Chromosoma (Berl) (1984) 89:212–217

CHROMOSOMA

Replication in *Drosophila* chromosomes

XI. Differences in temporal order of replication in two polytene cell types in *Drosophila nasuta*

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Abstract. The temporal order of replication of specific sites in polytene chromosomes from salivary glands and gastric caeca of *Drosophila nasuta* larvae was compared using ^{3}H-thymidine autoradiography. Labelling of different cytological regions in segments of chromosome *2R* (section 47A to 49C) and chromosome *3* (section 80A to 82C) was examined in detail in nuclei showing late S-period labelling (2D and 1D types) in both cell types. The different labelling sites (22 on the *2R* segment and 38 on the chromosome *3* segment) are cytologically similar in the two cell types. However, there are profound differences in the labelling frequencies of certain sites in polytene nuclei from salivary glands and gastric caeca during the late S-phase. This suggests that even though a comparable number of chromosomal replicating units operates in the two polytene cell types, the temporal order of completion of replication differs.

Introduction

The temporal order of replication of different chromosome regions and the replicon properties have been shown to vary in a tissue-specific manner in diploid cells (Callan 1972; Blumenthal et al. 1973; Farber and Davidson 1977; Steinemann 1981a, b; Sheldon and Nichols 1981). Polytene chromosomes of Diptera, with their distinct banding patterns and puffs, are, however, more suited for an analysis of the organization of replicating units and the temporal order of their replication in different cell types of an individual. In spite of the numerous reports on polytene chromosome replication, this aspect has received little attention since nearly all studies have been restricted to polytene nuclei of only one cell type, i.e., the larval salivary glands.

The constancy of polytene banding patterns in different cell types of a species (Beermann 1962) and the belief that a band is the unit of replication (Pelling 1966) suggest that in spite of cellular differentiation, the replicative organization may remain similar in different polytene cell types. A recent study by Redfern (1981) on polytene replication in larval salivary gland and ovarian nurse cells of *Anopheles* has tended to support this. However, other recent studies, using DNA fibre autoradiographic techniques (Steinemann 1981b; Lakhotia and Sinha 1983), have shown that the operational replicating units in polytene nuclei are larger than one band and thus the replicative organization may vary in different cell types despite the constancy of the banding patterns.

To examine this in greater detail, we are analysing the replicative organization in various organs of *Drosophila* larvae that either normally display polytene chromosomes or may be induced to develop an improved polytene chromosome morphology by modifying the developmental and genetic conditions. In the present study, we compared the temporal order of termination of replication in specific chromosome segments in polytene nuclei of salivary glands and of gastric caeca of *D. nasuta* larvae.

Material and methods

The flies and larvae of a wild-type strain of *D. nasuta* (Varanasi) were reared on the standard food medium. The larvae were grown under uncrowded conditions at 20° ± 1° C since the polytene chromosome morphology in gastric caeca is optimal at this breeding temperature. Under these conditions of growth, pupation occurs about 180 h after oviposition.

Gastric caeca and salivary glands of the same mid-third instar larvae (about 145–150 h after oviposition) and of a few late third instar larvae were dissected in a modified Poels' medium (see Sinha and Lakhotia 1980) and pulse labelled with ^{3}H-thymidine (250 μCi/ml, sp. act., 15.8 Ci/mM; BARC, Trombay) for 10 min in separate depression slides. After the radioactive pulse, the tissues were washed in radioisotope-free medium, stained with 2% acetocarmine and squashed in 50% acetic acid. After the removal of the coverslips, the preparations were processed for autoradiography with 1:1.5 diluted Ilford K5 nuclear emulsion. In gastric caecum chromosome preparations, a considerable amount of cytoplasm obscures polytene chromosomes in squash preparations. Therefore, prior to autoradiography the gastric caecum chromosome preparations were treated with 0.2% pancreatic RNase (E. Merk) in 2 × SSC (sodium chloride/sodium citrate) at 37° C for 30 min to remove the stainability of cytoplasm. To control whether the RNase treatment affects autoradiography of ^{3}H-thymidine-labelled chromosomes, some preparations of labelled salivary glands were also exposed to the RNase treatment. From this experiment we concluded that the RNase treatment has no effect on the autoradiography of ^{3}H-thymidine labelled chromosomes. After a 10- to 12-day exposure at 4°–6° C, the autoradiograms were developed, fixed, washed, and stained with Giemsa. After mounting the slides with D.P.X., they were examined for chromosomal labelling.

