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Inactivation of whole chromosomes in mammals and coccids: some comparisons*

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SUMMARY

Genetic systems involving developmental inactivation of entire chromosomes occur in two widely different groups of organisms: mammals and coccids (Homoptera: Insecta). The two groups show several similarities and some interesting contrasts with respect to this unusual cytogenetic phenomenon. Although mammalian *X* chromosomes and coccid paternal sets are components of different genetic systems, comparisons between them nevertheless suggest approaches that might prove to be of value. Further, the occurrence of facultative heterochromatization in these two wholly unrelated taxa must mean that this type of heterochromatization represents a fundamental capacity of chromosomes.

The tremendous current interest in mechanisms controlling gene activity in multicellular organisms is in part the result of the theory that one of the two *X* chromosomes in cells of mammalian females is genetically inactive. This theory, which is frequently called the 'Lyon hypothesis' (Lyon, 1961; Russell, 1961), is such an attractive explanation of the hitherto puzzling behaviour of *X*-linked genes that it has stimulated a great deal of discussion and experimental work. These developments, in turn, have given rise to the hope that an understanding of the mechanism of inactivation of the mammalian *X* might contribute to a more general understanding of control of gene action in complex organisms (Sutton, 1965).

One consequence of this interest in the mammalian *X* has been that it is no longer unfashionable to speak of 'heterochromatin' and 'euchromatin' – terms which were until recently primarily in the domain of the traditional cytologist. Since much of the early work on heterochromatic chromosomes and chromosome regions was done on insects (particularly *Drosophila* and the coccids (Coccoidea: Homoptera)), several recent reviews have attempted to relate work on heterochromatin in these organisms to that in mammals (e.g. Lyon, 1968; Ohno, 1969). While all these reviews are excellent in coverage and discuss the more striking findings in coccid cytogenetics, some of the less well-known results have not received similar scrutiny. It is the purpose of this paper to draw attention to a wide range of cytogenetic phenomena among coccids which may be pertinent to analogous events in mammals.

Since comprehensive reviews of coccid cytogenetics are available (Brown & Nur,

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1964; Brown, 1969), only a brief account will be given here. The mealybug or lecanoid system is the one most relevant to the situation in mammals and in this system both males and females start development as diploids. As in other Hemiptera, the chromosomes of the coccids have no localized centromere; that is, they are holokinetic (Hughes-Schrader & Ris, 1941). Chromosome behaviour is conventional in the female except for the inverted meiotic sequence (Hughes-Schrader, 1948; Chandra, 1962). In male embryos, on the other hand, the paternal complement turns heterochromatic during early embryogeny and remains so in most tissues throughout development. It is eliminated immediately after meiosis and only the maternal chromosomes, all of which remain euchromatic, form sperm. Both radiation-induced damage and tests with genetic markers have indicated that the heterochromatic set is substantially inert. Indeed, no evidence to date suggests that it is active in any way other than in its own replication. However, in some tissues the heterochromatization of the paternal set is reversed and genetic activity is restored (Nur, 1967).

1. CONSTITUTIVE AND FACULTATIVE HETEROCHROMATIN

Although many puzzles still shroud the cytological phenomena collectively called heterochromatin, it has recently become possible (Brown, 1966) to discriminate between two major types of heterochromatin. This discrimination is of significance because the two types would be expected to have quite different genetic and evolutionary implications.

In the familiar case, which Brown has named *constitutive heterochromatin*, the chromosome region in question will, in the specified tissue, appear as heterochromatin regardless of prior history. In diploid cells the heterochromatic region would be expected to express itself in both the homologues normally present.

On the other hand, a specified chromosome or region may regularly appear heterochromatic in one of the homologues and euchromatic in the other. Heterochromatin appearing under such circumstances has been called *facultative* (Brown & Nelson-Rees, 1961; Brown, 1966). In the coccids, it has been possible to trace the origin of the heterochromatic set and to show that its prior history in development was different from that of the euchromatic set (Brown & Nelson-Rees, 1961). In the other, now well-known example of facultative heterochromatin, that of the mammalian *X* chromosome, differentiation between or among homologues is apparently random during early embryogeny. It is a fact worth noting that the timing of facultative heterochromatization of the mammalian *X* is very similar to that of the paternal complement in mealybug embryos: it begins, in both cases, when the embryos are still very young and undifferentiated.

