AUTOSOMAL DOSAGE COMPENSATION IN INTERSPECIFIC HYBRIDS OF *DROSOPHILA*: TRANSCRIPTIONAL ACTIVITY IN DIPLOID AND TRIPLOID HYBRIDS

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ABSTRACT

Transcriptional activity of a triploid-2L segment has been compared in an interspecific hybrid (I) (compound-2L of *Drosophila melanogaster* ♀ × *D. simulans* ♂) and an intraspecific hybrid (II) (compound-2L of *D. melanogaster* ♀ × Oregon R^+ ♂). The results reveal that although one of the three homologues in hybrid I has been contributed by *D. simulans*, the segment 21A-22F of 2L shows a level of transcriptional activity equal to that in hybrid II, where all the three homologues are from *D. melanogaster*. In the asynapsed region of 2L(21ABC) of hybrid I, the transcriptional activity of the two homologues derived from *D. melanogaster* is double that of the single homologue from *D. simulans*, and two-thirds of that of the two homologues of Oregon R^+. The total transcriptional activity of this asynapsed region (21ABC) in hybrid I is equal to that in hybrid II and the diploid Oregon R^+. These results corroborate the existence of autosomal dosage compensation as proposed earlier and strongly suggest that, in contrast to dosage compensation of X-coded products, autosomal dosage compensation operates through suppression of activity per gene at the level of transcription.

INTRODUCTION

THE concept of autosomal dosage compensation was introduced by Devlin *et al.*[1] In studies on transcriptional activity of hyperploid autosomal arms (2L and 3L) of *Drosophila melanogaster*[2–5], it was revealed that several hyperploid autosomal segments have the ability to equalize their transcriptional product to that of the corresponding autosomal segments of diploid Oregon R^+ strain flies. In contrast, in haplo-condition, the smallest chromosome, 4, of *D. melanogaster* does not show hyperactivity in comparison with those of diploid sibs, implying that the mechanism of autosomal dosage compensation might be different from that of the haplo-X in male *Drosophila*[5].

The present study was undertaken to examine the transcriptional behaviour of a segment of triploid autosomal arm (2L) in an interspecific hybrid in which two of the three homologues were contributed by *D. melanogaster* female parent and one homologue came from *D. simulans* male parent. When *D. melanogaster* females are crossed with *D. simulans* males, the hybrid progeny are normally all female but they are sterile[6]. However, using the rescue gene (*lhr*)[7], otherwise nonviable male hybrids appear among progeny of the cross.

The results of analysis of transcriptional activity in trisomic-2L hybrids of *D. melanogaster* and *D. simulans* are presented here. This study has some special significance. It has already been proposed that autosomal dosage compensation in *D. melanogaster* is achieved by suppression of activity per gene. In the hybrid of compound-2L strain of *D. melanogaster* and *D. simulans*, some of the regions of the hyperploid arm remain asynapsed, and provide an opportunity to study the transcriptional activity of the chromosomal elements separately and conjointly. This is an ideal situation for examining the transcriptional activity of an autosome in one, two or three doses.

MATERIALS AND METHODS

The following strains were used:

i) Oregon R^+ (wild-type strain of *D. melanogaster*).
ii) C(2L) SHI; F(2R) *bw* (compound-2L strain of *D. melanogaster*).
iii) *D. simulans* (wild type).

Generation of hyperploid strains

To generate the interspecific trisomic-2L strain, *D. melanogaster* C(2L) SHI; F(2R) *bw* females were

crossed with *D. simulans* males; the progeny arising in F_1 were called hybrid I. The intraspecific trisomic-2L strain (hybrid II) was obtained by crossing C(2L) SM1 I (2R) *bw* females to Oregon R^+ males or vice versa. Both hybrids (I and II) were lethal at the late pupal stage.

The stocks were maintained at 20±1 C in a bacteriological incubator and cultured on sterile *Drosophila* culture medium containing maize powder, agar sugar, propionic acid and Nipagin.

