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Replication in *Drosophila* Chromosomes

VII. Influence of Prolonged Larval Life on Patterns of Replication in Polytene Chromosomes of *Drosophila melanogaster*

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Abstract. Prolongation of larval life in *Drosophila melanogaster*, by growing wild type larvae at lower temperature, or in animals carrying the X-linked mutation *giant* is known to result in a greater proportion of nuclei in salivary glands showing the highest level of polyteny. We have examined by autoradiography the patterns of ^{3}H-thymidine incorporation during 10 min or 1 min pulses in salivary gland polytene chromosomes of older *giant* larvae and of wild type late third instar larvae of *D. melanogaster* grown since hatching either at 24° C or at 10° C. The various patterns of labelling and their relative frequencies are generally similar in glands from the warm- (24° C) or cold (10° C)-reared wild type larvae, except the interband (IB) labelling patterns which are very frequent in the later group but rare in the former. The IB type labelled nuclei in cold-reared wild type larvae show labelling ranging from only a few puffs/interbands labelled to nearly all puffs/interbands labelled. In warm-reared wild type larvae, very low labelled IB patterns are not seen. In older *giant* larvae, the ^{3}H-thymidine labelling patterns are in most respects similar to those seen in cold-reared wild type larvae. In 1 min pulsed preparations from all larvae, the IB patterns are relatively more frequent than in corresponding 10 min pulsed preparations. No nuclei with the continuous (2C or 3C) type of labelling pattern, with all bands and interbands/puffs labelled, were seen in 1 min pulsed preparations from cold-reared wild type or in *giant* larvae, and only a few nuclei in 1 min pulsed preparations from warm-reared wild type larvae exhibited the 2C labelling pattern. Analysis of silver grain density on specific late replicating sites in late discontinuous (1D) type labelled nuclei suggests that the rate of DNA synthesis per chromosomal site is not different at the two developmental temperatures. It is suggested that correlated with the prolongation of larval life under cold-rearing conditions or in *giant* larvae, the polytene replication cycles are also prolonged. It is further suggested that the polytene S-period in these larvae is longer due to a considerable asynchrony in the initiation and termination of replication of different sites during a replication cycle.

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Introduction

Different nuclei in the salivary glands of larvae of *D. melanogaster* grown at 24° C attain varying levels of polyteny, with the posteriormost nuclei achieving the highest polyteny (8 cycles) and the anterior nuclei stepwise decreasing polytenic levels (Rodman, 1967; Rudkin, 1972). Larvae grown at lower temperatures (10° C–15° C) take 5 to 7 times longer to pupate and yet the highest polyteny levels achieved by any nucleus in these cold-reared larvae is the same as in warm-reared larvae (Rodman, 1967; Hartmann-Goldstein and Goldstein, 1979a). However, at lower growth temperatures, the overall polyteny levels in salivary glands are somewhat higher since most of the other nuclei undergo more endoreplication cycles than at 24° C (Hartmann-Goldstein and Goldstein, 1979a). A comparable change is seen in polytene nuclei of salivary glands in larvae homozygous for the X-linked mutant allele *giant* (*gt*, Lindsley and Grell, 1968) grown at 24° C; in these larvae pupation is delayed and the polytene chromosomes appear thicker and morphologically similar to those seen in wild type larvae grown at 10° C. Presumably, the overall polyteny levels in *giant* larvae are also increased as in cold-reared wild type larvae. Thus the growth temperatures and certain genotypes appear to influence the replicative programme of polytene nuclei. In order to understand the underlying mechanisms which may bring about these changes, we have examined the autoradiographic patterns of ^{3}H-thymidine labelling of polytene chromosomes in salivary glands from wild type late third instar larvae grown at 10° C or at 24° C and in *giant* larvae grown at 24° C. In this communication, we present our data on the differences in general patterns of ^{3}H-thymidine labelling of polytene chromosomes in relation to growth temperatures and genotypes. Our observations suggest that the polytene S-period, particularly its initial part, is considerably prolonged in cold-reared wild type and in *giant* larvae. Apparently, this prolongation of an S-period is not brought about by a slower rate of replication, but by a greater asynchrony in initiation and termination of replication of different sites in a nucleus.

Materials and Methods

A wild type strain (Oregon R^{+}) of *Drosophila melanogaster* and a mutant stock, *gt* w^{a} (for details, see Lindsley and Grell, 1968) were used for the present study. The flies were raised at 24° ± 1° C and eggs were collected from healthy flies in agar-cornmeal-brownsugar food at intervals of 1 h and allowed to hatch at 24° C. Immediately after hatching (about 22 h post oviposition), one group of wild type larvae was transferred to 10° ± 2° C and grown there (cold-reared larvae) while the *giant* and other wild type larvae were grown at 24° ± 1° C (warm-reared larvae). The larvae were cultured in Petri dishes under uncrowded conditions, and the food was periodically supplemented with live yeast. In the cold-reared group, the polytene nuclei from salivary glands suitable for autoradiography can be obtained from the 28th to 32nd day after hatching. Some heterogeneity in the development of different cold-reared larvae exists, since different larvae pupate between the 32nd to 35th day and a few fail to pupate even after 35 days. Imagos do not hatch if pupae are kept at 10° C, but when fresh pupae are transferred to 24° C, healthy and fertile flies can be obtained. Different *giant* larvae grown at 24° C pupate between the 5th and 15th day. For autoradiographic studies, salivary glands were excised from wild type third instar larvae (112–116 h after oviposition in the case of warm-reared and 30 days after hatching in the case of cold-reared ones); *giant* larvae were dissected on 8th and 12th day after oviposition. Freshly dissected sister

salivary glands from warm-reared wild type and *giant* larvae were separated and pulse labelled with ^{3}H-thymidine (Sp. Act. 22 Ci/mM, BARC, Trombay) for 10 min (act. 250 μCi/ml) or for 1 to 2 min (act. 1,000 μCi/ml) at 24° C. Similarly, freshly dissected salivary glands from cold-reared larvae were pulse labelled with ^{3}H-thymidine for 10 min or for 1 to 2 min at 24° C; in this case sister glands were not used for the two pulse durations. The medium used for pulse labelling contained only the inorganic salts and the organic acids of Poels' medium (1972). The labelled glands from cold- and warm-reared larvae were fixed and squashed in the routine manner. All preparations were processed for autoradiography with Ilford L4 or K5 emulsion, as described earlier (Lakhotia and Roy, 1979). The autoradiographic exposures were 9 days (10 min pulse) or 10 days (1 min pulse) at 4–6° C.