Since it is the large segments of heterochromatin, constitutive or facultative, with which the cytologist is most familiar and since it is apparently only at this level that it is necessary and feasible to differentiate between the two types, the following discussion will be restricted to such large segments.

Brown's recognition of two types of heterochromatin has received striking

support in the recent findings that apparently only constitutive and not facultative heterochromatin fluoresces brightly after exposure to acridine dyes and certain other fluorochromes (Caspersson *et al.* 1969; Pearson, Bobrow & Vosa, 1970; George, 1970). Furthermore, in what promises to be an exciting series of investigations, Pardue & Gall (1970), and Jones (1970) have shown that satellite DNA from the mouse binds selectively to centric constitutive heterochromatin. Rae (1970) has obtained similar results in *Drosophila melanogaster*. Satellite DNA's from different organisms appear to vary greatly in base composition. Some are rich in adenine-thymine while others are not. But all of them consist of tandem repeats of identical sequences as long as 150–300 base pairs (Corneo, Ginelli & Bernardi, 1968; Walker, 1968) or shorter (Southern, 1970).

The high degree of variability of the satellite DNA associated with centric constitutive heterochromatin is intriguing. Since we appear to be at the threshold of some important discoveries and possibly a few surprises, at present it would seemingly be safe to say only that DNA of genetically inert chromosome regions would not be subject to the stringent selection necessary to maintain specific codons and would be free to vary within thermodynamic limitations or other unknown limitations. It is thus not surprising that such regions appear to be highly variable in their chemical composition.

In facultative heterochromatin the region heterochromatized has a full comportment of genes which, up until that moment, have been subject to the usual types of selection. Since heterochromatic regions may also deheterochromatize (Chandra, 1963; Nur, 1967) and resume genetic activity (Nur, 1967), the genetic information appears to have been masked rather than altered. Similarity of DNA base ratios before and after heterochromatization (that is, females vs males in the case of mealybugs) tends to confirm this concept (Loewus, Brown & McLaren, 1964). It also appears that facultative heterochromatization in mealybugs is not related to quantitative or qualitative variation in histone fractions resolvable by electrophoretic methods (Pallotta, Berlowitz & Rodriguez, 1970).

The euchromatic region homologous to the facultative heterochromatin is solely responsible for the continuation of the activity of genes of this region, and hence any interference in the activity of the euchromatic region would therefore be highly deleterious. Thus, the mechanism by which facultative heterochromatization is originally induced must be precisely controlled. As Brown (1966) has suggested, at least part of the control mechanism would be expected to be external to the chromosome to assure differentiation between homologues. In fact, some authors have gone as far as suggesting episomes or episome-like structures as inductive agents (Morishima, Grumbach & Taylor, 1962).

2. ANEUPLOIDY, POLYPLOIDY AND HETEROCHROMATIZATION

In mealybugs, matings between triploid females ($3n = 15$) and diploid males give embryos with varying numbers of chromosomes from the mother and a normal haploid complement from the father (Chandra, 1962). The only surviving classes

are diploid and triploid females, with all chromosomes euchromatic, and diploid males with 5 eu- and 5 heterochromatic chromosomes; no triploids with 10 eu- and 5 heterochromatic chromosomes survive. Male embryos with 6, 7, 8 and 9 chromosomes show respectively 1 euchromatic (E) + 5 heterochromatic (H), 2 E + 5 H and 4 E + 5 H chromosomes. Embryos with more than 5 E chromosomes, including 10 E + 5 H chromosomes, are also seen. But none of these constitutions is recovered as adults. Aneuploidy and triploidy (i.e. 10 E + 5 H) thus seem to have no obvious effect on heterochromatization itself, although these constitutions are not compatible with life. Similarly, among human females trisomic for autosomes, normal sex chromatin patterns have been observed. As Ohno (1969) has noted, the presence of *Y* chromosomes also seems to have no influence on heterochromatization of an *X* in *XXY* and *XXYY* individuals. Similar data are available for doubly trisomic individuals (48, *XXY*, trisomy 18; 48, *XXY*, trisomy 21).