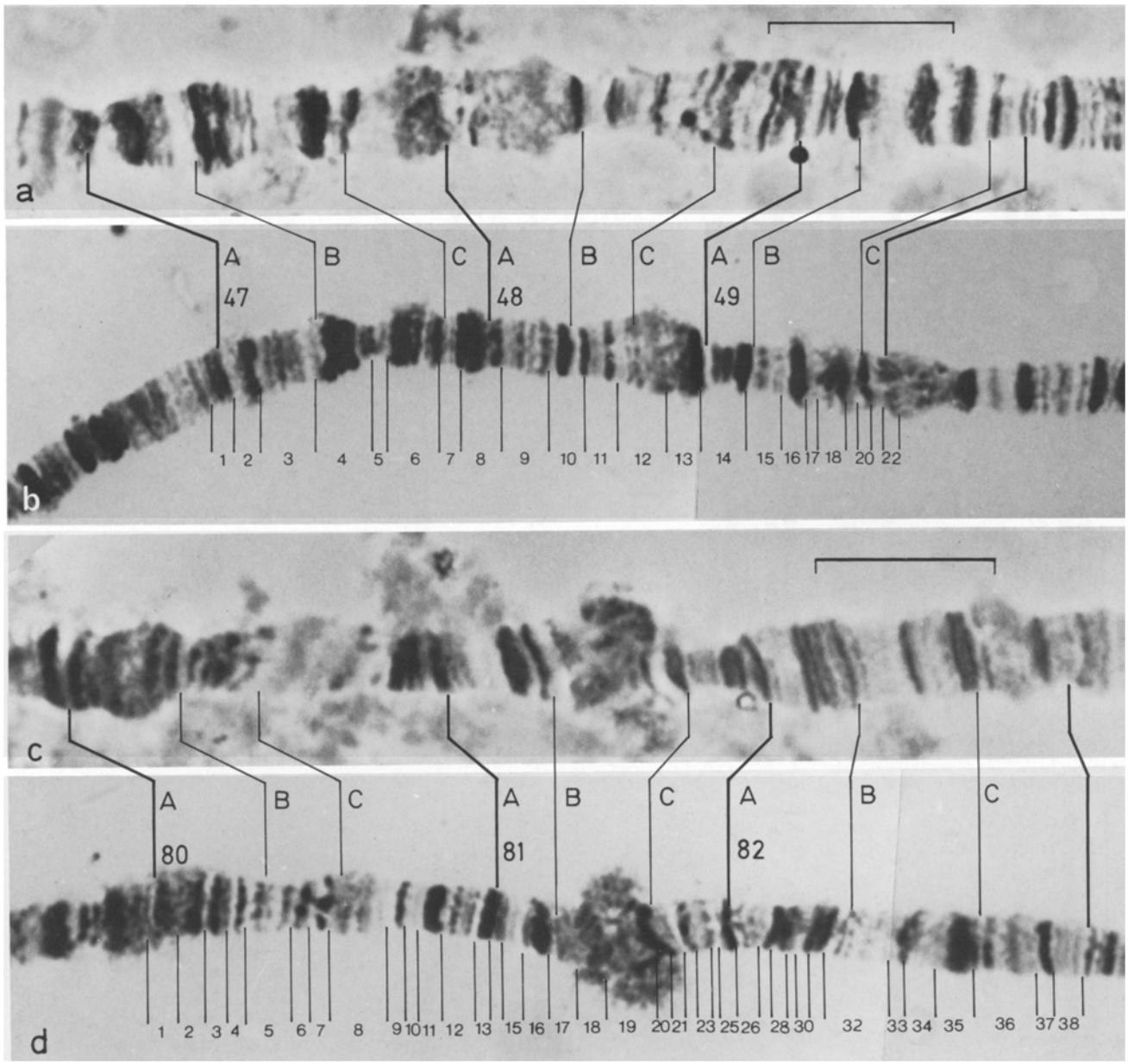

Fig. 1 a–d. Cytology of the segments of polytene chromosomes *2R* (**a** and **b**) and *3* (**c** and **d**) from salivary glands (**a**, **c**) and gastric caeca (**b**, **d**), analysed for ^{3}H-thymidine labelling. The cytological map positions (following the photographic map of Roy and Lakhotia 1981) for the two segments are indicated in the middle panels while the independently labelling sites identified in this study are shown in the lower panels. Bar represents 10 μm

Results

Polytene chromosomes in gastric caeca of D. nasuta larvae

Polytene chromosomes in gastric caeca from mid or late third instar larvae are generally of lower polyteny than those in salivary glands. Also, the number of cells with polytene chromosomes adequate for analysis is lower in caeca and these chromosomes disintegrate much earlier than those in salivary glands before puparium formation. The banding pattern in the caecum polytene chromosomes is, in general, similar to that in salivary glands (detailed comparison not presented, but see Fig. 1).

Patterns of ^{3}H-thymidine labelling in gastric caecum polytene chromosomes

The autoradiographic patterns of chromosomal labelling in ^{3}H-thymidine pulse-labelled gastric caecum nuclei are generally of the same types as in salivary gland polytene nuclei of *D. nasuta* (see Roy and Lakhotia 1981). In mid-third instar gastric caeca, about 30%, and in late third instar gastric caeca only 0.5%–1% nuclei are labelled with ^{3}H-thymidine. Among the labelled nuclei in mid-third instar gastric caeca, a majority show the late S-period discontinuous type labelling (3D: 16.5%, 2D: 16.5% and 1D: 54.4%, in more than 1,200 nuclei examined) while the remaining 12.6% are of the interband and continuous types

