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REPLICATION IN *DROSOPHILA* CHROMOSOMES

I. REPLICATION OF INTRANUCLEOLAR DNA IN POLYTENE CELLS OF *D. NASUTA*

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SUMMARY

The organization and replication of intranucleolar DNA in polytene cells of *Drosophila nasuta* have been examined. Normal Giemsa and fluorescent (Hoechst 33258 and quinacrine mustard) staining reveal that in *D. nasuta* polytene cells, the intranucleolar DNA is organized into condensed and disperse forms and very often shows connexion to the alpha heterochromatin of the chromocentre. Results of [^{3}H]thymidine autoradiography indicate that the known underreplication of rDNA sequences in polytene cells is due to the slower replication of the intranucleolar DNA. Some aspects of the organization of condensed and disperse intranucleolar DNA are discussed.

INTRODUCTION

In polytene cells of larval salivary glands of *Drosophila* and a few other Diptera, it has been suggested that the rDNA is underreplicated compared to the euchromatic segments of the chromosomes (Hennig & Meer, 1971; Spear & Gall, 1973; Gambarini & Lara, 1974) and this underreplication is apparently under a precise regulation since the level of final polyteny achieved for rDNA sequences is the same irrespective of the initial dosages of nucleolar organizer regions (Spear & Gall, 1973). The exclusive localization of rDNA cistrons within the nucleolus of polytene cells of *Drosophila*, as shown by *in situ* hybridization studies (Pardue, Gerbi, Eckhardt & Gall, 1970) provides an opportunity to examine the relationship between the replication cycles of chromosomes and intranucleolar rDNA. Underreplication of rDNA in polytene nuclei could be achieved in one of several ways. In early polytenic replication cycles, the chromosomes and rDNA may replicate synchronously, but in nuclei with higher polyteny, the rDNA may not replicate; alternatively, part of the redundant rDNA sequences may replicate synchronously while others may not replicate at all. A third possible way is that all rDNA sequences may replicate but their replication may be slower compared to the chromosomal replication cycles and thus the rDNA level remain underreplicated. In order to understand some aspects of the mechanism of rDNA underreplication, we have analysed autoradiographically the replication of

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intranucleolar DNA in salivary gland polytene nuclei of *D. nasuta* larvae. Our results indicate that in these nuclei, the replication cycles of chromosomes and intranucleolar DNA are different, the latter probably replicating slowly compared to the former.

MATERIAL AND METHODS

A wild strain of *D. nasuta* (Varanasi), maintained in our laboratory for the past 3 years, has been used. Eggs were collected at 30-min intervals in Petri dishes containing *Drosophila* food. Larvae were grown at 24 ± 1 °C. Third instar larvae of *D. nasuta* show changes in colour of the anterior and posterior pair of spiracles. The posterior pair of spiracles which are initially brown, turn black; this change has been used to synchronize the age of the larvae. At 24 °C, the posterior spiracles begin to turn black at 148 h after egg laying and remain black till pupation. The larvae whose posterior spiracles are just turning black are referred to as O-h black spiracle stage. Salivary glands were dissected from larvae between O- and 24-h posterior black spiracle stages.

Cytological procedures

For analysing the morphology of intranucleolar DNA, the salivary glands were fixed in 1:3 aceto-methanol and squashed in 50% acetic acid. After the removal of coverslips the preparations were either stained with Giemsa or with quinacrine mustard (QM, 500 μg/ml for 10 min) or Hoechst 33258 (H, 5 μg/ml for 5 min) and examined for fluorescence patterns in a Carl Zeiss 'Fluoval' microscope.

[^{3}H]*thymidine labelling and autoradiography*

Salivary glands were dissected in *Drosophila* Ringer solution and labelled for 10 min with [^{3}H]thymidine (250 μCi/ml, sp. act. 6·4 Ci/mM; BARC, Trombay). Glands were fixed and squashed by the usual aceto-methanol-orcein-carmine method. Coverslips were removed, and preparations were dehydrated through ethanol grades and air dried. Slides were then treated with 5% TCA at 4 °C for 10 min, again passed through ethanol grades and dried. The dried slides were coated with Ilford L4 emulsion (1:1 dilution with water) and exposed in the dark at 4 °C for 9 days. They were then developed in D19b, fixed, washed in running water, rinsed in distilled water and after drying, restained with Giemsa, mounted and observed under a 100 × oil-immersion objective.