Autoradiography

Salivary glands from the larvae of both hybrids (I and II) and Oregon R^+ were dissected out and immediately transferred into *Drosophila* Ringer containing [^{3}H]uridine (500 μCi/ml, sp. activity 12,000 μCi/mM, obtained from BARC, Bombay) and incubated for 10 min. The procedure for chromosome preparation and processing for autoradiography was as described previously[8]. Photomicrographs and grain counts were obtained on a Zeiss Photomicroscope III.

Grain count analysis

[^{3}H]Uridine-induced silver grains over the 21A-22F region of 2L in both hybrids (I and II) and Oregon R^+ were counted under a 100× (oil-immersion) objective. In all cases, the number of grains on the tip of 2R (59A-60F) were counted for use as internal standard. Besides, grains were counted on a specific region of 2L, viz. 21ABC in hybrid I, as this region remains in an asynapsed

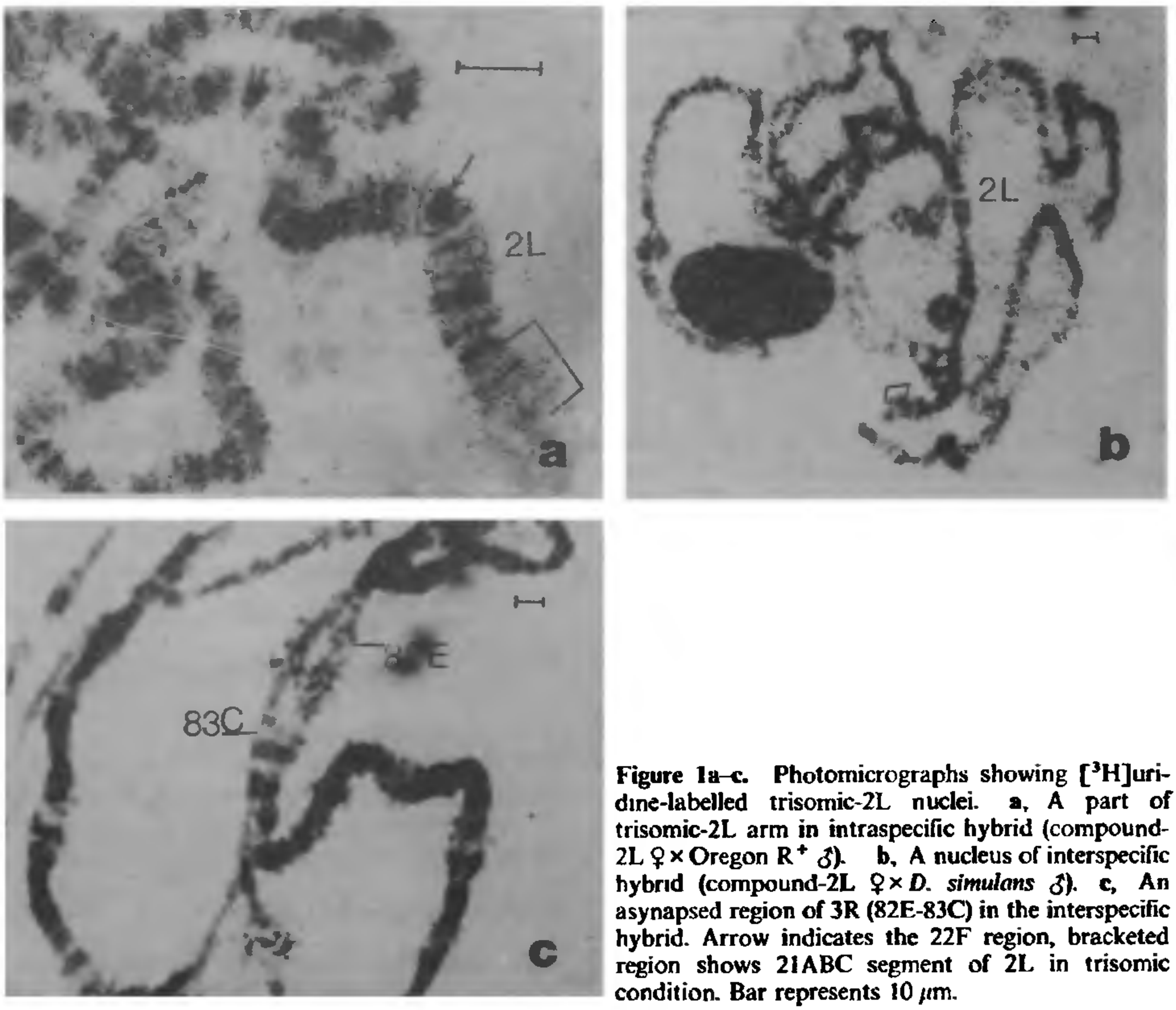

Figure 1a–c. Photomicrographs showing [^{3}H]uridine-labelled trisomic-2L nuclei. **a**, A part of trisomic-2L arm in intraspecific hybrid (compound-2L ♀ × Oregon R^+ ♂). **b**, A nucleus of interspecific hybrid (compound-2L ♀ × *D. simulans* ♂). **c**, An asynapsed region of 3R (82E-83C) in the interspecific hybrid. Arrow indicates the 22F region, bracketed region shows 21ABC segment of 2L in trisomic condition. Bar represents 10 μm.

