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Chromosome structure and DNA replication in nurse and follicle cells of *Drosophila melanogaster*

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Abstract. In the nurse cells of *Drosophila*, nuclear DNA is replicated many times without nuclear division. Nurse cells differ from salivary gland cells, another type of endoreplicated *Drosophila* cell, in that banded polytene chromosomes are not seen in large nurse cells. Cytophotometry of Feulgen stained nurse cell nuclei that have also been labeled with ^{3}H-thymidine shows that the DNA contents between S-phases are not doublings of the diploid value. In situ hybridization of cloned probes for 28S + 18S ribosomal RNA, 5S RNA, and histone genes, and for satellite, copia, and telomere sequences shows that satellite and histone sequences replicate only partially during nurse cell growth, while 5S sequences fully replicate. However, during the last nurse cell endoreplication cycle, all sequences including the previously under-replicated satellite sequences replicate fully. In situ hybridization experiments also demonstrate that the loci for the multiple copies of histone and 5S RNA genes are clustered into a small number of sites. In contrast, 28S + 18S rRNA genes are dispersed. We discuss the implications of the observed distribution of sequences within nurse cell nuclei for interphase nuclear organization. – In the ovarian follicle cells, which undergo only two or three endoreplication cycles, satellite, histone and ribosomal DNA sequences are also found by in situ hybridization to be underrepresented; satellite sequences may not replicate beyond their level in 2C cells. Hence the pathways of endoreplication in three cell types, salivary gland, nurse, and follicle cells, share basic features of DNA replication, and differ primarily in the extent of association of the duplicated chromatids.

Introduction

Many organisms have cells in which several rounds of DNA replication occur without cell or nuclear division. These endoreplications result in large cells whose nuclei contain many copies of each chromosome (see Nagl 1978). The most striking endoreplicated cells are those in which the many copies of each chromosome remain tightly bundled together, producing banded polytene chromosomes (see Beermann 1962). Polytene chromosomes have often been used as a model system for studying chromosome structure, replication and transcription. The bundling together of 1,000 or more copies of each locus permits cytological analyses that would be difficult in mitotically dividing cells with only two or four copies.

However, most endoreplicated cells do not have polytene chromosomes. In some cases chromosomes clearly separate from one another after duplication, leading to endopolyploidy. Separation almost always occurs when the chromosomes go through cycles of condensation and decondensation (endomitosis: see Geitler 1953). In contrast, the salivary glands of several Cecidomyiidae contain cells whose banded polytene chromosomes break up into smaller bundles at a specific developmental stage (Ashburner 1980) without endomitotic condensation. Environmental and genetic factors can both affect transitions between polyteny and endopolyploidy (Ribbert 1979; King et al. 1981; see Nagl 1978).

The ovarian nurse cells of *Drosophila* are mitotic sisters of the oocyte. They appear to synthesize most of the RNA and protein that is important in programming the egg for early embryonic development (see King 1970; Mahowald and Kambysellis 1980). Multiple rounds of DNA replication take place in nurse cells without intervening nuclear division, but the ensuing chromosome organization is unlike that of other endoreplicated *Drosophila* cells, such as salivary glands cells, that have banded polytene chromosomes. Very young nurse cell nuclei have distinct polytene chromosomes (Brun and Chevassu 1958), but chromosomes cannot be distinguished in larger nuclei.

Our observations reveal striking similarities with polytene cells, despite differences in chromosome organization. We measured the total DNA content of nurse cells in *D. melanogaster* by Feulgen staining and cytophotometry. Cells engaged in DNA synthesis were identified by radiolabeling with ^{3}H-thymidine before staining. Hence we could determine the total DNA content of cells that were between S-phases, and learn whether or not these DNA contents formed a doubling series.

Heterochromatic and rDNA sequences in polytene *Drosophila* nuclei replicate less than the rest of the DNA (Hennig and Meer 1971; Spear 1977). Euchromatic sequences within a single polytene nucleus may also be replicated to slightly different extents (Laird 1980; Lifschytz 1983). To investigate the replication of specific sequences in nurse cells and follicle cells, we followed the replication of satellite, 28S and 18S rDNA, 5S DNA, histone and telomere sequences using quantitative in situ hybridization.

