DOSAGE COMPENSATION OF SERINE-4 TRANSFER RNA IN *DROSOPHILA MELANOGASTER*

JAMES A. BIRCHLER,[1] R. KEITH OWENBY AND K. BRUCE JACOBSON

The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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ABSTRACT

A dosage series of the X chromosome site for serine-4 transfer RNA consisting of one of three copies in females and one to two in males was constructed to test whether transfer RNA expression is governed by dosage compensation. A dosage effect on the level of the serine-4 isoacceptor was observed in both females and males when the structural locus was varied. However, in males, each dose had a relatively greater expression so the normal one dose was slightly greater than the total female value and the duplicated male had the highest relative expression of all the types examined. Serine-4 levels in males and females from an isogenic Oregon-R stock were similar. Thus the transfer RNA levels conform to the expectations of dosage compensation.

DESPITE the dosage differential of the X chromosome in the two sexes of Drosophila, most of the sex-linked genes exhibit a more nearly equal expression than expected from the number of copies of the gene present. This phenomenon of dosage compensation was first recognized by MULLER (1932) and it is characterized by the following rules: A gene on the X chromosome exhibits a dosage effect in a short segmental aneuploid series that gives one, two or three doses in females and correspondingly one or two doses in males. The total activity of two doses of an X-linked gene in a female, however, is roughly equivalent to the activity of a single dose in a male, and the total activity of two gene copies in duplicated males is twice as great as that in either the one-dose male or the two-dose female.

Dosage compensation has been demonstrated to apply to numerous phenotypic mutants (MULLER 1950), enzyme activity (SEECOF, KAPLAN and FUTCH 1969; LUCCHESI and RAWLS 1973; MARONI and PLAUT 1973; LUCCHESI, RAWLS and MARONI 1974, 1977), protein level (KORGE 1970), and rates of synthesis of nascent chromosomal RNA along salivary gland polytene chromosomes (MUKHERJEE and BEERMAN 1965; MARONI and PLAUT 1973; LUCCHESI, RAWLS and MARONI 1974; LUCCHESI, BELOTE and MARONI 1977). An exception is the 18 and 28S ribosomal RNA which is encoded in the proximal heterochromatin of the X chromosome and on the Y and thus does not achieve equal expression in the same manner (RITOSSA and SPIEGELMAN 1965; SPEAR and GALL 1973). The rDNA

[1] Present address: Department of Genetics, University of California, Berkeley, California 94720.

is transcribed in eukaryotes by RNA polymerase I, whereas messenger RNA is formed by polymerase II (see ROEDER 1976). The present study involves a test of whether X-linked transfer RNA loci, transcribed by polymerase III, follow the same principles of compensation as messenger RNA or whether, owing to considerable functional differences, a unique mechanism governs their expression.

Studies on the genetic localization of transfer RNA species in Drosophila have indicated the presence of a major site for serine isoacceptors 4 and/or 7 at 12DE in the X chromosome (DUNN *et al.* 1979a; HAYASHI *et al.* 1980). Individually purified 4 and 7 hybridize to common sites on the polytene chromosomes, the major regions being 12DE and 23E on chromosome 2. These two isoacceptors are distinct transfer RNAs possessing different anticodons but with quite similar nucleotide contents (WHITE *et al.* 1975) and sequences (see HAYASHI *et al.* 1980). For this reason, the two RNA species will cross-hybridize to the structural genes in the chromosomes and a distinction of whether one or both types might be encoded at any one locus is not possible (DUNN *et al.* 1979a). However, since the 12DE region is the major site of hybridization, it probably contains multiple gene copies in random array as to other tRNA gene clusters in Drosophila (e.g., HOVEMANN *et al.* 1980). In this regard, it was reasoned that if the locus exhibits a gene dosage effect, it should be demonstrable, as is the case with the autosomally encoded valine tRNAs (DUNN *et al.* 1979b). The dosage study would determine whether the cluster contains functional copies of 4 or 7 or both.

