made, however, is that the analysis does not rule out the possibility of existence of a deletion as the cause of its slight early finishing.

Figures 2a and b present an initial and a terminal labelling pattern, respectively, of the paired X chromosomes in In(1)d149/+ heterozygote. No study on the homozygous female bearing the inversion has been possible as the inversion bearing X also carries a lethal on the tip of it. In figure 2a, which shows an early pattern, the unit 4DEF, being late replicating is unlabelled in both homologues, whereas 11EF, being early replicating is labelled completely across the bands. In contrast, in Figure 2b (in late phase of replication) the 4D is completely labelled on both homologues, while 4F-4E1.11F3 segment is labelled on the inverted segment but in the normal homologue the 11EF part is unlabelled. The 4EF section is also labelled in the normal homologue (hidden under 4D in the band of the loop in Fig. 2b).

The histogram of the frequencies of labelling [obtained from 22 labelled nuclei of middle to late pattern of replication, each in control and 1(1)J1, In(1)d149/+ genome] presented in Figure 3, corroborates the observations on the autoradiogram. Here in the inversion bearing chromosome, the frequency of 4D1-7 (to the left of the arrow on the segment 4) remains unaltered, whereas that of 4EF.11F3 drastically increases by a factor of about 3. Thus, it appears that in this case the late replicating property is inherent in the 4EF section. It may be noted that some other sites (namely 6DEF, 7F, 9C, 10B, 10DEF and 11B) also show some difference in frequency from normal.

No data on intensity of site-wise labelling has been presented for this inversion heterozygote, as such intensity data may be incorrect due to introduction of change in geometrical configuration of the segments involved which might arise out of pairing in the inversion heterozygote. However, a total grain count data over 1A-4BC, which is outside the inversion, and that over 4D-12A which includes the inversion, has been presented in Figures 4a and b. It is interesting to note there that when analysis is made considering all middle to terminal pattern labelled chromosomes (3D-1D type), the regression slopes do not show significant deviation. However, when the analysis is delimited by considering only the grain range of 0-90, a limit up to which no super-imposition or "coincidence" is detectable, the regression slope of grain number in +/+ and the inversion heterozygotes significantly deviated from each other (Fig. 4b). This finding is a strong evidence to support that the alteration in the sequence of the constituent subunits of the replicating unit affects the terminal patterns more significantly than the initial patterns. The results of the present investigation have therefore revealed that the rearrangement of polytene chromosomes might affect the kinetics of DNA synthesis by establishing new patterns of control.

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Effect of α-amanitin on the DNA synthesis in the polytene chromosome of **Drosophila melanogaster.**

In the giant cells of dipteran larvae, α-amanitin, a potent inhibitor of eukaryotic RNA polymerase II, inhibits chromosomal RNA synthesis but not the nucleolar RNA synthesis at a concentration range of 20 to 30 µg/ml (Beermann 1971; Chatterjee & Mukherjee 1982, 1984). α-amanitin is also known to inhibit DNA synthesis in eukaryotes (Montecuccoli et al. 1973; Chatterjee & Mukherjee 1977). It has been suggested that the toxin, α-amanitin inhibits the initial and middle part of S phase probably through the inhibition of the primer RNA. An investigation was therefore undertaken to find out whether the inhibition of DNA synthesis by α-amanitin is causally related to the inhibition of RNA polymerase II by the inhibitor. For this reason, parallel pulse labelling with ^{3}H-UR and ^{3}H-TdR of the two contralateral glands of a pair was performed.

For this series of investigations, salivary glands from late third instar larvae of **Drosophila melanogaster** were dissected out in buffered Drosophila Ringer (pH 7.2) and incubated for 20 minutes in α-amanitin (Conc. 20 µg/ml). One gland from the pair was transferred to ^{3}H-UR (300 µCi/ml, Sp. activity 7500 mCi/mM obtained from BARC, Bombay, India) containing Ringer and the remaining one was transferred to ^{3}H-Tdr Ringer (Conc. 500 µCi/ml, Sp. activity 6500 mCi/m mole, obtained from BARC, Bombay, India). Cytological preparations of chromosomes were then made and processed for autoradiography.

