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Transcription in *X*-chromosomal segmental aneuploids of *Drosophila melanogaster* and regulation of dosage compensation

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SUMMARY

Transcription of *X* chromosomal DNA has been examined autoradiographically in various $1X2A$ and $2X2A$ normal larvae and $1X2A$ ($+X$ fr) and $2X2A$ ($+X$ fr) segmental aneuploid larvae of species *Drosophila melanogaster*. The segmental aneuploids contained duplications for the segment 9A–11A and 15D–18A of the *X* chromosome. Results show that in the aneuploid male containing 9A–11A duplicaton both the homologous segments involved in the aneuploidy are autonomously hyperactive; their combined activity, measured by X/A grain ratio, is found to be nearly 70% more than the activity in normal male and about 100% more than that in diplo-*X* female. In the aneuploid female, containing the aneuploid segment 15D–18A and having three doses of the segment of the *X* chromosome, the activity was over 100% more than the diplo-X activity. The per gene dose activity for the two segments in the aneuploid male and female, respectively, is also significantly higher than their male and female counterparts. The possible role of lack of contiguity of the genetic segments and an intra-nuclear variation has been ruled out by appropriate analysis. We, therefore, interpret these findings to be due to an autonomous expression of the *X* linked compensatory genes, resulting from a primary modulation in the organization of the entire *X* chromosome. The autosomal signal then renders the individual genetic locus hyperactive.

INTRODUCTION

Dosage compensation is a phenomenon by which males with one dose of *X*-linked genes produce the same amount of *X* coded gene product as females with two doses of the same (Muller, 1950). It has been shown by Mukherjee & Beermann (1965), and others (Mukherjee, 1966; Kazazian, Young & Childs, 1965; Lakhotia & Mukherjee, 1969; Seecof, Kaplan & Futch, 1969; Korge, 1970*a*, *b*; Chatterjee, S. N. & Mukherjee, 1971; Lucchesi, Rawls & Maroni, 1974), that dosage compensation in *Drosophila* is effectively accomplished by hyperactivity of the *X* chromosome in the male *rather than by repression of the* X's *in the female*. This mechanism essentially

implies a positive control device operating through hyperactivation of the transcription of the *X* chromosomal DNA in the male (Mukherjee, 1966; Lucchesi, 1973; Mukherjee, 1974), as opposed to a negative control mechanism operative through the female. The latter type of control was originally proposed by Muller (1950) and is known to be operative in mammals (Lyon, 1961; Brown & Sharat Chandra, 1973).