To analyse the replication patterns in prepupal salivary glands from the cold-reared group, fresh white prepupae were dissected on the 32nd day. Their salivary glands were pulse labelled with ^{3}H-thymidine for 10 min (act. 250 μCi/ml; sp. act. 22.0 Ci/mM) and processed for autoradiography as above. The patterns of labelling of different nuclei in the autoradiograms were examined under 100X oil immersion objective. All the preparations were coded before examination.

Results

1. Frequencies of Different ^{3}H-Thymidine Labelling Patterns in Warm- and Cold-reared Wild Type Larvae

As has been noted by earlier workers (Hartmann-Goldstein and Goldstein, 1979a), the polytene chromosomes from cold-reared larvae show superior cytological qualities in their spreading behaviour, width and banding patterns as compared with those from warm-reared larvae. In our autoradiographic preparations from ^{3}H-thymidine pulse labelled salivary glands of warm- as well as cold-reared late third instar larvae, we have observed that the nuclei with larger chromosomes (i.e., with higher polyteny levels) appear to be labelled as often as those with lower polyteny levels.

For comparing the frequencies of different ^{3}H-thymidine labelling patterns of polytene chromosomes from 10 min or 1 min pulsed glands, nuclei with very low polyteny levels have not been scored. The labelled nuclei have been classified into seven different categories of labelling patterns following Rodman (1968) and Roy and Lakhotia (1979, 1981). The data presented in Table 1 reveal interesting differences in the frequencies of different patterns, particularly the interband patterns between cold- and warm-reared larvae.

It is seen that in 10 min pulsed preparations, the frequency of interband (IB) labelling patterns in cold-reared larvae is considerably higher than in warm-reared larvae. Apart from this increased frequency, different grades of IB labelling, in terms of the number of labelled sites and their labelling intensity, are also observed in cold-reared larvae. Two major categories of IB patterns are discernible at the low developmental temperature. One group shows labelling over only 10–40 puff and/or interband sites in the whole nucleus with a low density of silver grains (Fig. 1) and in the other group, many to nearly all of the puffs and interbands, along with some faint bands are labelled with moderate to heavy grain density. These two categories, with reference to the autoradiographic labelling patterns observed in polytene chromosomes of *D. kikkawai* and *D. nasuta* (Roy and Lakhotia, 1979, 1981) are referred to as mid (MIB) and heavy (HIB) interband patterns, respectively. In view of the present

Table 1. Frequencies of different autoradiographic labelling patterns after 10 or 1 min ^{3}H-thymidine 10° C, and *giant* larvae reared at 24° C

Genotype	Larval age	Rearing temperature	Pulse duration	Total no. of nuclei observed[a]	No. of labelled nuclei (%)[b]
Wild type	112–116 h	24° C	10 min	1345 (12)	927 (68.9)
"	"	"	1 min	1214 (12)	313 (25.8)
"	30 days	10° C	10 min	2497 (18)	1241 (49.7)
"	"	"	1 min	2362 (15)	393 (16.6)
"	32 days (prepupae)	"	10 min	1339 (10)	344 (25.7)
giant	8 days	24° C	10 min	292 (4)	69 (23.6)
"	"	"	1 min	303 (4)	34 (11.2)
"	12 days	"	10 min	460 (6)	35 (7.6)
"	"	"	1 min	406 (6)	24 (5.9)

[a] Figures in parentheses indicate the number of larvae examined
[b] Figures in parentheses are % values

concept of the temporal order of the different labelling patterns during a polytene S-period (Hägele, 1973; Chatterjee and Mukherjee, 1975; Roy and Lakhotia, 1979), it is assumed that amongst the nuclei showing the IB labelling pattern, the fewer the number of labelled puff/interband sites in a nucleus, the earlier is its position in the polytene S-period. Only one nucleus with MIB pattern of labelling has been seen in the 10 min pulsed glands from warm-reared wild type larvae. The earliest initial patterns generally observed in these preparations of warm-reared larvae correspond to the HIB stage.

Taking advantage of the presence of such early labelling patterns and the good cytological qualities of polytene chromosomes in cold-reared larvae, we have identified the sites which initiate a polytene S-period in salivary glands of cold-reared larvae. The labelled sites in different chromosome arms of 15 well-spread female nuclei showing MIB patterns have been scored. For any site to be labelled the presence of 3 silver grains at the site was taken as the minimum. The frequencies with which the different interband or puff regions have been seen to be labelled in these 15 MIB type nuclei are given in Table 2. On the basis of these frequencies it would appear that in cold-reared larvae, a polytene replication cycle is initiated by DNA synthesis at the 2B, 71DE, 74EF, 75B and 78D puff sites. The interbands and puffs which are seen to be labelled with progressively lower frequency among the MIB type nuclei, apparently start replicating later in more advanced MIB patterns.