However, there is evidence that the *X* inactivation mechanism becomes upset in triploid human foetuses. Some *XXY* triploids are sex-chromatin-positive (Mittwoch, Atkin & Ellis, 1963) while the majority are not (Boue', Boue' & Lazar, 1967). The majority of *XXX* triploids have only one sex chromatin body whereas *XXX* diploids almost always have two. In a 48, *XXYY*/71, *XXXYY* mosaic Schmid & Vischer (1967) did not find more than one sex chromatin body per nucleus and radioautographic studies showed that only one of the three *X*'s was late-replicating and not two as would have been expected. In the only other radioautographic study yet made of triploid cells, Schindler & Mikamo (1970) did not find any late-replicating *X* chromosomes among cultured fibroblasts from a 69, *XXY* infant. It thus appears that the relative dosages of *X* chromosomes and autosomal sets have an effect on heterochromatization of the mammalian *X*. The possibility should also be kept in mind that there may be a difference in origin between triploids with and without sex chromatin.

In mealybugs, reversal of heterochromatization occurs in haploid (Chandra, 1963), in diploid (Huang, 1970; Kitchin, 1970) and possibly also in polyploid cells (Nur, 1967) of certain tissues as part of normal developmental processes. Hence, it is probably premature to conclude that heterochromatization of the mammalian *X* is an irreversible change of state. I have suggested elsewhere (Chandra, 1970) that the inactive *X* might well revert to an active state in response to special developmental situations such as those to be found in *XO* embryos. It might even be that reversibility is a basic property of facultative heterochromatin.

3. TRANSLOCATIONS BETWEEN EU- AND HETEROCHROMATIC CHROMOSOMES

The distinction between the E and H chromosomes of mealy-bugs is dramatic and therefore it is often possible to do detailed cytological studies of such chromosomes to an extent not possible with those of mammalian systems. On the other hand, the spectrum of genetic experiments possible in the mouse are not yet feasible in the mealybug largely on account of a paucity of genetic markers.

During the past few years *X*-autosome translocations in the mouse have contributed a great deal of information on the behaviour of the inactivated *X* chromosome (Russell & Montgomery, 1969). Unfortunately, the coccid chromosome systems do not permit easy manipulation of the heterochromatic set because it is discarded rather than transmitted to the offspring. At least in the simple mealybug system, a haploid complement, once it turns heterochromatic, is eliminated at spermatogenesis and thus from genetic continuity. Perhaps because of this peculiarity, attempts to recover transmissible E–H translocations have thus far not been successful (S. W. Brown, unpublished). However, Nur (1970) has recently obtained some interesting data on the meiotic behaviour of E–H translocations which had been induced by irradiating first instar larvae. He found that in such translocations the border between the E and H regions was usually sharp, but occasionally it appeared that short E segments adjacent to the break point might have become heterochromatic. An analogous behaviour of euchromatic segments experimentally shifted next to heterochromatic regions is known in *Drosophila* (Schultz, 1965). In the mouse, Russell (1963) has suggested that heterochromatization of autosomal E segments as a possible cause of the variegated phenotype produced by certain loci when the latter are translocated on to an *X* chromosome.

