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Genetic Activity of Sex Chromosomes in Germinal Cells [and Discussion]

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Genetic activity of sex chromosomes in germinal cells

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If I adhere strictly to the title proposed for me and speak only of the genetic activity of the sex chromosomes in germ cells, there is very little to say. The evidence is necessarily indirect and includes, first, examples of differential behaviour of germ cells of different sex chromosome constitution in situations where competitive proliferation is a possibility, as in some mosaics and chimaeras; and secondly, exceptional species in which the sex chromosome constitution is normally different in germ cells and soma. The species concerned are all mammals.

An instance of the first kind is provided by observations made on a 39, X/41, XYY mosaic mouse discovered by chance in the course of an irradiation experiment (Evans, Ford & Searle 1969). All the spermatogonia and spermatocytes examined contained 41 chromosomes, including two Y chromosomes, whereas bone marrow (the only other tissue examined) was mosaic, the probability of difference being due to sampling error being very low. The question, then, was whether the failure to detect mosaicism among the germ cells was a consequence of chance exclusion of the 39, X cell type from the germ line during development, or of differential proliferation and/or survival of 41, XYY germ cells in the testicular environment. The latter interpretation was favoured on the grounds: (1) A 39, X/41, XYY mosaic is likely to have originated by non-disjunction of the Y chromosome at the first cleavage division of a 40,XY zygote, since other theoretically possible modes of origin would require the combination of rare events or other implausible assumptions. (2) Primordial germ cells of the constitution 39, X are capable of reaching the developing gonad and subsequently forming functional oocytes as evidenced by the fertility of 39, X female mice (Russell, Russell & Gower 1959). (3) Nearly all half-and-half coat colour mosaic mutants are also germ cell mosaics (Russell 1964), implying that when two distinct cell lines are present very early in development both lines are likely to be represented among the germ cells.

A second example is provided by fertile human 47, XYY males. Thompson, Melnyk & Hecht (1967) have reported a sibship of six 46, XY sons and one 46, XX daughter sired by one such male. Four other children of 47, XYY males whose chromosomes have been examined are quoted in a review by Court Brown (1968) and three more are reported by Court Brown, Price & Jacobs (1968) and by Lisker, Zenzes & Fonesca (1968). There were four 46, XY sons, two 46, XX daughters and one 47, XX, 21+ daughter with Down's syndrome. Four classes of progeny, including also 47, XYY sons and 47, XXY sons, would be expected on the basis of meiotic segregation of an XYY complex, the aneuploid offspring being as frequent as the euploid offspring. Even with the small numbers involved the deviation from expectation is very highly significant. The explanation may be provided by other information furnished by Thompson *et al.* (1967). They reported finding spermatocytes with only a single Y chromosome in testicular preparations from another 47, XYY male whose cultured blood lymphocytes revealed no sign of mosaicism. This observation, which has been confirmed on six further XYY subjects (Melnyk, Thompson, Rucci, Vanasek & Hayes 1969) raises the possibility that 46, XY germ

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cells arise spontaneously from 47, XYY progenitors by non-disjunction or loss of one Y chromosome and are subsequently favoured in the testicular environment of the human 47, XYY male.

The best evidence of differential behaviour of germ cells with different sex chromosome constitutions is provided by the studies of Mystkowska & Tarkowski (1968) on artificial mouse chimaeras produced by the fusion of independent embryos. Three of these mice were mixtures of 40, XX and 40, XY cells, two of them anatomically normal males and one a true hermaphrodite, yet the results of direct chromosomal examination and breeding tests alike showed that the germ cells were all of the constitution 40, XY. Chance exclusion of 40, XX cells from the germ lines of all three animals seems very unlikely. A competitive advantage of 40,XY germ cells over 40, XX germ cells after they had reached the testicular rudiments is therefore the preferred explanation.

Foremost among the exceptional species mentioned is the vole *Microtus oregoni*. Ohno, Stenius & Christian (1966) found that the females of this species originate from XO zygotes, but nevertheless acquire XX oocytes in consequence of non-disjunction of the single X during the oogonial mitoses. Other examples are provided by the marsupial genera *Parameles* and *Isoodon* (Hayman & Martin 1969). In *Parameles* the somatic tissues (other than the stromal cells of the gonads, which have not been examined) of both males and females have an XO constitution, although the germ cells themselves are normal XY and XX respectively. In *Isoodon* the situation is similar, but the loss of the one sex chromosome appears to be confined to tissues of mesodermal origin.