The different continuous and discontinuous types of ^{3}H-thymidine labelling patterns do not show much difference in their frequencies in 10 min pulsed glands from cold- and warm-reared larvae (Table 1) except for the somewhat lowered frequency of 1D patterns in cold-reared as compared with that in warm-reared larvae (see Table 1). However, we cannot say if these differences

pulse labelling of salivary glands of *D. melanogaster* larvae and prepupae reared at 24° C or at

Frequencies (%) amongst labelled nuclei						
Interband (IB)		Continuous (C)		Discontinuous (D)		
Mid (MIB)	Heavy (HIB)	Mid (2C)	Heavy (3C)	Heavy (3D)	Mid (2D)	Low (1D)
0.1	1.3	5.1	4.6	7.4	7.1	74.2
2.5	6.1	1.9	–	11.5	8.0	70.0
2.0	5.9	3.7	9.8	12.3	13.4	52.9
7.9	22.7	–	–	24.2	15.3	30.0
–	1.2	0.9	2.9	13.7	9.0	72.4
2.9	10.1	15.9	5.8	17.4	17.4	30.4
26.5	20.6	–	–	26.5	5.9	20.6
5.7	14.3	17.1	5.7	20.0	11.4	25.8
20.8	33.3	–	–	25.0	4.2	16.7

in the 10 min pulsed preparations have any real significance or just reflect the variability in relation to larval ages.

In 10 as well as 1 min pulsed glands from cold-reared larvae and in 1 min pulsed glands of warm-reared larvae, a few labelled nuclei classified as 3D or 2D, differed in some respects from the simultaneously present typical 3D and 2D type labelled nuclei (Fig. 2a–d). In these variant types of 3D and 2D type nuclei seen in cold-reared larvae, the number of labelled or unlabelled sites are similar to those in the typical patterns of respective categories, but the grain density at the labelled sites is significantly lower. Thus, in the variant nuclei with 3D type of labelled sites distribution, the grain density is comparable to that typically seen in late 2D (Fig. 2a–b), while in the variant 2D nuclei, the grain density is like that in typical 1D nuclei (Fig. 2c–d).

The frequency of labelled nuclei in 1 min pulsed glands in warm- as well as cold-reared larvae has been seen to be much lower than in 10 min pulsed sister (warm-reared) or non-sister (cold-reared) glands. However, with respect to the distribution of labelled sites, the labelled nuclei in 1 min pulsed preparations are very closely similar to those seen in 10 min pulsed preparations. A comparison of the sites labelled in IB and 1D type labelled nuclei from 10 and 1 min pulsed preparations confirms the identity of different labelling patterns after the two pulse timings. Representative examples of labelling on the 56A–60F segment of 2R in IB and 1D type labelled nuclei seen in 10 or 1 min pulsed preparations from warm- and cold-reared larvae are shown in Figures 3 and 4. These examples very distinctly show that the labelling patterns seen after 1 min pulse in this study are not aberrant patterns resulting from the brief duration of the pulse but are typical patterns similar to those seen after the usual 10 min pulse.

Compared to 10 min pulsed preparations, the relative frequency of labelled nuclei showing the IB labelling patterns among the labelled nuclei is considerably

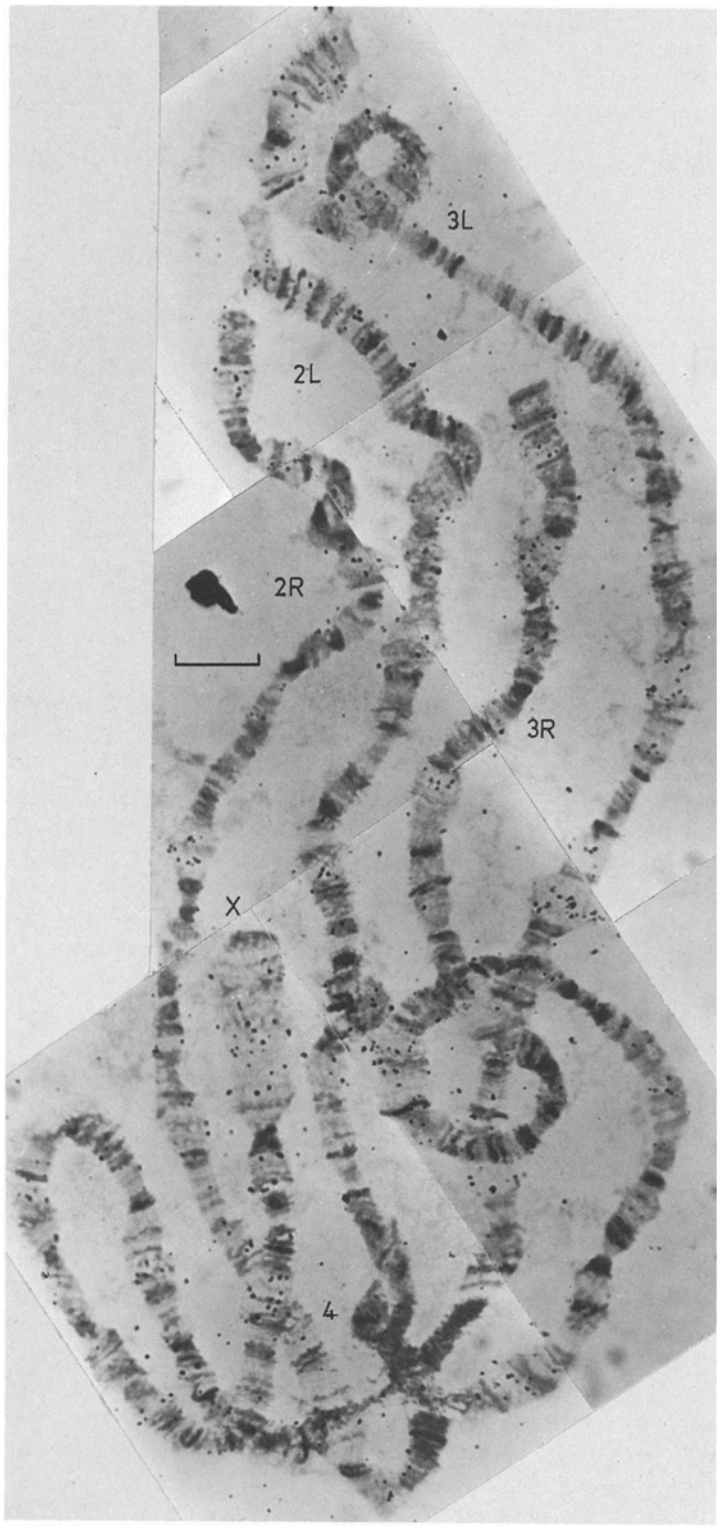

Fig. 1. A mid-interband type labelled nucleus from 10 min ^{3}H-thymidine pulsed preparation of cold-reared wild type larva. Note the low labelling of a few puff and interband sites. The horizontal bar in this and other figures represents 10 μm