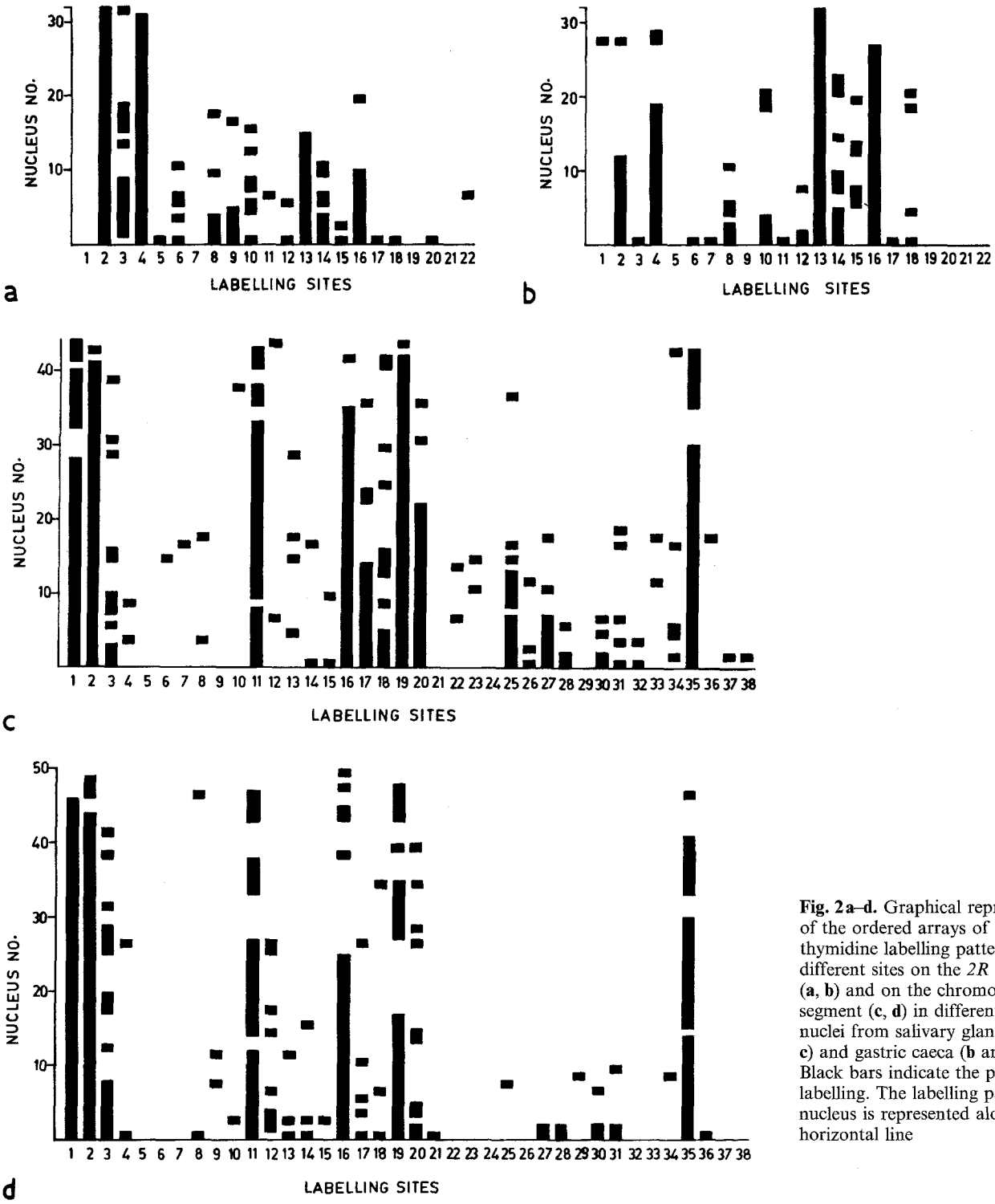

Fig. 2a–d. Graphical representation of the ordered arrays of ^{3}H-thymidine labelling patterns of the different sites on the *2R* segment (**a**, **b**) and on the chromosome *3* segment (**c**, **d**) in different 1D-type nuclei from salivary glands (**a** and **c**) and gastric caeca (**b** and **d**). Black bars indicate the presence of labelling. The labelling pattern of a nucleus is represented along one horizontal line

(for nomenclature of the discontinuous labelling patterns, see Rodman 1968). In late third instar gastric caeca, nearly all of the labelled nuclei are of the 1D type (latest replication stage with only few bands labelled), while a few are of 2D type. In contrast, in salivary glands of these larval ages, 60%–70% or more nuclei show ^{3}H-thymidine incorporation (Roy and Lakhotia 1981). Thus it appears that, compared to salivary glands, the last polytene replication cycle in gastric caecum nuclei is completed at an earlier stage of larval development.

Temporal order of termination of replication of specific sites in polytene nuclei from salivary glands and gastric caeca

To compare the temporal order of termination of replication in the two tissues, the labelling of the segments 47A to 49C and 80A to 82C of chromosomes *2R* and *3* in 2D- and 1D-type-labelled nuclei of mid third instar larvae was examined (for cytological maps, see Roy and Lakhotia 1981 and Fig. 1). A total of 22 discontinuously labelled

