LACK OF DOSAGE COMPENSATION
FOR AN AUTOSOMAL GENE
RELOCATED TO THE X CHROMOSOME
IN DROSOPHILA MELANOGASTER

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ABSTRACT

Aldehyde oxidase activity has been measured in flies with the structural
gene for this enzyme translocated to the X chromosome. These measurements
are presented as experimental evidence that, in Drosophila melanogaster, an
autosomal gene relocated to the X chromosome is not dosage compensated.

The expression of genes on the X chromosome is found to be equal in males
and females of many animals having heterogametic sexes, a phenomenon
which has been called dosage compensation. The need to equalize the expression
of X-linked genes in sexes having one or two X chromosomes is a general regulatory problem which has different solutions in different organisms. In mammals,
such equalization is achieved by the inactivation of one of the two X chromo-
somes in female somatic cells (Lyon 1961). Relatively early during develop-
ment, an X chromosome becomes condensed, heterochromatic and genetically
silent. A potential consequence of this occurrence is the inactivation of autosomal
segments translocated to the X chromosome. The study of several such trans-
locations in the mouse has led to the following general conclusions: (1) there
may exist an inactivation center(s) present on the X chromosome which is
called into play in one of the two X chromosomes of female somatic cells; (2)
when an autosomal segment is inserted into (e.g., fd transposition) or trans-
located (e.g., translocations R3, R4, R5, R6) to the X chromosome, it may come
under the influence of the inactivation center, with the loci closer to the break-
point in the inactivating chromosome affected more strongly; (3) the comple-
mentary X fragment appears, in some instances (e.g., translocation R2), to lack
the power of inactivation (Russell and Montgomery 1970; see Eicher 1970,
and Cattanach 1975 for additional references and a discussion of these
manifestations).

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In Drosophila, both X chromosomes seem to remain active in the female soma (KAZAZIAN, YOUNG and CHILDS 1965). Dosage compensation is thought to be achieved by equalizing the transcriptional level of the two X chromosomes in females to that of the single X chromosome in males (for reviews of the evidence leading to this conclusion, see LUCCHESI 1973, 1976). The activity of X-chromosome fragments translocated to the autosomes is unaltered and retains dosage compensation. This was most clearly substantiated by TOBLER, BOWMAN and SIMMONS (1971), who reported that males carrying one dose of the X-chromosome structural gene for tryptophan oxygenase translocated to the third chromosome exhibit the same enzyme activity as females homozygous for the translocation, i.e., with two doses of the relocated gene.

The following question remained unanswered in Drosophila: would an autosomal segment transposed to the X chromosome come under the regulatory influence of this chromosome? The present paper offers evidence that this is not the case; the genetic activity of an autosomal segment is unaltered by its translocation to the X chromosome and is not modulated by the regulatory mechanism responsible for dosage compensation.

MATERIALS AND METHODS

Drosophila stocks and genetic procedures.

The translocation we used was T(1;3)ry85, cu ry85 kar, which has a third chromosome segment 87C-E to 91B-C on the salivary gland chromosome map) translocated to the X chromosome. Since this translocation is mutant for rosy (52.0 on the genetic map), the left breakpoint is in or very near the ry locus. Furthermore, the translocated segment contains the locus for Ubx (58.8 on the genetic map). Therefore, this segment should contain Aldox+, the structural gene for the enzyme aldehyde oxidase, which is located at 56.7 on the genetic map of chromosome 3 (DICKINSON 1970). The recessive mutants cu (curled wings), ry85 (rosy eye color) and kar (karmoisin eye color) are associated with the translocation. In the stock, the translocated chromosome is maintained in heterozygous condition with T(2;3)apX*, which is marked with the dominant allele apterus-Xasta and serves as a balancer. The translocated autosomal segment (87C-E to 91B-C) is viable as a duplication while heterozygous deficiency for this segment is inviable.