Table 2. Labelling frequencies of different dispersed (puff or interband) regions in 15 MIB type nuclei in 10 min ^{3}H-thymidine pulsed preparations of cold-reared female larvae

Labelling frequency class (%)	Sites
96–100	2B, 71DE, 74EF, 75B, 78D
81–95	34F, 50CD, 58DE, 82ABC
51–80	15DEF, 21A, 36F, 56D, 61A, 72D, 82DEF, 85F, 88EF, 93B, 95B, 95F
31–50	23C, 42A, 43E, 44AB, 47A, 60E, 62A, 84BC
11–30	4A, 10A, 22B, 25BC, 28D, 32CD, 33B, 35B, 83E
5–10	3F, 98F

higher in 1 min pulsed preparations from cold- as well as warm-reared larvae. MIB type labelled nuclei are also seen in 1 min pulsed glands from warm-reared larvae. They are comparable to the more advanced MIB type nuclei seen in cold-reared larvae. In 1 min pulsed glands from cold- as well as warm-reared larvae, the increase in the frequency of IB patterns is accompanied by the *total* absence of 3C (heavy continuous) patterns and significantly, in 1 min pulsed salivary glands of cold-reared larvae, the 2C (mid continuous) patterns are also absent (Table 1).

In 10 min pulsed salivary glands from cold-reared prepupae, nearly 26% nuclei appear labelled (see Table 1) and among these while the 1D patterns are most common (72%), nuclei showing HIB and continuous types of labelling are also seen.

2. Frequencies of Different ^{3}H-Thymidine Labelling Patterns in Giant Larvae

In the older *giant* larvae reared at 24° C the ^{3}H-thymidine labelling patterns appear similar to those in cold-reared wild type larvae. The absolute frequencies of labelling of polytene nuclei in 8 and 12 days old *giant* larvae (Table 1) are rather low varying from about 24% (10 min pulse, 8 days old larvae) to about 6% (1 min pulse, 12 days old larvae). However, among the labelled nuclei, the MIB and HIB patterns are common as in cold-reared larvae, and in salivary glands pulsed for only 1 min these patterns become predominant since the 2C as well as 3C patterns are entirely absent. In 10 as well as 1 min pulsed preparations from *giant* larvae, some of the 3D and 2D nuclei are of the variant type as seen in preparations from cold-reared wild type larvae.

3. Rate of ^{3}H-Thymidine Incorporation in Polytene Chromosomes from Warm- and Cold-reared Larvae

In general (except, as noted above, the few variant 3D and 2D type nuclei seen in cold-reared larvae), the overall grain densities in nuclei showing different