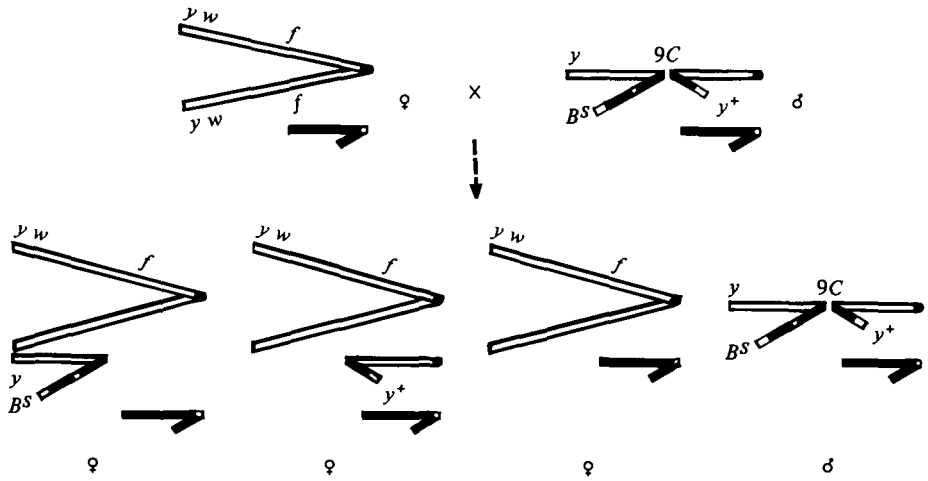
Several models have been proposed to explain the various features of dosage compensation and its possible regulation (Mukherjee, 1974; see the recent review by Stewart & Merriam, 1980). Lucchesi *et al.*'s (1974, 1977) works on euploids (*2X2A*, *1X2A*), metamales (*1X3A*) and metafemales (*3X2A*), support the existence of an autosomal regulation of hyperactivity in the euploid male. Nevertheless, several sets of evidence, namely cellular and locus-wise autonomy of hyperactivity of the haplo-*X* (Lakhotia & Mukherjee, 1969; Chatterjee, S. N. & Mukherjee, 1971) maintenance of hyperactivity of the *X in situ* by exogenous RNA polymerase (Khesin & Leibovitch, 1974; Chatterjee, R. N. & Mukherjee, 1980), and the results of Chatterjee, R. N. & Mukherjee (1978) and Mazumder, Chatterjee & Mukherjee (1975), challenge the validity of the autosomal regulation hypothesis. Stewart & Merriam (1975) showed that duplication of either one or both sites of G6PD (18D-E) and 6PGD (2D–E) resulted in elevated levels of the enzymes, but superfemales (*3X2A*) had the same enzyme level as their euploid (*2X2A*) sibs. They argued that the positive activator type control through autosomal factor(s) is not sufficient to account for the regulation of hyperactive transcription in the male *Drosophila* and that at least one step of the regulation would involve the entire *X* chromsome.

We, therefore, attempted to test the validity of Stewart & Merriam's (1975) proposition by examining the situation *in situ*. For this purpose, transcription on the aneuploid segment of the *X* chromosome in partially aneuploid male and female *Drosophila* was examined autoradiographically. If the hyperactivity of the *X* chromosome in males is regulated by a positive control 'signal' from the autosome(s) alone, one should expect lack of hyperactivity for each of the homologous segments involved in the aneuploid duplication in the male and a lower than diploid activity for the partially hyperploid female, as observed in metafemales (*3X2A*) by Maroni & Plaut (1973) and Lucchesi *et al.* (1974, 1977), the regulation being presumably determined by the *X* to autosomal ratio. If, on the other hand, the hyperactivity results from a combined interaction between the autosomal signal and certain autonomously acting factors immanent to the *X* chromosome segments, addition of an extra piece of the *X* chromosome to an otherwise haplo-*X* condition (as in male) should not alter the level of activity, which is already determined consequent to the modulation of the male's *X*-specific factors. Similar addition to an otherwise diplo-*X* condition (as in female) should show at least a dosage effect. The results presented support the second of the two alternatives and demand a reformulation of the models.

2. MATERIAL AND METHODS

(i) *Synthesis of stocks used*

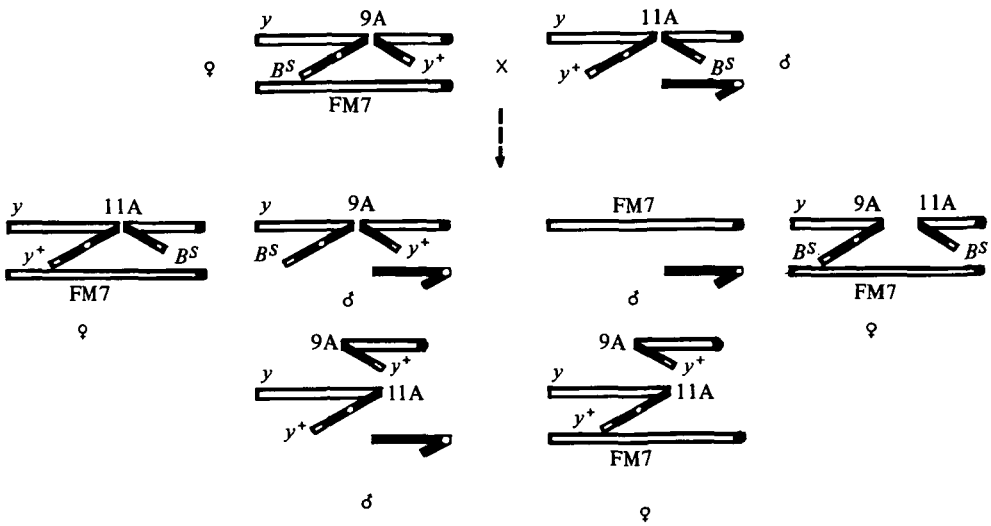
Female and male aneuploids were constructed from $X;Y$ translocation stocks of *Drosophila melanogaster* by the method of Lindsley *et al.* (1972). The stocks used were T(X; Y)9C, T(X; Y)9A and T(X; Y)11A. Males from these stocks carried a free Y chromosome in each case besides the one involved in the translocation. The X chromosome was marked with the recessive body colour mutant, yellow (y). The Y^{L} was marked with B^{S} and the Y^{S} with y^{+}.



