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REPLICATION AND TRANSCRIPTION PATTERNS IN DROSOPHILA POLYTENE CHROMOSOMES AND MOLECULAR BASIS OF DOSAGE COMPENSATION[1]

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Mukherjee and Beermann(1) and Mukherjee(2) and Mukherjee et al(3) have shown that the single X-chromosome of male (1X2A)/ salivary glands in Drosophila synthesizes twice as much RNA as the individual X's of the female, in spite of the presence of as much DNA in the former as in the latter(4). This phenomenon of accelerated rate of RNA synthesis implies that (a) the male X in the giant salivary gland cells should be in a permanent 'puffy' condition and (b) the DNA replication pattern of the male's single X should be different from that of the female's paired X's. The reality of these predictions have been corroborated by the observations that there is practically no difference either in the size of the 1X of the male and the 2X's (conjugated) of the female(3) or in the puffing activity(5) and RNA synthesis in different puffed regions between the two sexes and that the single X of the male may be early replicating as compared to the females's double X's as well as autosomes(6, 7). It has been shown earlier that this hyper-activity of the male X chromosome in salivary glands of Drosophila does not depend a priori on the physiology and development of the zygote as male but is determined autonomously by the 1X2A condition alone(8). Apart from the developmental significance of this autonomy of the hyperactive X, this fact suggests that a genetic control exists that may be switched off or on depending on the dosage of the genes in the X-chromosomes.

It is the purpose of the present report to provide further evidence to the molecular basis of dosage compensation in Drosophila. The following data show that the precise control underlying the phenomenon of chromosomal basis of dosage compensation lies in the replicative and informative organization of the genes themselves.

MATERIALS AND METHODS

DNA replication and RNA transcription patterns in the polytene chromosomes of larval salivary glands of Drosophila have been examined by use of autoradiography in three species, D. melanogaster, D. hydei and D. ananassae. Since detailed work on DNA replication and

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RNA synthesis has been made so far on D. melanogaster and D. hydei, respectively, the data will be presented from the work on them. The wild type Oregon R + stock of melanogaster and the wild type hydei stock obtained from Tubingen (by the courtesy of Dr. O. Hess) have been used. Both of them were raised on Drosophila culture medium and reared at 24° ± 1°C.

Salivary gland chromosomes from the larvae of specific age group have been prepared by our conventional technique[8] and observed under a phase contrast microscope or an ordinary light microscope with properly adjusted illumination. Puffs have been identified under the phase contrast from temporary squash preparations. Banding patterns of D. melanogaster have been identified from Bridges' map[9] and bands and puffs of D. hydei have been located by the use of the reference maps of Berendes[10, 11].

For DNA replication, the radioactive precursor ^{3}H-thymidine (^{3}H-TdR) having specific activity 5.7 Ci/mM, conc. 150 Ci/ml, and for RNA synthesis, ^{3}H-Uridine (^{3}H-UR) having specific activity 3.27 Ci/mM, conc. 250 μCi/ml, have been used (both the radioactive isotopes were obtained from Bhabha Atomic Research Centre, India). Salivary glands were incubated for 20 or 10 minutes in ^{3}H-TdR or ^{3}H-UR, respectively, and the slides were processed for autoradiography in usual manner[8]. Kodak AR 10 stripping film has been used throughout the various experiments.

OBSERVATIONS

The patterns of ^{3}H-TdR incorporation in the salivary gland chromosomes of D. melanogaster, D. ananassae and D. hydei have been examined in respect of their degree of synchrony in the two sexes supposedly both belonging to the same age group. As first pointed out by Berendes[6], in the case of D. hydei, certain proportion of cells which are continuously labelled, in-so-far as the autosomes are concerned, show discontinuous labelling in the X-chromosomes in the males of all three species. It has been further observed that the male nuclei in which autosomes are also discontinuously labeled have still fewer labeled sites in their X-chromosomes. A detailed analysis of this aspect has been made on D. melanogaster, and will be presented below. For this purpose the labeling patterns in the terminal part of the right arm of the second chromosome (2R and a part of the X) have been critically examined in the two sexes. The 2R chromosome arm (section 56F to 60F of Bridges' map) has been arbitrarily divided into 20 sites and the X (from 1A to 12E) into 45 sites. Whenever all 20 sites in the 2R segment in a nucleus were found to be labelled it was considered to have continuous labeling.

