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SEX CHROMOSOME ACTIVATION DURING SPERMATOGENESIS

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INACTIVATION of chromosomal elements is a process which takes place in various organisms, cell types, and cell cycle stages. The reasons for chromosome inactivation, which is superimposed on the more specific level of gene control, are different for the various systems. Dosage compensation in female mammals *vs.* mitotic chromosome shut-off are the extreme cases. Since in many systems every chromosome may be in an active or inactive form, it is reasonable to assume, for the time being, that the molecular mechanism in different organisms is similar, thus justifying generalization. The role and behaviour of sex chromosomes during gametogenesis provide a striking example of differentiation of chromosomal elements and by inference reflect chromosome function.

Precocious inactiviation of the *X* chromosome during seprmatogenesis together with activation of fertility factors on the *Y* in some organisms are of particular interest. In view of cytological and genetic observations we suggest a working hypothesis according to which the *X* choromsome is inactivated during a critical stage of spermatogenesis in all male heterogametic organisms. As the inactivation is an essential control and not a compensatory step, any interference with this process will change the developmental course of the spermatocyte leading to dominant male sterility (LIFSCHYTZ and LINDSLEY 1972).

In the course of this paper, the observations that support or lead to this view will be presented. Several experimental approaches we have undertaken to study further the genetics of the phenomenon, as well as its relation to *Y* chromsome activation, will be discussed.

Cytological evidence

The relevant cytological observations have been known for years. Without exception, all adequately tested male heterogametic organisms exhibit allocycly, or heterochromatinization of the *X* chromosome during the first meiotic prophase (HENKING 1891; WILSON 1928). In both insects and mammals, heteropycnosis is correlated with late replication of *X* and early cessation of RNA synthesis (KOFMAN-ALFARO and CHANDLEY 1970; MONESI 1965; ODARTCHENKO and PAVILLARD 1970). Of particular interest is the example provided by the creeping vole (*Microtus oregoni*) in which nondisjunction, a regular feature of gonial mitosis, results in *YO* spermatogonia which are the only ones to produce primary spermatocytes, thus, giving rise to *YA* and *OA* sperm (OHNO, TAINCHILL and STENIOUS

1963)—confirmation of the dispensability of the *X* chromosome in mammalian male gametogenesis. The early stage at which the *X* is no longer essential, however, (i.e., spermatogonia) is surprising indeed.

To establish that the observed allocycly or inactivation is an important control mechanism, it is necessary to find genetic alterations of this phenomenon that lead to male sterility. Analysis of male sterile mutations is therefore the logical method of obtaining genetic evidence for the role of *X* chromosome in spermiogenesis. It should be stated that we distinguish between the role of the *X* chromosome in sex determination and its role in spermiogenesis. Normal sex determination is necessary but not sufficient for normal gametogenesis; *XO* males in Drosophila and *XXY* mammals are examples.

Genetic Analysis

Genetic causes of male sterility fall into two major categories, genic (specific) and chromosomal (non-specific). Genic sterility results from the mutation of specific genes, the products of which are needed at one time or another (directly or indirectly) for normal spermiogenesis. These genes are scattered throughout the genome, including the *Y* chromosome (in Drosophila), are readily allocated to specific loci, and are generally recessive. About 200 EMS-induced *X*-linked male steriles that we have recently analyzed apparently fall into this category. Chromosomal sterility on the other hand generally involves the *X* chromosome, is not readily attributed to the effects of particular gene loci, and is dominant. We will discuss two types of chromosomal sterility, *X*-autosome translocations and *X*-chromosome deficiencies.

Males carrying reciprocal *X*-autosome translocations are known to be sterile, both in Drosophila and the mouse (RUSSELL and MONTGOMERY 1969). Furthermore, females heterozygous or homozygous for *X*-autosome translocations have been shown to be fertile in Drosophila. Some types of autosomal translocations also cause sterility when heterozygous in male mice. Such an effect has not been observed in Drosophila. About 75% of an unselected sample of *T(X;A)*'s were shown by WARTER and LINDSLEY (see LINDSLEY 1965) to be male sterile. Translocations that interchange chromosome tips and some of those having one break in the proximal heterochromatin of the *X* are fertile or semi-fertile (Figure 1). This pattern, as well as the observation that *X*-autosome translocations interfere with sperm head elongation, and that as the translocation breakpoints approach chromosome ends sperm head morphology becomes more nearly normal, suggests a chromosomal rather than a specific gene effect.