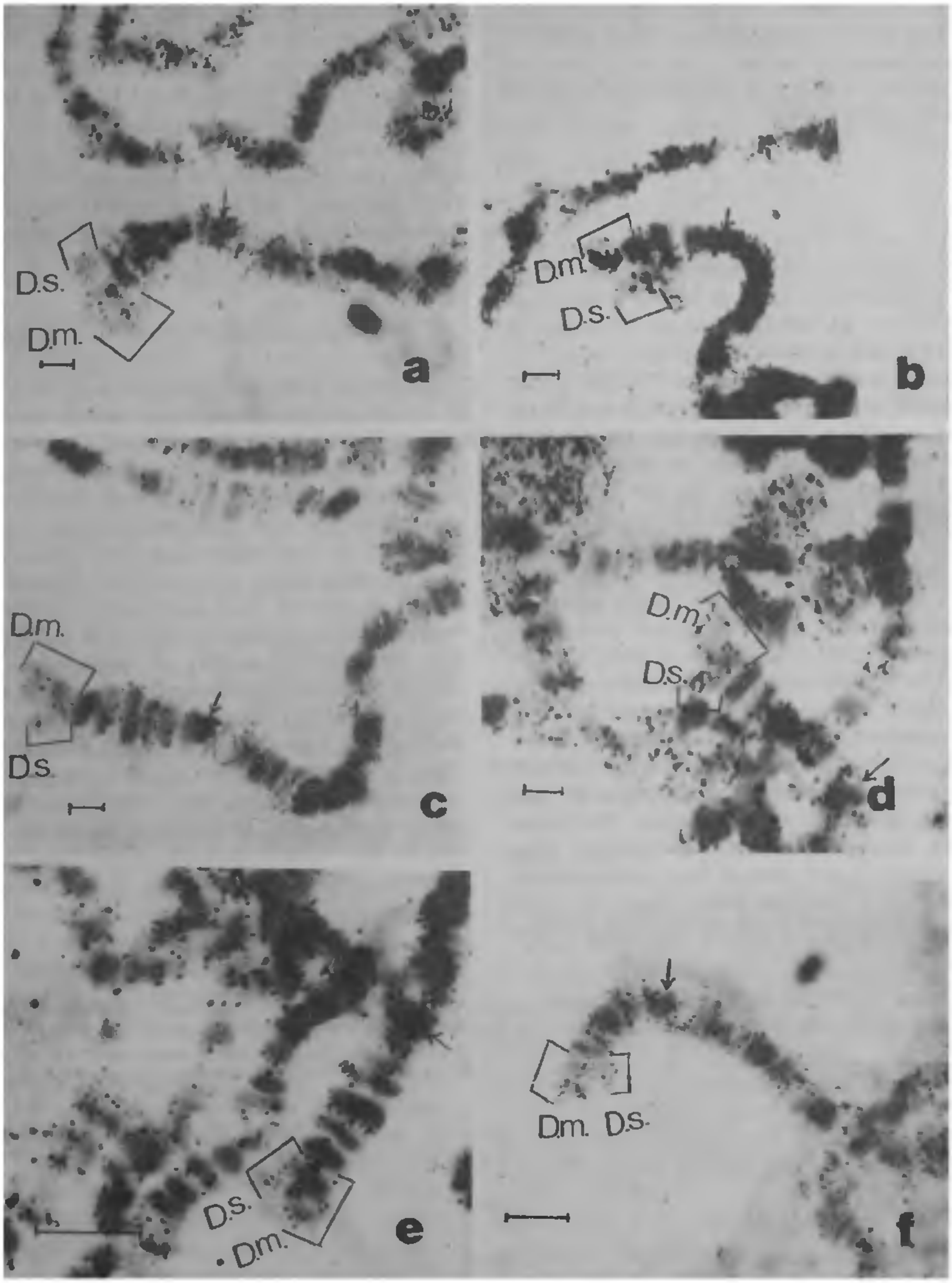

Figure 2a–f. Photomicrographs showing [^{3}H]uridine incorporation in an asynapsed region of 2L (21ABC) in different nuclei of the interspecific hybrid (compound-2L ♀ × *D. simulans* ♂). Bracketed regions show the asynapsed 21ABC region. D.m., *D. melanogaster* homologue; D.s., *D. simulans* homologue.

condition in the majority of nuclei. We also counted the number of [^{3}H]uridine-induced silver grains on the homologues of *D. melanogaster* and *D. simulans* in an asynapsed region (82E-83C of 3R) of a diploid autosomal arm in hybrid I.

RESULTS

In both hybrids (I and II) the hyperploid-2L arm can be distinguished from diploid counterparts by its wider diameter (figure 1a, b). In hybrid I, the tip of 2L (21ABC), i.e. the distalmost segment, remains in asynapsed condition (figure 1b and figure 2a–f).

The mean number of grains on 21A-22F of 2L and 59A-60F of 2R in the two hybrids (I and II) are presented in table 1. The data reveal that although one of the three homologues in trisomic-2L of hybrid I is from *D. simulans*, the 21A-22F region shows a level of transcriptional activity comparable to that of the trisomic-2L of hybrid II, where all three homologues are derived from *D. melanogaster* (21A-22F/59A-60F ratio of grain counts of hybrid II/21A-22F/59A-60F ratio of grain counts of hybrid I=0.93, table 1). The data in table 2 reveal that in hybrid I the transcriptional activity of the 21ABC region of the two homologues derived from *D. melanogaster* is twice as much as that of the single homologue from *D. simulans* and two-thirds as much as that of the two homologues of Oregon R$^+$ (see also figure 2a–f). This finding suggests that transcriptional