To test whether the serine transfer RNA genes follow the rules of dosage compensation, genetic crosses were made using X;Y translocations that result in progeny with one, two or three doses of this region in females and one or two doses in males. If the tRNA loci are regulated by the same dosage compensation mechanism that controls most other X-linked genes, then the level of gene product would be expected to be directly proportional to the dosage of the short chromosomal segment in each sex, but the expression in males would be approximately twice as great per copy. If the transfer RNA loci are not affected by this mechanism, then a dosage effect would be expected in direct relation to the gene dosage regardless of sex unless a feedback of some other control is operative.

Considerable advances in elucidating the processes involved with transcription by RNA polymerase III have recently been made (e.g., TELFORD *et al.* 1979; DEFRANCO, SCHMIDT and SOLL 1980; SAKONJU, BOGENHAGEN and BROWN 1980). It would add to an understanding of transfer RNA expression to determine whether these genes are governed by dosage compensation. In addition, a clearer understanding of the compensating mechanism would come from a test of whether genes transcribed by both polymerase II and III are regulated by this system.

MATERIALS AND METHODS

Genetic crosses: A dosage series of the 12DE region of the X chromosome was constructed using the X;Y translocations induced by STEWART and MERRIAM (1974, 1975). These translocations were formed between a *y* marked X and a Y carrying y^+ on the short arm and B^s on the long. The two

translocations used were B166 and J1. B166 is broken in the X at 12A and in the short arm of the Y. J1 is broken at 13A and in the Y long arm. Thus, the distal portion of the X in B166 is $y\ B^s$ and the proximal segment is y^+. With J1, the proximal and distal markers are reversed. The translocations were obtained from the Mid-America Drosophila Stock Center, Bowling Green, Ohio; the breakpoint determinations were made by B. R. STEWART and J. MERRIAM.

The dosage series of 12A-13A was constructed as follows. Males of *T(1;Y)B166* were mated with females homozygous for the X-chromosome balancer *FM7*, $y^2\ v\ B$. The F_1 females were crossed to the *T(1;Y)J1* males. Females inheriting *FM7*, the distal element of *B166*, and the proximal portion of *J1* have a deficiency for the segment of the X between the breakpoints and are phenotypically yBB^sB^s. Females that are *FM7/J1* or *B166/J1* are y^+BB^s or $y^+B^sB^s$ and have two doses of all regions. Those progeny receiving the distal element of *J1* and the proximal from *B166* as well as *FM7* are $y^+y^+B/+$ females that contain three doses of the tRNA locus.

Among the male progeny, the *FM7*, $y^2\ v\ B$, and *B166* (y^+B^s) flies are readily distinguished from the duplication males of $y^+y^+B^+$ phenotype. Thus, six distinct classes are produced and, with the exception of the two types of euploid males, have different doses of the 12A-13A region of the X chromosome. Zero to 2-day-old flies were collected, sorted into the respective six classes, and frozen at −80° until extraction of tRNA.

To compare profiles of wild-type males and females, an isogenic Oregon-R stock was grown, collected, sorted according to sex and frozen at −80° until time of extraction.

Isolation of transfer RNA: Flies of a particular genotype were homogenized in 88% redistilled phenol and buffer [10 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate and 100 mM NaCl] at a ratio of 5 ml phenol:5 ml buffer per gram of flies. After centrifugation at 10,000 rpm for 10 min in a Sorvall SS-34 rotor, the aqueous layer was removed, brought to 1 M NaCl, and the nucleic acids were precipitated by the addition of 2.5 volumes of 95% ethanol (−20°) and stored in the freezer for at least 2 hr. The precipitate was collected by centrifugation at 10,000 rpm and dissolved in the extraction buffer. The redissolved nucleic acids were applied to a DEAE-23 column equilibrated with 10 volumes of the same buffer. The column volume was 2–3 ml/g of flies initially extracted. The column was then washed with buffer [10 mM Tris HCl (pH 7.5), and 10 mM magnesium acetate] that was 250 mM NaCl. Subsequently, the transfer RNA was eluted with 1.0 M NaCl buffer. Fractions absorbing at 260 nm were pooled and the RNA was precipitated by the addition of 2.5 volumes of 95% ethanol and stored at −20° overnight. The precipitate was collected by centrifugation and the pellet dissolved in 10 mM Tris (pH 7.5), and 10 mM Mg acetate. RNA was diluted 1/50 to 1/200 to determine the A_{260}:A_{280} ratio.