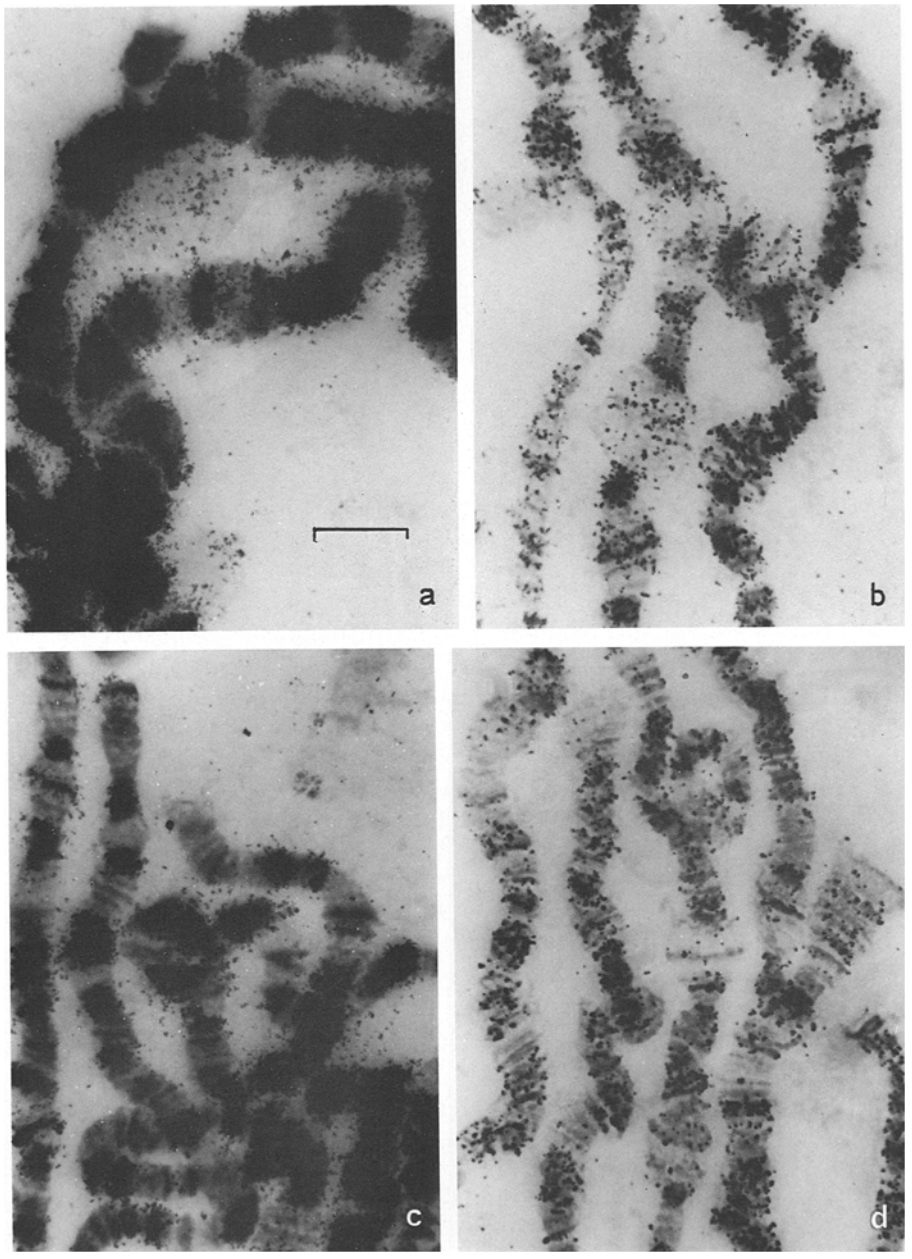

Fig. 2a–d. Examples of the typical (**a, c**) and the variant (**b, d**) 3D (**a, b**) and 2D (**c, d**) types of labelling patterns seen in ^{3}H-thymidine pulsed salivary glands of cold-reared larvae. In the variant types the silver grain distribution is as in respective typical patterns, but the grain density is considerably lower

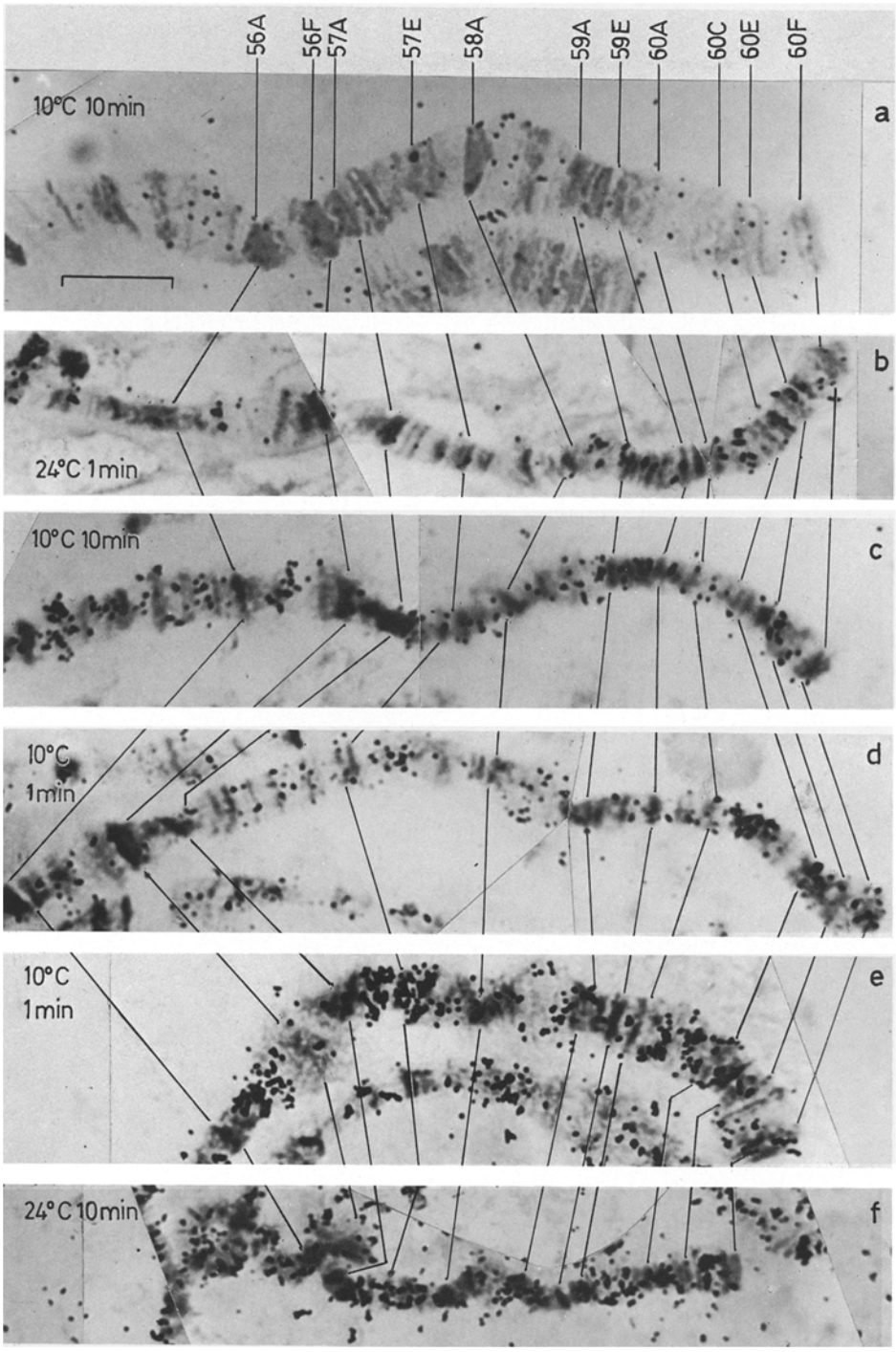

Fig. 3a–f. 56A to 60F segments of 2R with interband type labelling from different preparations of wild type larvae reared and labelled as indicated in each case. The different cut outs have been arranged to indicate the temporal sequence of labelling of different dispersed regions from MIB to HIB patterns. Note the labelling of same disperse sites in the 10 and 1 min pulsed preparations from warm- or cold-reared larvae. Some of the major bands in this segment of 2R are indicated