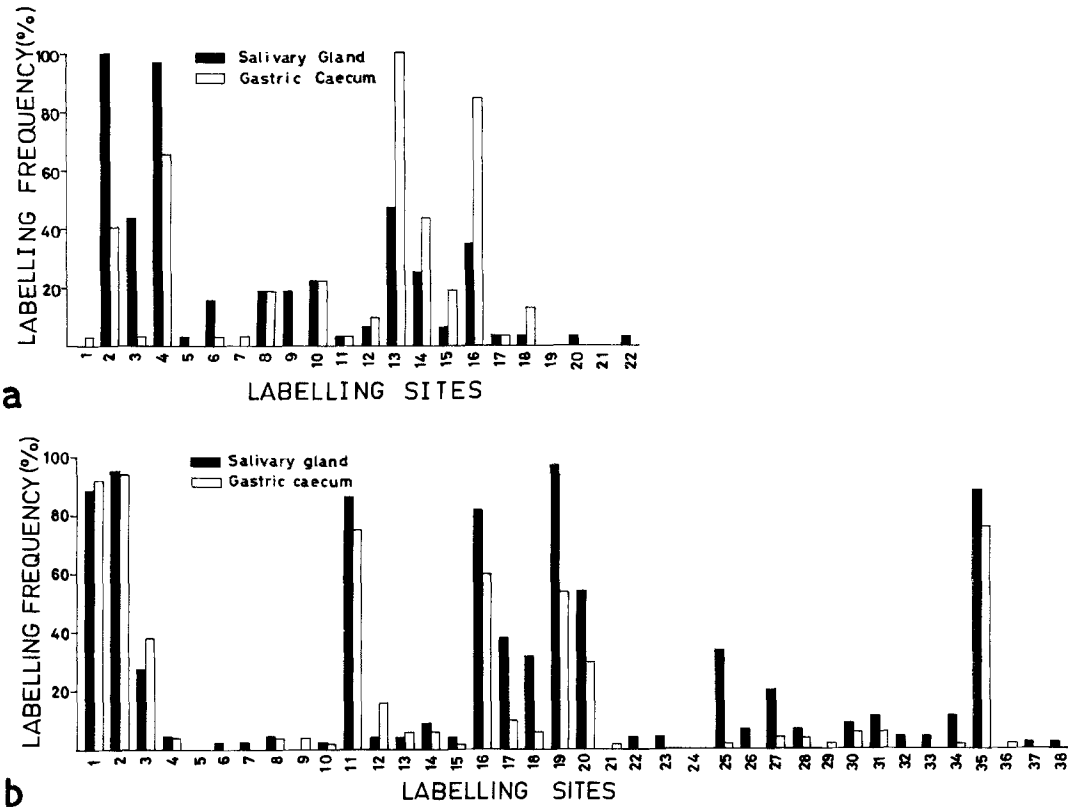

Fig. 3a, b. Comparison of the labelling frequencies of different sites on chromosome *2R* (**a**) and *3* (**b**) in 1D-type-labelled polytene nuclei from salivary glands and gastric caeca. Data taken from Figure 2