We have also used in situ hybridization to investigate the spatial distribution of these sequences within nurse cell nuclei. In *Drosophila*, several phenomena suggest the existence and functional significance of interphase chromosome organization, including ectopic pairing (Kaufmann

and Iddles 1963), the association of homologues in mitosis and somatic recombination (Becker 1976), the zeste-white interaction (Jack and Judd 1979), and transvection (Lewis 1954). However, all these phenomena only indirectly reveal the distribution of sequences in intact interphase nuclei. Direct studies are possible in mitotic nuclei (Lifschytz and Hareven 1982; Heslop-Harrison and Bennett 1983), in polytene nuclei (Agard and Sedat 1983), and in nurse cell nuclei, as described here.

Materials and methods

Details not given here were described in Hammond and Laird (1985).

Preparation of slides. Drosophila melanogaster (strain Canton-S) were raised on standard cornmeal medium at 25° C under uncrowded conditions.

Ovaries were dissected in Ephrussi-Beadle Ringer's solution from adult females 24–72 h after eclosion. Several isolated egg chambers (stage 8 or older) or strings of younger egg chambers were transferred to a drop of Ringer's solution on a siliconized cover slip, which was then picked up with a slide "subbed" by dipping in 0.1% gelatin, 0.01% chrome alum. In some control experiments, egg chambers were prefixed for 3 min in 45% acetic acid. When necessary, egg chambers were staged before squashing (according to the criteria given in Table 1). Squashing was accomplished by first gently tapping the cover slip to rupture the egg chambers and release nurse cell nuclei, and then drawing off most of the liquid with filter paper. Final flattening required gentle pressure on the cover slip. The squashing process took 1–2 min. Cover slips were removed after freezing in liquid nitrogen. Slides for cytophotometry were fixed overnight at 4° C in ethanol:formaldehyde:acetic acid (85:10:5), while slides for in situ hybridization were fixed for 10 min at 22° C in ethanol:acetic acid (3:1). Following fixation, slides were rinsed in 95% ethanol and air dried. These preparations contained, beside nurse cell nuclei, the follicle cells that form an epithelium around the nurse cell-oocyte complex. First leg imaginal discs were dissected from female white prepupae and prepared similarly, except that squashing was accomplished by hard thumb pressure.

Salivary glands were dissected from late third instar larvae, and fixed for 2–4 min in ethanol:acetic acid (3:1). Nuclei were broken open and squashed in 45% acetic acid between a subbed slide and a siliconized cover slip. The preparations were fixed in ethanol:acetic acid (3:1) for 10 min, rinsed in 95% ethanol and air dried.

Cytophotometry. The DNA contents of nurse cells were measured with the aid of a Vickers M85 microdensitometer after acid hydrolysis and staining with Schiff's reagent. To relate the DNA contents measured in machine units to multiples of the haploid *Drosophila* genome (multiples of C), we compared nurse cells to 4C imaginal disc cells.

The data presented are partially corrected for glare, but not for residual distribution error. Fully corrected values for salivary gland cells would differ from the values we present, relative to disc cells, by less than 12% in all cases, and less than 7% for all except some of the 50C and 100C nuclei (see Hammond 1984).

Labeling with ^{3}H-thymidine. To label cells synthesizing DNA, ovaries or discs were incubated for 20 min at 22° C in a drop of Ringer's solution containing methyl-^{3}H-thymidine (New England Nuclear) 80 Ci/mmol at a concentration of 40 μCi/ml, then rinsed four times with Ringer's solution. Subsequent preparation, fixation, staining, and cytophotometry of nuclei were as described above.

In control experiments, deoxyribonuclease treatment (DNase I, Sigma) reduced labeling of nuclei to background levels, while ribonuclease treatment (RNase A, Worthington) had no discernible effect on labeling.

