[*Genetic regulation in eukaryotes is one of the fascinating subjects in genetics and molecular biology. Various techniques available during the last decade have helped clearing the web of complexity of chromatin organisation and functional aspects of DNA sequences. Dosage compensation forms a unique system for the study of genetic regulation in eukaryote. Mukherjee's demonstration in 1965 on the hyperactivity of the X chromosome in male Drosophila has opened up a new avenue for searching the regulatory elements. It parallels to a great extent the demonstration of inactive X in mammals by Mary Lyon four years earlier. Dr. Mukherjee has analysed in this article the results of different aspects of studies on dosage compensation in Drosophila* and predicts the possible scope of progress in the field]

DOSAGE COMPENSATION AS A MODEL SYSTEM FOR GENETIC REGULATION IN EUKARYOTES

A. S. MUKHERJEE
Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700019, India

INTRODUCTION

The prime consequence of evolution of the X chromosome in higher animals is the establishment of tolerance of the haplo-X condition. While natural selection had favoured the process of tolerance of haplo-X, means to restore the balance for haplo-X in male against diplo-X in female had to be promoted simultaneously. The balance requires a stringent control of gene expression, and was termed dosage compensation[1]. Stern[2,3] showed that the restoration of the balance of X coded gene products in the two sexes (XX♀ *vs.* XY♂) is related to the tolerance of the haplo-X. Recently, Lucchesi[4] analysed the data available on the sex determination and evolution of sex chromosome and concluded that the evolution of the mechanism of sex determination and that of dosage compensation might have been convergent.

When we look through the dark glass into the panorama of evolution we can find several lines of animal groups in which precise difference in the sex chromosome between male and female was established, and a need for dosage compensation followed as an obligatory consequence. Obviously, although evolved simultaneously, the sex determining genes and compensated genes maintained an antiparallel relation, the former tended to retain a dosage effect, the latter a compensatory effect.[5]

While the dosage effect can be conceived by assuming a simple relation of quantum of gene product as a function of the dosage of structural genes, dosage compensation requires stringent control prior to the determination of the amount of translatable mRNA. Evidently, this control mechanism involves a system of regulaton of the production of genetic message and hence an important aspect of eukaryotic genetic regulation in general[6–8].

In this review, it is the author's intention to highlight the key features of dosage compensation in *Drosophila* and their implications in terms of genetic regulation. Then I wish to synthesize the available results into one or more possible models of regulation.

GENETICS OF DOSAGE COMPENSATION IN DROSOPHILA AND MAMMALS

As early as 1929 H. J. Muller recognized the equality of the phenotypic effect of certain X

linked genes in the two sexes. Muller[9] proposed the existence of '+' and '−' type modifiers in the X chromosome which in female with double dose cancel each other and thereby results in identical phenotype for the sex linked genes in both sexes. Stern[2] suggested the concept of genetic balance and showed that such a balance is an inherent property of the dosage difference of X linked genes in the two sexes. This conclusion was arrived at by the demonstration that *bb* mutant gene does not reveal dosage compensation as it has loci in both X and Y chromosomes.

All earlier studies were based on visual estimation of phenotypic effect like eye pigment. Smith and Lucchesi[10] and later others[11,12] demonstrated identical expression of X coded gene products by actual measurement of the product in both wild type and mutant (relocated X linked gene) male and female and thus established that dosage compensation is in actuality an expression of balance of wild type genes of the X chromosome.

It has been observed that mammals such as cat or mice heterozygous for the colour gene show mosaic patches of coat colour[13,14]. Lyon[15] showed that in female mammals with two X chromosomes, there is always only one active X chromosome. The inactive X chromosome can be seen in cytological preparation to form the sex chromatin body or Barr body. A direct proof that only one of the two X chromosomes in female mammal remains active has been given by Ray *et al.*[16] by the demonstration of a high positive correlation between G6PD inactivity and late replication of one of the X chromosomes in fibroblast clones from a female mule.