activity of the two homologues from *D. melanogaster* is at a reduced level in the triploid-2L of hybrid I. On the other hand, the total transcriptional activity of the 21ABC region of the three homologues of hybrid I is equal to that of hybrid II as well as to that of the diploid Oregon R$^+$, suggesting that this region is dosage-compensated in both the hybrids (I and II) at the level of RNA synthesis (table 2).

It is evident from table 2 and figure 1c that in hybrid I the transcriptional activity of the 82E-83C of 3R (asynapsed region) of a single *D. melanogaster* homologue is the same as that of a single *D. simulans* homologue.

DISCUSSION

Studies on whole-arm trisomy of 2L and 3L in *D. melanogaster* suggested that several autosomal segments of a hyperploid autosomal arm synthesize RNA at a level comparable to that synthesized in the diploid condition[3,4]. The present results indicate that the level of activity of the triploid-2L segment of each of the homologues in hybrid I (two derived from *D. melanogaster* and one from *D. simulans*) is similar to that of each homologue of hybrid II, where all three homologues are from *D. melanogaster*. The transcriptional activity of this segment (21A-22F) in the triploid condition (in hybrid I) is shared equally by the two homologues from *D. melanogaster* and the one from *D. simulans*. It is likely that the regulatory sequences involved in transcription of autosomal arms have been conserved in these two species of *Drosophila*. Chatterjee and Ghosh[9] made such a contention while discussing the transcriptional behaviour of the X chromosome in female hybrids of *D. melanogaster* and *D. simulans*. Their results indicated that in the female hybrid, the