A comparison of different labeling patterns in male and female X in relation to the labeling patterns on the 2R reveals a striking difference in the number of replicating sites at any stage of replication. Fig. 1 presents the number of labeled sites on the X in different nuclei of male and female in relation to comparable patterns of 2R in the two sexes. The arbitrary line connecting the points indicate the ordered sequence on the basis of the assumption that DNA synthesis at any particular site follows an uninterrupted sequence. The data show that excepting a few patterns in both sexes (which may be compared with the unconventional patterns described by Nash and Bell[12] all others fit this uninterrupted ordered sequence. Such a comparison clearly shows that except in some continuously labeled nuclei (initial points in the figure), i. e. when the autosomal segment shows labeling in all 20 sites, the number of labeled sites on the X is invariably less than in female, in spite of their having identical patterns of labeling on the 2R segment.

It should be noted that with any particular pattern on the 2R, one may have several labeling patterns on the X-chromosome in male as well as female. But as regards the number of labeled sites on the X the overall patterns are always different in the two sexes without any apparent over-lapping. As for example, with 5 labeled sites on the 2R (viz., 56F, 57AB, 58A, 59CD and 60F), the female X may show labeling patterns varying from 19 labeled sites to only nine; in the male, on the other hand, with the same sites labeled on 2R, the range of labeled sites on the X vary from 7 to 3. These facts not only support the idea that both X's and the autosomes start replicating simultaneously at all points[13] but also suggest that the male X replicates faster, both in comparison with the female X's of different nuclei as well as the autosomes of the same nucleus. Moreover, it is evident from Table I that for specific patterns in the higher labeled-site groups of the X, the 2R/X grain ratios in the male and female are similar but when the labeled site classes fall below 50% the ratios sharply deviate, with much higher ratios in the male. The observed effect is clearly in conformation with the expectation based on the idea that the male X-chromosome completes its replication cycle faster than the autosomes, and therefore, also faster than the diploid X-s in the female.

That this differential replication of the X in the male and female has its counterpart in the synthesis of RNA has been shown by identical incorporation of ^{3}H-UR in the male's single X and the females double X's[1] (Mukherjee and Beermann, 1965). A further puff-wise analysis of absolute ^{3}H-UR grain number as well as the pattern of rank distribution of the grains on different puffs of the X-chromosome incoporate reasonably similar amount of the precursor in both sexes (Table II). Table II gives the total number of grains in different puffs of the male and female glands and also the puff class or classes which form the peak of distribution of ^{3}H-UR grains in the

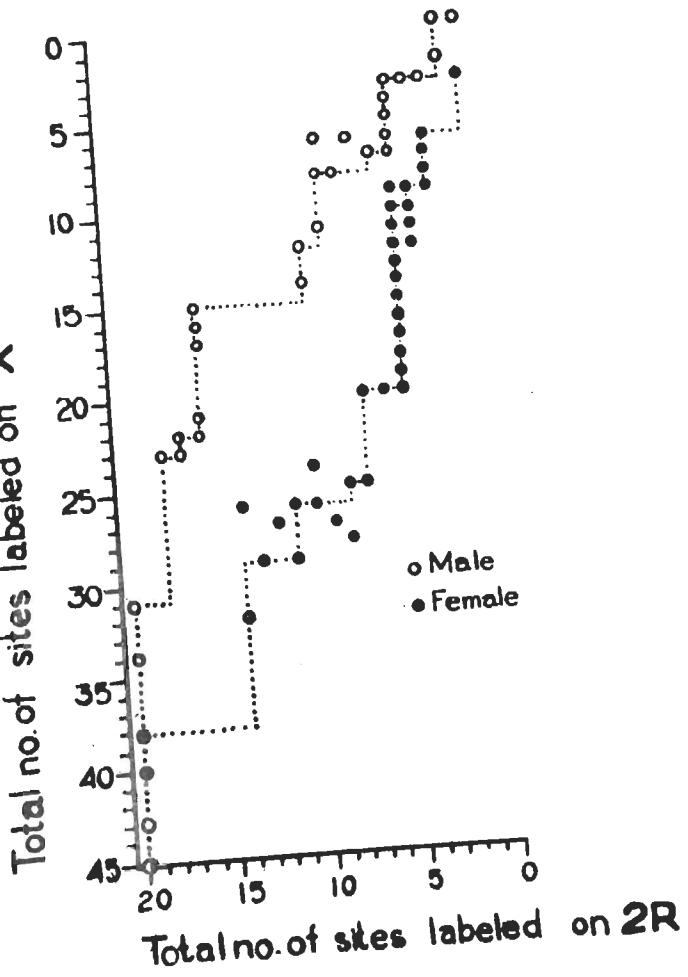
0
5
10
15
20
25
30
35
40
45
Total no. of sites labeled on X
o Male
• Female
20
15
10
5
0
Total no. of sites labeled on 2R