4. DATA FROM MEALYBUG INTERSPECIFIC HYBRIDS

Interspecific hybridization experiments among mealybugs have shown that the chromosomes of one species can be heterochromatized in the eggs of another, even though the two species belong to different genera (Nur & Chandra, 1963). However, no such cross gave survivors beyond the first larval stage. If the heterochromatic complement were completely inactive, then the hybrid male embryos would be expected to develop normally and resemble the males of the maternal species. The failure of such hybrids to develop beyond the first instar led us to consider the following interpretations as possible causes of lethality. (1) The heterochromatic set performs certain functions and the death of the male embryos, like that of the female embryos, is due to incompatibility between sets of chromosomes from different species. (2) When heterochromatization takes place in a foreign cytoplasm the process is abnormal, either incomplete or exaggerated, and thus the heterochromatic set would be either more or less active than it is in normal males. (3) The possible genetic activity of the paternal set before heterochromatization, which takes place only after the fifth or sixth cleavage division, is sufficient to lead to the death of the hybrid embryos.

One of the still unresolved issues in regard to the Lyon hypothesis is the existence of a number of developmental abnormalities in *XO* women and in other individuals with an abnormal number of *X* chromosomes. Interpretations similar to those suggested above to account for the lethality of male interspecific hybrids of mealybugs (see above, and Nur & Chandra, 1963) have been advocated in attempts to reconcile the Lyon hypothesis with the presence of obvious anomalies in *XO* women. For example, Lyon (1963) has suggested that both *X* chromosomes may

be necessary for normal development before inactivation occurs, thus implying that the two X's are active prior to heterochromatization. Incomplete inactivation of the X has also been suggested as a possible cause (Lyon, 1963). Recent studies by Steele (1970) on the X-linked enzyme glucose-6-phosphate dehydrogenase in human embryos and newborns would support this idea. His data indicate that some loci may escape inactivation at least during the early developmental stages.

In cells of some mealybug interspecific hybrids Nur (1970, p. 382) observed what appeared to be intermediate stages in heterochromatization which raises the possibility that the second interpretation mentioned above might be valid in some instances.

Sabour's (1970) studies on RNA and protein synthesis during early embryogenesis in a mealybug have shown that the onset of nuclear RNA synthesis (as detected by [H^3]uridine radioautography) parallels the appearance of heterochromatization. There was no evidence of RNA synthesis prior to heterochromatization. If only a very few genes would be active prior to heterochromatization then their activity might not be detectable by current radioautographic methods, but there is no evidence for such activity from work on aneuploidy and induced lethality.

Nur (1967) on the basis of more recent evidence has reinterpreted the lethality of hybrid mealybug males as being largely the result of reversal of heterochromatization of the paternal complement in some tissues. Certain tissues of male mealybugs do not have a heterochromatic set (Brown & Nur, 1964). Nur (1967) has shown that the lack of a H set in these tissues is the result of reversal of the paternal complement to an euchromatic state. Further, he could show that in F_1 male embryos of interspecific crosses, those tissues in which the H set reverses show gross developmental disturbances. In contrast, tissues in which the H set does not reverse – such as the hypodermis and its derivatives – show no such anomalies. These data indicate a relationship between reversal of heterochromatization and developmental damage resulting from an apparent resumption of genetic activity by the paternal complement. These results were confirmed by intraspecific studies in which the effects of heavily irradiated paternal complements were similarly followed in the two types of tissue – those with and without reversal of the H set. These results paralleled and confirmed those derived from the hybridization experiments. Bregman (1968) has extended these studies and confirmed Nur's conclusions and has, in addition, shown that the reversal of the H set in cells of the serosa (or embryonic covering) of hybrid males is under the control of maternal genes or cytoplasm.

Finally, it is worth recalling that the distribution of sex chromatin in mammalian organs and tissues has not been studied with the same thoroughness as the heterochromatic set in mealybugs. This is largely because of the size and complexity of mammals and also because sex chromatin is a relatively small body visible only during a brief period of interphase. In spite of these drawbacks careful cytological investigation of this problem might prove rewarding. We may reasonably infer from our experience with coccids that in certain organs or tissues of female

mammals the heterochromatic X might revert to an euchromatic state. Indeed, the XX bivalent in meiotic cells of female mammals is reported to be euchromatic (Ohno, Kaplan & Kinosita, 1961). It is not known if this is the result of reversion or whether the X escapes inactivation in female germ line cells.