OBSERVATIONS

Morphology of nucleolar DNA

The polytene nuclei of *D. nasuta* have 4 long arms (X, 2L, 2R and 3) and a short 4th chromosome (Ranganath & Krishnamurthy, 1973–4). The chromocentre is small but with a prominent alpha heterochromatin (Kumar & Lakhotia, 1977) which is very often attached to the base of the 3rd chromosome. In the late third instar stage, a large, often rounded, nucleolus can be seen in about 25% of the squash preparation nuclei. The shape and size vary somewhat in different nuclei. With Giemsa the entire nucleolus appears to be stained nearly homogenously except for a densely stained mass near its centre (Fig. 1). This densely stained mass is usually a rounded, compact structure (Fig. 1A) but occasionally may have a different morphology – elongated (Fig. 1B), branched, or even slightly disperse (Fig. 1C). The size of the condensed mass varies to some extent in different nucleoli. In some cases, the nucleolus does not

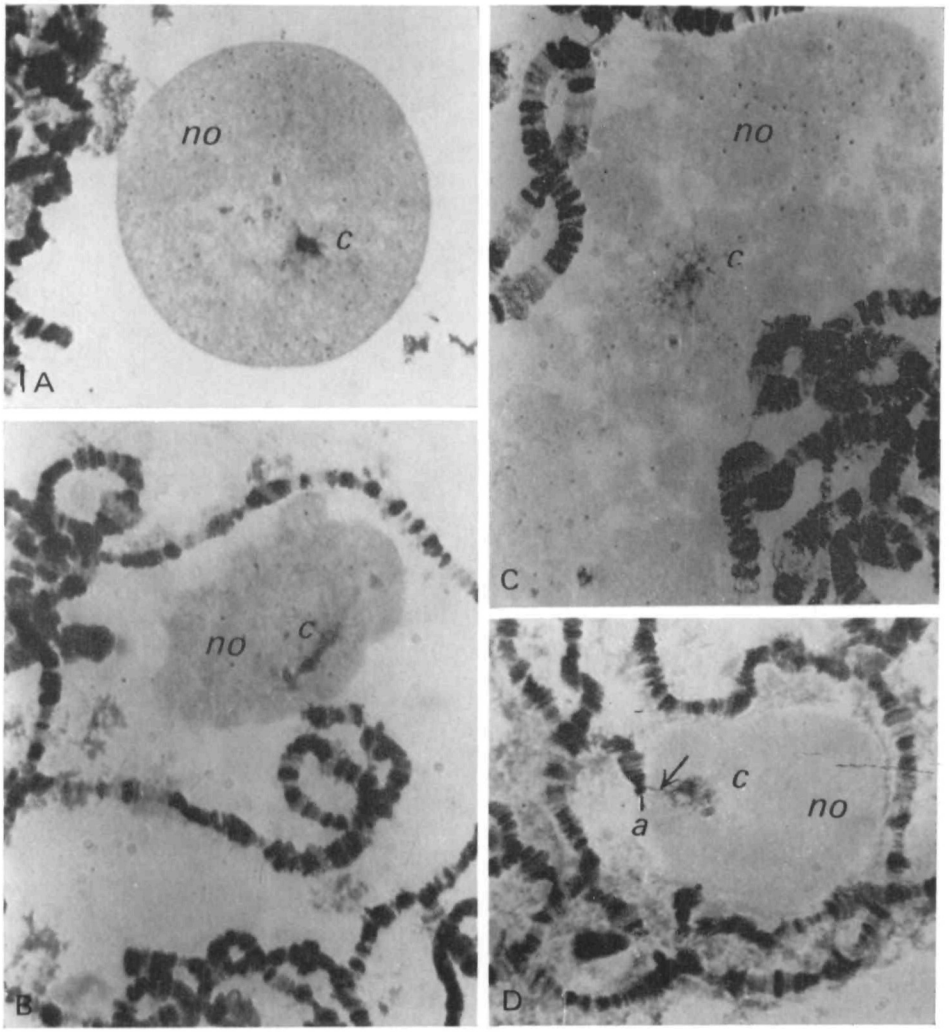


Fig. 1A–D. Giemsa-stained squash preparations of salivary glands of *D. nasuta* to show the variable morphology of condensed DNA mass (*c*) in the nucleolus (*no*). In 1D, a fine connective (arrow) may be seen between the condensed mass and the alpha heterochromatin (*a*) attached to the base of the third chromosome. Some of these microphotographs are taken from autoradiographic preparations and, therefore, some silver grains are visible. × 960.

seem to have a discrete condensed mass. Whether this absence is artifactual or due to a real change in organization of the condensed mass is not known.

When examined under the fluorescence microscope, the nucleolus gives a dull fluorescence with QM (Fig. 2A). However, with H the body of the nucleolus remains totally non-fluorescing (Fig. 2B). Within the nucleolus, the condensed mass fluoresces with moderate brightness with QM as well as with H. However, the fluorescence of the condensed mass is not as bright as that of the alpha heterochromatin. In addition, a network of fluorescing fibrils can also be made out in the nucleolar body.

As with Giemsa staining, the morphology of the condensed mass varies in preparations stained with QM or H.

