their dose in males is half that in females. He postulated that each locus is modulated by its own constellation of modifiers called compensators. According to Muller, the compensators are themselves sex-linked; they are uncompensated, and their activity is nonlinearly related to their dose, suggesting that they in turn are regulated in some way. Perhaps it is easier to view dosage compensation in *Drosophila* as more closely related to X-chromosome inactivation than previously imagined. A unifying hypothesis is that basic control of dosage compensation in *Drosophila* is by the modulation of sex-linked-gene activity at the chromosomal level and is a special modification of the phenomenon of X-chromosome inactivation seen during spermatogenesis and in somatic cells of female mammals.

Although the behavior of the X chromosome during spermatogenesis is of great interest by itself, we wish to emphasize that if our interpretations are correct, the phenomenon provides a model system of great value for investigating problems of control and coordination on the chromosomal level. The defined stage of occurrence, the favorable cytology, and the possibility of using male-sterilizing mutations will be of great advantage in such studies.

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