5. DEVELOPMENTAL EFFECTS

Heterochromatic chromosomes have long been considered to harbour genes for quantitative variation. In this regard, the recent finding by Penrose (1967) that there is an effect of heterochromatin of the sex chromosomes on the total ridge count of the fingers on the human hand is very interesting. But it is not yet clear whether Penrose's results are due to an effect of heterochromatin *per se* or whether other mechanisms might also be involved.

Also relevant in this regard are the results of Berlowitz, Loewus & Pallotta (1968) which show that male mealybugs (with one heterochromatic set) have 1·47 times more DNA as females and that in the same tissues males have 1·45 times more cells than females. This might merely be an indirect effect of physiological haploidy, but the haploid condition is the result of heterochromatization. Berlowitz *et al.* have suggested that the increase in cell number is one way by which male mealybugs compensate for having organs with only one set of active, euchromatic chromosomes. More such studies are needed – in coccids as well as in mammals – and they take on special relevance in view of recent speculations that sex differentiation in man and other mammals might involve little more than differential cell growth and kinetics and that this differential growth is under the influence of heterochromatic chromosome regions (Mittwoch, 1969; see also Hamerton, 1968).

6. GENETIC INFLUENCES ON INACTIVATION

An interesting test of the randomness or otherwise of X inactivation in the mouse was made by Cattanach & Isaacson (1965). Among female mice heterozygous for an X-autosome translation and a recessive albino gene linked to it, selection for eight generations for smaller and for larger total albino areas in the mosaic coat gave no decrease in area and only a moderate increase, from a base value of about 30 % to about 50 %. In effect, Cattanach and Isaacson were trying to select for genes controlling or influencing heterochromatization of the X. An experiment similar in principle to this one was made several years ago by Mrs L. Weigmann (personal communication), who selected for high and low sex ratio in mealybug cultures. Since maleness is intimately associated with heterochromatization, she was attempting to alter the frequencies of genetic factors, if any, that controlled or influenced heterochromatization. In spite of several generations of selection, she was unable to get a significant response in either direction. Several generations of inbreeding also did not affect the sex ratio. But one has to keep in mind that even under the most controlled conditions there are rather wide fluctuations in the sex ratio of these insects, and hence very minor genetic components, if they exist, would not have been detected.

In man the only comparable study on genetic control of heterochromatization appears to be the twin study of Brewer *et al.* (1967), who compared monozygotic and dizygotic female twins heterozygous for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency for frequency of G-6-PD-positive cells in the blood. In addition, the erythrocytes of these twins were assayed for G-6-PD activity. In both types of test monozygotic twins showed less 'within pair' variation than dizygotic twins. Indeed, in six of the eight monozygotic pairs, the agreement between twins in regard to the proportion of inactivation of paternally derived versus maternally derived alleles was so close that the character must be under complete genetic control. In the two twin pairs which did not show such close agreement, one pair did not have identical karyotypes: one of the twins was an XX/XO mosaic. The other twin pair could not be similarly studied.

7. CONTROLLING ELEMENTS

Brown (1969) has drawn attention to the fact that there appear to be no chromosomal genes (analogous to the X inactivation centre in the mouse) controlling heterochromatization of mealybug chromosomes. Thus, chromosomes broken into several fragments as a result of paternal irradiation turn heterochromatic along with normal unbroken chromosomes. These results make it unlikely that there are localized inactivation centres on each chromosome.

It also appears unlikely that there are heterozygosity-dependent mechanisms operating in the female mealybug to ensure heterochromatization of the paternal complement in her sons. A number of indirect lines of evidence supporting this conclusion were obtained by Chandra (1963). At about the same time, Nur (1963) found a small percentage of male embryos in a parthenogenetically reproducing coccid which showed typical heterochromatization of a haploid set (in spite of the fact that the two haploid complements, one eu-, the other heterochromatic, were derivatives of a single egg pronucleus and thus had to be, barring new mutations, genetically identical). These and other results indicate that heterochromatization in coccids is under some kind of subtle developmental control and it appears to be closely linked to the general problem of sex determination in these insects (Brown, 1969).

