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CHROMOSOMAL BASIS OF DOSAGE COMPENSATION IN *DROSOPHILA*. X. ASSESSMENT OF HYPERACTIVITY OF THE MALE X *IN SITU*

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SUMMARY

The results of examination of the template activity of the fixed polytene chromosomes of *Drosophila hydei*, monitored by ^{3}H-UTP, under *in situ* assay conditions, upon the use of endogenous *Drosophila* polymerase, exogenous *Escherichia coli* RNA polymerase (holoenzyme) and exogenous *Drosophila* RNA polymerase II (or B) have been presented. Analysis of the data reveals that the transcription patterns with the 3 enzymes are not strictly comparable with the pattern obtained under *in vivo* conditions. Yet, with each of the 3 conditions of assay, there is a reasonable concordance between the template activity on the single X chromosome of the male and the paired Xs of the female, as observed under *in vivo*. There is also, in every case, a high positive correlation between the ^{3}H-UMP incorporation into the X chromosome and that into a specific autosome. A site-wise analysis of ^{3}H-UMP labelling under the 3 assay conditions also reveals that for most of the regions, the sites which are highly active *in vivo* also show high labelling *in situ*, and the proportionality is maintained in both sexes. These results have been interpreted to have suggested that the hyperactivity of the male X vis-a-vis dosage compensation in *Drosophila* is primarily a property of the inherent organization of the X chromosome itself and is achieved through modulation in the organization, rather than exclusively through autosomal factor(s), although a secondary level of autosomal regulation has not yet been ruled out.

INTRODUCTION

Previous studies have shown that the X chromosome of larval salivary glands of male *Drosophila* is hyperactive with respect to transcription and translation as compared to the individual Xs of the female (Mukherjee & Beermann, 1965; Mukherjee, 1966; Mukherjee, Lakhotia & Chatterjee, 1968; Lakhotia & Mukherjee, 1969; Korge, 1970; Chatterjee, S. N. & Mukherjee, 1971; Lucchesi, 1974; Lucchesi, Rawls & Maroni, 1974), replicates its DNA earlier (Berendes, 1966; Lakhotia & Mukherjee, 1970; Chatterjee, S. N. & Mukherjee, 1973) and at a faster rate (Chatterjee, R. N. & Mukherjee, 1977) than the autosomes, and conversely than the female's X chromosomes. These male-specific activities of the X chromosome are considered to be the counterparts of dosage compensation. Works of Lucchesi and his co-workers (Lucchesi, 1974; Lucchesi & Rawls, 1973; Lucchesi *et al.* 1974) have shown that there is in fact a higher net synthesis of the X-linked enzyme (G6PD) per gene dose in the male, metamale (1X3A) and 2X3A intersex than in the diploid or triploid female,

which implies that at least one of the determining factors for equalization of the gene products of the X-linked genes might be present in the autosome. Maroni & Plaut (1973) and Mukherjee (1974) suggested an inherent organizational change in the male X chromosome (in addition to an autosomal regulation) to account for the hyperactivity of the X. Stewart & Merriam (1975) opened up the issue and questioned the autosomal regulation and Prasad *et al.* (in preparation) have confirmed their finding. In order to examine this issue more directly, re-examination of the transcription of the X chromosome and autosome *in situ* should be profitable since in such conditions mobilization of remotely located factor is virtually prevented (Gross, Luse & Beer, 1977).

Earlier, Khesin & Leibovitch (1974) and Leibovitch, Belyaeva, Zhimulev & Khesin (1976) using *E. coli* RNA polymerase, showed that the utilization of RNA polymerase by the single X of male *Drosophila* is nearly twice as great as by individual X chromosomes of female *Drosophila*. However, *Drosophila* RNA polymerase II (or B), which is responsible for the non-ribosomal RNA transcription, differs from *E. coli* RNA polymerase in many respects (Phillips & Forrest, 1973; Natori, Wortori, Boshes & Ristow, 1973; Greenleaf & Bautz, 1975; Gross & Beer, 1975). Moreover, considerable endogenous *Drosophila* RNA polymerase activity can be detected under *in situ* conditions, that is after fixation, though only for a short period (Fisher, 1968; Lezzi & Robert, 1972; Morcillo, de la Torre & Gimenez-Martin, 1976; Pages & Alonso, 1976). The present investigation was therefore undertaken to examine the relative transcriptive activity of the X chromosome *in situ* in male and female with endogenous RNA polymerase, *E. coli* RNA polymerase and *Drosophila* RNA polymerase II.

MATERIALS AND METHODS

For all cytological preparations, larvae from the wild-type stock of *Drosophila hydei* (Tubingen) were used. All developmental stages including adults were reared in standard *Drosophila* medium enriched by addition of live yeast at 24 ± 1 °C.

Cytological preparations of chromosomes

Salivary glands from mid third-instar larvae of *Drosophila hydei* were dissected out by hand in buffered Ringer's solution at pH 7·2 (Berendes, 1973) fixed in ethanol:acetic acid (3:1) and squashed in 50 % acetic acid. Coverglasses were removed by freezing on dry ice. The preparations were then processed as required. Photomicrographs were taken under Zeiss Photomicroscope III, using 100 × /1·30 oil-immersion objective.