Preparation of aminoacyl-tRNA synthetases: Crude synthetases were prepared by the method of TWARDZIK, GRELL and JACOBSON (1971). Twenty-five grams of frozen Oregon-R flies were homogenized in a Waring Blendor in 100 ml of extraction buffer [10 mM Tris HCl (pH 7.5), 10 mM β-mercaptoethanol, 10 mM magnesium acetate and 10% glycerol] for 2 min. The homogenate was centrifuged at 10,000 rpm in a Sorvall SS34 rotor for 20 min. The supernatant was filtered through Miracloth and centrifuged at 79,000 × g for 90 min in a Spinco ultracentrifuge. The supernatant was applied to a DEAE-23 cellulose column equilibrated with extraction buffer. The column was washed with extraction buffer until the bulk of the nonbinding proteins was eluted ($A_{280} < 0.10$). Step elution of synthetase was achieved with extraction buffer at 0.30 M NaCl. Peak fractions according to A_{280} were assayed for seryl-tRNA synthetase activity. Fractions with greatest activity were pooled and dialyzed at 2° against 20 volumes of buffer containing 50% glycerol. Synthetase preparations were stored at −80°.

Aminoacylation: Aminoacylation of tRNA was performed in a reaction mix of 50 mM Tris-HCl (pH 8.0), 10 mM Mg acetate, 10 mM KCl, 4 mM ATP and 4 mM β-mercaptoethanol at 22° for 30 min. ^{14}C-labeled serine (162 mCi/mmol, New England Nuclear) was at a concentration of 20 nm/ml and ^{3}H-valine (7 Ci/mmol, Schwarz/Mann) at a concentration of 10 nmol/ml. Crude RNA was present at either 5 or 10 A_{260} units/ml. Sufficient synthetase was present to completely aminoacylate the RNA in 20 min.

The limits of each synthetase preparation were tested by aminoacylation reactions containing 5, 10, 15 and 20 A_{260} units/ml of tRNA. In all cases, the level of aminoacylation was directly proportional to the quantity of RNA present in the reaction.

For determination of total acceptance of amino acid by transfer RNA, the reaction volume was 60 μl. At the end of the 30-min incubation period, 50 μl was withdrawn and applied to a numbered

3 MM filter paper disc (2.3 cm in diameter), allowed to dry 30 sec, and immersed in cold 10% trichloroacetic acid (TCA) for 15 min followed by two rinses (15 min each) in cold 5% TCA. The discs were then rinsed with 95% ethanol for 5 min, followed by 5 min in ether with subsequent drying under a heat lamp. Discs were transferred to scintillation vials and 5 ml of 0.4% 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene (BBOT) in toluene was added for scintillation spectrometry.

RNA destined for RPC-5 separation was aminoacylated by the above procedure with the following modifications to recover the labeled tRNA. The reaction was stopped at 30 min by the addition of one-fourth of the reaction volume of 2.0 M sodium acetate (pH 4.6). To remove the synthetase and other proteins, the reaction mix was applied to a DEAE-23 column after dilution with 10 mM Mg acetate and 10 mM Na acetate (pH 4.6) to lower the ionic strength. The column size was 2–3 ml/g of flies originally extracted and was equilibrated with 10 column volumes of 50 mM NaCl, 10 mM Na acetate, and 10 mM Mg acetate buffer. After addition of the acylation mixture to the column, it was washed with 20 column volumes of 250 mM NaCl, 10 mM Na acetate, and 10 mM Mg acetate buffer to elute proteins. Finally, the aminoacylated RNA was eluted with 1.0 M NaCl, 10 mM Na acetate, and 10 mM Mg acetate (pH 4.6). Elution was monitored by absorbance at 260 nm. Peak fractions were pooled and a 50-μl sample was taken to determine the cpm/ml.