Lately there have been a few reports of human males and females heterozygous for deleted X chromosomes in whom it appears, albeit on the basis of somewhat indirect evidence, that it is the *normal* X which is heterochromatized. It is difficult to conceive of any selective advantages for such cells over those in which the deleted X was inactive unless one assumes that in these cases the normal X carried deleterious alleles of some 'critical' genes whose normal alleles were on the deleted X. This of course is not the only possible explanation. The presumed lack of inactivation of the abnormal X's in these individuals may also be attributed to the existence of a locus (or loci) controlling X inactivation which has become deleted in the abnormal X chromosomes.

Although there is, as yet, no direct evidence for the presence of an X inactivation

centre on the human X (or elsewhere in the complement), data from deleted X chromosomes should permit a test of such ideas. With this in view, data on sex chromatin and other aspects were taken from 21 case reports in the literature and these will be evaluated here. Among these, the short arm was affected ($Xp-$) in 12 cases and the long arm ($Xq-$) in the remaining nine. There is very little that is unexpected in the behaviour of the $Xp-$ cases. Of the 12 cases, in 8 there was evidence of sex chromatin formation (as indicated by the size of the s.c. body) and/or late replication by the deleted X. For three no data were presented on the size of the sex chromatin (s.c.) body or about the replicating properties of the deleted X. In only one case (London *et al.* 1964) was a size difference in s.c. looked for and not found; radioautography was not attempted in this case. In apparent contrast, there are some puzzling observations involving several $Xq-$ cases. In three (Grouchy *et al.* 1961, cases 1 and 2; Nielsen, 1966), the authors specifically searched for smaller s.c. bodies but were unable to find any. Even though they would have been expected to be positive on karyotypic grounds, the two cases reported by Miles *et al.* (1962) and by Valencia *et al.* (1964) (respectively $45,XO/47,XXq-Y$ and $47,XXq-Y/46,XXq-/46,XY$) were sex chromatin negative in spite of repeated examinations of multiple tissues. Similarly, Crawfurd's (1961) case ($47,XXq-Y/48,XXXq-Y$) had only one s.c. body per nucleus although again the expectation was far at least an occasional cell with two bodies. Indeed the lack of s.c. in the first two cases and the presence of only one body in Crawfurd's case has, in the past, raised doubts as to whether these involved X deletions at all (Nielsen, 1966). These unexpected observations and what appears to be a slight difference between the behaviour of $Xp-$ and $Xq-$ cases can be interpreted in at least three ways. (1) There obviously may have been an undetected mosaicism. (2) In the $Xq-$ chromosome, the remaining short arm may become heterochromatized, but the s.c. body thus formed is so small that it is not easily distinguishable from the surrounding chromatin. Although this interpretation does not appear likely, radioautography of such deleted chromosomes should provide valuable evidence in this regard. (3) There is an X inactivation centre on the long arm, and its loss makes the X incapable of becoming inactivated.

One would expect data on isochromosomes derived from the X to provide crucial evidence on the problem. Long-arm isochromosomes have so far always proved to be late replicating and form sex chromatin, a fact consistent with the idea of an X inactivation centre on the long arm. There are very few examples of isochromosomes for the short arm of the X and in one such probable case (Fraccaro & Lindsten, 1964) the abnormal X was late-replicating, thus contradicting the hypothesis. But it is conceivable that isochromosomes pose special dosage problems and hence are always inactivated. Genetic data on the Xg blood group are in accordance with this view (Polani *et al.* 1970). The fact that *ad hoc* assumptions of this nature are necessary obviously means that the inactivation mechanism involves something more than a straightforward single locus difference (or, less likely, that the abnormal X's resulted from complex rearrangements shifting the locus into new regions).

In view of these ambiguities, experimental studies of sex chromatin, late-replication and related properties of structurally altered X chromosomes (deletions; X-autosome translocations) are urgently needed, particularly in exceptionally good cytological material such as that of the marsupials.

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