After cytophotometry, slides were exposed to Kodak NTB-2 emulsion, diluted 1:1 with water, for 6–8 days at 22° C and developed in Kodak D-19 for 2 min at 22° C. Grains over nuclei were counted and a correction was made for background. The background was defined as the average grain number over a tissue-free area of equal size: for disc nuclei this was <1 grain, for nurse cell nuclei it varied from 8–35 grains, depending on the size of the nucleus and the preparation. Nuclei were classified as "unlabeled", "moderately labeled", or "heavily labeled". "Unlabeled" nuclei had less than twice background labeling, while "moderately labeled" nuclei had <10 grains (disc), <100 grains (small nurse cell nuclei), or <250 grains (large nurse cell nuclei).

In situ hybridization. Radiolabeled probes for 28S+18S rRNA, 5S RNA and histone genes, and for telomere and 1.705 g/cm^{3} satellite sequences were prepared as described in Hammond and Laird (1984). The probe for copia sequences was cRNA made from plasmid cDm 351 (Finnegan et al. 1978). Hybridization, autoradiography, quantitation of the amount of hybridization, and control experiments with deoxyribonuclease pretreatment were performed as described in Hammond and Laird (1984).

Table 1. Criteria for staging egg chambers in adult *D. melanogaster* based on King (1970)

Stage	Shape	Yolk	Follicle cells	Size (μm)
4	Spherical			<45 × 40
5	to	Absent		<55 × 50
6	oval		Columnar	<75 × 65
7				140 × 85[a]
8		<1/3 e.c.[b] volume		190 × 95[a]
9	Elongate	<1/2 e.c.[b] volume	Squamous over part to all of nurse cell region	350 × 140[a]
10A		1/2 e.c.[b] volume		450 × 160[a]

[a] Typical size, classified on other criteria
[b] e.c. = egg chamber

Results

Cytophotometric and labeling experiments

Nurse cells were labeled with ^{3}H-thymidine to detect those nuclei engaged in DNA replication, and then Feulgen stained to measure their DNA content. The distribution of DNA content and amount of labeling for individual nurse cell nuclei is shown in Figure 1. The DNA contents

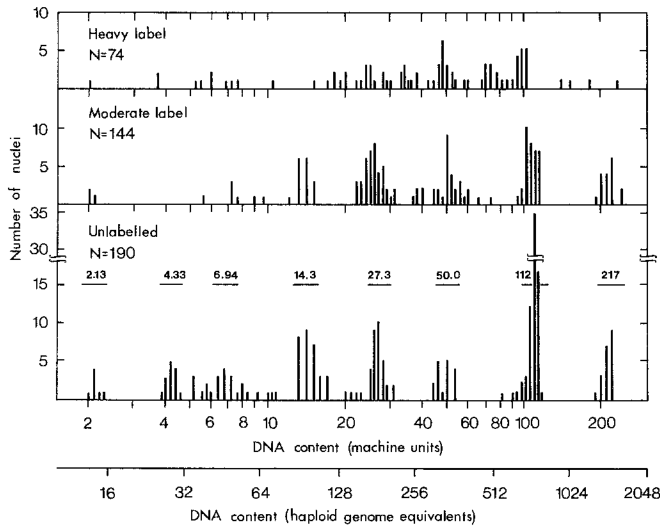

Fig. 1. DNA content and ^{3}H-thymidine incorporation in nurse cells. Ovaries were labeled with ^{3}H-thymidine, then egg chambers were isolated and squashed in Ringer's solution. Preparations were fixed and Feulgen stained, and the DNA content of each nucleus was determined cytophotometrically. The extent of labeling of each nucleus was then determined autoradiographically (see Materials and methods). Nuclei are plotted according to extent of labeling and DNA content. Numbers above the peaks of unlabeled nuclei are mean peak DNA contents (see Table 2). The data are from seven preparations stained in three different sessions. Data from different sessions were combined after normalizing to the DNA content of 4C disc cells

of unlabeled nuclei fall into discrete peaks. The nuclei represented in these peaks presumably came from cells that were between S-phases. The DNA contents of most of the heavily labeled nuclei fall between the peaks in the distribution of unlabeled nuclei. The pattern observed is that expected if DNA replication in nurse cells takes place in discrete S-phases: unlabeled nuclei in the main peaks were between S-phases, while labeled nuclei between the peaks were engaged in S-phase DNA replication.