The central dense mass is very often seen to be connected by one or more thread-like connective/s to the alpha heterochromatin or to the base of the third chromosome (Fig. 1 D). In some cases, the thread-like connective may extend to the base of the X-chromosome or more rarely to other chromosomal regions. As will be noted below [^{3}H]thymidine incorporation occurs both in the condensed mass as well as in the bulk of the nucleolar body; therefore, we recognize 2 types of organization of intranucleolar DNA: condensed intranucleolar DNA (c-No-DNA) and disperse intranucleolar DNA (d-No-DNA).

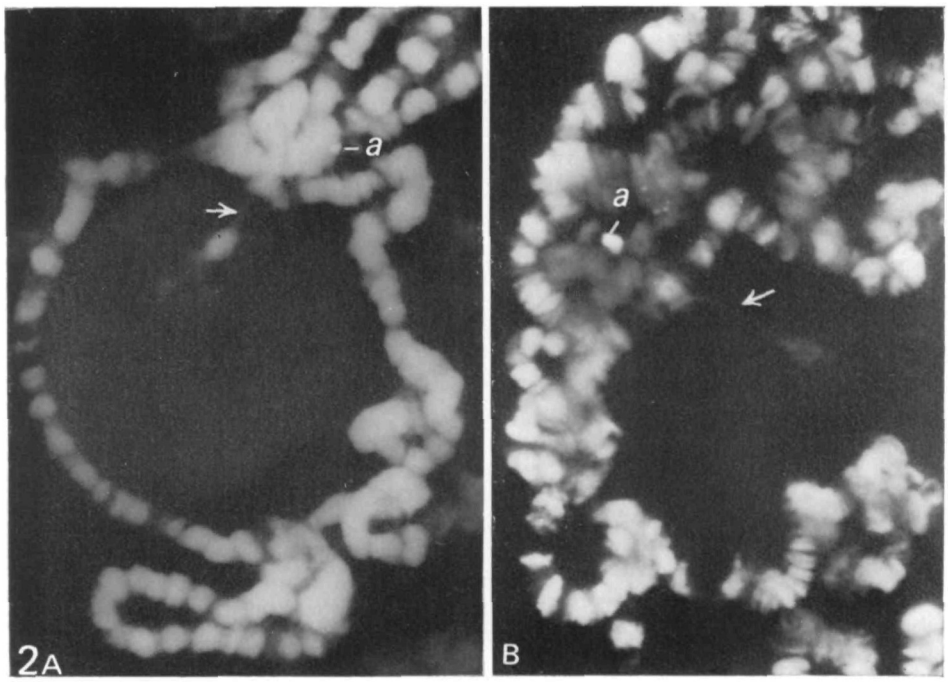


Fig. 2 A, B. Florescence patterns of nucleolus in squash preparations of salivary glands of *D. nasuta* stained with quinacrine mustard (A) and with Hoechst 33258 (B). Note the brightly fluorescing condensed mass and associated fibrillar network in the nucleolus. In both A and B, a fine thread extending from the condensed mass to the chromocentre can be seen (arrow). The brightly fluorescing alpha heterochromatin (*a*) can also be seen in both cases. The contrast in the photographs has been deliberately reduced to show details of intranucleolar fluorescence, particularly in A. × 1200.

[^{3}H]*thymidine labelling patterns of the intranucleolar DNA*

A total of 6081 nuclei (excluding nuclei of low polyteny) were examined from 91 late third instar larvae of 0–24 h posterior black spiracle stages. Among these, only 1672 nuclei had a well defined nucleolus. Occasionally isolated nucleoli could also be seen in the slides; these are probably a result of squashing. It would appear that the absence of a nucleolus in many of the nuclei is also due to loss during squashing and/or

coverslip removal etc. The present analysis is based on the 1672 nuclei observed which had a distinct nucleolus. Data presented in Table 1, show that the nucleolus incorporated [^{3}H]thymidine (either only in d-No-DNA or in d-No-DNA as well as in c-No-DNA) in 89·20% of these nuclei. Whenever the chromosomes are labelled the nucleolus appears to be labelled, except in 3 nuclei, where the chromosomes show low labelling but the nucleoli are unlabelled. However, in 28% of all nuclei, the nucleolus was labelled even though the chromosomes were unlabelled. Further analysis revealed that the labelling of d-No-DNA or c-No-DNA is not always similar and the degree of labelling of these regions varies considerably in these nuclei. Therefore, the labelling of d-No-DNA and c-No-DNA has been analysed in detail.

Table 1. *[^{3}H]thymidine labelling of intranucleolar and chromosomal DNA in different polytene nuclei of D. nasuta*

	No. of nuclei with chromosomes		
Nucleolus	Labelled	Unlabelled	Total
Labelled	1023 (61·2)	469 (28·0)	**1492** (89·2)
Unlabelled	3 (c·2)	177 (10·6)	**180** (10·8)
Total	**1026** (61·4)	**646** (38.6)	**1672** —

Values in parentheses indicate percentages of different types in the total number of nuclei examined.