Text-fig. 1: Experimental protocol used to generate segmental aneuploid females. Attached-X ($y\ w\ f$: = /Y) females were crossed to T(X; Y)9C/Y males, which yielded two types of aneuploid females, first and second progeny from bottom left in the figure ($y\ w^{+}\ fB^{S}$ female was aneuploid for distal segments 1–9C; $y^{+}\ w\ f^{+}$ female was aneuploid for proximal segments 9C-20F), euploid female $y\ w\ f$: = /Y (third from bottom left) and parental type male, T(X,; Y) 9C/Y (the right most progeny). Hollow bars, with dark spot as centromere, represent X-chromosome, and black bars with open circle as centromere represent Y-chromosome.

To obtain partially aneuploid (hyperploid) females, $y\ w\ f$ attached-X virgins were mated to T(X; Y)9C/Y males. F_1 progeny of this cross yielded females of three genotypes (Text-fig. 1). Females with yellow body colour (y) and B^{S} were hyperploid for the 1–9C region while females otherwise normal but with white-eyes were hyperploid for the 9C–20 F region. The latter class of females were recognized in larval stages by their black mouth parts and colourless malpighian tubules, while the former type of larvae had yellow mouth parts. Autoradiograms were prepared from both types of hyperploid females but since in 1–9C hyperploids the chromosomes were either not well spread or overlapping, the 9C–20F hyperploids were mainly used.

Male aneuploids were generated by crossing T(X; Y)9A/FM7 females to T(X; Y)11A/Y males. This cross yielded, among others, males aneuploid for the region 9A–11A as shown in Text-fig. 2. Aneuploid males were recognized by their near-normal appearance. These males were selected and crossed to $y\,w\,f$ attached-X virgin females. All male progeny of the cross were aneuploid for 9A–11A. Although this cross also yielded females aneuploid for the distal (1–11A) and proximal (9A–20F) segments, in addition to euploid females, males were of only one genotype. Therefore, it was easy to select the aneuploid males at larval stages for autoradiography. All markers are described in Lindsley & Grell (1968).



Text-fig. 2: Experimental protocol used to generate segmental male aneuploids. T(X;Y)9A/FM7 females were crossed to T(X;Y)11A/Y males, which yielded six types of progeny. Counting from left, the fourth progeny in the figure was female heterozygous for the deficiency for the segment 9A–11A. The fifth and sixth progeny in the figure were aneuploid for the segments 9A–11A. The symbols for the X and Y chromosome are the same as in Fig. 1.

(ii) *Autoradiography*

Standard autoradiographic technique was followed, using Kodak AR10 stripping film. Late third instar aneuploid larvae, raised at 20 ± 1 °C, were selected for the cytological preparation of chromosomes. Two pairs of salivary glands (either female or male as the case may be) were incubated in [^{3}H]uridine (200 μCi/ml; sp.act. 13600 mCi/mM; obtained from Bhabha Atomic Research Centre, Bombay) for 10 min, fixed in acetic acid : methanol (1 : 3) mixture and squashed on 50% acetic acid. Coverslips were removed, and the preparations were covered with Kodak AR 10 stripping film, exposed for 14–15 days in light-proof bakelite boxes, and developed in the developer D19b at 10 °C. Slides were stained in 1% toluidine blue in 30% alcohol.