Autoradiographic procedure

In vivo transcription of polytene chromosomes was assayed by conventional autoradiography using [^{3}H]uridine (^{3}H-UR) as the precursor. Excised glands were incubated in 10 μl of *Drosophila* Ringer containing 3 μCi ^{3}H-UR for 10 min (sp. act. 50 Ci/mM; conc. used, 1 mCi/ml; obtained from Amersham, England). Cytological preparations of the pulse-labelled glands were made as described above. Coverglasses were removed by dry freezing. Slides were processed for autoradiography following the usual method (Lakhotia & Mukherjee, 1969) using Kodak AR 10 stripping film. Exposure time was 26 days. Autoradiograms were developed in D19b at 10–12 °C for 11–12 min, washed in cold water and fixed in X-ray film fixative. The slides were washed in cold running water for 20–30 min. The preparations were stained with toluidine blue.

For autoradiography of the cytological preparations for *in situ* transcriptions the transcription

assay was carried out in standard mixture in presence of ^{3}H-UTP, and with endogenous or exogenous RNA polymerase, as detailed below and thereafter the preparations were covered with Kodak AR 10 film, exposed for 26 days and developed, fixed and stained as above.

Isolation of Drosophila RNA polymerase II (B)

DNA-dependent RNA polymerase II was isolated and purified from the cells of Echalier KL213 embryonic cell line by a slight modification of the method of Roeder & Rutter (1969) and Phillips & Forrest (1973). Two forms of RNA polymerase were obtained from DEAE cellulose (DEAE-32) column.

The *Drosophila* RNA polymerase thus isolated was further fractionated on a DEAE cellulose (Whatman DE 32) column by $(NH_4)_2SO_4$ linear gradient. RNA polymerase II was rechromatographed on DEAE cellulose column. The polymerase II, purified in this way, was free of ribonuclease as judged by kinetics of ^{3}H-UMP incorporation (data not included).

In vitro assay for RNA polymerase activity

The standard assay mixture (75 μl final volume) contained 3·5 μmol Tris-HCl, pH 7·9; 0·13 μmol $MgCl_2$; 0·07 μmol $MnCl_2$; 0·02 μmol ATP; 0·02 μmol GTP; 0·02 μmol CTP; 0·005 μmol UTP; 100 pmol ^{3}H-UTP (sp. act. 50 Ci/mM; obtained from Amersham, England), 12·5 μg calf thymus DNA or *Drosophila melanogaster* DNA (isolated from Echalier KL213 cells) and 25 μl of the enzyme obtained from the column. After incubation for 15 min at 30 °C, the reaction was stopped by chilling to 0 °C in an ice-water bath, and pipetting the incubation mixture directly onto the dried DEAE cellulose square filter paper (Whatman DE 3 mm) which had previously been soaked in 0·5 % yeastolate (Difco)/0·5 % crude yeast RNA (Sigma Chemicals). Unincorporated nucleoside triphosphates were freed from the filters by repeated wash either in 5 % (w/v) Na_2HPO_4 according to the procedure of Litman (1968) or in 5 % (w/v) cold TCA containing 0·1 M $Na_4P_2O_7$. 10 H_2O (Merck). The filters were rinsed in water, dried and counted in toluene-based scintillation fluid (POP-POPOP). The counting was performed in a Phillips liquid scintillation analyser pw 4500/01 which was equipped with a computer program for computation of dpm and E.S. ratio.

In situ assay of chromatin template activity

Chromatin template activity with endogenous RNA polymerase. For measuring template activity with endogenous RNA polymerase, salivary glands were dissected out and were fixed in aceto-ethanol (1:3 v/v) for just 30 s at 0 °–4 °C and squashed in 50 % acetic acid (Pages & Alonso, 1976). Coverglasses were removed by dry freezing and the preparations were successively passed through the grades of ethanol-water (3:1) containing 1 % NaCl and then through a 1:3 grades of ethanol-water containing 1 % NaCl for 5 min each at 4 °C. Thereafter, the preparations were washed thoroughly in distilled water and transferred for 10 min to Tris buffer containing 0·01 M Tris-HCl, pH 7·9; 1·5 mM $MgCl_2$; 0·01 mM EDTA; 0·5 mM DTT and 25 % glycerol. The whole procedure was carried out within 40–60 min of cytological preparation of glands. The reaction for *in situ* assay of endogenous RNA polymerase activity was then carried out by adding 25 μl of standard assay mixture to each preparation. The standard assay mixture contained in 25 μl, 1·2 μmol Tris-HCl, pH 7·9; 0·04 μmol $MgCl_2$; 0·02 μmol $MnCl_2$; 0·02 μmol ATP; 0·02 μmol GTP; 0·02 μmol CTP and 3 μl ^{3}H-UTP (sp. act. 50 Ci/mM; 1 mCi/ml; obtained from New England Nuclear).