The foregoing examples all favour the view that the sex chromosome constitution of the germ cells can influence their behaviour in the mammalian gonad. But they fall short of proof. More information is needed and it would now be of particular interest to learn something of the behaviour of germ cells with different sex chromosome constitutions in a shared ovarian environment.

It is a legitimate extension of my topic now to quote evidence that bears on the more general problem of genetic activity in germ cells.

First, in the testis of a mouse given a sufficient dose of radiation, all the seminiferous elements are destroyed except for a few type A spermatogonia. When regeneration occurs it can only be from the few survivors. If the spermatocytes of an irradiated mouse are examined subsequent to the full restoration of spermatogenesis, *clones* of cells characterized by specific types of multivalent configurations can be identified (Searle, Evans, Ford & West 1968). Although the proportion of type A spermatogonia that survive irradiation under the conditions of these experiments is low (Oakberg & Clarke 1961), the total number is likely to be of the order of thousands if not tens of thousands. It follows that if all survivors proliferated at an equal rate, the probability of identifying a clone in a relatively small random sample of spermatocytes from the mixed contents of a whole testis would be negligible. It may therefore be inferred that there is strong differential colonal proliferation of the surviving spermatogonia, which could reflect specific mutant genotypes induced by the irradiation.

Secondly, it is known that heterozygosity for specific reciprocal translocations may lead to defective spermatogenesis and sterility in male mice. Evidence obtained recently by Cattanach, Pollard & Isaacson (1968) from sterile males bearing reciprocal translocations newly induced by ethane methane sulphonate suggests that the breakdown of spermatogenesis may occur at different stages, specific to the individual translocation concerned. The description of sterile mice in radiation-induced translocation stocks by Lyon & Meredith (1966), on the other hand,

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points to a progressive breakdown of spermatogenesis with fewer identifiable cells at each successive stage. Further information is required to resolve this apparent disparity.

Finally, a recent experiment has shown that about half the reciprocal translocations induced in spermatogonia of mice by irradiation that are identifiable cytologically in the derived spermatocytes, are not capable of being transmitted to the progeny (Ford, Searle, Evans & West 1969). A selective process of some kind evidently occurs between the primary spermatocyte stage and fertilization. The numbers of dominant lethals are also reduced to about half the numbers expected. This result is best explained if about half the spermatocytes with translocation configurations do not give rise to viable sperm, that is, if selection operates on heterozygous diploid genomes rather than the haploid genomes of the spermatids or sperm. Now if the failure during spermatogenesis attributable to some reciprocal translocations is an autonomous property of individual germ cells bearing these translocations, some degree of elimination is to be expected. The surprise is that it should be so high. For the present purpose the important point is that here is more evidence associating reciprocal translocations with failure during spermatogenesis. In formal genetic terms the disturbance of spermatogenesis could be attributed either to position effect or to a local change (duplication, deletion or 'point' mutation) associated with one or both of the break points. Since the break points of reciprocal translocations in the mouse are widely distributed over the whole genome (compare Carter, Lyon & Phillips 1955, 1956) and since so many different translocations are evidently associated with failure in spermatogenesis, it would appear that very many independent genetic loci are concerned in spermatogenesis.

To sum up, there is some evidence that the sex chromosomes themselves influence the capacity of the germ cells to proliferate; and there is other evidence suggesting that a large part of the total genetic information in the diploid genome is in some way concerned with the process of spermatogenesis.