Oregon R^+ females and the *y w f* attached-*X* euploid sibs of the crosses mentioned (Fig. 1) were used as the controls for 9C-20F aneuploid females. For 9A–11A aneuploid males, Oregon R^+ males were used as controls. Aneuploid males were also compared with males having translocated *X* chromosome from the T(X; y^+Y^L)11A/*yf*: = stock in order to check whether change in the normal sequence of the *X* chromosomal genetic order causes any deviation in the transcriptive activity. Intensity of grains was examined over the segments involved in the aneuploidy and over outside segments. The grains on the outside segments and those on the segment 56E–60F of the autosome arm 2R of the corresponding nuclei served as the reference. The relative transcriptive activity was recorded as the

Table 1. *Mean X/A ratio of grains* (±S.E.) *on different segments of the* X-*chromosome in* Drosophila melanogaster

Strain	1–3C	1–11A	11B–20F	Whole *X*
Oregon R^+ ♂	0·42±0·017 (41)	2·04±0·08 (20)	1·31±0·06 (20)	3·42±0·09 (41)
Oregon R^+ ♀	0·52±0·020 (31)**	2·14±0·07 (12)	1·62±0·12 (12)**	3·76±0·15 (12)
T(X;*y*Y^L)11A ♂	0·51±0·020 (21)**	2·10±0·07 (31)	1·57±0·05 (31)**	3·59±0·13 (23)
y w f: = ♀	0·61±0·020 (19)	2·38±0·14 (12)	1·17±0·07 (12)	3·50±0·16 (13)

Numbers in parentheses indicate the numbers of respective segments examined.
**$P < 0·01$.

ratio of grains of specific segments of the *X* chromosomes to those on the autosomal segment.

Photomicrographs were taken under oil immersion objective (100×) in Zeiss Photomicroscope III with bright field transmitted or phase contrast illumination.

3. RESULTS

The purpose of this investigation was to examine the pattern of transcription in phenotypic and functional males and females in which the increase in the dose of sex-linked genes was restricted to only a very small quantum, large enough for detecting the change in transcription and in which the chromosomal sex determination was undisturbed. The aneuploid males and females synthesized for this purpose are perfectly viable and fertile, although they are partially 2*X*2*A* (male) or partially 3*X*2*A* (female).

Since the two segments of the *X* chromosome forming the partial aneuploid were attached to two non-homologous chromosomes or centromeres, and are therefore topographically separated from each other, it was desirable first to examine the possibility whether disruption of the linear arrangement and continuity of the genes might influence the transcriptive property. For this purpose, transcription has been compared autoradiographically in the wild type male (1*X*2*A*), wild type female (2*X*2*A*), *X*; *Y* translocation male (1*X*2*A*) and attached-*X* female (2*X*2*A*).