sites on the *2R* segment (Fig. 1a, b), and 38 sites on the chromosome *3* segment (Fig. 1c, d) were identified (see Rudkin 1972). The cytological borders of these independent labelling sites are similar in both cell types. For any site the presence of three silver grains was taken as the lower limit for indicating replication. The distributions of silver grains on the segments of chromosome *2R* and *3* in different salivary gland or gastric caecum nuclei with 1D type-labelling were arranged in matrices to define the temporal order of completion of the replication cycle of each labelling site (Rudkin 1972; Mishra and Lakhotia 1982). These arrays are shown in Figure 2. The relative frequencies of labelling of each site are presented in Figure 3. The labelling patterns are ordered in the matrices in Figure 2 such that there are minimum discontinuities in the later replicating sites (with higher labelling frequencies). However, no matter how the ordered arrays are arranged, the labelling of many sites in both tissues does not remain uninterrupted in the matrices (Fig. 2). Comparison of the labelling frequencies (Fig. 3a, b) and the ordered arrays (Fig. 2) reveals significant differences in the temporal order of replication of homologous labelling sites in the two tissues. In a χ^2-test (see Redfern 1981), the labelling frequencies of the 22 sites on the *2R* segment and of the 38 sites on the chromosome *3* segment in 1D-type nuclei in salivary glands and gastric caeca are found to differ significantly ($P<0.001$ for *2R* and $P<0.01$ for the chromosome *3* segment). Some examples of the differences in the terminal labelling patterns on the *2R* segment in the two tissues are illustrated in Figure 4. In salivary gland polytene nuclei, sites 13 and 16 of the *2R* segment are relatively early replicating while labelling sites 2 and 4 are the last to complete their replication cycle since they show 100% labelling frequency (Figs. 2a and 3a) and are labelled even in very late 1D-type salivary gland nuclei. In contrast the temporal order of termination of these sites is reversed in gastric caecum polytene nuclei. Sites 13 and 16 show higher labelling frequencies (Figs. 2b and 3a) and appear labelled in very late 1D gastric caecum nuclei when sites 2 and 4 are unlabelled (see Fig. 4). Sites 3 and 9 of the *2R* segment also show notable differences. In salivary glands, site 3 shows a labelling frequency higher than 44% (Figs. 2a and 3a) whereas it is labelled in less than 5% of 1D-type gastric caecum nuclei (Figs. 2b and 3a). Similarly, site 9 (the 48A puff site) is labelled in 18% of 1D-type salivary gland nuclei but has not been seen to be labelled in any of the 1D nuclei in gastric caeca (see Figs. 2a, b and 4).

Similar, though less marked, differences between the two cell types are noted in the labelling frequencies of different sites on the chromosome *3* segment (Figs. 2c, d and 3b). Sites 16, 17, 18, 19 (the 81B puff site), and 25 of the chromosome *3* segment are labelled significantly more often in salivary gland than in gastric caecum polytene nuclei. However, the very late replicating sites in this segment (e.g., sites 1, 2, 11, and 35) show similar labelling frequencies in the two tissues (Fig. 3b).

Discussion

The 1D (or spot-labelling) patterns of ^{3}H-thymidine labelling in polytene chromosomes of *Drosophila* represent the very late S-period of a polytene replication cycle (Rodman 1968; Rudkin 1972). Thus, a comparison of the sites labelled in 1D-type nuclei permits us to identify at the cyto-