Thus, in both *Drosophila* and mammals most X linked genes show dosage compensation. However, the sex-determining genes are somehow excluded from such compensation effect[5,17]. Lyon[15,18] proposed a basic model for the operation of dosage compensation, which has been validated later by cytological and genetic demonstration[16,19,20]. According to this hypothesis (Lyon hypothesis), the dosage difference for X linked genes in male and female mammals is balanced by keeping only one of the two X chromosomes active at a time in any cell. Although, generalization of this hypothesis has met with some criticism as exceptions have been found[20], the essential feature of the concept remains valid[5]. It is generally true that the number of Barr bodies is invariably one less than the total number of X chromosomes. In contrast, in *Drosophila* unlike mammals females heterozygous for X linked (compensated) genes do not show any mosaicism for their expression. This is generally true for all X linked body colour mutants tested[21,22] as well as for X coded gene products[23]. Kazazian *et al.*[23] have shown by the use of electrophoresis that while one each of the two electrophoretic variants, one slow and another fast, of 6 phosphogluconate dehydrogenase can be obtained in two distinct mutants, *viz.* Pgd^B/Pgd^B and Pgd^A/Pgd^A respectively, the F_1 heterozygote for the two mutants (Pgd^B/Pgd^A) yields an intermediate form in addition to the two parental forms. This finding is a strong evidence toward the activity of both X chromosomes in cells bearing two X chromosomes. Furthermore, the Barr body like structure is never seen in cells with two or more X chromosomes.

These facts suggest that while the phenomenon of dosage compensation has been found as one successful way to defend haplo-insufficiency for the X chromosome, the means of achieving this result has been evolved independently.

HYPERACTIVITY OF MALE X : CONCEPT OF OPERATION OF DOSAGE COMPENSATION IN DROSOPHILA THROUGH MALE

Dobzhansky[24] examined the hybrids of *Drosophila tropicalis* × *D. insularis* and observed that in hybrid males as in parent

males, the single X chromosome in giant salivary glands is pale stained and twice as wide as the individual autosomes, all of which in the hybrids remain unpaired. He concluded that the inflated X chromosome in the hybrid male might be a cytological manifestation of dosage compensation. The prediction of Dobzhansky was proved to be correct by the demonstration that the polytenic X chromosome in *Drosophila melanogaster* male transcribes twice as much RNA as each of the X chromosomes of female[25, 26]. Mukherjee and Beermann[25] arrived at two important conclusions : (a) that dosage compensation operates through the male by hyperactivation of the X linked genes rather than through female by suppressing one set of the genes, (b) that the regulatory process leading to dosage compensation acts at the level of transcription. The former implies that the regulation is a positive control system. The latter requires that the regulation system must prepare the template sufficiently usable for the hyperactivity of the X chromosome. The hyperactivity of the X linked genes of the male was shown to be valid by various workers both at the level of transcription as well as at the level of enzyme activity[17,27,28].

Mukherjee and his co-workers examined the validity of this theory in several different species of *Drosophila* and showed that in all of them, regardless of the evolutionary distance, the X chromosome was hyperactive in male[29,32].

Maroni and Plaut[33] and Lucchesi and his co-workers[27] have corroborated that dosage compensation in *Drosophila* indeed operates through hyperactivity of the male X. By measuring transcription and/or enzyme activity in normal female and male as well as various hyperploids, they have claimed further that this phenomenon is causally related to the X : A ratio.

It was quite clear by these investigations that contrary to the original proposition by Muller[1] who suggested the operation of dosage compensation through female by negative control and in contrast to dosage compensation in mammals, in *Drosophila* it operates through male by a positive control mechanism.

CELLULAR AUTONOMY AND DEVELOPMENTAL CONTROL

Soon after the discovery of hyperactivity of the X chromosome, Komma *et al.*[34] and Lee[35], raised the issue of difference of developmental rate and physiology between the two sexes as a possible explanation to the phenomenon of dosage compensation and claimed that the effect might be the resultant of difference in the development and physiology of sexes. A definite answer to this polemic was provided by Lakhotia and Mukherjee[22] who upon the use of unstable ring-X chromosome produced XX-XO gynandric mosaics. Estimation of ^{3}H-uridine labelling density in the X chromosomes of XX and XO cells showed unambiguously that the X chromosome in XO cells was invariably hyperactive regardless of the proportion of XO to XX cells in the mosaic glands. Clearly, this piece of work demonstrated a cellular and developmental autonomy of hyperactivity of the male X.

The implication of the autonomy is quite important from the regulatory aspect. It implies that the hyperactivity is set in with the haplo-X cellular organization.

Results on the studies on site-wise activity of segments of the X chromosome[29] revealed further. that the determination event of hyperactivity evokes an autonomous modulation of perhaps segments or individual genes of the X chromosome. Consequently, transposition or translocation of segments of the X chromosome to autosomes fails to alter the autonomous hyperactivity of the X chromosomal segment. Similarly, translocation of autosomal segments to X chromosome fails to evoke hyperactivity to the segment translocated[36].