We determined the mean DNA contents of cells that were between S-phases by taking the mean of all the unlabeled nuclei with DNA contents within 10% of the modal class within each peak. After the 32C stage, these mean peak values are less than would be expected if the complete genome present in diploid cells were duplicating in each S-phase (Table 2). The values fit much better with the DNA contents expected if part of the genome fails to replicate during nurse cell growth. Hence the data suggest that some sequences replicate little during successive S-phases beyond 32C in nurse cells.

We have also determined the DNA contents of nurse cells from staged egg chambers of various ages (Table 3). Some stage 7 chambers contain nuclei from three different classes. By stages 9 and 10A, most chambers contain 4 nuclei with about 1,500 times, and 11 nuclei with about 750 times the haploid amount of DNA. Moreover, by stage 10A, nuclei that incroporate ^{3}H-thymidine are very rare and this 4 and 11 distribution seems to be the final state before nurse cell breakdown.

Table 2. DNA contents of nonreplicating nurse cell nuclei, and a comparison with values predicted if all, or only 75% of the genome is replicating beyond 32C stage

Predicted by full replication		Predicted by 75% replicating[b] (m.u.)	Observed[c] (m.u.)
(C-value)	(m.u.)[a]		
4	0.586±0.01[d]	0.586	—
8	1.17	1.17	—
16	2.34	2.34	2.13±0.07
32	4.69	4.69	4.33±0.11
64	9.37	8.2	6.94±0.24
128	18.7	15.2	14.3 ±0.29
256	37.5	29.3	27.3 ±0.41
512	74.9	57.4	50.0 ±1.7
1024	150	113	112 ±0.74
2048	300	226	217 ±3.6

Nonreplicating nuclei are those unlabeled after incubation with ^{3}H-thymidine

[a] m.u. = machine units
[b] 25% of genome fails to replicate beyond 32C
[c] Mean peak values for unlabeled nurse cell nuclei ±(standard error × 1.96). Calculated by taking the mean of all values ±10% from the modal class in each peak (see Fig. 6)
[d] 4C value is mean ±(standard error × 1.96) of the 4C class of unlabeled disc nuclei. Data from Hammond and Laird (1985)

Table 3. Classes of DNA content in staged nurse cell nuclei

Stage[a]	Classes[b]							
4	(1)	2	3					
5		(2)	3	4				
6			(3)	4	5			
7					5	6	(7)	
8							7	8	
9/10A								8	9

[a] See Materials and methods, Table 1
[b] Number of replication cycles beyond 4C; ( ) indicates infrequent class

Replication of defined sequences

The cytophotometry suggests that some DNA sequences do not replicate fully during nurse cell growth. To determine the extent of replication of defined sequences, cloned probes representing 28S + 18S rRNA, 5S RNA and histone genes, satellite sequences, a telomeric sequence, and copia (a dispersed middle repetitive element) were hybridized in situ to stage 9 or 10A nurse cells. The two classes of nurse cells (765C and 1480C) can be distinguished reliably in squash preparations on the basis of nuclear size, enabling determination of the extent of hybridization in each (Fig. 2).