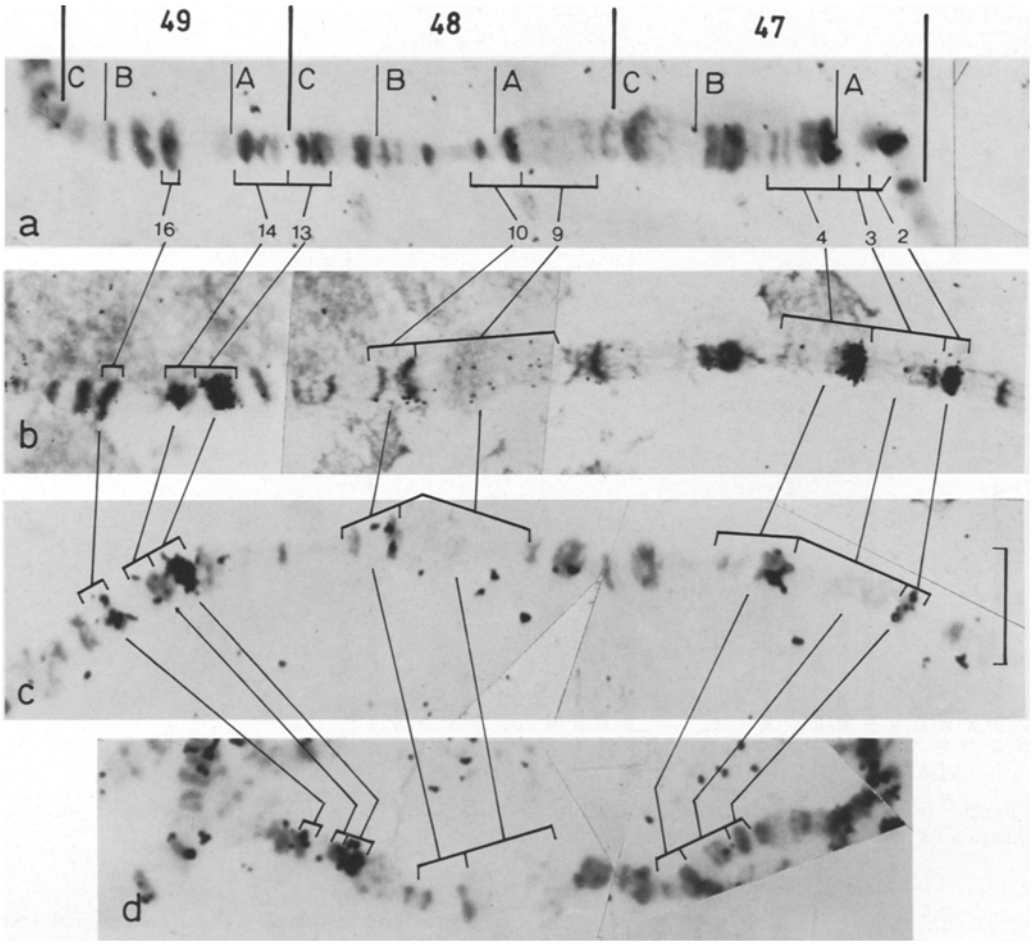

Fig. 4a–d. Examples illustrating differences in the terminal labelling patterns of different late replicating sites on the *2R* segment in salivary glands (**a**, **b**) and in gastric caeca (**c**, **d**). **a** and **d** are from the very late 1D salivary gland and gastric caecum nuclei, respectively; **b** and **c** represent slightly earlier stages from each tissue. Bar represents 10 μm

logical level the late replicating sites and their programme of termination of replication (Mishra and Lakhotia 1982). The cytological identity of the independently labelling late replicating sites in the two polytene cell types is remarkable. Although the relationship between the units of replication that is identifiable at the polytene banding pattern level (independently labelling sites) and at the DNA molecule level (active replicons) appears complex and is not yet well understood (see Steinemann 1981b; Lakhotia and Sinha 1983), the similarity at the chromosomal level between replicating units in polytene nuclei of two different cell types has implications for the spatial organization of active replicons.

The matrices of ordered arrays (Fig. 2) reveal temporal discontinuities in the labelling of many sites and these persist no matter how the matrices are arranged. This does not agree with the usual belief of temporal continuity of labelling of a polytene chromosome site (Rudkin 1972). This aspect has been discussed elsewhere (Mishra and Lakhotia 1982; Lakhotia and Sinha 1983) and we believe that the temporal discontinuities in the labelling are not artifacts of autoradiography but reflect a basic feature of the replicative organization of polytene chromosomes.

The relative frequency with which a given labelling site appears labelled in 1D-type nuclei provides information about the relative time that it takes to complete its replication cycle in relation to the other replicating sites showing higher (later replicating) or lower (earlier replicating) labelling frequencies (Rudkin 1972). Thus, the observed differences in the relative labelling frequencies show that the temporal order of completion of polytene replication cycle of different labelling sites is different in the salivary gland and gastric caecum cells. However, if a given site is late replicating in one cell type, i.e., is labelled in more than 20%–30% of the 1D-type nuclei, it generally remains so in the other cell type as well. Only the temporal order (rank order of labelling frequency) in which it completes its replication differs. This suggests that the late replicating nature of a given site may be inherent in its DNA sequence composition and organization, but the precise time sequence of its replication within the late S-period may be modulated in a tissue-specific manner.