The demonstration by Berendes[37] that parallel to this event of hyperactivity of male X, the X chromosome of male completes its replication earlier than the rest of the chromosomes of the complement. This finding added a new vista to the research on dosage compensation. Lakhotia and Mukherjee[38] made a critical evaluation of this early replication of the male X of *Drosophila* and suggested its relation to dosage compensation. In all species of *Drosophila*, wherever hyperactivity of male X has been established, one could predict and actually demonstrate early replication of the X in male[30,32,39]. Two additional findings have added support to the possible relation between early replication and dosage compensation. Firstly, it has been found in this laboratory that in *Drosophila miranda* male, a section of the second X (X_2) which has evolved rather lately from one of the autosomes of *Drosophila melanogaster*, shows an intermediate transcriptive activity and also shows lack of early completion of replication (Das *et al.*, in preparation). The second set of observations is the change in the duration of replication of the male X in homozygous sex specific lethal mutant (*mle*), an autosomal mutant at 56·8 of the 2nd chromosome[40]. Belote and Lucchesi[8] showed that in mle^{ts}/mle^{ts} (temperature sensitive male specific lethal) males, the X chromosome is distinctly thinner than +/+ male X, and the property of hyperactivity of the X is lost. Subir Ghosh (unpublished) in this laboratory has shown that the "early replication" property of the X in such males is also lost (Fig. 1). These findings strongly suggest that the process of dosage compensation is guided by a developmental control system.

Chatterjee and Mukherjee [41], again using unstable ring X produced XX-XO mosaic salivary gland, demonstrated a cellular and developmental autonomy of early replication of the male X. They have argued that this early replication is due to a faster rate of chain growth. What it essentially

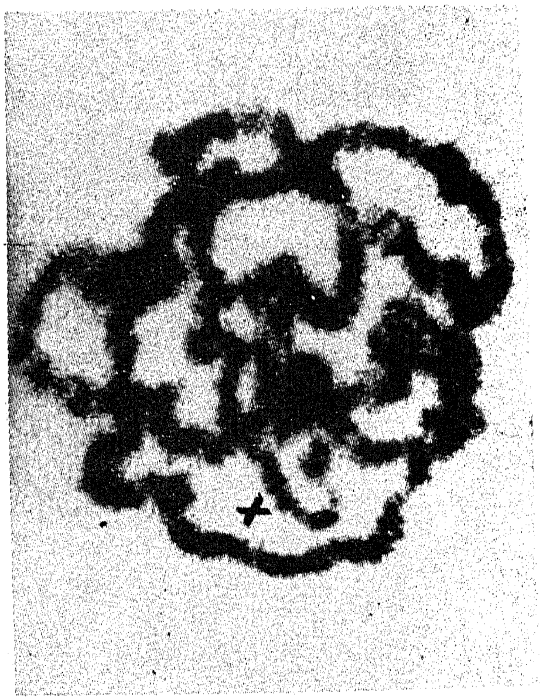

Figure 1. Autoradiogram showing ^{3}H- thymidine labelling (3C-3D type labelling pattern) in mle^{ts}/mle^{ts} male. The X chromosome is narrow and shows nearly the same labelling pattern as the autosomes, and does not show the typical asynchrony as found in +/+ male.

implies, is that the whole process of equality of X-coded gene product is guided by certain developmental clock system within the cell which includes interactions of the DNA sequences, organizational proteins, DNA- and RNA- polymerases.

X-CODED GENE ACTIVITY IN CHROMOSOMAL ANEUPLOIDS

Maroni and Plaut[33,42] examined X chromosomal transcription and enzyme activity for certain X chromosomal genes in 2 × 3 A intersexes and triploids (3 × 3A) and Lucchesi *et al.*[27] examined that in metamale (1 × 3A) and metafemale (3 × 2A) of *Drosophila melanogaster*. Their results revealed that the primary message as well as the enzyme activity were dependent upon the ratio of the number of X chromosome and the autosomal sets. Furthermore, it was evident from the data that while within the sex, the product of the X linked genes

showed a dosage effect, that between the sexes showed compensation of the X linked genes[7,21]. This conclusion was so invariable that one could predict the net amount of X coded product in a male or female by finding out the dosage of the X linked genes and the sets of autosomes. It was clear therefore, that certain autosomal signals must be playing a role in guiding the phenomenon of dosage compensation in *Drosophila*.