In experiment I, translocation-bearing males were crossed to females from the stock C(I)RM, Y U bbb+/Y; Aldox+/Aldox+. These females are homozygous for an aldehyde oxidase negative allele which does not allow synthesis of cross-reacting material (DICKINSON 1970) and exhibits less than 2% the activity of wild type in our assay conditions; the stock was kindly supplied to us by Dr. JOHN WILLIAMSON. The cross yielded males with one (translocated) or two (translocated and autosomal) doses of active Aldox+, and females with one dose (autosomal) of this gene. The two-dose males and the females are phenotypically apterus-Xasta. The reciprocal cross yielded males and females with a single dose (either translocated or autosomal) of Aldox+. The one-dose (translocated) males and one-dose (translocated) females have normal wings while all other classes are apterus-Xasta. These crosses and the specific progeny classes used in this study are diagrammed in Figure 1.

In experiment II, translocation males were crossed to females from the stock C(I)RM, Y f+/Y. This cross yielded males with two or three doses and females with two doses of Aldox+. The three-dose males and the females are phenotypically apterus-Xasta. The reciprocal cross yielded females with two doses of Aldox+, differing from previous two-dose females in that the autosomal Aldox+ was on a normal third chromosome rather than on the apXa balancer. These crosses and the specific progeny classes used are diagrammed in Figure 2. The fundamental
Figure 1.—(a): C(1)RM, y v bb/Y; Aldox\textsuperscript{n1}/Aldox\textsuperscript{n1} attached-X females are crossed to T(1;3)ry\textsuperscript{a8}, cu ry\textsuperscript{a8} kar/Y; T(2;3)ap\textsuperscript{xa} males. Thick solid lines represent the X chromosome, cross-hatched symbols and open symbols are chromosomes 2 and 3, respectively; thin I-shaped symbols are Y chromosomes; + = Aldox\textsuperscript{+}, = Aldox\textsuperscript{n1}, brackets indicate the presence of inversions in T(2;3)ap\textsuperscript{xa}. F\textsubscript{1}'s include one-dose (translocated) males and two-dose (translocated and autosomal) males. The former have normal wings, the latter exhibit the abnormal wing phenotype associated with ap\textsuperscript{xa}.

(b): Reciprocal cross. F\textsubscript{1}'s include one-dose (translocated) females and males with normal wings.
Figure 2.—(a): C(1)RM, γ v f/Y attached-X females are crossed to T(1;3)ry²⁸, cu ry²⁸ kar/Y; T(2;3)ap²⁸ males. All symbols are as in Figure 1. F₁'s include two- and three-dose males with normal wings and aperous-Xasta phenotype, respectively.

(b): Reciprocal cross. F₁'s include two-dose females.
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difference between this experiment and the previous one is the presence of Aldox+ instead of Aldoxn1 on the normal third chromosomes.

A more detailed description of the chromosomes described above and of the mutants they bear is provided in LINDSLEY and GRELL (1968). Flies were raised at 25 ± 1° on standard cornmeal-molasses-agar medium containing propionic acid and tegosept-M as mold inhibitors. The food was seeded with dry yeast.

Preparation of extracts.

In experiment I flies were collected every six hours (or occasionally every 12 hours) and aged for 72 hours; heads and thoraces with attached legs and wings were removed and frozen in liquid nitrogen. After thawing, the thoraces were homogenized at a concentration of 8 thoraces/0.1 ml distilled water in 1.5 ml Brinkman-Eppendorf polypropylene microtest tubes using a Teflon homogenizer specially designed to fit the tubes. The extracts were allowed to stand on ice for 20 minutes and were centrifuged for 40 minutes at 12,000 × g in a Sorvall RC2-B refrigerated centrifuge. The supernatant was used as the extract in the enzyme assays.

In experiment II, flies ranging in age from one to seven days were pooled. Extracts were prepared by homogenizing thoraces at a concentration of 75 thoraces/ml of distilled water in a ground glass conical homogenizer and treated as described above.