Most important, translocation sterility is dominant, as we were unable to restore fertility by the addition of a duplication for the segments of the *X* in which *T(X;A)* breakpoints are located. This was tried with more than twenty translocations with or without associated lethal mutation. In the latter case viability was restored, but in no case was fertility restored.

Taking together the precocious condensation and inactivation of the *X* chromosome on the one hand and the genetic behaviour of *X*-autosome translocations on the other, we suggest that interference with the normal inactivation process is the

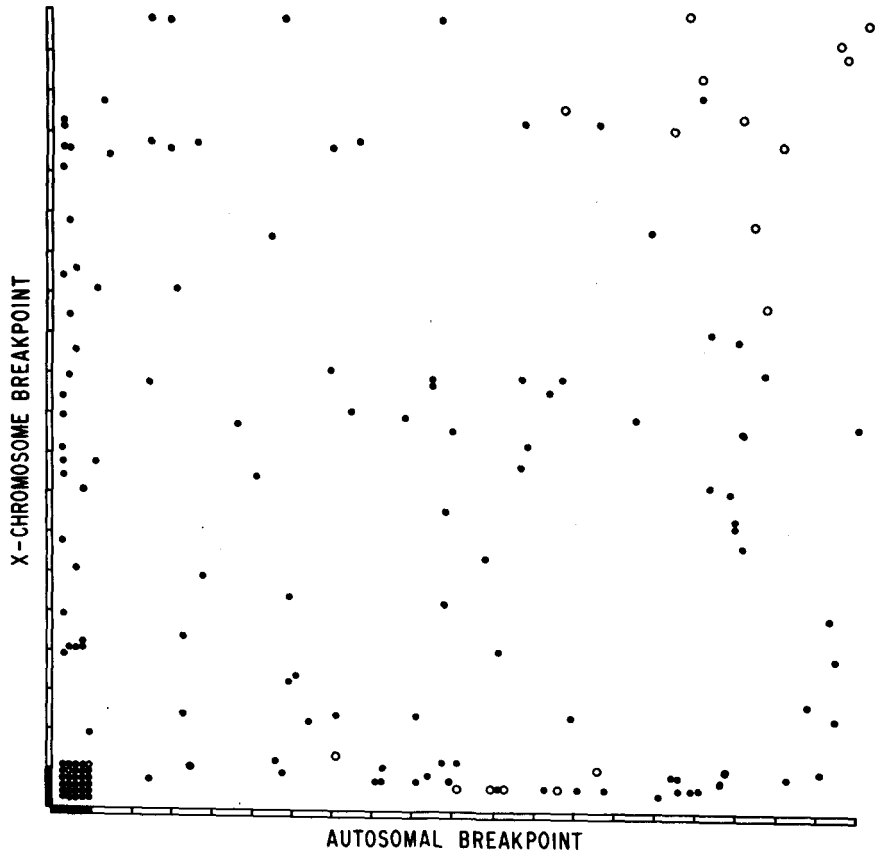

FIGURE 1.—The breakpoints of all two-break male-viable translocations between the *X* chromosome and chromosome *2* or *3*. The ordinate represents the *X* chromosome of the salivary gland; the terminal division is at the top and the proximal-most division, which is heterochromatic and at the centromere, 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.

cause of sterility. If the *X* and the autosomes are in different phases or under asynchronous control, disruption of this relation by means of reciprocal translocations could interfere with the required precocious inactivation of all or part of the *X*, and would be expected to be dominant. The analysis of *T(X;A)*'s in female mice provides us with some very good evidence bearing on changes imposed by a translocation on chromosomal allocycly (LYON 1972).

In contrast to *T(X;A)*-associated sterility, the sterility of some *Y*-autosome or *X–Y* translocations in Drosophila is recessive and can be restored by the addition of normal *Y* (BROSSEAU 1960; NICOLETTI and LINDSLEY 1960; LINDSLEY *et al.* 1972). Thus, although the dominant sterility of *X*-autosome translocations is not associated with the breakpoint per se, the recessive sterility of translocations involving the *Y* chromosome is attributable to breakpoint-associated deficiency or

malfunction of *Y*-linked male fertility factors. In either case, since the *Y* is metabolically active in primary spermatocytes, fertility is restored by a normal *Y*, i.e., sterility is recessive. In fact, several *X–Y* but not *Y–A* translocations in *Drosophila hydei* have been shown by HESS (1968) to exhibit an inhibition of the typical lampbrush structures of the *Y*.