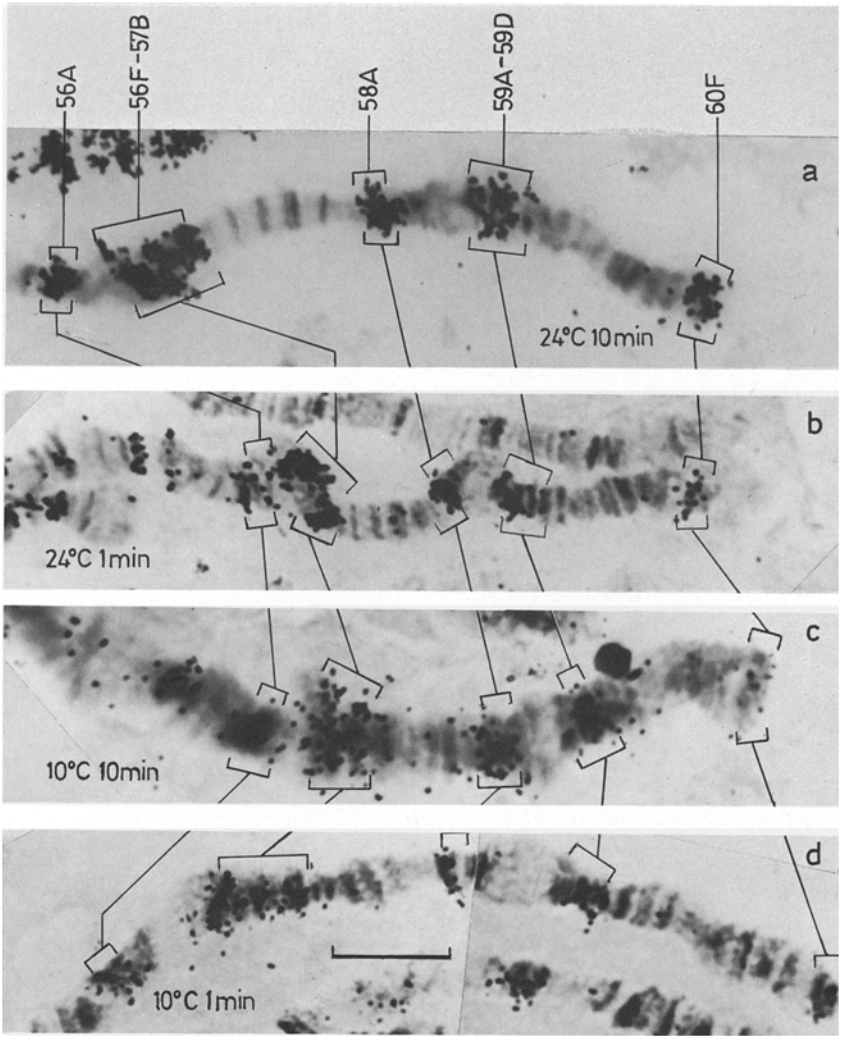

Fig. 4a–d. 56A to 60F segment of 2R with 1D type labelling from different salivary gland preparations from wild type larvae reared and labelled as indicated in each case. In each case the same set of late replicating bands (56A, 56F-57B, 58A, 59CD and 60F) are characteristically labelled irrespective of the pulse duration

types of labelling patterns in cold-reared larvae, but pulsed at 24° C (10 or 1 min), appear similar (Figs. 3 and 4) to those seen in nuclei with corresponding labelling patterns in warm-reared wild type larvae. To more specifically ascertain this aspect, we have counted the number of silver grains present on two late replicating sites viz., 3C on X-chromosome and 75C on 3L in 1D type labelled nuclei after 10 min pulses (at 24° C) with glands from male and female larvae grown at 24° C and at 10° C. The data are presented in Table 3. It is seen that in male as well as in female for both the sites, the mean grain counts

Table 3. Mean number of silver grains on two late replicating sites in 1D type labelled nuclei in 10 min ^{3}H-thymidine pulsed preparations from warm- and cold-reared larvae

Sex	Rearing temperature	Mean (±S.E.) no. of silver grains on	
		3C	75C
Male	24° C	7.17±0.64 (36)[a]	11.59±0.63 (39)
	10° C	12.02±0.94 (41)	16.83±1.27 (36)
Female	24° C	12.06±0.90 (30)	14.79±1.24 (24)
	10° C	16.95±1.05 (61)	18.37±1.17 (66)

[a] Figures in parentheses indicate the number of nuclei examined in each case

On Student's "t" test analysis, the mean number of silver grains in cold-reared larvae is found in each case to be significantly different (higher) from the corresponding value for warm reared larvae.

in preparations from cold-reared larvae are higher than the corresponding counts in warm-reared larvae.

Discussion

Before discussing the significance of the present observations, it may be pointed out that salivary glands from cold-reared larvae show similar ^{3}H-thymidine incorporation patterns whether they are labelled at 24° C or at 10° C; in a separate study (data not presented here) we have seen that qualitatively as well as quantitatively, the different labelling patterns are closely comparable in cold-reared glands labelled at 24° C or at 10° C. A comparison of silver grain densities in 1D type nuclei in these preparations of cold-reared salivary glands shows that the rate of ^{3}H-thymidine incorporation at the two incubation temperatures is not different i.e., the mean number of grains at 3C and 75C regions in 21 1D type nuclei in cold-reared glands labelled at 24° C were 10.38± 1.05 and 12.76±0.92, respectively, while those in 22 1D type nuclei from 10° C labelled glands have been found to be 10.80±0.95 and 11.27±1.01, respectively. Similarly, on the 56F to 60F segment of 2R, the rate of ^{3}H-thymidine incorporation at the two incubation temperatures is similar in 1D type labelled nuclei: the mean grain count on 16 2R segments from 24° C labelled glands and on 10 2R segments from 10° C labelled glands are 135.4±13.3 and 123.7±9.3, respectively. Thus, it appears that temperature between 10° C and 24° C is not rate limiting for ^{3}H-thymidine incorporation in salivary glands of cold-reared larvae. In view of the absence of any apparent effect of incubation temperatures on the rate of ^{3}H-thymidine incorporation and the labelling patterns in cold-reared glands, in the present study we have labelled all cold-reared glands at 24° C because of practical limitations of labelling at 10° C.