Reversed phase chromatography: Separation of serine isoacceptors by RPC-5 was accomplished as follows. The column dimensions were 0.9 x 55.0 cm. Chromatography was carried out at 45°. Both the column length and temperature were required for complete separation of the five major peaks, in accordance with previous observations (WHITE *et al.* 1975). Aminoacylated tRNA was applied to the column in 0.5 M NaCl. Development of the column was accomplished with a 200-ml linear gradient (0.68–0.78 NaCl) in a buffer of 10 mM Na acetate (pH 4.6), 10 mM Mg acetate, and 1 mM disodium ethylenediamine tetraacetic acid and saturated with Adogen 464 (Ashland Chemical Co.). The initial 12.5 ml, including the column void and a fraction of the gradient, was removed, after which 1-ml fractions were collected at a rate of 30/hr. The elution profile was determined by adding 10 volumes of triton toluene BBOT and counting each vial for 10 min in a Searle scintillation spectometer. Background subtraction, $^{3}H/^{14}C$ discrimination, and the plotting of the chromatographic profile were performed by computer.

Normalization of serine isoacceptors to total valine: As a means of standardization of the individual peaks of serine tRNAs separated on the RPC-5 columns, each value was corrected to the total valine in the same class of flies. To accomplish this, the respective peaks were divided by the total serine recovered from the same column run and this value was divided by the valine/serine ratio for flies of the same genotype determined in total charging experiments (see Table 3). Valine was chosen as a control since all three major isoacceptors have been used in tRNA gene localization studies and no sites of hybridization to the X chromosome are known (HAYASHI *et al.* 1980). The values from this calculation correct for variation in separate column runs and normalizes each class to a standard independent of the total serine or the individual isoacceptors. It is of note that serine-4 (the species that responses to the dosage of 12A-13A) shows the same relative expression in the various genotypes whether the normalization is to total valine, serine isoacceptor 5a, 6 or 7 (ratios not shown for latter three).

RESULTS

The analysis of the chromatographic separation of the serine isoacceptors from the dosage series of the chromosomal region surrounding 12DE is given in Table 1. When the amount of radioactivity in each of the six major peaks is calculated and normalized to the total valine in each class of the dosage series, peak 4 changes in relationship to the dosage of the varied region. This species of RNA corresponds to isoacceptor-4, which was purified and localized to the 12DE region by HAYASHI *et al.* (1980).

It should be noted that in the nomenclature of WHITE *et al.* (1973), the serine isoacceptors were named for their elution order from reversed phase columns developed at 37°. However, when chromatography was conducted at 45°, the order of elution of 4 and 5 was reversed (see WHITE *et al.* 1975). Based on the

relative peak heights of tRNA from Oregon-R mixed sexes, this reversed elution was also observed in our experiments, which were performed at 45°. The Oregon-R stock produced five major peaks that correspond in elution and relative expression to isoacceptors 2, 5, 4, 6 and 7 of WHITE *et al.* (1975). However, in the stocks used to produce the dosage series, an additional peak was resolved between 5 and 4 and the relative quantities of these two were changed. It is possible that these effects are caused by subdivision of the original peak 5, and for this reason the designations 5a and 5b have been used. Otherwise, the nomenclature, as shown in Figure 1 (one-dose females), corresponds to that used by WHITE *et al.* (1975).

As a means of internal standardization, the radioactivity in peak 4 was normalized to the total valine in the respective class of flies as described in MATERIALS AND METHODS. When the means of the ratios from multiple column runs are compared, it is clear that the level of isoacceptor-4 is correlated with the one to three dosage in females. The dosage effect does not follow an integral 1, 2, 3 dosage relationship, nor was it expected, since other sites of serine-4 genes are present in the genome and would also contribute to the total amount of the isoacceptor (DUNN *et al.* 1979a; HAYASHI *et al.* 1980). The relative level of isoacceptor-4 in the one-dose male [*T(1;Y)B166*] was slightly greater than the two-dose female. The two-dose male shows an even higher level and exhibits the greatest relative expression of all the genotypes. In addition to isoacceptor 4, the shoulder on peak 2 (Fig. 1) responds to the dosage of the 12A-13A region in both sexes. Also, this species of tRNA is consistently present at a higher level in males than in females. Examples of the chromatographic profile of each of the six genotypes are shown in Figure 1.

The quantitative comparison of the chromatographic profiles from the isogenic Oregon-R stock is presented in Table 2. There is a similar expression of serine-4 in the male and female. The equal expression observed between the two sexes, when contrasted to the one- *vs.* two-dose female ratio of 0.79, indicates that this isoacceptor exhibits dosage compensation.