The preparations were then placed in a moist chamber and incubated at 30 °C for 20 min. Reactions were terminated by placing the slides in 5 % (w/v) cold TCA containing 0·1 M $Na_4P_2O_7$. 10 H_2O. The unincorporated nucleoside triphosphates were removed from the chromosomes by repeated washing in 5 % cold TCA (w/v) containing 0·01 M $Na_4P_2O_7$. 10 H_2O for 1 h. The preparations were processed for autoradiography as described.

Chromatin template activity with exogenous RNA polymerase. In situ transcription by exogenous RNA polymerase on fixed cytological preparations was carried out using 2 different types of DNA-dependent RNA polymerases: (1) Purified *E. coli* RNA polymerase (holoenzyme), obtained from Sigma Chemicals, and (2) *Drosophila melanogaster* RNA polymerase II, isolated from Echalier KL 213 embryonic cell line, and purified as described above (Roeder & Rutter, 1969; Phillips & Forrest, 1973). Cytological preparations were processed as described for

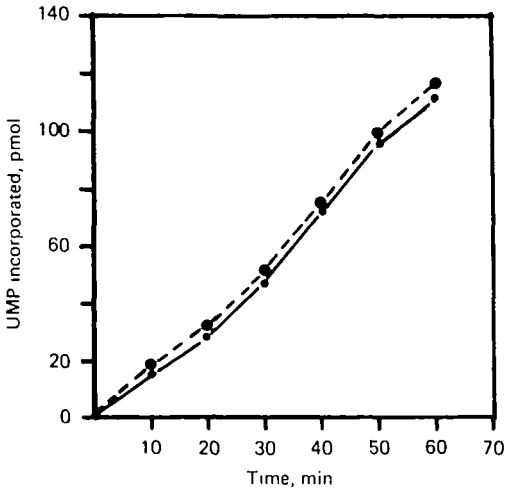

Fig. 1. Kinetics of RNA synthesis by partially purified *Drosophila* RNA polymerase (——). The DNA templates were either isolated from the cultured cells of Echalier KL 213 embryonic cell line or obtained from B.D.H., England, as commercially prepared calf thymus DNA (- - -). Enzymes were assayed under standard assay conditions as mentioned in Materials and methods.

endogenous polymerase except that the preparations were kept overnight in ethanol for better fixation. Thereafter, the reaction for *in situ* transcription was carried out on the fixed chromosome preparations by addition of 3 units of purified *E. coli* RNA polymerases (3 to 4 μl) or 3 units (10 μl) of *D. melanogaster* RNA polymerase II to each slide in combination with the assay mixture (as described above). One unit of enzyme activity catalyses the incorporation of 1 nmol UMP in 15 min at 30 °C under standard assay condition. For controls, 25 μl of the standard assay mixture (without polymerase) were added to the cytological preparation. The preparations were thereafter processed as described above.

RESULTS

Relative efficiency and specificity of RNA polymerases

The efficiency and specificity of *Drosophila* RNA polymerase were checked by an *in vitro* RNA synthesis using both calf thymus and *Drosophila melanogaster* DNA as template. Data on time kinetics of incorporation of ^{3}H-UMP into the RNA show that partially purified *Drosophila* RNA polymerase can utilize both calf thymus and *Drosophila* DNA as template and synthesize RNA on both the templates with equal efficiency (Fig. 1).

Previously, the activity of *E. coli* RNA polymerase to utilize heterologous DNA as template in transcription has been shown by a number of workers (Natori *et al.* 1973; Tsai & Saunders, 1973). Our observation shows that the *E. coli* RNA polymerase (holoenzyme) used in these experiments can also utilize *Drosophila* DNA as template for *in vitro* RNA synthesis. These results, therefore, suggest that RNA polymerases are relatively non-specific under our conditions and can be used for interspecific transcription.