The validity of comparing the autoradiographic labelling patterns observed after 1 min and 10 min pulses may also be considered before discussing the results. The significantly lowered frequency of labelled nuclei in 1 min pulsed glands may raise doubts that the labelled nuclei in 1 min pulsed preparations do not show a representative distribution of silver grains on chromosomes

due to the brief period of availability of the labelled precursor. However, all the labelled nuclei in 1 min pulsed preparations from the four sets of larvae could be unambiguously assigned to one of the seven categories of typical labelling patterns seen after a 10 min pulse and secondly, as shown in Figures 3 and 4, the distribution of silver grains on specific chromosome sites in nuclei with corresponding labelling patterns, is similar, if not identical. This means that during the brief pulse period all the replicating sites in a labelled nucleus incorporate ^{3}H-thymidine to be adequately recorded in the autoradiograms. Moreover, we have found (data not presented here) that a longer autoradiographic exposure (up to 20 days) for one min pulsed preparations neither alters the quality of patterns of labelled nuclei, nor their frequencies. Thus, while the reduction in the absolute frequencies of labelling in 1 min pulsed preparations is probably due to lack of transport of the labelled precursor to many nuclei, those nuclei which show labelling, do incorporate the precursor in the typical manner. Thus we believe that the labelled nuclei in 1 min pulsed glands showed true labelling patterns and a comparison of the frequencies of different patterns *among labelled nuclei* in 10 min and 1 min pulsed glands is valid.

As noted in the Introduction, inspite of the large differences in the duration of larval period under cold- and warm-rearing conditions, the highest level of polyteny achieved by salivary gland nuclei has been reported to remain the same (Rodman, 1967; Hartmann-Goldstein and Goldstein, 1979a). Our autoradiographic observations show that in salivary glands of cold-reared wild type late third instar larvae, the ^{3}H-thymidine labelling frequency can be as high as in corresponding larvae grown at 24° C and nuclei with largest sized polytene chromosomes are labelled in all groups of larvae. These observations indicate that under cold-rearing conditions the last polytene replication cycles in different nuclei of salivary glands are progressing or initiated during the late third instar stage just as in warm-reared larvae (Rodman, 1967, 1968). Thus while in warm-reared larvae eight cycles of endoreduplication in some nuclei are completed during the 5 days of larval life, in cold-reared larvae the same number of replication cycles are spread over a period of 30–35 days. A. priori, this suggests that under cold-rearing conditions the duration of polytene replication cycles (synthetic + intersynthetic period) is longer than at 24° C. The polytene S-period may be lengthened at 10° C due to one or more of the following factors which influence the progression of replication in specific ways (Prescott, 1976):

(i) there are fewer origin points at 10° C than at 24° C, resulting in an increased average replicon size and longer time to complete the S-period;

(ii) the rate of replication, i.e., the rate of fork movement is slower at 10° C;

(iii) the number of origin points and the rate of fork movement is the same under the two developmental conditions, but the initiation and termination of replication of different 'replicons' are more asynchronous at 10° C so that the total S-period is longer.

Our present and other (manuscript in preparation) observations suggest that in cold-reared larvae, the polytene S-period is prolonged mainly due to a greater asynchrony in initiation and termination of replication of different sites of a nucleus. Although a more definitive answer about these molecular aspects

must be obtained by the application of DNA-fibre autoradiography techniques, the unique band-interband organization and the large size of polytene chromosomes permits, to some extent at least, the analysis of molecular aspects at the chromosomal level. In the present study, we have noted a general similarity in the quality of different patterns of chromosomal labelling in preparations of cold- and warm- reared wild type larvae. As can be noted from Figures 3 and 4, the number and the cytological extent of independently replicating sites, resolvable at chromosomal level (Rudkin, 1972) in specific segments of polytene chromosomes are closely similar in cold- and warm-reared larvae (a more detailed analysis of replication of specific sites in warm- and cold-reared larvae will be presented elsewhere). These observations suggest that the number of 'replicating units' is not reduced at low temperature as would be the case if the prolongation of polytene S-period were due to a reduced number of origin points. The present observations seem to suggest that the rate of ^{3}H-thymidine incorporation in polytene nuclei in general and also at specific replicating units is not necessarily lower in cold-reared larvae. Thus, at the 3C as well as 75C sites, the mean grain counts in 10 min pulsed preparations from cold-reared larvae were found to be significantly higher than in warm-reared wild type larvae. The higher grain counts in cold-reared larvae could be presumably related to the overall higher levels of polyteny attained under cold-rearing conditions (Hartmann-Goldstein and Goldstein, 1979a), and the consequent higher number of templates at any site, since the relative increase in mean grain counts (Table 3) at a site in cold-reared larvae is comparable to the earlier reported relative increase in the DNA content in these larvae (Fahrig et al., 1968; Hartmann-Goldstein and Goldstein, 1979a). However, the variant 3D and 2D types of labelled nuclei seen in salivary glands of cold-reared larvae may indicate a reduced rate of DNA synthesis at lower temperatures. But since these variant nuclei were fewer than the typically labelled 3D and 2D type nuclei, it is also possible that the relative low degree of ^{3}H-thymidine incorporation seen in them is due to some unspecific effect such as a lower rate of transport of the labelled precursor into some cells in salivary glands. The presence of similar variant 3D and 2D nuclei in 1 min pulsed preparations of warm-reared wild type larvae and also in 10 min pulsed preparations of *giant* larvae supports the later explanation. If the above interpretation about similar rates of DNA synthesis in warm- and cold-reared larvae is valid, the prolongation of the polytene S at low temperatures would be brought about by a greater asynchrony of initiation and termination of replication of different sites in a nucleus. Our observations provide evidence for this. The IB patterns seen in cold reared larvae are comparable to those seen in warm-reared larvae of *D. kikkawai* and *D. nasuta* (Roy and Lakhotia, 1979, 1981), although patterns comparable to the Low Interband type (with only 3–4 puffs/interbands labelled) seen in the latter two species have never been found in *D. melanogaster*. Assuming that the interband patterns of ^{3}H-thymidine labelling of polytene chromosomes represent the initial phases of a polytene S-period (Hägele, 1973; Chatterjee and Mukherjee, 1975; Roy and Lakhotia, 1979, 1981), the scarcity of interband patterns in polytene chromosomes of warm-reared wild type larvae of *D. melanogaster* may be ascribed to the relatively brief interval between initiation of