However, the problem seems to be more complex than this simple rule of thumb, when one examines the segmental or partial heteroploids, that is duplication for X chromosomal or autosomal segments. Stewart and Merriam[43] measured the enzyme activity in a number of different X chromosomal heteroploids and showed that the enzyme activity varied from one group of heteroploids to another and this variation could not in every case be explained upon the X to A dosage ratio. They claimed that their data could be best explained upon alteration at the level of integrity of the entire X chromosome. Maroni and Lucchesi[44] and Prasad *et al.*[45] examined the validity of Stewart and Merriam's findings and conclusions in segmental aneuploids and both groups had one common fact in their data that is when the transcription in duplicated segments was measured autoradiographically both segments in the partially heteroploid male were hyperactive while the transcription of the entire X (corrected for duplicated region) was not significantly different from that in 1 × 2A normal male. Maroni and Lucchesi[44] concluded that the transcriptive activity is distributed in such a way that the net amount is the same in both types. On the other hand, we[45] proposed a preset modulation of the entire X chromosome or part thereof as long as the major part of the chromosome is haplo-X. In a sense, we supported Stewart and Merriam's[43] conclusion.

A rather recent investigation in our laboratory using a tandem duplication (confluens) for the segment 3C1–3D5 and a transposition of segment 16A1–20D, revealed that each component segment of the two duplications was hyperactive (Bose and Mukerjee, unpublished). This provides further support to our earlier conclusion. On the other hand, when transcription was monitored by ^{3}H-uridine autoradiography in segmental heteroploid for autosomal segment (88B–92A) the activity on the X chromosome was significantly greater in both female and male ($p < 0{\cdot}05$) but more so in the male ($p < 0{\cdot}01$) (Ghosh and Mukherjee, in preparation).

EXPRESSION OF X-LINKED hsp GENE IN DROSOPHILA PSEUDOOBSCURA

The heat shock protein genes (hsp) are known to be present on several autosomal sites of *Drosophila melanogaster* [*viz.*, 63C, 64F, 67B, 70A (on 3L) and 87A, 87C, 93D and 95D (on 3R)][46]. In *Drosophila pseudoobscura* one of them has been evolutionarily shifted to the X chromosome[47] and occupies the site 23 on XR. Pierce and Lucchesi[47] have shown that the X chromosomal hsp gene in *Drosophila pseudoobscura* is also dosage compensated.

This piece of finding, in the author's opinion, implies, apart from the selective advantage of the phenomenon in evolution, that an inherent modulation system in the X must have to be established before it can respond to any regulatory signal for dosage compensation.

EVIDENCE TO MODULATION OF CHROMATIN TEMPLATE IN HAPLO-X SYSTEM

A few years ago Chatterjee *et al*[48] examined the binding of nonhistone protein to X chromosome of male and female by scanning cytospectrophotometry and showed that the male X binds more nonhistone protein than does female's individual X chromosome[48]. Chatterjee *et al.* and Chatterjee and Mukherjee[49,50] then

examined the chromatin template activity by *in situ* transcription method on immobilized chromatin preparations using exogenous RNA polymerase from *E. coli* without and with extraction in high salt and showed that the chromatin template activity of the male X is altered differently from the female X's following the extraction. They concluded that this difference is due to the inherent difference in the organization of the male X from female X's which are differentially extracted by high salt.

Recently, we have come across with an X-linked mutant strain, called 'reinverted mosaic'. The X chromosome of most of the cells of larval salivary glands of such mutant male is nearly twice as much inflated as that in the normal male. Ghosh in our laboratory has shown that the X chromosome in the mutant male is hyper-hyperactive (figure 2) but its increased hyperactivity is not always proportional to its inflated size. Further, it appears from her observation that the locus for this mutant may be in the region of 16A-20D as its effect is suppressed by Barr duplication (B^S.Y) (Ghosh, unpublished). It is suggested that this mutant is a mutation for the modulator-type gene that induces altered organization of the X chromosome in the male.