Although both cell types from each larva were labelled, the limited numbers of analysable 1D-type-labelled nuclei available in preparations of tissues of a single larva, particularly the gastric caeca, made it impossible to restrict the comparison of the temporal programme of replication to the same individual. Therefore, each of the two kinds of labelled nuclei were pooled from several mid-third instar larvae. This raises the possibility that the differences in the replication programmes were due to interlarval rather than to intertissue differences. However, previous studies on rep-

lication of specific sites in salivary gland polytene nuclei of *D. melanogaster* have demonstrated a remarkable constancy in the temporal order of replication (see review in Rudkin 1972). The temporal programme of replication of different sites in a given tissue type, therefore, does not appreciably differ between larvae reared in different laboratories under comparable conditions. Moreover, Hägele and Kalisch (1980) have shown that the temporal order of replication of a given site in salivary gland polytene nuclei remains constant in third instar larvae and prepupae. Thus the differences in the ^{3}H-thymidine labelling patterns of certain sites in polytene chromosomes from salivary glands and gastric caeca of *D. nasuta* larvae observed in this study are unlikely to be influenced by sampling methods. In a comparison of temporal order of replication of different sites in segments of chromosomes *X* and *2* in gastric caecum and salivary gland polytene nuclei of *D. hydei* we also have found significant differences in the labelling frequencies of certain sites in the two cell types. Thus, differences in the programmes of replication in different polytene cell types may be a common feature.

In earlier studies on replication in polytene chromosomes in salivary glands of *D. nasuta* larvae reared at 24° C (Sinha and Lakhotia 1980; Roy and Lakhotia 1981), it was noted that the 48A puff site (no. 9) on *2R* was always labelled even in the very late 1D-type nuclei. However, during the present study with larvae reared at 20° C, we found the 48A puff to be unlabelled in most 1D nuclei. Significantly, even in the larvae reared at 24° C (as in the earlier studies) we now do not find the 48A puff site to be labelled in all 1D-type salivary gland nuclei. Another unexplained fact is that whereas during our earlier studies (Sinha and Lakhotia 1980; Roy and Lakhotia 1981) the larvae reared at 24° C were pupating about 156–170 h after egg laying, for the past year or so, the *D. nasuta* larvae under identical rearing conditions (24° C) have been pupating about 130–140 h after oviposition. It is possible that this change in the replication programme of the 48A puff site in salivary gland polytene nuclei is related to the change in the larval developmental pattern since alteration in the developmental programme of *D. melanogaster* larvae has been found to modify the temporal order of replication of some sites in salivary gland polytene nuclei (Mishra and Lakhotia 1982).

Our results differ from those of Redfern (1981) who concluded that the temporal order of termination of the replication cycle of different labelling sites of *X* chromosomes in polytene nuclei from larval salivary glands and from adult ovarian nurse cells of *Anopheles* is similar. It is, however, to be noted that in the study of *Anopheles* chromosomes (Redfern 1981), the entire *X* chromosome has been divided into only 23 labelling regions. Hence, each of these large sections may actually comprise more than one replicating unit resolvable even at the chromosomal level, and thus finer differences in the temporal order of the termination of replication cycle in the two cell types of *Anopheles* could be overlooked.

The general similarities in the banding pattern and in the cytological definitions of the independently labelling, late replicating sites in polytene chromosomes in different cell types of a species suggest that these two aspects are interrelated. The functional significance of the differences in the temporal order of replication is not known. Presumably, this may be related to differences in the programmes of gene activity in the different cell types. This becomes significant in the context of the possible existence of domains of variable levels of polyteny along the length of a chromosome (Laird 1980) and a different organization of the active replicons in the late replicating regions (Lakhotia and Sinha 1983). This aspect needs further analysis.

Acknowledgements. This work has been supported by a grant from the University Grants Commission, New Delhi (Career Award Project) to SCL.

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Received December 15, 1982 / in revised form November 4, 1983
Accepted by M.L. Pardue