[^{3}H]*thymidine labelling of disperse intranucleolar DNA*

In all nuclei with a labelled nucleolus, the d-No-DNA was labelled although in many the c-No-DNA was not labelled. The number of silver grains over the nucleolus (except those on the condensed mass) was counted in each of the labelled nucleoli and on the basis of this number, 4 categories of nucleolar labelling have been made: (i) *low* – with 6–75 grains; (ii) *mid* – with 76–150 grains; (iii) *heavy* – with 151–300 grains; and (iv) *very heavy* – with more than 300 grains over the disperse nucleolar areas. It must be admitted here that this grouping of d-No-DNA labelling into 4 categories is subjective. Nonetheless, this grouping provides some basis for comparing the degree of nucleolar labelling with the chromosomal labelling patterns. Of the 1492 nuclei observed with labelled nucleolus, 34·7% had low, 32·2% had mid, 21% had heavy and 12·1% had very heavy labelling of d-No-DNA (see last column of Table 2). In order to ascertain whether the different categories of d-No-DNA labelling are related to a specific type of chromosomal labelling, each of the nuclei was also scored for chromosomal labelling pattern.

The [^{3}H]thymidine-labelling patterns of *D. nasuta* polytene chromosomes can be grouped, as in other species (Hägele, 1973; Chatterjee & Mukherjee, 1975) into 3 groups: (i) *interband (IB) types* – with a preferential labelling of the puffs and

interbands; (ii) *continuous (C) types* – with a uniform labelling of all bands, interbands and puffs; and (iii) *discontinuous (D) types* – with a preferential labelling of bands and chromocentre. Within each of these groups, we can further recognize subclasses. Thus nuclei with interband type of labelling can be subdivided into low (LIB), mid (MIB) and heavy (HIB) interband types, on the basis of the numbers of puffs and interbands labelled in a nucleus. The continuous and discontinuous types of labelling have been further subdivided, following Rodman (1968) into medium (2C), heavy continuous (3C), heavy (3D), mid (2D) and low (1D) discontinuous types, respectively. A detailed account of these chromosal labelling patterns in *D. nasuta* will be presented elsewhere, but some of the patterns are illustrated in Figs. 3–10.

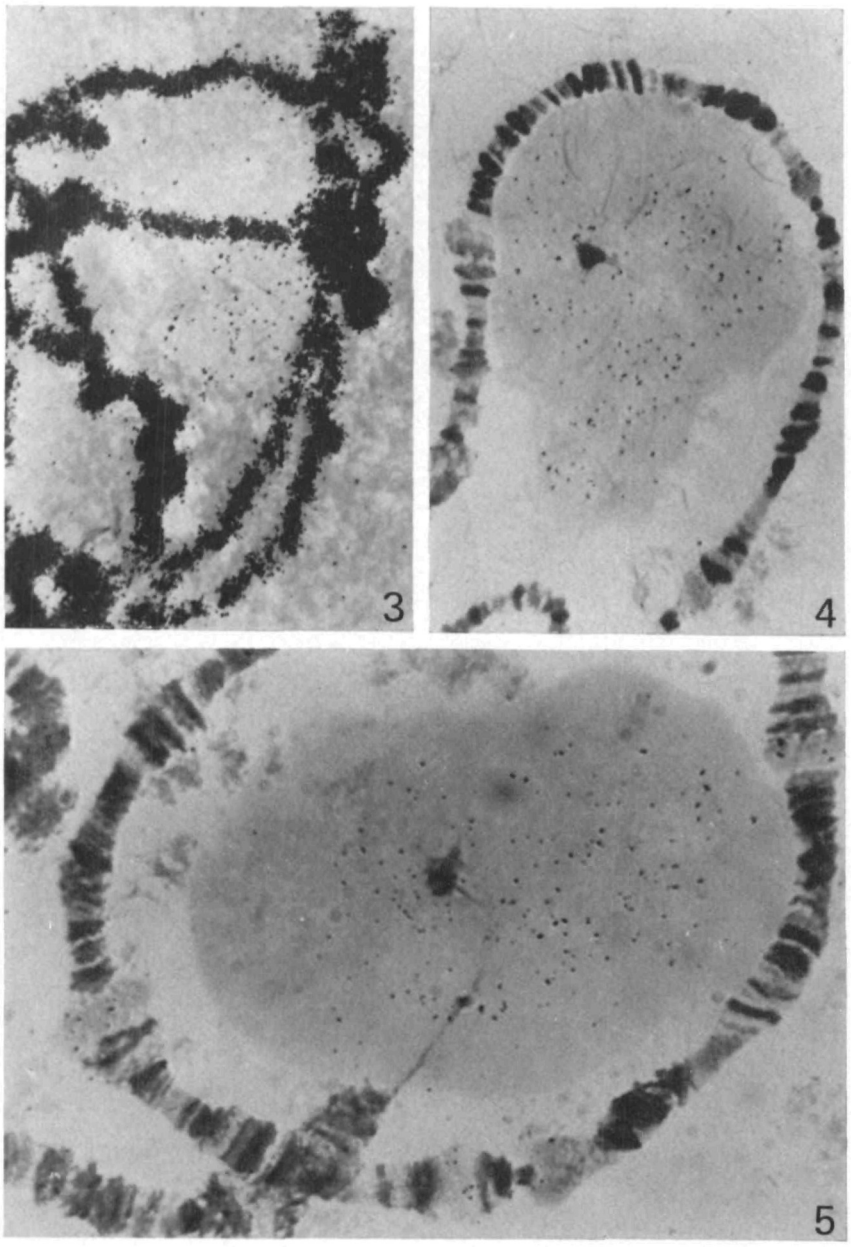
Table 2. *Different types of [^3H]thymidine labelling patterns of disperse-intranucleolar DNA(d-No-DNA) in relation to chromosomal labelling patterns in polytene cells of D. nasuta*