The level of total acceptance of serine and valine per A_{260} unit in each class of flies was determined (Table 3). As noted above, valine was chosen as the control since there are no sites of hybridization of valine tRNAs on the X chromosome (HAYASHI *et al.* 1980). In the one- to three-gene dose comparison in females, there is a negative correlation between picomoles accepted per A_{260} unit and the segmental dosage. This effect is observed for both serine and valine and is therefore thought not to be a secondary effect of varying a class of serine genes *per se*. The one- *vs.* two-dose male comparison also shows a slight negative effect on the acceptance of both amino acids. This effect obscures any minor differences that might be expected from the dosage response of serine, when expressed in this way, and might be analogous to a similar phenomenon observed on the level of proteins in segmental dosage series (see BIRCHLER 1979; BIRCHLER and NEWTON 1981).

However, when the total serine acceptance is expressed as a ratio relative to the total valine, this response is cancelled and a dosage effect on serine is observed in the one-, two- three-dosage series in females. The one-dose male

TABLE 1

Analysis of chromatographic separation of serine isoacceptors from the 12DE dosage series

Class	Chromatography column no.	Isoacceptor 2	5a	5b	4	6	7	Total	Mean (serine isoacceptor/total valine) ± S.E. 2	5a	5b	4	6	7
One-dose females	1	7104	5637	2806	7884	5263	11338	40032	244 ± 12	198 ± 5	82 ± 8	274 ± 8	181 ± 3	388 ± 2
		(243)	(193)	(96)	(269)	(180)	(387)		(1.08)	(0.89)	(0.92)	(0.79)	(0.96)	(0.94)
	2	7152	6659	2155	9299	5978	12567	43810						
		(223)	(208)	(67)	(290)	(187)	(392)							
	3	8040	5894	2559	8015	5379	11692	41579						
		(265)	(194)	(84)	(264)	(177)	(385)							
Two-dose females	1	6071	5816	2857	8984	5288	11486	40502	226 ± 4	223 ± 9	89 ± 6	345 ± 8	189 ± 2	412 ± 4
		(222)	(213)	(105)	(329)	(194)	(421)		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
	2	6156	5854	2387	9708	5006	11290	40401						
		(226)	(215)	(88)	(357)	(184)	(415)							
	3	6384	5774	2079	9714	5058	11095	40104						
		(236)	(214)	(77)	(359)	(187)	(410)							
	4	4570	5169	1806	6901	3968	8344	30758						
		(220)	(249)	(87)	(333)	(191)	(402)							
Three-dose females	1	6755	5941	2067	12181	5708	13514	46166	222 ± 7	211 ± 7	67 ± 3	399 ± 5	198 ± 3	437 ± 8
		(224)	(197)	(69)	(405)	(190)	(449)		(0.98)	(0.95)	(0.75)	(1.16)	(1.05)	(1.06)
	2	5587	5323	1896	10274	5323	11358	39761						
		(216)	(205)	(73)	(396)	(205)	(438)							
	3	7010	6737	1863	11226	5832	12034	44702						
		(241)	(231)	(64)	(385)	(200)	(413)							
	4	3637	3701	1075	7138	3419	7843	26813						
		(208)	(212)	(61)	(408)	(196)	(449)							
FM7 males	1	7831	4959	2305	7996	2933	4886	30910	406 ± 4	267 ± 3	133 ± 6	413 ± 5	156 ± 2	258 ± 1
		(414)	(262)	(122)	(423)	(155)	(258)		(1.80)	(1.20)	(1.49)	(1.20)	(0.82)	(0.63)
	2	7355	4930	2514	7439	2804	4677	29719						
		(404)	(271)	(138)	(409)	(154)	(251)							
	3	4350	2918	1503	4403	1723	2812	17709						
		(401)	(269)	(139)	(406)	(159)	(259)							