FIG. 1

TABLE I

Mean 2R/X H^3-TdR grain ratio in male and female in different labeling patterns of X-chromosome in D. Melano Gaster

Sex	Mean 2R/X Grain Ratio No. of Sites Labeled on X - Chromosome				
	31 to 45	21 to 30	11 to 20	6 to 10	1 to 5
Female	0.41 (5)	0.38 (14)	0.38 (31)	0.44 (14)	0.32 (1)
Male*	0.37 (2)	0.45 (4)	0.60 (7)	0.87 (13)	0.15 (40)

* The 2R/X Grain Ratios in Male have been halved for a direct comparison with the ratios in female.

The numbers in parentheses indicate the number of nuclei observed in different labeling groups.

TABLE II

Puffwise distribution of H^3-uridine grains in the X-chromosome of male and female Larval Salivary Glands of D. Hydei

Puff site	Absolute grain number in the X		Highest labeled class or classes in different puff sites	
	Male	Female	Male	Female
1A	32.2	30.6	E	F
1 BC	25.6	37.6	F	F
1 D	9.0	16.8	C	E
2 A	12.2	14.1	C	C
3 B	27.6	34.9	E, F	F
4 CD	54.4	55.7	G	F
6 C	13.9	18.9	C, D	E
8 A	22.1	22.3	E	E
8 D	13.9	9.1	A, E	B
8 C	21.2	24.2	E	E
12 BC	47.1	57.3	G	G
13 C	34.7	36.7	F	F
16 C	27.9	41.1	F	F
18 D	33.5	29.4	F	F
19 B	25.9	23.9	E	E
19 D	30.7	34.3	F	F
20 B	23.4	22.0	E	E
20 D	50.3	45.6	G	F

Class A = 1 to 5 grains; B = 6 to 10 grains; C = 11 to 15 grains; D = 16 to 20 grains; E = 21 to 30 grains; F = 31 to 50 grains; G = 51 to 70 grains.

two sexes. Use of this absolute grain number as a parameter for the comparison of the extent of similarity of RNA synthesis in the X-chromosomes of male and female is based on the fact that the grain numbers in various puffs of the diploid autosomes are also similar. As for example, the 93C puff of the chromosome IV has mean grain numbers 37.9 and 36.0, in the male and female, respectively (ratio = 1.05) and the 85A of the same chromosome has the mean grain numbers 38.3 and 42.4, respectively (ratio=0.90).

The puff site 1BC, 1D and 12BC of the X-chromosome are the three exceptional regions which exhibit relatively higher grain numbers in the female than in the male. A comparison of the highest labeled class of puffs also shows fairly good correspondence between the two sexes (Table II).

DISCUSSION

The phenomenon of dosage compensation has been known to have a significant role on accuracy and precision of genetic adaptation[14] After a long gap of silent phase the chapter has been opened again (after the orginal discovery of Stern[15] and Muller[16] following several important findings along this line in mammalian cytogenetics[17-22]. The molecular aspects of the mechanism of dosage compensation have been sought first in mammalian system. It has been shown that in mammals the sex chromatin whenever formed, normally in the female and exceptionally also in the male (e.g. in XXY) represents one of the X's which is genetically inactive and consists of compacted DNA which shows late replication and very little transcribing activity[20-24]. Thus it is apparent that in mammals dosage compensation operates by inactivation of one of the X's in females. Genetically, this inactivation means repression of all (or some) genes borne by the inactive X; cytologically, this phenomenon manifests itself by the inactive X; cytologically, this phenomenon manifests itself by the formation of compact sex chromatin; and physiologically, it implicates and actually shows delayed synthesis of DNA and suppression of transcription. It was claimed that in Drosophila also the compensation operates by repression of the activityof the two sets of genes in females to the level of activity of only one set in the male[14,25]. Cytologically, no compaction of chromatin is, however, demonstrable in the female Drosophila, neither in ordinary somatic cells nor in polytene nuclei of larval salivary glands. On the contrary, the X-chromosome in the male larval salivary glands shows just the opposite morphological expression, that of de-compaction. Indeed, as shown earlier by Dobzhansky[26], Rudkin[27] and Mukherjee et al.[3], the male X-chromosome in giant salivary glands is enlarged to nearly double the width of the individual X's of the female, and gives a puffy appearance by its pale stainability and diffuse banding pattern, Correspondingly, the single male X synthesizes almost twice as mcuh RNA as the individual female X's[1,3]

and the individual puffing activities in the single male X is very much similar to those in the two paired X's of the female[5] also.