Enzyme assays.

Substrate solutions for aldehyde oxidase (AO) have been described by DICKINSON (1970). Activity was monitored as a decrease in absorbance at 600 nm in a Gilford 250 spectrophotometer. In addition, glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), α-glycerophosphate dehydrogenase (α-GPDH), and NADP-dependent isocitrate dehydrogenase (IDH) were measured. Substrate solutions and assay conditions for the dehydrogenases are those described by LUCCHESI and RAWLS (1973). In experiment I, extract was added to 0.2 ml of substrate solution (20λ for G6PD and 6PGD, 10λ for IDH, 4λ for AO and α-GPDH). Reactions were monitored in a microcuvette maintained at 29°. In experiment II, 0.2 ml of extract and 2.0 ml of substrate solution were used for G6PD and 6PGD, 20λ of extract and 2.18 ml of substrate solution for AO, and 0.1 ml of extract and 2.0 ml of substrate solution for IDH. Assays were carried out in a standard cuvette maintained at 29°. Extract protein concentration was determined according to the method of LOWRY et al. (1951). Enzyme activities are expressed as ΔO.D./min/mg protein.

RESULTS

Using the Aldox+ mutant, we were able to generate both males and females with only one copy of the Aldox+ gene capable of producing active enzyme and relocated to the X chromosome. Enzyme activity measurements are presented in Table 1 and include both X-linked (G6PD, 6PGD) and autosomal (IDH, α-GPDH) enzyme measurements for control purposes. For a particular enzyme, no significant differences exist between males and females for any of the activities reported. Note that the single dose males were produced by both crosses of experiment I; since there was no difference in the enzyme activities of these two sets of males, their data was pooled for inclusion into the table.

To establish that we would be able to detect any significant difference in activity between males and females that might occur, we compared enzyme activity in males with one and two doses of Aldox+. The latter exhibit a 1.8-fold increase in AO activity over single dose males and no significant difference in the level of control enzymes (Table 1).
### TABLE 1

*Enzyme activities* of progeny of C(1)RM, y v bb/+/Y;Aldox\(^n1\)/Aldox\(^n1\) ×
T(1;3)ry\(^{ss}\), cu ry\(^{ss}\) kar/Y;T(2;3)ap\(^{xa}\)

<table>
<thead>
<tr>
<th>Genetic constitution†</th>
<th>Aldox</th>
<th>G6PD</th>
<th>GPD</th>
<th>IDH</th>
<th>α-GPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(1;3)ry(^{ss})/Aldox(^n1) ♀ ♀ (one dose Aldox(^{+}))</td>
<td>2.7 ± .12 (19)</td>
<td>3.1 ± .16 (19)</td>
<td>2.2 ± .2 (15)</td>
<td>4.9 ± .3 (19)</td>
<td>15.2 ± 1.5 (5)</td>
</tr>
<tr>
<td>T(1;3)ry(^{ss})/Aldox(^n1) ♂ ♂ (one dose Aldox(^{+}))</td>
<td>2.8 ± 1 (14)</td>
<td>3.2 ± .2 (14)</td>
<td>2.0 ± .14 (12)</td>
<td>4.3 ± .2 (14)</td>
<td>17.3 ± 1.2 (5)</td>
</tr>
<tr>
<td>Dp(3;1)ry(^{ss})/ap(^{xa})/Aldox(^n1) ♂ ♂ (2 doses Aldox(^{+}))</td>
<td>4.9 ± .24 (13)</td>
<td>3.7 ± .19 (13)</td>
<td>2.3 ± .15 (11)</td>
<td>5.1 ± .27 (13)</td>
<td>16.4 ± 1.5 (6)</td>
</tr>
</tbody>
</table>

* Enzyme activities are expressed as ΔO.D./min/mg protein × 10\(^2\) ± S.E. Extracts were from adult flies minus abdomens. The number of determinations are indicated in parentheses.
† These genotypes are diagrammed in Figure 1.
TABLE 2