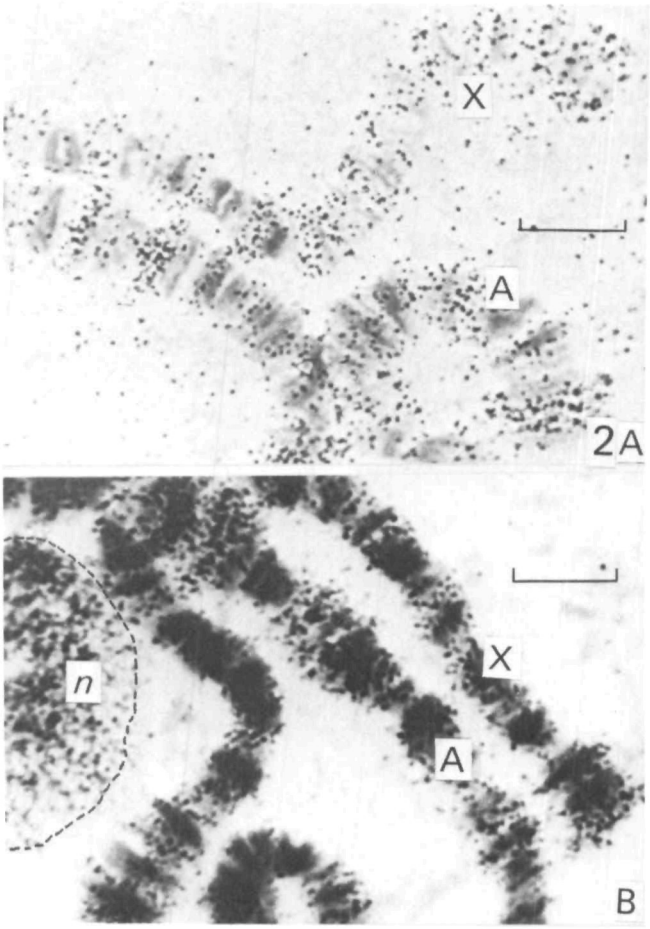

Fig. 2. [^{3}H]uridine autoradiograms of larval salivary glands of *Drosophila hydei* showing pattern of transcription *in vivo*. A, [^{3}H]uridine labelling over the X chromosome and autosomes in a nucleus from male larval gland; B, that in a nucleus from a female larval gland. X, X chromosome; A, autosome; *n*, nucleolus. Bar, 10 μm.

Autoradiographic visualization of chromatin template activity in situ

When *in vivo* transcription of polytene chromosomes is monitored by [^{3}H]uridine, the nucleolus and major puffsites label intensely while the lightly stained regions have disperse label and some dark bands have low or little label (Fig. 2A); this has also been reported by earlier workers (Pelling, 1972; Ananiev & Barsky, 1978; Lakhotia, 1979). Furthermore, the density of label on the single X chromosome of the male (Fig. 2A) is close to that on the paired Xs of the female (Fig. 2B).

When chromatin template activity was assayed with endogenous RNA polymerase on squashed cytological preparations of polytene chromosomes fixed for 30 s, very low labelling intensity was observed over both the nucleolus and chromosomes, and the label was mainly restricted to bands (Fig. 3). The ^{3}H-UMP labelling in male, like the *in vivo* transcription, is generally similar to that of the two Xs of the female.

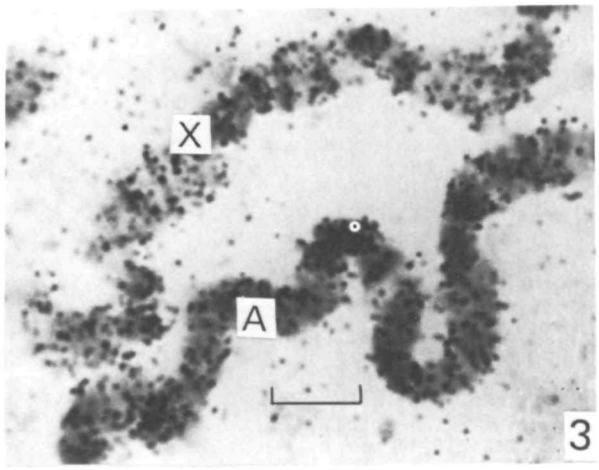

Fig. 3. Autoradiogram showing endogenous RNA-synthesizing activity of briefly fixed polytene chromosomes of *D. hydei* male larval gland *in situ* by ^{3}H-UTP. Symbols and scale as in Fig. 2.

When fixed chromosomal preparations were assayed for template activity with *E. coli* RNA polymerase (holoenzyme) as described in Materials and Methods, ^{3}H-UMP was incorporated into the chromosomes as was the [^{3}H]uridine in *in vivo* transcription, except that in this case silver grains were distributed in a more disperse rather than localized manner (Fig. 4A, B). Yet, the chromatin template activity on the X chromosomes of the male and female is comparable implying that the hyperactivity of the male X is maintained even under conditions of *in situ* transcription. Furthermore, the nucleolar labelling is mainly restricted to the nucleolar threads (Fig. 4C) and not scattered over the nucleolus as found *in vivo*.

Results *in situ* of chromatin template activity assay with exogenous *Drosophila* RNA polymerase II (from Echalier KL 213) show almost no labelling on the nucleolus; the chromosomes show label mainly over dark bands, and moderately on puffs and interbands. Also, the X chromosome of the male utilizes more RNA polymerase than the individual X chromosome of the female resulting in an equal grain intensity over the single X of the male and the paired Xs of the female (Fig. 5A, B).

Comparison of the relative efficiency of chromatin template activity in situ

Comparison of the intensity of silver grains resulting from ^{3}H-UMP incorporation into chromosomes and nucleolus of male and female larval glands of *D. hydei* in the presence of exogenous RNA polymerase from *Drosophila* (*Drosophila* RNA polymerase II) and *E. coli*, together with results obtained in *in vivo* transcription and under *in situ* endogenous RNA polymerase assay conditions are presented in Tables 1, 2. The segments 18A-20D of the X and 91A-94C of the 4th chromosome of *D. hydei* have been used as reference.