TABLE 1—*Continued*

Class	Chromatography column no.	Isoacceptor							Mean (serine isoacceptor/total valine) ± S.E.					
		2	5a	5b	4	6	7	Total	2	5a	5b	4	6	7
One-dose males	1	7004	4382	2336	6943	3802	7670	32137	311 ± 14	203 ± 4	112 ± 4	368 ± 19	186 ± 3	355 ± 8
		(335)	(209)	(112)	(332)	(182)	(367)		(1.38)	(0.91)	(1.26)	(1.07)	(0.98)	(0.86)
	2	6117	4200	2553	8450	3982	7694	32996						
		(285)	(196)	(119)	(393)	(185)	(358)							
	3	4581	2995	1542	5560	2791	4992	22461						
		(313)	(205)	(105)	(380)	(191)	(341)							
Two-dose males	1	6925	4800	2103	9777	3048	5887	32540	393 ± 17	233 ± 8	117 ± 6	495 ± 12	170 ± 6	302 ± 4
		(364)	(252)	(110)	(514)	(160)	(309)		(1.74)	(1.04)	(1.31)	(1.43)	(0.90)	(0.73)
	2	6814	4004	2234	8743	2811	5329	29935						
		(389)	(229)	(128)	(499)	(161)	(304)							
	3	8823	4760	2081	9200	3628	5802	34294						
		(440)	(237)	(104)	(459)	(181)	(289)							
	4	3962	2256	1321	5298	1862	3203	17901						
		(378)	(215)	(126)	(506)	(178)	(306)							

All isoacceptor values represent the summation of the cpm minus background from each fraction within the respective peaks. Although peak 2 is clearly divisible in some genotypes (see Figure 1), the limits of the two portions cannot be discerned; therefore it has been treated as a single peak. Isoacceptor values in parentheses represent normalization to total valine as described in MATERIALS AND METHODS. Values in parentheses below the means indicate the expression of each isoacceptor relative to the respective female value.