Labelling of d-No-DNA	Chromosomal labelling patterns*									
	LIB	MIB	HIB	2C	3C	3D	2D	1D	Un-labelled	Total†
Low	112	2	0	1	8	2	32	57	304	**518 (34·7)**
Mid	162	23	2	2	28	6	35	83	140	**481 (32·2)**
Heavy	114	28	3	0	24	6	28	85	25	**313 (21·0)**
Very heavy	20	42	27	0	18	1	24	48	0	**180 (12·1)**
Unlabelled	1	1	0	0	0	0	0	1	177	**180**
Total	**409**	**96**	**32**	**3**	**78**	**15**	**119**	**274**	**646**	**1672**
Percentage	**24·5**	**5·7**	**1·9**	**0·2**	**4·7**	**0·9**	**7·1**	**16·4**	**38·6**	

* For abbreviations of chromosomal labelling patterns, see text.

† The figures in parentheses indicate the percentages of different d-No-DNA labelling types among labelled nucleoli.

The data on the different categories of d-No-DNA labelling in relation to the chromosomal labelling patterns are presented in Table 2. Some representative examples of nucleolar labelling are shown in Figs. 3–10. As can be seen from the figures, the degree of d-No-DNA labelling varies independently of the nucleolar size. The examples in Figs. 3–10 and data presented in Table 2 also show that there is no precise relationship between the degree of d-No-DNA labelling and the types and degrees of chromosomal labelling. Thus the overall degree of labelling of the chromosomes in the LIB, MIB and 1D patterns is low; however, the d-No-DNA labelling in these nuclei varies from low to very heavy (Figs. 5, 6). Likewise, in nuclei with very heavy chromosomal labelling (2D, 3D or 3C types), the nucleolar labelling again varies from low to very heavy (Figs. 3, 9, 10). Furthermore, even in nuclei with unlabelled chromosomes, low to heavy labelling of d-No-DNA has been observed (Fig. 4). However, with unlabelled chromosomes, very heavy labelling of d-No-DNA has not been seen. It may be noted here that the frequency of different types of chromosomal labelling patterns observed in these nuclei (see last row in Table 2) selected for nucleolar labelling is similar to the overall frequency of these patterns in late third instar larvae of *D. nasuta* (unpublished). Thus the low frequency of HIB, 2C and 3D



Figs. 3–10. Autoradiographs of squash preparations of [^{3}H]thymidine-labelled salivary glands of *D. nasuta* larvae to illustrate some of the different types of nucleolar and chromosomal labelling patterns. Magnification of all autoradiographs (unless otherwise stated) is × 1200.

Fig. 3. Chromosomes with heavy continuous (3C) labelling; d-No-DNA with low labelling; c-No-DNA not discernible.

Fig. 4. Chromosomes unlabelled; d-No-DNA with mid type labelling, c-No-DNA condensed and unlabelled.

Fig. 5. Chromosomes LIB type; d-No-DNA with mid type labelling; c-No-DNA unlabelled Note the connective between c-No-DNA and the base of the third chromosome.