Results of these experiments are presented in Table 1. Since it was noted that the amatoxin fails to produce a selective inhibition of DNA synthesis only in male or only in female nuclei, the data from both sexes have been pooled together. In Table 2, the glands receiving ^{3}H-TdR were designated as 'A' (contralateral gland) and those receiving ^{3}H-UR, in the contralateral counterparts were coded as 'B'. Data presented in Table 1, show that as it was observed earlier (Chatterjee & Mukherjee 1977), α-amanitin

Table 1. Analysis of the frequency of ^{3}H-TdR labelled chromosomes and nucleoli in the contralateral glands of **D.melanogaster** after in vitro treatment with alpha-amanitin.

	Number of ^{3}H-Tdr labelled nuclei						Number of ^{3}H-UR labelled nuclei		
Gland number	Total number of nuclei observed	Only nucleoli labelled pattern (NL)	Early pattern (DD-2C)	Mid pattern (3C-3D)	Terminal pattern (2D-1D and chromocentre labelled)	Gland number	Total number of nuclei observed	Only nucleoli labelled pattern	Both chromosomes/nucleoli and nucleolus labelled
1A	123	--	--	9 (7.31%)	3 (2.45%)	1B	125	27 (21.60%)	23 (18.40%)
2A	90	1 (1.11%)	19 (21.11%)	2 (2.22%)	6 (6.67%)	2B	72	12 (16.44%)	14 (19.15%)
3A	53	1 (1.88%)	--	3 (5.66%)	3 (5.66%)	3B	74	24 (32.43%)	18 (24.32%)
4A	100	--	--	1 (1.00%)	2 (2.00%)	4B	26	--	--
5A	66	--	--	41 (62.12%)	17 (25.75%)	5B	21	14 (66.62%)	7 (33.33%)
6A	43	--	--	2 (4.65%)	--	6B	19	--	3 (15.79%)
7A	98	--	--	--	--	7B	86	--	1 (1.16%)
8A	104	--	2 (1.92%)	24 (23.08%)	37 (35.58%)	8B	50	2 (4.00%)	3 (6.00%)
9A	90	1 (1.11%)	2 (2.22%)	5 (5.55%)	11 (12.22%)	9B	134	44 (32.84%)	42 31.34%)
10A	58	9 (15.51%)	3 (5.17%)	2 (3.45%)	1 (1.72%)	10B	181	44 (24.31%)	84 (46.41%)
11A	71	1 (1.11%)	4 (5.63%)	8 (11.27%)	24 (33.80%)	11B	104	50 (48.08%)	52 (50.00%)
12A	56	--	--	--	--	12B	55	--	1 (1.82%)
13A	55	--	--	2 (3.64%)	1 (1.82%)	13B	45	2 (4.44%)	14 (31.11%)
14A	71	--	--	--	--	14B	121	17 (14.05%)	5 (4.13%)
15A	90	--	--	9 (10.00%)	7 (7.79%)	15B	35	6 (17.14%)	4 (11.13%)
16A	133	--	--	12 (9.02%)	8 (6.02%)	16B	79	6 (7.59%)	4 (5.06%)

drastically inhibits the initial phase of replication in the majority of the glands. Only in a few cases (see Table 1, Gland Nos. 2, 3, and 9-11), a small amount of early phase of DNA replication was observed. It was observed that the percentage of ^{3}H-UR labelled nuclei (both chromosome and nucleolus) ranged from approximately 1 to 48 (Table 1). Detailed analysis of the data presented in Table 1 reveals that there is no correlation between the ^{3}H-UR labelling on the nucleolus and the ^{3}H-TdR nucleolus labelled (NL) pattern. This is expected, since α-amanitin does not inhibit the activity of RNA polymerase I, which is responsible for rRNA (nucleolar RNA) synthesis. On the other hand, in the majority of cases, there is a reasonable correlation between the frequency of ^{3}H-TdR labelled nuclei and the frequency of ^{3}H-UR labelled cells (both chromosome and nucleolus labelled) in α-amanitin treated salivary glands (see Gland Nos. A and B, 1, 6, 7, 9, 12-16). In two pairs (Nos. 5 and 8), lack of correlation between ^{3}H-UR labelled chromosomes and ^{3}H-TdR labelled cells, have been noted. In the two glands there was a higher frequency of 3C-1D pattern of labelling in ^{3}H-thymidine labelled glands. This result is interesting because it appears from the data that α-amanitin, a RNA polymerase II inhibitor, is unable to interfere with the late phase of replication.