We also determined the extent of hybridization to diploid prepupal disc cells. The representation of each sequence is shown in Table 4. Satellite sequences are greatly underrepresented in nurse cells. Histone genes are present at less than half the level expected from full replication, while telomere and 28S + 18S rDNA sequences appear to be underrepresented to a lesser extent. In contrast, 5S genes appear to be fully replicated in nurse cells. There are some possible sources of error in quantitative comparisons of in situ hy-

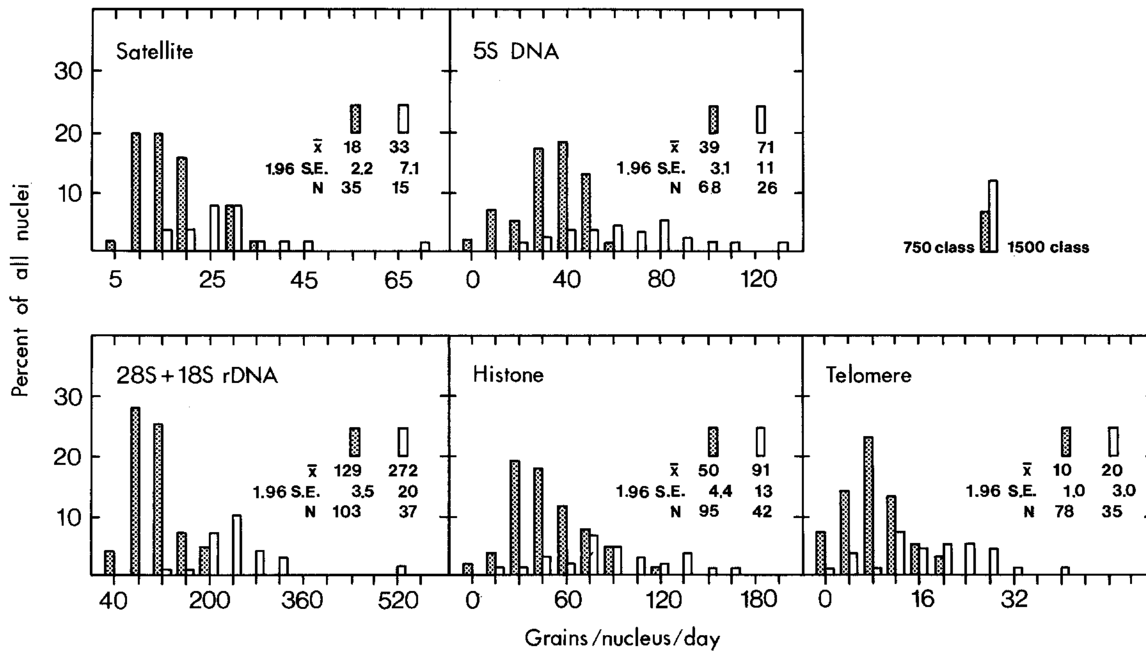

Fig. 2. In situ hybridization to nurse cells. Tritiated probes for satellite, 28S+18S rDNA, 5S, histone, and telomere sequences were hybridized in situ to squashed nurse cell nuclei from stage 9 or 10A egg chambers. The amount of probe bound was determined autoradiographically, after various exposure times (see Materials and methods). The panels show the number of grains per nucleus per day of exposure. Separate distributions are shown for the two size classes of nuclei in stage 9/10A egg chambers. 750C (*shaded bars*) and 1500C (*white bars*). Calculations of the mean ($\bar{x}$) and 95% confidence limits ($\pm 1.96\times$ the standard error) were performed separately for each class

Table 4. Comparison of sequence representation in nurse, follicle, and disc cells

Probe	Mean grain number/cell/day				Average DNA content[d]			Other data[d]
	Disc[a]	Nurse cells[b]		FC[c]	Nurse cells (stage 9/10A)		FC	
		Small	Large		Small	Large		
Total DNA (Feulgen)	–	–	–	–	765C	1480C	14.3C	–
Satellite (1.705)	1.90	18	33	1.23	36.4C (4.8%)	66.7C (4.5%)	2.5C (17%)	–
28S+18S rDNA	0.78	129	272	1.39	635C (83%)	1339C (90%)	6.8C (49%)	(85%)
Histone	0.67	50	91	1.11	287C (37%)	521C (35%)	6.17C (44%)	–
Telomere	0.083	10	20	0.17	463C (60%)	925C (63%)	7.9C (55%)	–
5S rDNA	0.18	39	71	0.97	832C (109%)	1515C (102%)	20.7C (145%)	(100%)