Results presented in Table 1 show that for both the long segment (1–11A) of

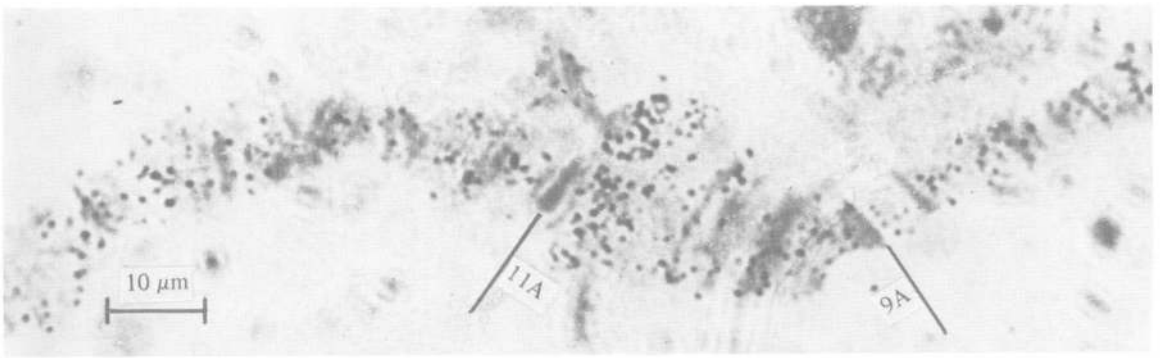
the X chromosome and the entire X chromosome, the X/A ratios of grain numbers are similar in the diploid ($1X2A$) male, diploid ($2X2A$) female, the X; Y translocation male and the attached-X female. The differences are not statistically significant. This finding rules out the possibility of an effect on transcription resulting from the lack of contiguity of the X chromosomal segments in the partial aneuploids. The X/A ratio for the sections 1–3C and 11B–20F, however, in the Oregon R^+ male is significantly different from that in the Oregon R^+ female as well as from those in the translocation male and attached-X female, respectively. Yet the ratios for neither the translocation male nor the attached-X female are different from that in the Oregon R^+ female. The differences are within the limit of variation reported earlier.

Table 2. *Mean X/A grain ratios* (±S.E.) *on the* X *chromosomal segments of the normal and aneuploid strains of* Drosophila melanogaster

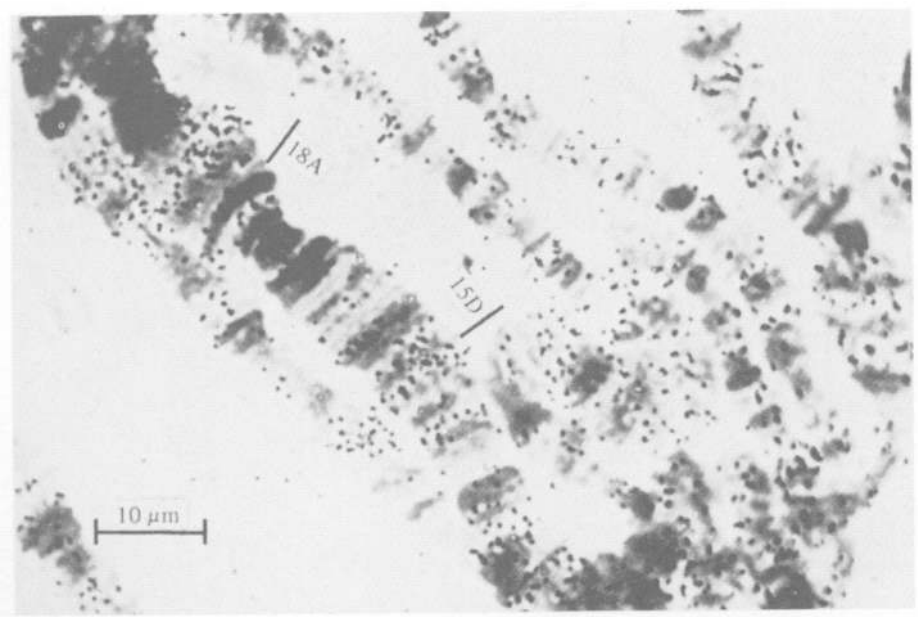
Strains	1–3C	9A–11A	15D–18A
Oregon R^+ ♂	0·42±0·017 (41)	0·44±0·02 (35)	0·36±0·03 (15)
Oregon R^+ ♀	0·52±0·020 (31)**	0·43±0·02 (14)	0·38±0·02 (20)
y w f: = ♀	0·61±0·020 (19)	—	0·41±0·02 (19)
Dp (9A–11A) ♂	0·46±0·010 (55)	0·89±0·04 (37)**	0·26±0·01 (25)
Dp (9C–20F) ♀	0·60±0·020 (28)	—	0·79±0·02 (28)**, (*)

Numbers in parentheses indicate the numbers of respective segments examined.
**$P < 0·01$, (*) $P < 0·05$ (*y w f*: = vs. aneuploid ♀).