A second group of mutations suspected of chromosomal sterility comprises deficiencies for a specific region in the proximal (centromeric) heterochromatin of the *X* chromosome. We observed that about 25% of deficiencies for suppressor of forked, [*su(f)*], are sterile in combination with a duplication covering the associated lethal effect. All these dominant steriles are also deficiencies for the bobbed, (*bb*) gene deep within the heterochromatin (ee Figure 2); some deficiencies exhibit semi-sterility and resemble partial deficiencies of *bb*. Deficiencies for *su(f)* or *bb* alone, regardless of their size, appear to be male fertile. This demonstrates cis dominance of the sterility but not the lethality. Since precedents for a role of heterochromatin in chromosomal control are well known (BAKER 1968; MCCLINTOCK 1951), it is tempting to consider this region as an inactivation center.

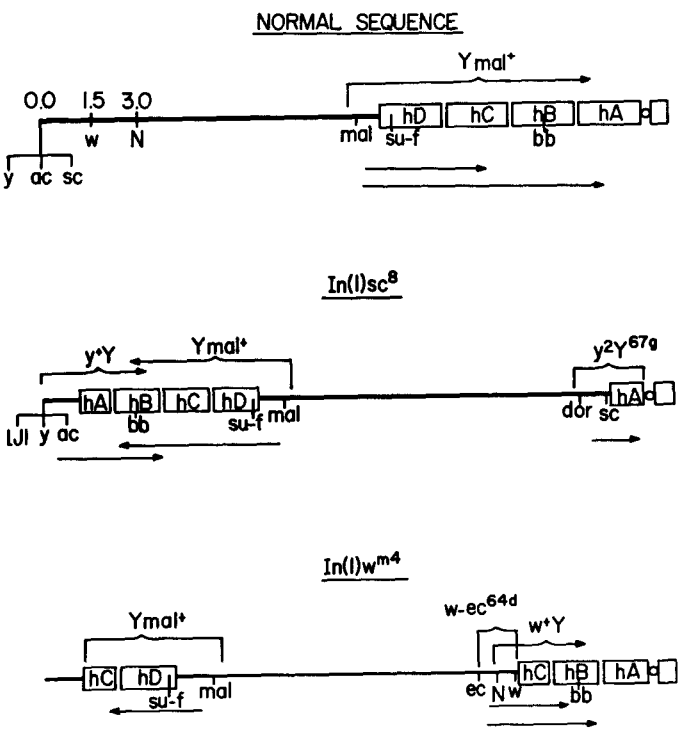

FIGURE 2.—Relative location of heterochromatic block in one normal and two inverted chromosomes. Arrows below figures represent the type of deficiencies that were selected from progenies of crosses between irradiated males bearing the relevant chromosome and appropriately marked females. The duplications used to cover deficiencies associated with lethality, or to study dominance of male steriles are indicated above the chromosomes.

In an attempt to clarify further the relation between the proximal heterochromatic region of the *X* chromosome and male fertility, heterochromatic segments were deleted from two different *X* chromosome inversions. In one of them [*In(1)sc*^{8}] the loci of both *su(f)* and *bb* are separated from the centromere, and in the other [*In(1)w*^{m4}] *su(f)* but not *bb* is separated from the centromere (see Figure 2 for further details). The preliminary results with the *w*^{m4} inversion are consistent with the observations on the normal *X* chromosome in that deficiencies of either distal or proximal heterochromatin, i.e., *su(f)* or *bb*, are male fertile. The observed male steriles studied so far are associated with *X*-autosome translocations.

Results with *In(1)sc*^{8} on the other hand are discordant with those from normal *X* chromosomes in that the four *su(f)–bb* deletions so far examined are fertile, as are three *y–bb* deficiencies. Thus the *su(f)–bb* segment appears to be more readily removed from its distal location in *In(1)sc*^{8} without leading to male sterility. In addition, 80% of the scute mutations induced by irradiation of *In(1)sc*^{8} are dominantly male sterile. Only one of nine, however, is not associated with *X*-autosome translocation. Thus, *X*-autosome translocations which are presumed to have breakpoints in the region of the centromere of *In(1)sc*^{8} can cause sterility; this observation suggests further study regarding the role of this region in dominant male sterility.