Table 1 shows that template activity of chromatin with endogenous short fixation is approximately only 20% of that observed *in vivo*. The endogenous activity is

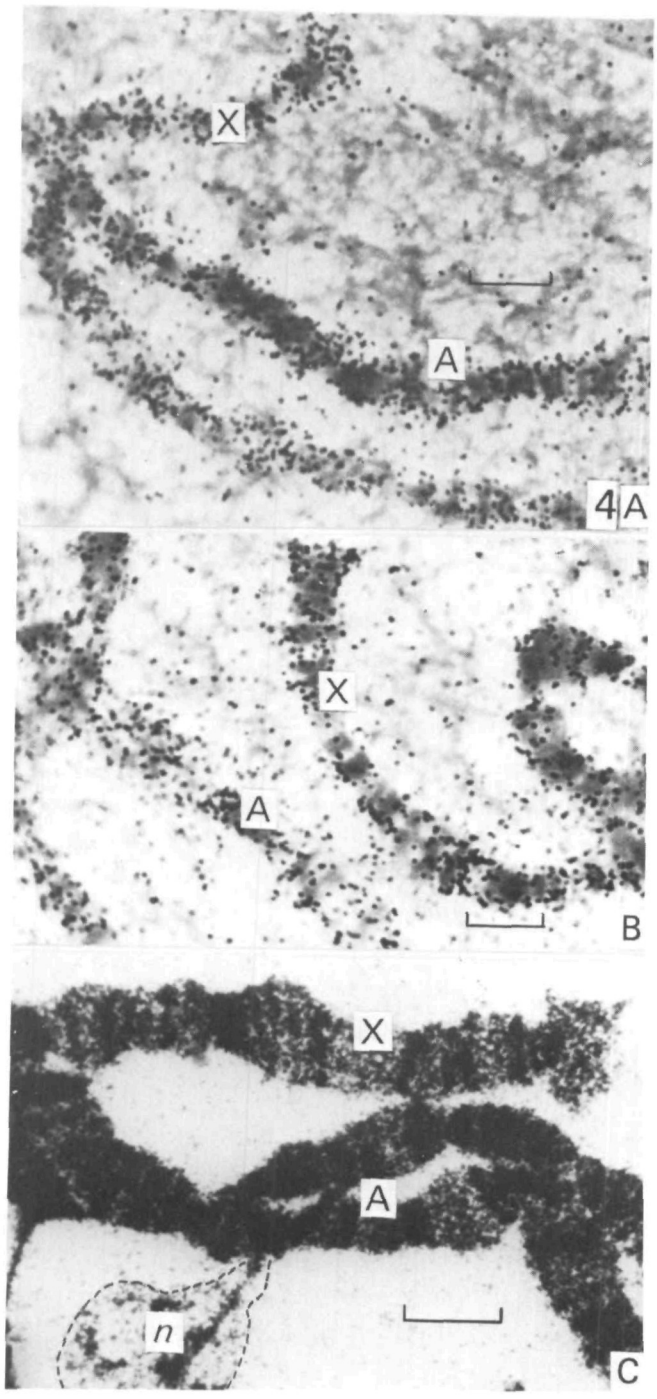

Fig. 4. Autoradiograms showing ^{3}H-UMP incorporation pattern over chromosomes and nucleolus of *D. hydei* after *in situ* transcription by *E. coli* RNA polymerase (holoenzyme) on cytological squash preparation of salivary gland chromosomes. A, ^{3}H-UMP labelling over the X chromosome and autosome in the male. B, ^{3}H-UMP labelling over the X chromosomes and autosomes in the female. C, ^{3}H-UMP labelling over the X chromosome, autosomes and the nucleolus of a nucleus from male larval gland with high intensity of grains. Note intense labelling on the nucleolar chromatin. Symbols and scale as in Fig. 2.