replication in dispersed (puffs and interbands) and elongation into condensed regions (bands) in this species (Hägele, 1973; Roy and Lakhotia, 1981). The results of 1 min pulse labelling of salivary glands from warm reared larvae confirms the brief duration of the interband labelling phase at 24° C, since after 1 min pulse, not only the frequency of IB patterns is higher but a few more MIB patterns are also seen in warm-reared larvae. In view of the synchronous replication in the sister salivary glands under comparable labelling conditions (Roy and Lakhotia, 1977), the differences in the frequencies of labelling patterns between 1 and 10 min pulsed sister salivary glands in warm-reared wild type larvae must reflect the effect of pulse duration. Since the increase in the frequency of IB patterns in 1 min pulse preparations in all the four sets of larvae is accompanied by a corresponding decrease in the frequency or absence of the C patterns seen after a 10 min pulse, the possibility may be raised that the observed IB patterns in 1 min pulsed glands are due to incomplete labelling. However, the IB patterns seen in 1 min pulsed glands cannot be due to incomplete labelling since in that case the dense band regions with their much greater DNA content would be expected to appear labelled more often than unlabelled and therefore, if incomplete labelling were the cause for the difference between 1 and 10 min pulsed preparations, there would have been a shift towards discontinuous rather than the observed shift towards IB type labelling patterns. Thus it appears that at 24° C the interval between the initiation of a replication cycle in disperse regions and in band regions is very brief. A longer pulse may obliterate the recording of many of the IB patterns, e.g., a nucleus which may have been at the IB stage when the pulse *began*, may proceed to 2C stage within the 10 min interval and thus after the 10 min pulse, it would be recorded in the autoradiograph as 2C; similarly a 2C may be recorded as 3C after the 10 min pulse. In 10° C reared wild type larvae and in older *giant* larvae, this interval is apparently considerably longer since even with a 10 min pulse, the IB patterns are more frequent and there is a wide spectrum of them, from only a few puffs/interbands labelled (MIB) type to the HIB type. This implies that unlike in warm-reared wild type larvae, in the other two cases the initiation of replication at different origin points occurs with considerable asynchrony, as has been found in warm-reared larvae of *D. kikkawai* and *D. nasuta* (Roy and Lakhotia, 1979, 1981).

The question why in 1 min pulsed glands of cold-reared wild type larvae and in *giant* larvae, neither 3C nor 2C type labelled nuclei are seen, remains open. The presence of HIB and 3D types of nuclei in the same preparations shows that the absence of both the categories of continuous patterns in 1 min pulsed preparations is not due to inadequate autoradiographic recording, but is likely to be due to a real absence of simultaneous incorporation of ^{3}H-thymidine in all bands and interband regions in *giant* and in cold-reared wild type larvae. The absence of simultaneous progression of replication in all band and interband regions in cold-reared wild type and in *giant* larvae may be related to the above discussed greater asynchrony with which the different replicating units in a given chromosome segment are activated in these two cases. As a result of such staggered activation, many of the early initiated replicating units may complete their replication cycle before some others have

initiated, so that the IB labelling stage is directly followed by D-patterns. In the above discussion it has been presumed that homologous origin points on the multiple lateral strands constituting a polytene chromosome are activated simultaneously and thus replication on all polytene strands progresses synchronously. However, the possibility that the corresponding origin points in the parallel lateral strands are activated sequentially cannot be ruled out, and if that be the case, the molecular basis of the C patterns normally seen at chromosomal level with 10 min pulse would be different. A resolution of the real significance of the C patterns must await data on the organisation of replicons in polytene chromosomes in relation to the different chromosomal labelling patterns. Studies in this direction are currently in progress in our laboratory.

The frequency of labelled nuclei in salivary glands of cold-reared prepupae is much higher than that observed earlier by Rodman (1968) in warm reared prepupae of *D. melanogaster*. Since some IB type labelled nuclei are also seen in cold reared prepupae, it appears that unlike the warm reared prepupae (Rodman, 1968), some nuclei initiate their last polytene replication cycle even at this late stage. The high frequency of labelled nuclei and the presence of IB patterns in cold-reared prepupae may be related to the prolongation of the duration of the polytene replication cycles and to the attainment of higher levels of polyteny by most nuclei under cold-rearing condition (Hartmann-Goldstein and Goldstein, 1979a).

The similarities observed in the replicative organisation of polytene nuclei in older *giant* larvae and in cold-reared wild type larvae are interesting. Under both conditions the larval period is extended and consequently, a greater number of nuclei of a salivary gland attain higher levels of polyteny. In both, the higher polyteny nuclei show a similar cytological appearance with thicker and shorter chromosomes. It would appear that the similarity in the patterns of polytene replication in these larvae is related to their nuclei achieving higher levels of polyteny than normal. Some changes in DNA-protein relationship have been implied (Hartmann-Goldstein and Goldstein, 1979b) in polytene nuclei of cold-reared larvae; whether the alterations in polytene replication patterns are related to these is not known.

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