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Discussion on papers by M. F. Lyon, p. 41 and C. E. Lord, p. 53

H. Grüneberg (*Department of Animal Genetics, University College, London*): The paper just read by Dr Mary F. Lyon prompts me to make a statement. I have examined the inactive-X hypothesis mainly on the basis of the phenotypes of heterozygotes and double heterozygotes for sex-linked genes in the mouse, and of heterozygotes for X–autosome translocations. I have also made a complete survey of comparable cases in other mammals, including man. The results of these investigations have been published in a series of papers since 1966. In not a single instance are these phenotypes in agreement with the consequences of the inactive-X hypothesis: throughout, various subsidiary hypotheses have to be invoked to account for the discrepancies, and on closer examination it can generally be shown that such *ad hoc* hypotheses do not in fact do the trick. These criticisms have remained unanswered until recently when Dr Lyon (1968) has devoted several pages of print to a reply. In that reply, important criticisms of logic have been completely ignored, obviously because they cannot be answered. In essence, Dr Lyon maintains that at the moment of inactivation (whenever that may be), the two cell types are present in about equal numbers and randomly distributed over the embryo; but subsequently, selective cell multiplication, regularities of cell lineage, non-autonomous development of tissues and other disturbing factors are supposed to supervene and thus to blur the original situation. There is no independent evidence for the reality of any of these phenomena; they are merely introduced *ad hoc* to 'explain' the discrepancies as and when required. These concepts of course make the hypothesis more permissive and hence less vulnerable, but also correspondingly less capable of being tested by observation. Dr Lyon has confined herself to these generalities. She has not attempted to refute my criticisms in detail; this is, perhaps, understandable as I had already shown that, confronted with hard facts, the various *ad hoc* hypotheses will not in reality resolve the discrepancies.

To suggest, as Dr Lyon does, that she has answered my criticisms cannot be seriously maintained. Detailed quantitative data cannot be countered by generalities. They require a detailed point-by-point reply, and this has not been attempted. I suggest that a hypothesis which fails to answer detailed criticisms cannot be regarded as a hypothesis in good standing. Nor does it help to point to other facts believed to be in agreement with the hypothesis.

All the known facts indicate that in heterozygotes for sex-linked genes, both alleles interact with each other as in the case of autosomal genes. To reconcile this situation with the need for dosage compensation, it has been suggested (Grüneberg 1967*b*, 1969) that the two X-chromosomes in the female jointly (but not necessarily equally) do the work of the single X-chromosome in the male. If the latter is equated to 100, the complemental-X hypothesis assumes that all relative activities from 100:0 to 0:100 can reversibly occur in individual cells, with no reason to assume that a 50:50 situation is favoured. If, beyond a critical point (say, 90:10 or perhaps 80:20), the less active X-chromosome shows signs of heterochromatinization (late labelling, sex chromatin formation, etc.), all the cytological facts hitherto regarded as such strong support for the inactive-X hypothesis are equally in agreement; in fact, they fit it marginally better. The cytological facts thus do not discriminate decisively between the two hypotheses.

M. F. Lyon: Most of Grüneberg's points were answered in a paper in the *Annual Review of genetics* in 1968. Nothing was mentioned in that paper about mice heterozygous for two different X-linked genes. This is why data on double heterozygotes were shown today. Professor Grüneberg claimed he could not see the effects that I could see, and I have produced photographs to prove my point. Professor Grüneberg's other points, about intermediate patches, unequal areas,

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presence of patterns, etc., were in fact dealt with in detail in the 1968 paper, and there was no need to go into them further today.

J. L. Hamerton (*Guy's Hospital Medical School, London*): Some data on the circumstantial relationship between the cytological and genetic evidence of inactivation at one gene locus, i.e. G_6PD in mules, have been recently obtained (R. V. Short, F. Gianelli and J. L. Hamerton, unpublished). Two things are needed for direct evidence of X-inactivation, first, two X chromosomes that are separately recognizable, and second an X-linked enzyme with differing electrophoretic mobilities in the two parent species. The X chromosome of the donkey is acrocentric and that of the horse is metacentric. Late-labelling studies on about 10 mules showed that in every case the X chromosome of the donkey parent was late-replicating in about 90 % of the cells. The fast electrophoretic variant of glucose-6-phosphate dehydrogenase comes from the donkey and the slower variant comes from the horse. Hardly any of the fast band is found in erythrocytes and fibroblasts of the mule. Therefore, there is complete correlation between late labelling of the chromosomes and the lack of expression of a gene on that chromosome. The analysis of clones from fibroblast cultures is needed to confirm this observation. Study of clones is made difficult by the low frequency of active X chromosomes of the donkey.