Another line of investigation derived directly from the observed dominance of chromosomal sterility, concerns itself with the problem of germinal selection. The inability of mice carrying *X*-chromosome translocation to initiate the first meiotic division might account for the observed shortage of such translocation, compared to autosomal exchanges, in the primary spermatocytes of irradiated mice (SEARLE *et al.* 1971). In order to examine the possibility that germinal selection is operating against dominant steriles, *su(f)* deficiencies were recovered from cells irradiated in premeiotic stages. So far 15 out of 15 are fertile, whereas more than 25% of those induced postmeiotically are male sterile. This suggests that the sterility of *su(f)* deficiencies is cell-autonomous even though all cells in a cyst are interbridged, and that the primary spermatocyte is an effective sieve through which male sterile deficiencies may not pass.

The relation between X-and-Y chromosome activity

There are three ways of rationalizing *X*-chromosome inactivation during spermatogenesis. One way is to suppose that many *X*-linked genes are associated with oogenesis, where both *X*'s are probably active. Another way is to imagine a mutual control relation between *X*-chromosome genes and autosomal genes during spermatogenesis. A third major possibility is to consider a relation between *X*- and *Y*-chromosome activity. The *Y* chromosome (excluding the nucleolus organizer region) is heterochromatic, inactive, and dispensible in somatic cells of both male and female Drosophila. Some repression or stimulation of the inactive *Y* is therefore required to initiate fertility gene activity during spermatogenesis. This suggests some control, positive or negative, of the *Y*-chromosome activity by *X* or autosomal genes. Since *X* inactivation is a major chromosomal

feature of spermatocytes *vis-à-vis* somatic cells we decided to investigate the possibility of *X* control of *Y* activity.

Our approach was to recover *X*-linked male sterile mutations following irradiation of mature sperm and to examine the primary spermatocytes for evidence of abnormal *Y* activity. *Drosophila hydei* was chosen because of the specific and very prominent lampbrush structures that are produced in their primary spermatocytes by the *Y* chromosome (MEYER, HESS and BEERMAN 1961). The indispensability of these structures for male fertility and their relative positions along the *Y* chromosome have been beautifully demonstrated by HESS and MEYER (1968). The appearance of *Y* structures was examined in 16 male sterile lines, and three were selected for further investigation. One of them is described below.

In the spermatocytes of males bearing the *X* chromosome, *ms(1)XL2 wy*, there are no threads, tubular ribbons or nooses (Figure 3), the pseudonucleolus is small and condensed and clubs are poorly organized. The normal order of structures as determined by HESS and MEYER is shown in Figure 4. Evidently, the missing structures do not arise from contiguous regions of the chromosome. Spermiogenesis does not proceed beyond the spermatid stage, and the testes are short as seen in *XO* males. The stock that now exists is the result of three generations of back crossing to unirradiated balancer bearing males [*In(1)f*^{s}]. Nevertheless, cytological analysis reveals a reciprocal translocation involving the heterochro-