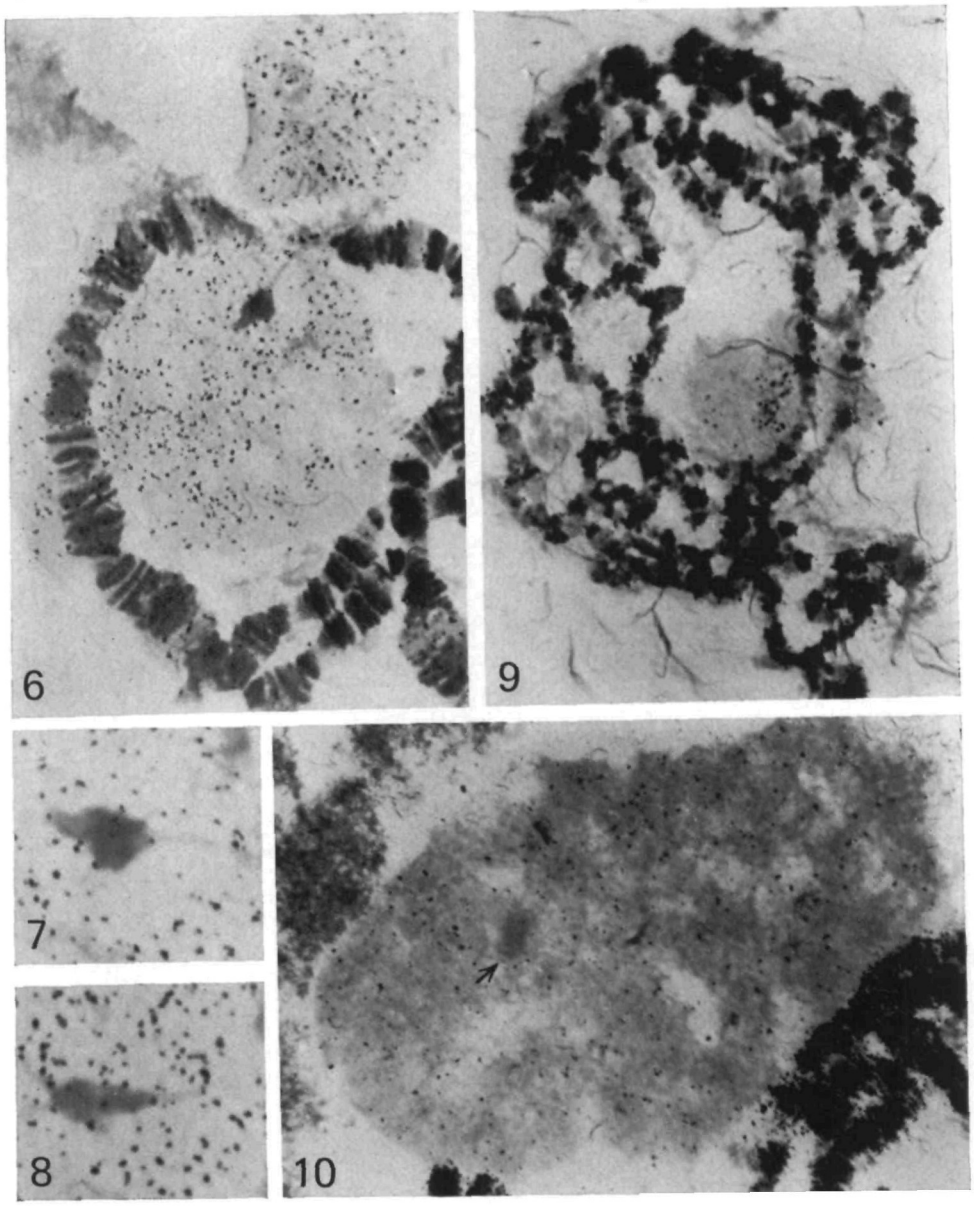


Fig. 6. Chromosomes MIB type; d-No-DNA very heavy; c-No-DNA lightly labelled (see Fig. 7).

Figs. 7, 8. Examples of low labelling (5–6 silver grains) of c-No-DNA region; the c-No-DNA region in Fig. 7 is same as in Fig. 6. × 2400.

Fig 9. Chromosomes 2D type; d-No-DNA very lightly labelled and c-No-DNA in a disperse form and heavily labelled.

Fig. 10. Chromosomes 3C type; d-No-DNA heavily labelled and the c-No-DNA although disperse (arrow) is, however, unlabelled (also see Fig. 1 c).

types of chromosomal labelling in the sample of nuclei with d-No-DNA labelled (Table 2) does not appear to be due to an infrequent occurrence of nucleolar labelling in these types.

[^{3}H]thymidine labelling in condensed intranucleolar DNA

Among the 1672 nuclei in which a nucleolus was seen, 1334 nuclei had a clearly identifiable c-No-DNA mass. The c-No-DNA mass was considered to be labelled only when a minimum of 3 grains were present over this region. Among the 1334 nuclei, in only 124 nuclei (9·3 %) had the c-No-DNA 3 or more silver grains and was, therefore, considered to be labelled. In these nuclei the number of silver grains over

Table 3. *[^{3}H]thymidine labelling of condensed-intranucleolar DNA (c-No-DNA) in relation to d-No-DNA and chromosomal labelling patterns in polytene nuclei of D. nasuta*