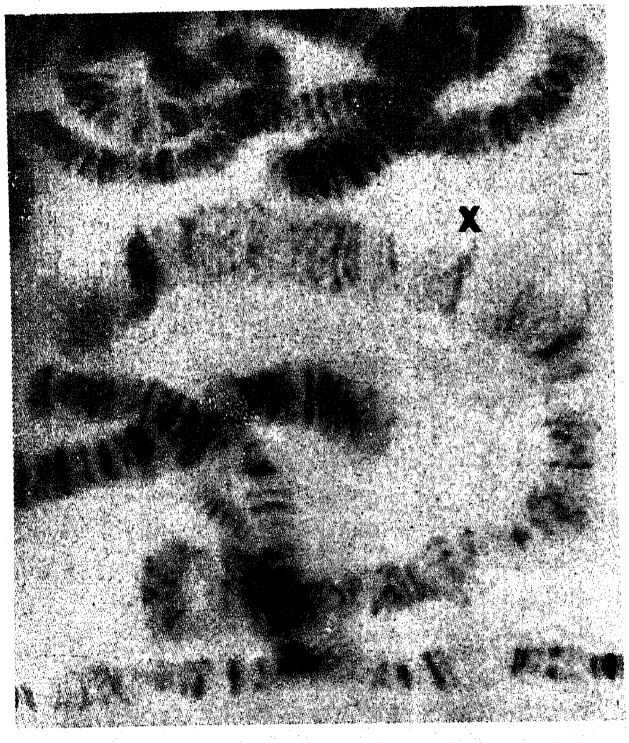

Figure 2. Morphology of the polytene chromosomes in In (1) BM_2 (re-inv) male showing the highly puffed out X chromosome.

SYNTHESIS OF THE MODEL OF GENETIC REGULATION AND CONCLUSION

On the basis of the results presented above a working model has been suggested for the observation of dosage compensation in *Drosophila* (figure 3). It is proposed that the

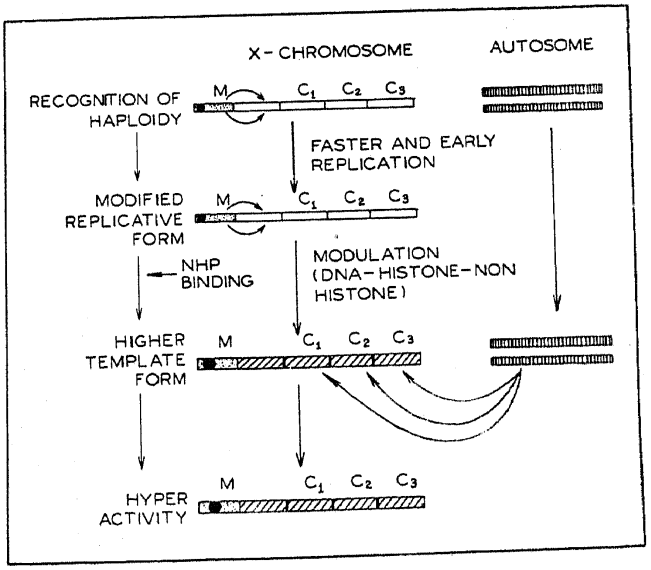

Figure 3. A model of genetic regulation of dosage compensation in *Drosophila*. M = the postulated modulator gene complex. C = Compensated genes. Hatched chromosome represents the modulated X chromosome.

regulation of dosage compensation is determined in two steps. First, a 'modulator' gene, existing in the X chromosome (or in an autosome, it really does not matter), recognizes the haploidy for the X in the male (the gene may be a simple single factor type or one or more complex loci). This recognition prepares the X chromosome for modulated organization through early (and/or faster) replication and nonhistone protein binding and evokes

a higher template form for the X. In the second step, certain autosomal signals stimulate higher activity on the higher template of the X so modulated. All results on the pattern of gene activity of X coded genes[7,21] can be explained by this model. The reason for the requirement of early completion of replication in the first step remains conjectural but appears obligatory. However, it may not be simply coincidental, as we know the reverse, that is transcriptive inactivity of the inactive X (in mammals) is also late replicating (facultative heterochromatinization).

In conclusion, the author would like to maintain that although much information has been obtained on different aspects of dosage compensation, it is necessary to understand the organization of the chromatin of the X chromosome before any model can be taken as useful. There is unending scope of research on this aspect. With the information on the process of compaction and decompaction of nucleosomal organization of the chromatin and role of nonhistone and histone proteins in such processes it should be possible to pinpoint the right model sooner than one can imagine. No doubt, more mutant genes of the kind of male specific and female specific lethals and of modulators would be the first step in reaching the goal. At the end, I may repeat the concluding sentence of Stern[3]: "Dosage compensation forms one chapter in the history of genetic systems. Its analysis is not yet a closed chapter".

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