R. G. Edwards: These data by Dr Hamerton, coming from excised tissue, do not resolve the debate between Grüneberg and Lyon since different parts of the two X chromosomes could be inactive. Only clones where there was 100 % all-or-none activity would suffice.

J. L. Hamerton: Clones are the final answer, but the present data provide a close co-relation between cytological behaviour of the X chromosome and genetic inactivation.

R. L. Gardner (*Physical Laboratory, Cambridge*): The typical patterns in mice with heterozygous sex-linked genes can be explained in embryological terms without much difficulty. When chimeras are formed by fusing two morulae carrying different colour genes, a sorting-out process occurs in an analagous manner, although the mechanism of sorting out is not understood.

C. D. Darlington: The sizes of patches in tortoiseshell cats, is variable. Does a larger patch indicate an early differentiation of the two lines, and the small patches a late differentiation? If so, is there some kind of genetic control of the time of separation of the lines, e.g. by the centromere or by a block of genes? Translocated chromosomes should be helpful in this study.

M. F. Lyon: In mice the patches in heterozygous animals are very similar to the patches in fusion chimeras. In both, large and small patches occur together in the same animal and it seems to be a question of the number of cells populating a particular area of skin. A simple postulate would be that two foundation cells populate an area, and if they both determine similar colours the patch is large, whereas if they determine different colours there are many small patches.

R. G. Edwards: Is there any information on whether discrete parts of an X could be inactivated in a cell as suggested by Grüneberg, based on autoradiographic or other studies?

P. E. Polani (*Guy's Hospital Medical School, London*): The best assessment of partial activity of an 'inactivated' X is likely to be biochemical rather than cytological. The use of current cytological techniques to assess DNA or RNA synthesis could certainly leave a margin of 10 % error.

H. Grüneberg: This is a considerable error. It could mean that both X chromosomes are active, one more so than the other, as suggested in my 'complemental-X' hypothesis,

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M. F. Lyon: Partial inactivation of the X-chromosome cannot be measured with cytological techniques detecting the time of DNA synthesis at particular parts of the X chromosome. Late-labelling DNA is associated with absence of mRNA synthesis, but knowledge of the causal connexions is lacking. It seems more than likely that neither phenomenon causes the other, but that both are associated through some common aetiology, such as the particular association between DNA and protein in the chromosome fibre. Therefore, one cannot assume that just because a certain chromosome region is early or late-replicating it is necessarily producing or not producing mRNA. In order to measure the degree of inactivation one needs not cytological studies but biochemical data on the gene products from the two chromosomes. Some of the techniques used on cloned cultures would have detected very small amounts of the products of the second X. Comings (1966), studying electrophoretic variants of G_6PD thought that 5% of the second electrophoretic type would have been noticeable, but could not detect any activity of the second X, even though he was applying treatments intended to provoke it. DeMars (1968) used a method capable of detecting G_6PD in single cells, and worked with a temperature-sensitive mutant. He found that some cells from deficient clones of heterozygotes showed faint activity, which he thought equal to about 1% or normal, whereas clones from males showed no such activity. Similarly, Salzmann, DeMars & Benke (1968), studying hypoxanthine-guanine phosphoribosyl transferase activity in individual cells, found some cells in deficient clones from heterozygotes which showed faint activity but no such cells in clones from mutant males. Neither DeMars nor Salzmann *et al.* found any definitely positive cells in their deficient clones. Thus, it seems that any possibility of the two X chromosomes having equal activities is excluded by these experiments, and if in any cells there is some action of the second X then it is only of the order of a small percentage.

R. G. Edwards: Some time ago, Russell postulated an inactivation centre on the X chromosome. Was there recent data in support of this suggestion?

M. F. Lyon: The inactivation centre described by Russell (1963, 1964) was based on work using X–autosome translocations which resulted in variegation for autosomal colour genes translocated to the X. In these cases, inactivation does peter out along the autosomes at some distance from the exchange, but it is perhaps not valid to argue from this case to the normal X chromosome. There could still be inactivation centres, of course, e.g. gene loci that become associated with proteins determining activity or inactivity, although how this is related to any possible gradient of inactivation is still an open question.

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