The configuration and [^{3}H]-uridine labelling of the partially aneuploid X chromosomes are shown in Plate 1. In the Dp (9A–11A) aneuploid male, the segment 9A–11A of the X chromosome is duplicated and is therefore represented twice in the genome (Plate 1*a*). It may be noted that the total width of the segment is almost twice as great as in the rest of the X chromosome and also considerably greater than in the diploid autosome. In Dp (9C–20F) aneuploid females, the segment 9C–20F of the X chromosome is present in three doses (Plate 1*b*). The width of the segment is, as expected, 1·5× as much as in the rest of the X chromosome. This was also true for the hyperploid 1–9C region (data not presented). This difference in the configuration between the two types of aneuploid by itself indicates some sort of regulation in the activity of the segment of the X chromosome. Data on the X/A ratios of grain numbers presented in Table 2 reveal that the ratio for the segment 9A–11A is twice as great in $1X2A$ ($+X$ fr, 9A–11A) males (0·89) as that either on the diplo-X segment of $2X2A$ females (0·43) or the haplo-X segment of $1X2A$ males (0·44). The differences are statistically significant at the 1 % level. This finding is also in agreement with the data reported in earlier literature (Korge, 1970*a*, *b*; See also Stewart & Merriam, 1980). This finding implies that addition of a small piece of the X to an otherwise $1X2A$ does not interfere with the hyperactivity of the individual segments of the X in the male, in spite of the presence of two sets of autosomes. On the other hand, the ratio for the aneuploid



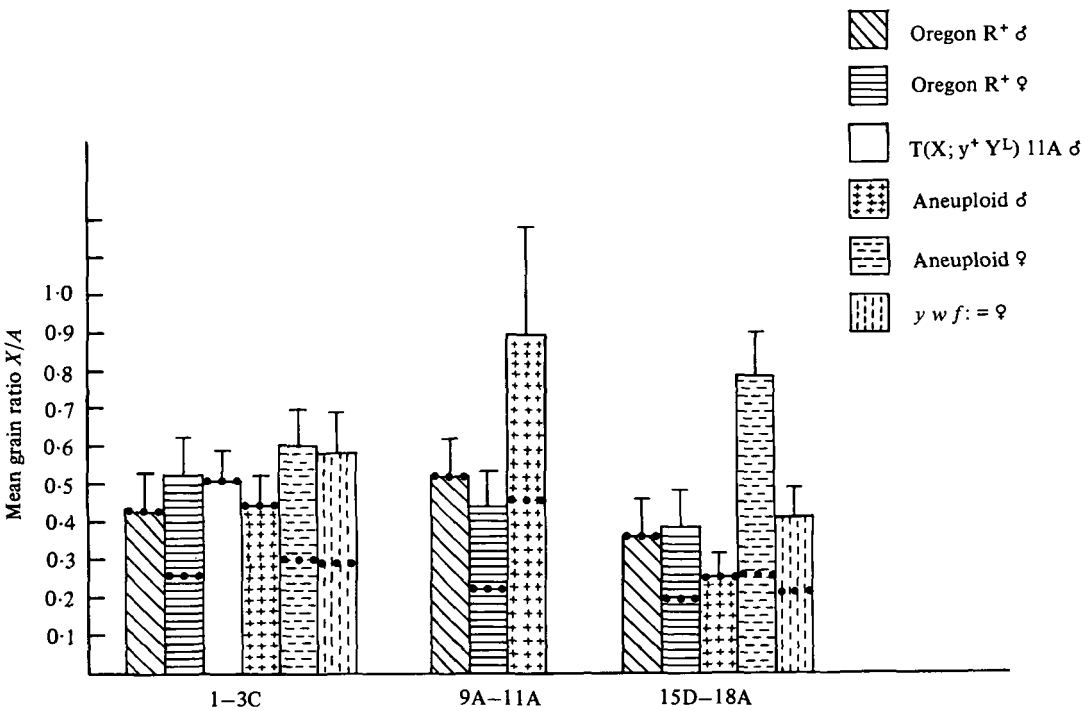
(*a*)