Labelling of		Chromosomal labelling patterns									
d-No-DNA	c-No-DNA	LIB	MIB	HIB	2C	3C	3D	2D	1D	Un-labelled	Total
Low	Labelled	4	0	0	0	1	0	5	2	1	**13**
	Unlabelled	75	2	0	1	2	2	15	36	260	**393**
Mid	Labelled	18	6	2	0	3	1	5	4	2	**41**
	Unlabelled	130	14	0	1	18	3	23	60	120	**369**
Heavy	Labelled	17	11	1	0	2	1	3	7	2	**44**
	Unlabelled	90	13	2	0	12	4	17	53	23	**214**
Very heavy	Labelled	1	10	1	0	2	1	7	4	0	**26**
	Unlabelled	17	26	21	0	9	0	10	20	0	**103**
Unlabelled	Labelled	0	0	0	0	0	0	0	0	0	**0**
	Unlabelled	0	1	0	0	0	0	0	1	129	**131**
Total	Labelled	**40**	**27**	**4**	**0**	**8**	**3**	**20**	**17**	**5**	**124**
	Unlabelled	**312**	**56**	**23**	**2**	**41**	**9**	**65**	**170**	**532**	**1210**

the condensed mass usually varied between 3 and 7 (Figs. 6–8), but in occasional nuclei up to 40 grains have been counted over the condensed mass. As noted earlier, the c-No-DNA appears as a rounded structure, but it may also assume various other forms (branched, elongated or relatively diffuse etc.). It was also observed that when the c-No-DNA was in compact rounded form, it was usually unlabelled (Figs. 4, 5) or labelled with very few grains only (Figs. 6–8). On the other hand, in a few nuclei in which the c-No-DNA showed heavier labelling (Fig. 9), it was always in a disperse state. However, in some nuclei, even though the c-No-DNA was in disperse state, it was unlabelled (Figs. 1 c, 10). In order to ascertain the relationship of the c-No-DNA labelling to d-No-DNA labelling and to the chromosomal labelling, the [^{3}H]thymidine incorporation patterns over these regions have been analysed and the data are presented in Table 3. It is seen that the c-No-DNA is always unlabelled in nuclei with unlabelled d-No-DNA. However, in the few nuclei in which the c-No-DNA was disperse and heavily labelled, the d-No-DNA appeared lightly labelled (Fig. 9). When

chromosomes are unlabelled, the c-No-DNA is mostly unlabelled, except for 5 nuclei in which the condensed mass is lightly labelled (with 3–4 grains) although the chromosomes are unlabelled. It is also seen from the data in Table 3, that the proportion of labelled c-No-DNA is lesser in nuclei with low d-No-DNA labelling. The thread-like connective between the c-No-DNA and the chromocentre, was not seen to be unambiguously labelled with [^{3}H]thymidine in any of the nuclei examined.

DISCUSSION

In the present autoradiographic study we have analysed the relationship between replication of intranucleolar and chromosomal DNA in salivary gland polytene nuclei of *D. nasuta*. Several observations in recent years have shown that the rDNA in polytene cells is underreplicated (Hennig & Meer, 1971; Spear & Gall, 1973; Gambarini & Lara, 1974); furthermore, regulation of the degree of underreplication has been suggested to be cell autonomous (Spear & Gall, 1973). Our present observations provide direct cytological evidence in support of these observations.

The polytene chromosomes undergo cyclic replication without intervening division stages. In these nuclei, each replication cycle is separated from the next by a non-synthetic (non-replicating) phase and it is believed that, with some possible variations, the temporal sequence of replication of different polytene chromosome regions is similar in consecutive replication cycles (Rudkin, 1972, 1973). Furthermore, the the different types of [^{3}H]thymidine labelling patterns seen in autoradiographs of these chromosomes have been related to the early, mid or late parts of a given polytenic replication cycle (*S*-phase); it is believed that the interband type of chromosomal labelling patterns occur in the initial part, the continuous labelling in mid, and the discontinuous labelling patterns in the later part of any replication cycle of polytene nuclei of *Drosophila* (Hägele & Kalisch, 1974; Chatterjee & Mukherjee, 1975; and our own unpublished data). Thus, an autoradiographic analysis of [^{3}H]thymidine incorporation in intranucleolar and chromosomal DNA of a nucleus may provide an understanding of the relationship between replication of the 2 regions and the mechanism regulating the underreplication of rDNA cistrons during polytenization.

Cytologically in *D. nasuta* polytene nuclei, 2 types of intranucleolar DNA organzation are seen – condensed and disperse. In view of the localization of rDNA cistrons within the nucleolus of polytene cells of *D. hydei* (Pardue *et al.* 1970), it could be suggested that in *D. nasuta* also, the rDNA cistrons in polytene cells are within the nucleolus. The condensed and disperse intranucleolar organization would appear to be related in some way to the organization of these sequences. The localization of nucleolar organizer region (NOR) in mitotic chromosomes in *D. nasuta* is not known, but as in other *Drosophila* species (Patterson & Stone, 1952; Hennig, Link & Leoncini, 1975), the NOR would probably be localized in the heterochromatic segments. The frequent connexions seen between the alpha heterochromatin and the intranucleolar DNA is in all likelihood related to the localization of NOR in the heterochromatic regions of mitotic chromosomes. Furthermore, we have observed that in the interphase nuclei from larval ganglia also the nucleolus is always attached to the heterochromatic chromocentre.