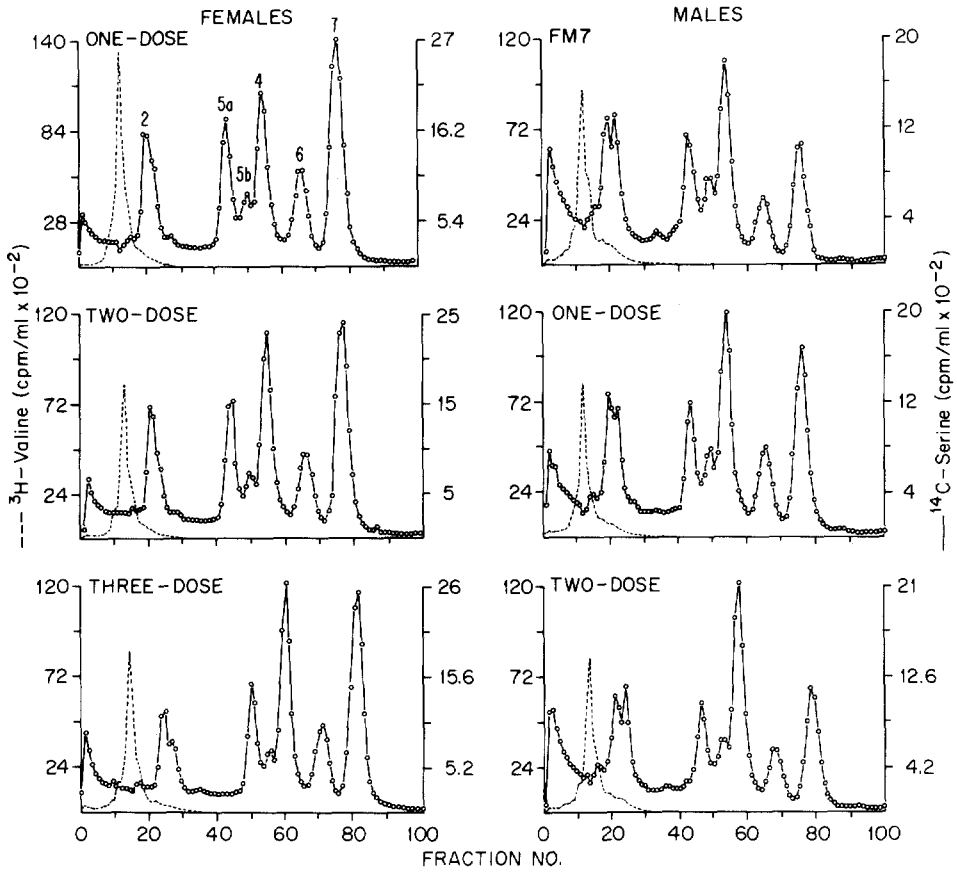

FIGURE 1.—Chromatographic profiles of the 12DE dosage series. Aminoacylated (^{14}C-serine and ^{3}H-valine) transfer RNA from each class of flies was chromatographed on RPC-5. Each point on the serine curve represents the cpm per fraction minus background. The peaks are numbered from left to right and correspond to those shown in Table 1.

(B166) has a slightly higher serine/valine ratio than the two-dose female and the duplication male has the highest ratio of all classes.

DISCUSSION

The results demonstrate that the transfer RNA serine-4 isoacceptors encoded at 12DE on the X chromosome exhibit the phenomenon of dosage compensation. The level of this RNA species relative to the total valine in each class of flies changes with the dosage of the 12A-13A region in both males and females, but the expression in normal males is similar to the female level. The duplication male produced the highest amounts. These observations are analogous to the phenomenology of most other X-linked genes in that they exhibit a dosage effect when only the structural locus is varied, but show a similar expression between the two sexes.

The values from the dosage series do not conform to those expected from a strict dosage response. This is the anticipated result from varying only a portion

TABLE 2

Analysis of chromatographic separation of serine isoacceptors from an isogenic Oregon R stock

Class	Chromatography column no.	Isoacceptor 2	5	4	6	7	Total	Mean (serine isoacceptor/total valine) ± S.E. 2	5	4	6	7
Males	1	4951	5466	5694	5151	10168	31430	78.33 ± 2.73	88.67 ± 0.88	93.33 ± 0.88	84.33 ± 1.45	165 ± 0.58
		(80)	(89)	(92)	(84)	(165)		(1.07)	(0.88)	(1.00)	(0.86)	(0.99)
	2	4362	5355	5651	5182	9747	30297					
		(73)	(90)	(95)	(87)	(164)						
	3	4758	5094	5424	4754	9679	29709					
		(82)	(87)	(93)	(82)	(166)						
Females	1	4562	6192	5780	5928	9972	32434	73.33 ± 1.2	100.68 ± 0.88	93.67 ± 0.67	98.33 ± 0.67	166.67 ± 1.76
		(75)	(102)	(95)	(97)	(164)		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
	2	4362	6092	5757	6089	10512	32812					
		(71)	(99)	(93)	(99)	(170)						
	3	4596	6263	5791	6137	10314	33100					
		(74)	(101)	(93)	(99)	(166)						

Isoacceptor values represent the summation of the cpm minus background from each fraction within the respective peaks. Peak 5b is not evident from the chromatographs of the Oregon R stock; otherwise the designations are as shown in Table I. Isoacceptor values in parentheses represent normalization to total valine. Values in parentheses below the means indicate the expression for each isoacceptor relative to the respective female value.

TABLE 3

Total acceptance of serine and valine by tRNA from each class of the 12DE dosage series and Oregon R males and females

Class	*n*	Serine (pmol) (mean ± S.E.)	Valine (pmol) (mean ± S.E.)	Ratio (Ser/Val)
One-dose female	6	67.19 ± 1.76	49.11 ± 1.77	1.37
Two-dose female	6	63.85 ± 2.66	43.06 ± 2.03	1.48
Three-dose female	6	49.28 ± 1.97	32.11 ± 1.32	1.53
FM7 male	6	41.26 ± 1.62	25.24 ± 1.40	1.63
B166 male	6	48.58 ± 1.92	31.63 ± 1.02	1.54
Two-dose male	6	46.33 ± 1.38	27.12 ± 0.83	1.71
Oregon R male	5	28.20 ± 1.50	55.22 ± 1.91	0.511
Oregon R female	5	30.23 ± 1.08	56.78 ± 1.04	0.532

All values represent the mean number of picomoles of amino acid accepted per A_{260} unit from *n* reactions for each class of RNA noted.

of the genes that contribute to the total serine-4 level; the autosomal loci contribute a background that reduces the magnitude of the effect.