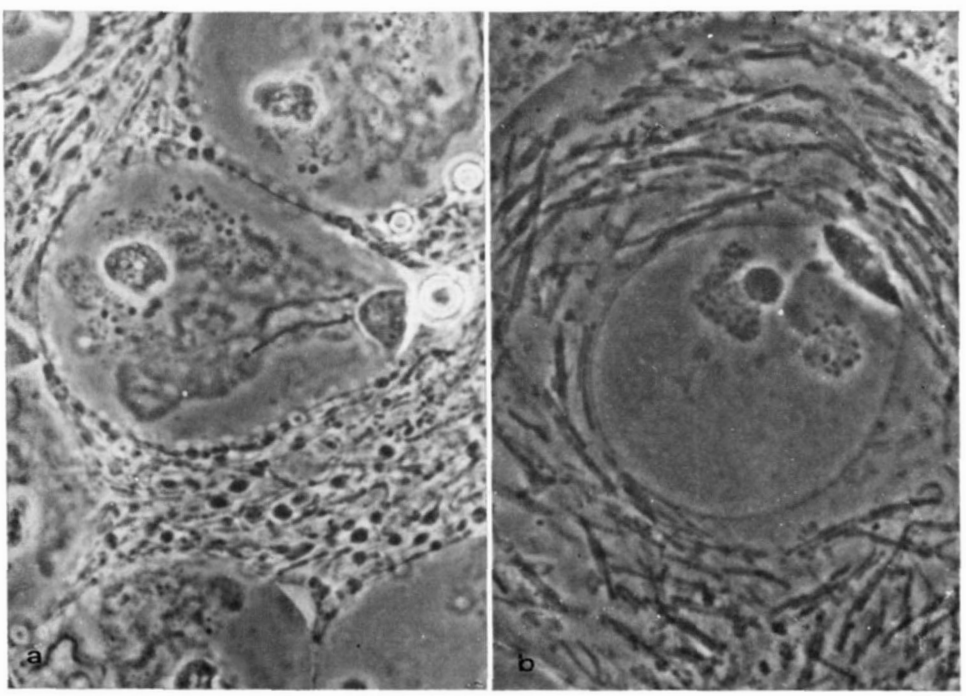

FIGURE 3.—Spermatocyte nuclei of *D. hydrei*:
(a) A normal nucleus,
(b) Abnormal nucleus of the *ms(1)XL2* stock.

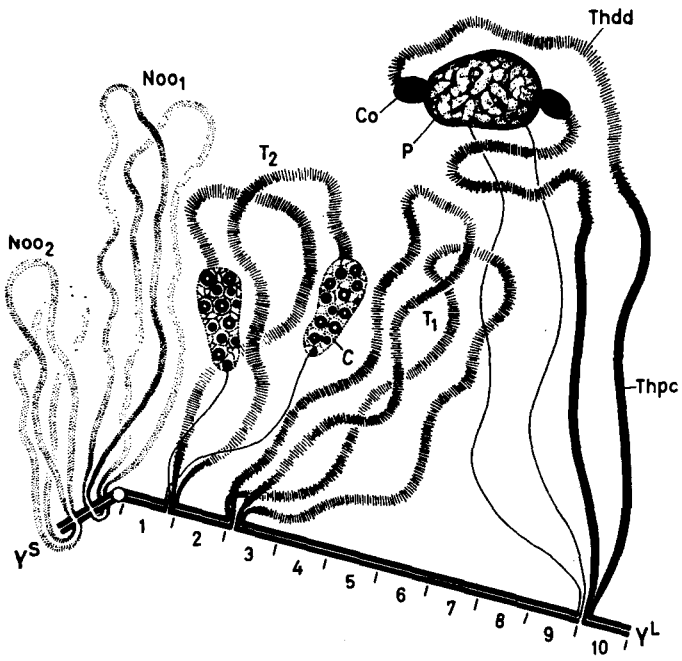

FIGURE 4.—Scheme and map of the lampbrush structures of the Y chromosome of *D. hydei* (From MEYER 1971).

matic arm of the metacentric *X* chromosome and the short arm of one of the telocentric autosomes segregating in the stock. In all cases male sterility appears to be completely linked to *wy*, and to cause abnormal functioning of genetically normal *Y* chromosomes.

To summarize, it appears as though there are *X*-linked elements controlling the activation of the *Y* chromosome during spermatogenesis by affecting the unfolding of the DNA–RNP lampbrush structures. If related to *X* inactivation during spermatogenesis, the *X*-chromosome sterile could be interpreted as mutation in an element controlling the inactivation of the *X* chromosome, so that inhibitory nucleoplasmic agents continue to flow in primary spermatocytes. If such an agent exists, we should be able to select for a *Y* chromosome unresponsive to the mutated *X* thus demonstrating the existence of *Y* chromosome receptors. Another plausible suggestion is that a gene, normally producing an activator agent for the *Y*, was mutated to an inactive form. More work is being done in both melanogaster and hydei to test the attractive model that *Y* inactivation in somatic cells depends on *X* activation, while *Y* activation in spermatocytes depends on *X* inactivation.

Some general evolutionary considerations

Three observations on sex-linked genes support the view that a non-random distribution of genes between the sex chromosomes and autosomes is a fundamental consequence of evolution: (a) Genes with special and related functions are located on the *Y* chromosome in both Drosophila and mammals; (b) The

genetic content of the *X* has been conserved during mammalian evolution (OHNO (1967)); (c) Coordinate control of sex-linked gene action is observed in the phenomenon of dosage compensation and during spermatogenesis. We postulate therefore that selection favored the location on *X* chromosome of only those genes that are able to function effectively when constrained by the mechanism of chromosomal control.

Whatever the evolutionary sequence, the relation between sex-linked and autosomal genes is a crucial feature of genome organization in heterogametic species. The extreme sensitivity of male germline to alterations in *X*-autosome relations, provides a powerful selective force for stabilizing this relation.

We would finally like to point out that although the behaviour of the *X* chromosome during spermatogenesis is of great interest by itself, the phenomenon provides a model system of great value for investigating problems of control and coordination on the chromosomal level.

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