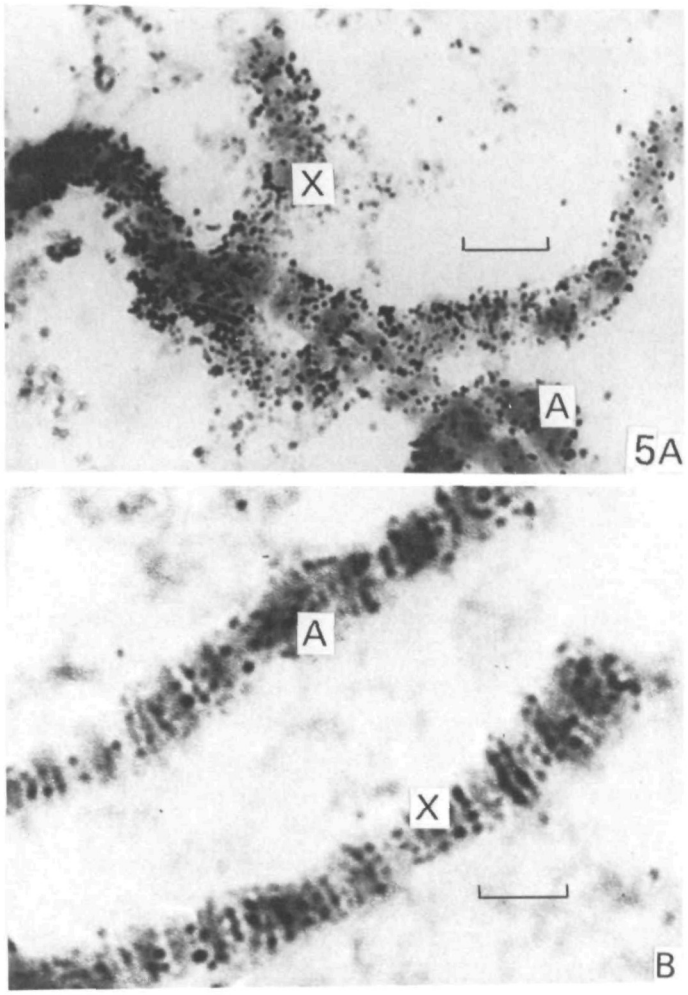

Fig. 5. Autoradiograms showing template activity of polytene chromosomes of *D. hydei* with exogenous *Drosophila* RNA polymerase II isolated from Echalier KL 213 embryonic cell line of *Drosophila melanogaster*. A, relative incorporation of ^{3}H-UMP into the X chromosome and autosome of a male nucleus. B, relative ^{3}H-UMP incorporation pattern over X chromosome and autosome of a female nucleus. Symbols and scale as in previous figures.

completely removed after longer fixation, e.g. 2 h. Therefore, the template activities of chromatin with *Drosophila* and *E. coli* RNA polymerase(s), *in situ*, can be safely taken as the net chromatin activity with the respective exogenous polymerases only. The activity with *Drosophila* RNA polymerase II is nearly 50–60% of that with *E. coli* RNA polymerase holoenzyme. This implies that *E. coli* polymerase transcribes more efficiently *in situ* than does *Drosophila* RNA polymerase II, all other conditons of the transcriptional assay being the same. The data from the autoradiograms reveal further that the ratio of the number of silver grains on the X chromosome segment (18A–20D) to that on the autosomal segment (91A–94C) is fairly concordant in the 2 sexes.

Table 1. *Data on the [^3H]uridine or ^3H-UMP incorporation pattern of the salivary gland X-chromosome and autosome (4th) of D. hydei in normal and in situ assay of template activity with endogenous polymerase after short fixation, or by in situ transcription by different groups of exogenous DNA-dependent RNA polymerase.*

Type of transcription	Sex	No. of silver grains with S.E.		X/A ratio	Intercept	Slope	r_i
		X chromosome (18A–20D)	4th chromosome (91A–94C)				
Transcription/*in vivo*	Female	182·24 (14) ± 17·11	150·64 (15) ± 12·73	1·21 ± 0·22	13·04	1·12 ± 0·17	0·87
	Male	141·06 (17) ± 12·55	129·13 (17) ± 10·13	1·08 ± 0·19	−3·70	1·12 ± 0·13	0·91
	Female/Male	1·29	1·17				
	t value	1·94	1·32				
Transcription/*in situ* by endogenous polymerase	Female	35·38 (18) ± 3·14	35·67 (18) ± 3·05	0·97 ± 0·20	2·15	0·93 ± 0·11	0·90
	Male	29·60 (15) ± 3·67	31·73 (15) ± 2·67	0·88 ± 0·32	−2·64	1·24 ± 0·15	0·90
	Female/Male	1·19	1·12				
	t value	1·18	0·97				
Transcription/*E. coli* polymerase exogenous	Female	89·67 (17) ± 11·11	77·64 (17) ± 7·96	1·12 ± 0·22	−11·58	1·30 ± 0·19	0·86
	Male	91·27 (17) ± 12·93	81·71 (17) ± 9·79	1·01 ± 0·28	−6·66	1·19 ± 0·13	0·91
	Female/Male	0·98	0·95				
	t value	0·09	0·21				
Transcription/exogenous *Drosphila* polymerase II	Female	53·25 (16) ± 2·58	48·94 (16) ± 2·04	1·08 ± 0·13	5·55	0·98 ± 0·20	0·77
	Male	46·88 (16) ± 3·80	48·25 (16) ± 3·89	0·99 ± 0·21	7·59	0·81 ± 0·12	0·86
	Female/Male	1·14	1·01				
	t value	1·39	0·16				

Values in parentheses denote the numbers of nuclei examined. X/A = ratio of grain number on the X-chromosome to that on the autosome
r_i = Correlation coefficient.

Table 1. *Data on the [^3H]uridine or ^3H-UMP incorporation pattern of the salivary gland X-chromosome and autosome (4th) of D. hydei in normal and in situ assay of template activity with endogenous polymerase after short fixation, or by in situ transcription by different groups of exogenous DNA-dependent RNA polymerase.*