Examination of the two types of one-dose males allows a control on any differences in tRNA expression that might exist between the *FM7* balancer chromosome and the one used for the induction of the XY translocations. The *FM7* males have a level of serine-4 that is greater than the other class of one-dose males. There are at least three possibilities to explain this difference: (1) the balancer chromosome is grossly rearranged and might be duplicated for the serine transfer RNA genes, either on a multigene level or only involving the serine cluster itself; (2) the level of expression of the serine genes in the *FM7* chromosome is different from the homologous cluster in the translocated ones; and (3) modifying genes exist along the X chromosome that affect tRNA expression differently in the two instances. In addition to the higher levels of serine-4, the FM7 chromosome also behaves as does the duplication male as judged by its effect on total serine and valine acceptance per A_{260} unit, by its negative and positive effects on serine acceptors other than 4 and by its dosage effect on the size of the shoulder on peak 2. The observation that the *FM7* chromosome has a greater expression of serine-4 than the other X chromosome used is consistent with the observation that as the dosage of 12DE increases in females there is not a strictly linear increase in serine-4 expression. This is the case because the increase in dosage is accomplished by the addition of less productive chromosomal regions. The one-dose female has a single copy of this region in an *FM7* chromosome. The two-dose female is heterozygous for *FM7* and *T(1;Y)B166* or for the two translocations and the three-dose class has *FM7* plus two copies of 12A-13A that originated from the X chromosome utilized in the construction of the translocations.

Regardless of the basis of this difference, we believe that it is inconsequential to the interpretation of dosage compensation. First of all, the one-dose females have less relative serine-4 than the euploid female even though the region present in the segmental monosomic has the greater level of expression of the two types in the diploid. Second, the three-dose to two-dose comparison in

females shows an increase that is accomplished with the addition of a segment that is identical to one of the two present in the heterozygous female and thus must truly represent a dosage effect. Third, the two doses in the duplication male are identical in origin to the one dose in *T(1;Y)B166* males, and there is clearly a response from the additional copy present. Fourth, the one-dose female has a single copy of this region in an *FM7* chromosome as do the *FM7* males and the latter has a greater relative expression of serine-4. Fifth, the ratios of total serine to valine acceptance in each of the six types of flies are consistent with the appropriate relative expressions as expected from dosage compensation. Finally, examination of an isogenic Oregon-R strain indicated equal serine-4 expression in males and females.

In the background used to construct the dosage series, the serine 2 and 5b values per total valine are routinely elevated in males and serine 7 per total valine is consistently reduced. Both the elevation and reduction appear to be enhanced in the respective directions by the duplication in the male. Although it is possible that such effects also operate on serine-4, the levels of this isoacceptor nevertheless follow the predictions of dosage compensation not only in the male, but also in the female dosage series as well. Moreover, the ratio of the total serine/valine acceptance is consistent with a dosage effect and compensation in the appropriate genotypes. Although the analysis is complicated by the fact that not all of the serine genes are present at 12DE, by the possibility that serine genes other than isoacceptor 4 might be present in this cluster and by the effect of the dosage of 12A-13A on the total acceptance of serine and valine per A_{260} unit, the fact that serine-4 responds to the dosage of this region, as do other tRNAs in structural gene dosage series, suggests that functional serine-4 genes are present and allows a test of dosage compensation.

Although the differences in serine-4 expression between genotypes in some instances are small, the six classes are aligned in the appropriate relationship expected from dosage compensation when both the individual isoacceptor values are compared or when the total serine/valine acceptance is examined. The probability that the six types would be arranged thusly as a matter of mere chance is 0.0014. In a statistical sense, this value represents a level of high significance ($P < 0.01$).

Since most X-linked genes are transcribed by RNA polymerase II to produce messenger RNA but the transfer RNA genes are transcribed by polymerase III, these observations suggest, but do not prove, that the mechanism of dosage compensation is indiscriminate as to the polymerase involved. The data do not rule out the alternative that dosage compensation of polymerase II and III genes is brought about by completely distinct mechanisms and the steady state levels of the gene products in both cases are only fortuitously similar.

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