Table 1 *[^{3}H]Uridine incorporation in the 2L segment (21A-22F) in hybrids of compound-2L strain and Oregon R$^+$ strain of D. melanogaster (hybrid II), hybrids of compound-2L strain of D. melanogaster and D. simulans (hybrid I), and Oregon R$^+$ flies*

Strain	Dose (2L)	Mean number of grains ± SE: 2L (21A-22F)	Mean number of grains ± SE: 2R (59A-60F)	Mean T2L/2R ± SE	Ratio of T2L/2R values: HII/HI	HII/OR	HI/OR
Compound-2L ♀ × Oregon R$^+$ ♂ (HII)	3	122.90 ± 11.76 (11)	100.20 ± 9.94 (11)	1.22 ± 0.017 (11)	0.93	1.01	1.09
Compound-2L ♀ × *D. simulans* ♂ (HI)	3	116.92 ± 12.26 (14)	89.64 ± 10.24 (14)	1.31 ± 0.03 (14)			
Oregon R$^+$ (OR)	2	41.58 ± 2.42 (13)	34.76 ± 2.57 (13)	1.20 ± 0.02 (13)			

Figures in parentheses are number of nuclei examined. HII, Hybrid II; HI, Hybrid I.

Table 2 *[^{3}H]Uridine incorporation in the 21ABC region of 2L in hybrid I, hybrid II and Oregon R$^+$*

Strain	Dose (21ABC)	Mean grain ratio of 21ABC and 59A-60F (2R) (21ABC/2R)	Ratio of 21ABC/2R values					Mean grain ratio of 82E-83C and 59A-60F (2R) (82E-83C/2R value ± SE)
			HII/HI	HII/OR	HI/OR	HIa/HIb	HIa/OR	
Compound-2L ♀ × Oregon R$^+$ ♂	3 (all D.m.)	0.48 ± 0.01 (HII) (11)	1.04	0.96	0.92	2.06	0.62	
Compound-2L ♀ × *D. simulans* ♂	3 a.2-D.m.	0.31 ± 0.02 (HIa)						0.38 ± 0.01*
	b.1-D.s.	0.15 ± 0.01 (HIb) (16)						0.36 ± 0.04* (12)
Oregon R$^+$	2 (all D.m.)	0.50 ± 0.01 (OR) (13)						

D.m., *D. melanogaster* homologue; D.s., *D. simulans* homologue.
Figures in parentheses are number of nuclei examined.
*Transcriptive activity of two homologues of an asynapsed region of 3R (82E-83C) in hybrid I nuclei.

X chromosomes of both *D. melanogaster* and *D. simulans* transcribe equally. In this case, X chromosome transcription is regulated by the combined action of the haploid autosomal complements of each species. Lakhotia *et al.*[10] studied the transcriptional behaviour of the haplo-X chromosome of *D. simulans* in a *D. melanogaster* background. They used the property of unstable transmission of the ring X chromosome of *D. melanogaster* to generate clones of XO cells in the salivary glands of female hybrids. Their results reveal that the single X of *D. simulans* maintains its normal hypertranscriptional behaviour in the *D. melanogaster* background.

It has been contended by earlier workers[2,5,11] that autosomal arms in hyperploid condition show regulation of transcription through a suppression of activity per gene. The present results reveal that transcription from 21ABC in 2L-trisomy is about the same in hybrids I and II and shows compensation for gene dosage when compared with transcription from the same region in diploid Oregon R$^+$ strain flies (see also Ghosh[3]). It is interesting that 21ABC of the two homologues derived from *D. melanogaster* in hybrid I is transcriptionally two-thirds as active as 21ABC of diploid Oregon R$^+$. This finding strongly supports the idea that equalization of gene product in hyperploid condition is achieved by the suppression of activity per gene at the level of transcription[2,5,11].

On the basis of the transcriptional behaviour of the triploid segment in hybrids I and II, it may be suggested that the regulatory sequences involved in autosomal gene regulation are conserved in *D. melanogaster* and *D. simulans* and that autosomal dosage compensation in hyperploid condition is achieved by the suppression of activity per gene rather than by hyperactivation as in the case of haplo-X of male *Drosophila*.

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