(*b*)

(*a*) Photomicrograph of segmental aneuploid *X* chromosome in male, duplicated for the segments 9A–11A, showing [^{3}H]uridine labelling. Note the increase in width of the aneuploid segment (nearly double that of haplo *X*).
(*b*) Photomicrograph of segmental aneuploid *X* chromosome in female duplicated for the region 9C–20F, showing [^{3}H]uridine labelling. Note the increase in width of the aneuploid segment (nearly 1·5 × the width of diplo *X*).

segment 15D–18A (that is the part of 9C–20F used for grain count) in otherwise $2X2A$ females shows almost twice the diplo-X value (0·38 and 0·41 vs. 0·79), while the expected value, on the basis of dosage effect, considering 0·19 and 0·20 as the basic value per segment, should have been $3 \times 0{\cdot}19 = 0{\cdot}57$ or $3 \times 0{\cdot}20 = 0{\cdot}60$. The differences between 0·57 and 0·79 and between 0·60 and 0·79 (calculated from the individual ratios) are statistically significant. This finding suggests an autonomous nature of hyperactivity rather than a dosage effect.



Text-fig. 3: Histograms of mean X/A grain ratios of different X chromosomal sites. Vertical line above each histogram indicates S.E. Black dots running over or across the histograms represent transcription activity per gene dose. The different genotypes (strains) are indicated in the key to the figure.

In Text-fig. 3 the X/A grain ratios and the relative level of transcription per gene dose have been presented histographically. It is evident that for the segment 1A–3C there is no excess dose in any of the strains, and hence the levels are in agreement with that corresponding to the normal $1X2A$ and $2X2A$ levels, respectively. The level of transcription per gene dosage in $1X2A$ males and the aneuploid males, for the segment 1A–3C, is nearly 1·5 to 2 times that in $2X2A$. In contrast, while the level per gene dose for the 9A–11A segment is over twice as high in the normal male as in the normal female, as expected, it is nearly twice as high in the $1X2A$ ($+X$ fr) aneuploid as in the normal $2X2A$ female, and almost the same as in the normal male. Thus, in spite of the same gene dose for the segment

9A–11A in the aneuploid male and normal female, the segment 9A–11A in the aneuploid male has a higher than diplo-*X* level of transcription activity. On the other hand, the level of transcription per gene for the segment 15D–18A in the duplication (aneuploid) female is nearly 50 % higher than that in the 2*X*2*A* female. These findings strongly suggest that the hyperactivity in the partial aneuploids is expressed autonomously, regardless of the difference in the ratio of the *X* to Autosome dosage (for that segment). This situation is not realized in 3*X*3*A* triploids (data not shown) or in 3*X*2*A* superfemales reported by others.

In order to check whether the differences observed among the 2*X*2*A* normal female, 2*X*2*A* (aneuploid 9A–11A) male and 3*X*2*A* (aneuploid 15D–18A) female is due to random variation in both *X* and Autosomes, the kinetics of the intensities of grains on the *X* and the Autosomes in every nucleus were examined to ascertain the intranuclear relationship between the *X* and Autosomes. The regression slopes (*X* vs. Autosomes) show that in all cases the kinetics of grain intensity follow a linear pattern and yet the corresponding slopes for the aneuploid segments in the aneuploid male and female and those for the same segments in normal male or female are distinctly different. This suggests that the autonomous hyperactivity in the segments 9A–11A and 15D–18A in the partially aneuploid male and female, respectively cannot be attributed to chance variation. However, in the case of the 15D–18A segment in the aneuploid female, due to lack of asynapsed chromosomes, the problem whether the excess activity was due to an excess labelling over only one of the three segments or to an increase of activity in all the three homologous segments remains open.