Table 2 presents statistical evaluation of the data in Table 1. The statistical evaluation of the data based on the correlation coefficient, clearly corroborates the above interpretation. The analysis of correlation was from the percent of frequencies of ^{3}H-TdR labelled cells and ^{3}H-UR labelled chromosome, as well as from the percent of frequencies of 3D-1D patterns. Results reveal that the measured values of correlation coefficients (r_i) of the three successive combinations (see Table 2) are 0.47, 0.35 and 0.93, respectively. All these values are positive and transcribe the fact that the correlation between the percent of ^{3}H-TdR labelled cells and that of ^{3}H-UR labelled chromosomes, as well as between the

Table 2. Analysis of the degree of correlation between ^{3}H-TdR and ^{3}H-UR labelling frequency in contralateral experiments of salivary gland chromosome of **Drosophila melanogaster** after in vitro treatment with alpha-amanitin.

Gland number	Frequency of ^{3}H-TdR labelled nuclei	Frequency of ^{3}H-UR labelled chromosomes	Frequency of mid to terminal pattern of ^{3}H-TdR labelled nuclei	Slope (a)	Intercept (b)	Correlation coefficent (r)	Multiple correlation coefficient ($r_{1.23}$)
1A/1B	9.75	18.40	9.75				
2A/2B	31.11	19.14	8.89				
3A/3B	13.21	24.32	12.22				
4A/4B	3.00	0.00	3.00				
5A/5B	87.88	33.33	87.88				
6A/6B	4.65	15.79	4.65	$b_{12.3}$=0.25	0.85	r_{12}=0.47	0.94
7A/7B	0.00	1.16	0.00	$b_{13.2}$=0.89		r_{23}=0.35	
8A/8B	60.50	6.00	58.66			r_{13}=0.93	
9A/9B	21.11	31.34	17.70				
10A/10B	25.86	46.41	5.17				
11A/11B	52.11	50.00	45.07				
12A/12B	0.00	1.00	0.00				
13A/13B	5.45	31.11	5.45				
14A/14B	0.00	4.13	0.00				
15A/15B	17.78	11.43	17.78				
16A/16B	15.04	5.06	15.04				

percent of ^{3}H-TdR labelled cells and that of 3C-1D patterns are significant at 5% level (Fisher's 'r' significant test Table). On the other hand, the correlation coefficient for the relation between the percent of ^{3}H-UR labelled chromosomes and that of 3D-1D patterns of ^{3}H-TdR labelling is not significant (r = 0.35). It appears therefore that there is a reasonably good correspondence between the proportion of ^{3}H-TdR labelled cells and that of ^{3}H-uridine labelled chromosomes. Conversely, therefore, the inhibition of ^{3}H-UR incorporation parallels the inhibition of ^{3}H-TdR incorporation by α-amanitin. A multiple correlation test on the percent of ^{3}H-uridine labelled chromosomes, the percent of all ^{3}H-thymidine labelled cells and the percent of 3D-1D patterns, yields a high positive correlation coefficient ($r_{12.3}$ = 0.94). Thus, the result shows clearly that the in vitro treatment of salivary glands with α-amanitin fails to intercept the labelling of late patterns (3D-1D), but drastically interferes with the initiation of replication. Therefore, the result of contralateral experiments suggest the inhibition of the initial pattern by α-amanitin is causally related to the inhibition of RNA polymerase II. So, on the basis of these observations, it may be suggested that the synthesis of RNA and initiation of DNA synthesis are closely coupled, and that disorder in the first process affects the process of initiation of DNA synthesis.

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Collins, M.F. and J.K. Hewitt. University of Birmingham, Birmingham, England. Correlations between the elements of male courtship behavior in a series of inbred lines of **Drosophila melanogaster** derived from the same population.

The male mating behavior of a series of 16 inbred lines of **D.melanogaster** derived the Texas (Barnes & Kearsey 1970) population was recorded using the time-sampling technique described in Collins, Hewitt & Gogarty (1984). The male courtship behavior in pairs of three day old male and female flies was scored, using Texas 15 females as a common tester genotype. The experiment was carried out in a fully randomised block design, consisting of ten blocks of five replicates. All courtships were recorded within the first four hours of a 12 hour day/night light cycle at 25°C.