Type of transcription	Sex	Mean grain no. over 4th (fra.) 91A–94C with S.E.	Mean grain no. on a particular unit area of nucleolus with S.E.	A/N	Intercept (a)	Slope (b)	r_i
Transcription	Female	150·64 ± 12·79 (15)	56·60 ± 4·61	2·66	36·10	2·11 ± 0·51	0·73
in vivo	Male	129·13 ± 10·13 (17)	40·93 ± 3·74	2·59	34·58	1·83 ± 4·09	0·69
Endogenous polymerase	Female	35·67 ± 3·05 (18)	2·29 ± 0·47	15·58	23·15	5·50 ± 0·81	0·85
in situ assay	Male	31·73 ± 2·67 (15)	1·87 ± 0·39	16·98	27·53	2·68 ± 1·63	0·39
E. coli polymerase *in situ*	Female	77·46 ± 7·96 (17)	10·24 ± 0·88	7·58	10·17	6·59 ± 1·50	0·73
transcription	Male	81·71 ± 9·79 (17)	10·29 ± 0·80	7·94	20·10	5·98 ± 2·25	0·54
Drosophila polymerase II	Female	48·49 ± 2·04 (16)	2·00 ± 0·32	24·47	46·48	1·23 ± 2·52	0·20
in situ transcription	Male	48·25 ± 3·89 (16)	2·31 ± 0·47	20·89	40·43	2·38 ± 1·88	0·31

Values in parentheses denotes the number of nuclei examined.
A/N = ratio of the grain number on the autosome to that on the nucleolus.
r_i = Correlation coefficient.

Analysis of the data also reveals a positive correlation between the grain number on the X chromosome and that on the 4th chromosome in the 2 sexes and the deviation between the 2 regression slopes is not statistically significant (Table 1).

Table 2 reveals that the template activity of nucleolar DNA with endogenous as well as exogenous *Drosophila* polymerases is drastically depleted compared to *in vivo* activity. Yet, the ratios of grain number on the segment of the autosome to that on the nucleolus are similar in the 2 sexes. This is expected, as in both sexes the autosomal genes as well as the nucleolar organizer (rDNA) are in double dose (the 2 Xs in the female and X and Y in the male contain the rDNA cistrons). On the other hand, with *Drosophila* RNA polymerase II, no significant incorporation of ^{3}H-UMP over nucleolar DNA was observed (Table 2). Interestingly, there is a low positive correlation between chromosomal and nucleolar RNA synthesis *in situ* with *Drosophila* RNA polymerase II. This is probably due to lack of sufficient incorporation of ^{3}H-UMP into nucleolar chromatin (Table 2).

Site-specific transcription activity of the polytene chromosomes with endogenous and exogenous RNA polymerases

Fig. 6 presents data on the relative template activity of specific sites under the 3 assay conditions namely, endogenous activity, activity with *Drosophila* RNA polymerase II, and with *E. coli* RNA polymerase, the *in vivo* transcription data serving as reference. As has been reported earlier, so here, the pattern of ^{3}H-UR incorporation *in vitro* into different sections of the chromosomes differed from *in vitro* ^{3}H-UMP incorporation into the chromosomes. The rate of RNA synthesis *in vivo* in different sections varies considerably (from 1- to 10-fold), whereas *in situ*, the rate of RNA synthesis of different sections varies only from 1- to 2-fold (e.g. 20D vs. 20AB, 90C vs. 94A etc.). It is evident from the data in Fig. 6 that for both the autosome and the X chromosome, there is a reasonable proportionality in the pattern of transcription. It is to be noted here that for most of the regions, those sites which are active *in vivo*, also show higher incorporation of ^{3}H-UMP *in situ* (e.g. 20D and 93C etc.) using exogenous polymerase. Certain exceptions to this general rule were, however, noted: e.g. sites 18AB of the X chromosome and 93AB of the 4th do not show similar proportionate depletion in activity with *E. coli* and *Drosophila* RNA polymerase II (Fig. 6).

The site-wise analysis confirms that alteration in activity is maintained proportionately in both sexes, implying that *in situ* also, the hyperactivity of the X chromosomal sites in male is maintained autonomously.

DISCUSSION

Natori *et al.* (1973) and Greenleaf & Bautz (1975) reported that *Drosophila* RNA polymerase II has no template specificity like yeast and mammalian RNA polymerase II. Kinetic studies also demonstrate that partially purified *Drosophila* RNA polymerase, extracted from cultured cells of the embryonic cell line (Echalier KL 213), can efficiently utilize both calf thymus DNA and *Drosophila* DNA.