Since in the majority of the polytene nuclei from late third instar larvae, the nucleolus was labelled with [^{3}H]thymidine, it is unlikely that the underreplication of rDNA in these nuclei is achieved by a complete block of replication of rDNA in nuclei with high polyteny. Therefore, the final underreplication of rDNA in polytene nuclei must be achieved by a slower and/or partial replication of rDNA sequences in these nuclei.

In an earlier autoradiographic study of replication of intranucleolar DNA in polytene cells of *Drosophila melanogaster*, Rodman (1969) suggested that the replication of intranucleolar and chromosomal DNA in a nucleus is synchronous. However, a few other autoradiographic observations (Jacob & Danieli, 1970; Berendes, 1973; Lakhotia, 1975) indicated that in polytene nuclei, the intranucleolar DNA may replicate independently of the chromosomal DNA. Results of the present detailed study in *D. nasuta* also do not agree with Rodman's (1969) observations. We have seen that the disperse nucleolar DNA can be labelled even when the chromosomes are unlabelled, resulting in a significantly higher labelling index of nucleolus compared to chromosomes. The simplest inference to be drawn from the higher labelling index of nucleolar DNA would be that the replication cycles of intranucleolar DNA (d-No-DNA) are more prolonged than the chromosomal replication cycles. Furthermore, the degree of [^{3}H]thymidine incorporation in the disperse nucleolar DNA is not in any way correlated to the degree or pattern of chromosomal labelling. Thus the d-No-DNA replication cycles appear to be slower and asynchronous with the chromosomal replication cycles.

The significance of the variable morphology of condensed nucleolar DNA mass is not immediately clear. Nevertheless, the fact that when in compact and rounded form it is very often unlabelled and when in a disperse state it may be heavily labelled, may indicate some kind of interrelationship between its morphology and replication. The very infrequent labelling (in 9·3% of nuclei) of c-No-DNA may be either due to a relatively brief duration of its replication cycle or to infrequent replication. Absence of c-No-DNA labelling in nuclei with d-No-DNA and/or chromosomes unlabelled (except in 5 nuclei, see Table 3) may indicate that the c-No-DNA incorporates [^{3}H]thymidine only when both chromosomes and d-No-DNA of the nucleus are also replicating. However, since as noted above, the replication cycles of chromosomes and d-No-DNA themselves are asynchronous, the replication of c-No-DNA may be related in a more complex manner with the chromosomal and d-No-DNA replication cycles. At present an independent replication cycle of the c-No-DNA cannot, however, be ruled out. Nevertheless, since the extent of rDNA replication in polytene nuclei is related to the level of polyteny of chromosomes (Spear & Gall, 1973) the replication of intranucleolar DNA would be ultimately regulated by chromosomal replication cycles.

We do not know how the rDNA copies are arranged in the nucleolus in polytene cells of *D. nasuta* and what the relationship is between the c-No-DNA and d-No-DNA. However, a few features are noteworthy in this context. The c-No-DNA usually remains darkly stained and brightly fluorescing with QM as well as with H. Results of our [^{3}H]thymidine incorporation studies do not rule out the possibility that the

condensed and disperse intranucleolar DNA may have independent replication cycles. More interestingly, we have also observed (unpublished) that when labelled with [^{3}H]uridine the d-No-DNA is labelled heavily, while the c-No-DNA mass in almost all nuclei remains totally unlabelled. This suggests that only the disperse nucleolar DNA is transcriptionally active (presumably for rRNA synthesis), while the c-No-DNA does not transcribe. These observations suggest one of two possibilities. Either, the condensed nucleolar DNA represents a part of the heterochromatin which surrounds the nucleolar organizer region in diploid cells; during polytenization this part may be drawn up within the nucleolar body and while the rDNA sequences ramify and replicate slowly in the body of the nucleolus, this region of heterochromatin remains as a centrally located condensed mass which is relatively less active in transcription and replication. Alternatively, it is also possible that both the c-No-DNA and d-No-DNA are largely comprised of rDNA, but in polytene cells a certain fraction of the redundant copies is transcriptionally more active than the others; the latter may remain as the condensed mass. These two fractions may also replicate differently in such a way that the total rDNA content of a polytene nucleus remains underreplicated compared to euchromatic DNA. Observations of Zuchowiski & Harford (1976*a*, *b*) on integrated and unintegrated ribosomal genes in polytene cells of *D. melanogaster* may be relevant in this context. Similarly, evidence for the presence of at least two kinds of rDNA repeat sequences (with or without an insertion in the 28S sequences) and the possibility of their differential participation in transcription in different cell types in *D. melanogaster* (Glover & Hogness, 1977; White & Hogness, 1977; Wellauer & Dawid, 1977) may also have a bearing on the present observation on the organization of intranucleolar DNA in condensed and disperse forms in polytene cells of *D. nasuta*. However, with the presently available information, it is not possible to positively interrelate these diverse observations. Further studies on the organization of rDNA cistrons in polytene cells of *D. nasuta* may be informative.

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