4. DISCUSSION

The results presented here reveal that the addition of a small piece of the *X* chromosome to an 1*X*2*A* genome fails to alter the hyperactivity either of the entire *X* or of the segment of the *X* chromosome (9A–11A) involved in the aneuploidy. Analysis of the results in terms of activity per gene dose strongly corroborates this finding. Since the levels of transcription in the normal and various rearranged *X* chromosomes of 2*X*2*A* and 1*X*2*A* used do not differ from each other (for either half (1–11A) or entire *X* chromosomes), the requirement of a physical continuity of the chromosome as a possible factor in the maintenance of hyperactivity can be easily ruled out (see also Stewart & Merriam, 1980). The slight but significant difference in the *X*/*A* ratios of grains for the two segments (1–3C and 11B–20F) between the normal and translocation male may be attributed to a localized genetic difference in the normal and rearranged forms. At any rate, the ratios in the male are certainly far greater than expected with a simple dosage effect. The findings obtained from the analysis of transcriptive activity in segmental aneuploids are also in agreement with those of Stewart & Merriam (1975), who assayed sex-linked enzyme activity in segmental aneuploids, although the segments assayed by them and by us were not identical. This implies that an autonomous regulation of dosage compensation exists at the level of transcription, as suggested earlier by Mukherjee & Beermann (1965) and by Lucchesi *et al.* (1977). Stewart

& Merriam (1980) however, have pointed out that published data do not always support the idea that each copy of an *X*-linked gene is twice as active in males as in females.

Our data concerning transcription of the two aneuploid segments in otherwise $1X2A$ or $2X2A$ genomes together with those of Stewart & Merriam (1975) argue against exclusively autosomal regulation of dosage compensation. They rather support the interpretation that 'dosage compensation is regulated more finely than just a male-level or a female-level' (Stewart & Merriam, 1980). Lucchesi (1977) attempted to explain all earlier data on transcription and translation products of *X*-coded genes by a simple model based on the ratio of *X* to Autosomes and predicted the expected levels of gene products in $1X2A$ and $2X2A$ segmental aneuploids (Fig. 1 of Lucchesi *et al.* 1973).

Maroni & Lucchesi (1980) have recently shown that the presence of a duplication fragment 'does not lead to proportionally elevated levels of total *X* chromosome transcription'. They interpreted their results to be due to uniform distribution of the activity over the genes on the entire *X* chromosome. In contrast to their approach, we measured the transcription on the duplicated segments. We believe that the elevated levels of transcription on the duplicated segments obtained by us, in otherwise $1X2A$ and $2X2A$ genomes, is due to a genuine autonomous expression of the genes involved.

These data and the findings of Maroni & Lucchesi (1980), lead us to propose that the hyperactivity of the *X* in male *Drosophila* is a consequence of an interaction between a signal(s) coming from an Autosome(s) and regulatory receptor sequences present on the *X* chromosome. This is also supported by increased binding of non-histone proteins to *X* chromosome DNA (Chatterjee *et al.* 1980) and hyperactivity of the *X* chromosome under *in situ* transcription conditions with exogenous RNA polymerase (Chatterjee & Mukherjee, 1980). It is also consistent with the data presented here and with those of Stewart & Merriam (1980). The presumed *X* chromosomal regulatory sequences may be located throughout the entire *X* chromosome as proposed by Stewart & Merriam (1975, 1980). The autosomal signals might only be required to switch on *X* chromosomal compensators or receptors the net activity per gene being determined by the genomic condition of the entire *X* chromosome.

We are grateful to Professor J. R. Merriam for providing us with some of the *X*; *Y* translocation stocks used in the experiments. We also thank the Bowling Green Stock Center, U.S.A., for supplying us with $T(X; y^{+}Y^{L})11A/yf$: = stock. We are specially thankful to Professor J. C. Lucchesi for the stimulating discussion on the paper during his visit to this laboratory. This work was supported by University Grants Commission Special Assistance to Zoology. One of us (J. P.) was a Junior Research Fellow in a pre-doctroal fellowship sponsored by University Grants Commission which we acknowledge.

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