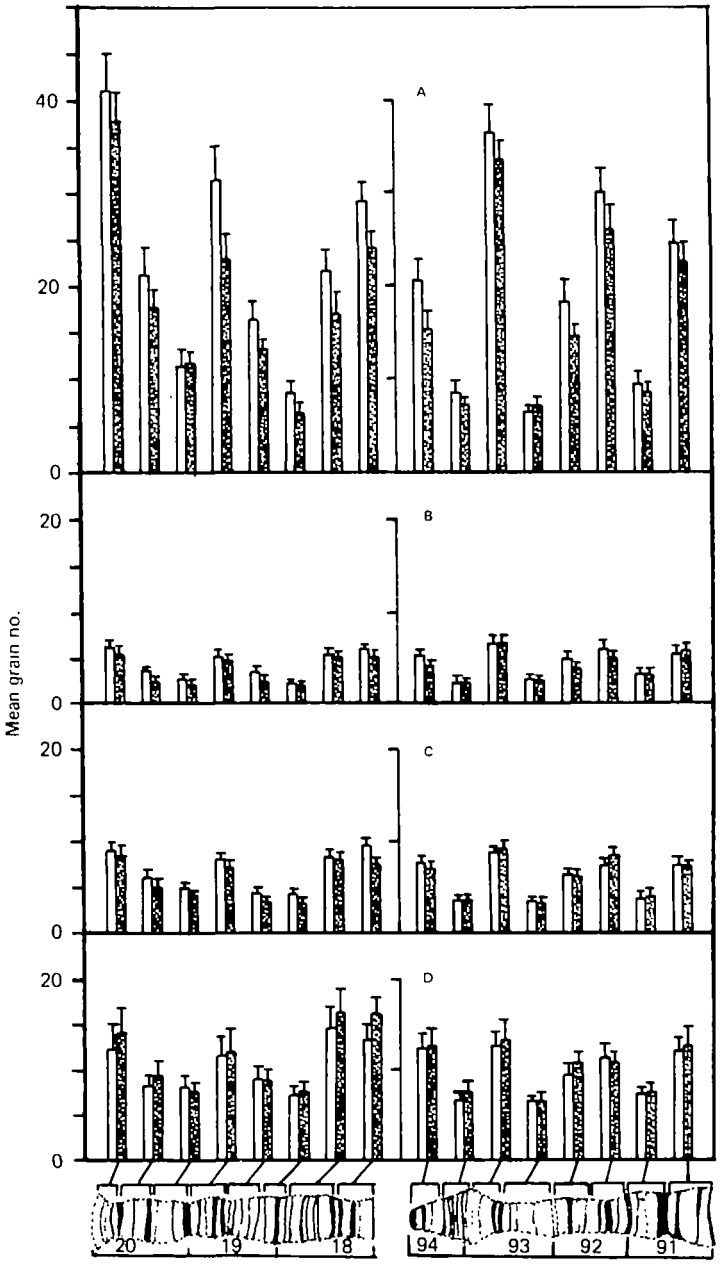

Fig. 6. Histograms showing the distribution of silver grains due to [^{3}H]uridine or ^{3}H-UMP incorporation over the X chromosome and autosomal segments of *D. hydei* polytene chromosomes under the four different transcriptional conditions. A, *in vivo* transcription; B, transcription by endogenous RNA polymerase *in situ*; C, transcription by *Drosophila* RNA polymerase II *in situ*; D, *in situ* transcription by *E. coli* RNA polymerase (holoenzyme). Hatched bars represent data for male and unhatched bars those for female nuclei. Histograms on the left part are from the data of the X chromosome segment (18A–20D) and those on the right part are from the data of the segment autosome 4 (91A–94C) of *D. hydei*. The respective bands of the 2 chromosome segments are shown diagrammatically at the bottom of the figure.

The work of Gross *et al.* (1977) indicates that *D. melanogaster* RNA polymerase II directs the synthesis of RNA only on the chromosomes and not on the nucleolus. This is substantiated by the present data on *in situ* transcription by *Drosophila* RNA polymerase II on *D. hydei* polytene chromosomes. On the other hand, when *E. coli* RNA polymerase (holoenzyme) activity was analysed on salivary gland preparations of *D. hydei* a considerable number of silver grains was observed on the nucleolus as well as chromosomes. RNA syntheses on chromosome and nucleolus with *E. coli* polymerase are positively correlated, as has been noted after *in vivo* transcription. The patterns of RNA synthesis *in situ* on the chromosomes with *Drosophila* RNA polymerase II and with *E. coli* RNA polymerase were not similar. Activity with *E. coli* RNA polymerase on fixed chromatin is relatively higher than with *Drosophila* RNA polymerase II. The reason for this difference between the 2 enzymes is not clear, but could possibly be related to difference in binding specificity (Tsai & Saunders, 1973) or activation (Chambon, 1975) of the 2 enzymes.

The data reveal that the template activity of the single X of the male observed *in situ* is twice as high as of an individual X chromosome of the female. The *in situ* hyperactivity of the single X chromosome of the male is comparable to the *in vivo* hypertranscriptive activity of the single X of the male which is considered to be the counter-part of dosage compensation (Mukherjee & Beermann, 1965; Mukherjee, 1966). Since chromatin-bound RNA polymerase activity was assayed *in situ* and there was no scope for active transfer or mobilization of extra-chromosomal material under the experimental conditions used, transfer of a regulatory signal for hyperactivity from the autosome is less likely.

These data, together with the finding that there is relatively more binding affinity of the X chromosome of the male with RNA polymerase than of the X chromosome of the female (Khesin & Leibovitch, 1974; Leibovitch *et al.* 1976), clearly suggest that organization of the X chromosome in *Drosophila* is prone to an inherent modulation in the 2 sexes and therefore that the regulatory phenomenon underlying dosage compensation in *Drosophila* is primarily a property of the organization of the X chromosome itself. The autosomal regulatory factor(s) if they exist must be operative through its autonomously operative organizational modulation in male and female Xs. The autonomy of the organization is supported by the data on the site-specific autonomy of the hyperactivity under *in situ* transcription conditions. The present data support the claim of Stewart & Merriam (1975) that hyperactivity of the X chromosome may be a property integrated with organization of the X in the male, although it does not rule out a secondary level of autosomal factor(s) playing a role in organizational modulation.

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