These facts then lead to the surmise that the dosage compensation operates in Drosophila through hyperactivation of the male X and not through repression in female as found for mammals. It is quite possible that different groups of animals have resorted to different means of compensation. And even this hyperactivation of the male X may not be the generalized rule for all insects[28].

We have presented here further evidence to show that the male in Drosophila has in fact the informative organization for the compensation for the lack of one dose of the sex linked genes at the level of information transfer which essentially involves, simultaneously, a faster rate of DNA replication and an overall higher rate of RNA synthesis. The implications of this early replication to the phenomenon of dosage compensation have been discussed with sufficient clarity in an earlier report[7,29]. That this faster rate of replication is inherent in their replicative organization of the genes themselves has been shown by their undisturbed pattern in an autosome-X insertion[29]. Thus in both normal genomic sequence as well as in the translocation, the number of labeled sites in the X and the 2R/X grain ratio maintain an inverse relationship. All these facts taken together may be considered to be sufficient evidence to support that the male X replicates faster than the female X's, although both may start replication synchronously at all sites.

Similarity in puffing patterns of the male's single X and female's paired X's have been shown in D. hydei[11] and in D. melanogaster[5]. This similarity in the grain distribution in the X-chromosomal puffs of the two sexes further support the earlier contention that the dosage compensation operates at the level of information transfer and attests the piecemeal mechanism of the phenomenon operating through the male[29] although the genetic control may be already set in as early as the replicative organization of the genes themselves. The close correspondence between the puffing activity in the two sexes as measured morphologically[5] and between the number of grains in different classes of puffs in males and females suggest strongly for the accuracy of the specific genetic control in the compensation phenomenon, since puffs are considered to be the cytological counterpart of genetic activity[30,31].

Several points are yet to be considered for the chain of reactions from the genetic information to gene expression underlying the identical gene activity involved in dosage compensation. The most important one of these is what triggers the accelerated replication in the male X. In the early works of Stern[15] and Muller[16], presence of plus and minus compensators has been assumed to have

caused the identical expression through a repression in the female This, however, does not explain the higher activityof the male X. Smith and Lucchesi[32] have proposed a "feedback mechanism" for the higher activity and the high rate of synthesis of protein by the compensable genes. While it is certainly one possibility, it may be added that a lack of threshold level of repressible control in the single male X may also initiate a higher rate of replication followed by a higher rate of transcription.

To sum up what we have come to know so far about the chromosomal aspects of dosage compensation in Drosophila is that the male X-chromosome in salivary glands is enlarged to the size of the paired X's of the female; the single X synthesizes twice as much RNA as the individual X's of the female and in this sense it is hyperactive; the hyperactivity of the male X is not directly dependent on the physiology or development of the sex; the hyperactivity is preceded by an early replication and is accurately controlled by a puffwise (i. e. individual locus-wise) precision for identical expression; and finally, the enlargement of the male X can be brought back to a lowered value upon the treatment of such agents which affect the nucleic acid metabolism[3]. The relation of all these facts to genetic dosage compensation is more of circumstantial nature rather than with direct onus of proff. But we are very much on the safe side to state that it is most likely that dosage compensation acts at the level of information transfer and its control lies in the replicative organizationof the genes themselves.

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DISCUSSION

C. R. Puttanna : I would like to add a note of comment on Dr. A. S. Mukherjee's interesting findings on the increased ~~amount~~ of X-chromatin in male. As he has shown there are many interesting things to come in Biology during the next 10 or 20 years. Contrary to the earlier view that genetic compensation between male and female Drosophila is brought about by the suppression of an extra chromosome in female, he has shown that one has to look at this from the view point of male. One could, then, actually show that the single X of male shows higher label of tritiated thymidine and about the double quantity of X-chromatin which compares well with the diploid autosomal chromation.

A. S. Mukherjee : I must correct the last statement of Dr. Puttanna that the male-X of Drosophila does not show higher label of H^3-thymidine but my data show early completion of replication as evident from fewer and fewer labeled sites as compared to the labeling in the autosoms at a particular time. Furthermore, the quantity of the X-chromatin is not doubled but it only enlarges to give an overall "puffy apperance", DNA remaining only half as expected.