<table>
<thead>
<tr>
<th>Genetic constitution+</th>
<th>Aldox</th>
<th>G6PD</th>
<th>6PGD</th>
<th>IDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(1;3)ry35/Aldox+ ♂♂</td>
<td>1.5 ± .08 (6)</td>
<td>3.0 ± .12 (6)</td>
<td>2.5 ± .12 (6)</td>
<td>4.1 ± .15 (6)</td>
</tr>
<tr>
<td>(2 doses Aldox+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T(1;3)ry35/Aldox+ ♀♀</td>
<td>1.6 ± .08 (6)</td>
<td>2.7 ± .12 (6)</td>
<td>2.4 ± .04 (6)</td>
<td>3.6 ± .16 (6)</td>
</tr>
<tr>
<td>(2 doses Aldox+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dp(3;1)ry35/apXa/Aldox+ ♂♂</td>
<td>2.1 ± .13 (5)</td>
<td>3.6 ± .36 (5)</td>
<td>2.6 ± .09 (5)</td>
<td>4.0 ± .04 (5)</td>
</tr>
<tr>
<td>(3 doses Aldox+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Enzyme activities are expressed as ΔO.D./min/mg protein × 10² ± S.E. Extracts were from adult flies minus abdomens. The number of determinations are indicated in parentheses.
† These genotypes are diagrammed in Figure 2.

The results of experiment II are presented in Table 2. The apparently lower AO activity in experiment II is largely the result of a different dilution factor for this enzyme in the standard assay versus the micro assay (see MATERIALS AND METHODS). Males and females with two copies of the Aldox+ gene, one on the normal third chromosome and one associated with the X chromosome, have comparable enzyme levels. Three-dose males exhibit a 1.4-fold increase in AO activity over males with two doses. In both experiments the activity of AO in males having the extra dose appears to be slightly less than expected on the basis of gene dosage. Whether this is the result of reduced activity of the Aldox+ gene associated with the apXa balancer or a general reduction in activity of all the alleles is not known. In either case it does not affect the critical male-female comparisons.

DISCUSSION

The modulation of X-linked gene activity in Drosophila could occur at the level of the individual gene or at the level of the whole X (or large fragments of the X) chromosome. If the former were true, autosomal genes translocated to the X chromosome should be unaffected by the mechanism of dosage compensation. However, if one or a few sites on the X chromosome were responsible for the dosage compensation of the entire chromosome, autosomal genes inserted in the X chromosome might become dosage compensated. This premise would lead us to expect a two-fold increase in activity in single dose males compared to single dose females (experiment I) and a 50% increase in activity in two-dose males compared to two-dose females (experiment II). Clearly, no such difference is evident in either case (Tables 1 and 2), leading us to conclude that autosomal segments, translocated to the X chromosome, exhibit autonomous genetic activity and do not become dosage compensated. This is consistent with the morphological autonomy of autosome-X translocations observed by Lakhotia (1970) in salivary gland nuclei of male larvae. He reported that a segment of chromosome 3 inserted into the X chromosome does not acquire the enlarged and pale appearance characteristic of the X chromosome that is indicative of
greater transcriptional activity. Rather, the translocated segment retains the morphology characteristic of its normal homolog.

The autonomous behavior of fragments in X-autosome translocations suggests that X-linked structural genes possess individual means of responding to the compensatory mechanism, whereas autosomal genes lack these means. A logical extension of this interpretation would be that control of transcription is mediated by special regulatory sequences associated with each gene, leading to equalization of X-linked gene products in males and females. The currently available evidence does not rule out some involvement of post-transcriptional controls in dosage compensation. In addition, the size of the translocated fragments in this and other studies does not allow us to distinguish between regulation of individual genes versus small clusters of genes. It remains for fine structure genetic analysis and nucleotide sequencing of X-linked genes to determine the nature of these putative adjacent controlling elements.

LITERATURE CITED


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