[a] Nuclei of first leg discs from female white prepupae. The mean DNA content of these cells is 3.84C (see Hammond and Laird 1985)

[b] Nurse cell nuclei; data presented for the two size classes present in stage 9 and stage 10A egg chambers

[c] Follicle cell nuclei; data presented for follicle cells from stage 9 and stage 10A egg chambers

[d] C values are calculated from the ratio of grain numbers for nurse cell and follicle cell nuclei compared with disc nuclei, times the average C value (3.84) for disc nuclei. Percentage values are calculated from the ratio of C values for the defined DNA sequence relative to total DNA. Percentage values listed under "Other data" were estimated as the percentage of sequence representation relative to that expected for uniform replication, using data of Renkawitz-Pohl (1978) for a mixture of nurse and follicle cells from *D. hydei*

bridization to different cell types (see Discussion and Hammond and Laird 1984). An independent way of checking the accuracy of quantitative in situ hybridization is to compare our results with results obtained using different methods (see Table 4). While the results are in good agreement, the available data are limited. Hence we cannot be certain that the slight underrepresentation we observe for telomere and 28S+18S rDNA sequences is significant.

When is the replication of the underrepresented sequences interrupted? All sequences appear to be replicating late in nurse cell life, as can be seen by comparing hybridization to the 750C and 1500C classes of stage 9/10A nurse cell nuclei (Fig. 2). In all cases, the larger class of nuclei hybridizes approximately twice as much probe. These results suggest that satellite replication is blocked early, and resumes late during nurse cell growth.

We have also analyzed the representation of each sequence in another endoreplicated cell type, the follicle cells of stage 9 and 10A egg chambers (Mahowald et al. 1979). We find that these nuclei have either about 13 or 26 times

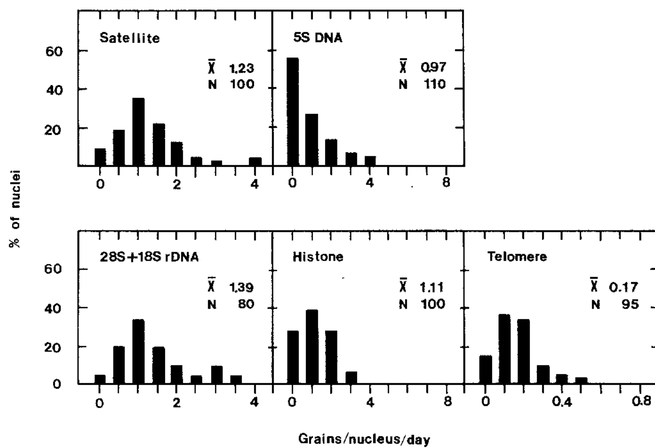

Fig. 3. In situ hybridization to follicle cells. Tritiated probes for satellite, 28S + 18S rDNA, 5S DNA, histone and satellite sequences were hybridized in situ to squashed follicle cell nuclei from stage 9 or 10A egg chambers. The amount of probe bound was determined autoradiographically after various exposure times (see Materials and methods). Panels show the number of grains per nucleus per day of exposure, the mean ($\bar{x}$) and the number of nuclei scored (*N*)

the haploid DNA content. Ninety percent have 13C so that the average DNA content is 14.3C. The extent of hybridization of the cloned probes to follicle cell nuclei is shown in Figure 3. Satellite, histone, 28S + 18S rDNA and telomere sequences all appear to be underrepresented compared to their representation in diploid cells (Table 4). Only 5S sequences appear to be fully replicated. The amount of satellite sequence is less than that in 4C disc nuclei.

Distribution of defined sequences

How are the many copies of each chromosome distributed within nurse cell nuclei? The distribution of a sequence in an intact nucleus can be inferred from the sites of hybridization within a squashed nurse cell nucleus. To ensure that under our hybridization conditions the probes were only reacting with the expected sequences, we also hybridized the same probes to salivary gland nuclei broken open before squashing, so that sites along chromosome arms could be distinguished. Hybridization patterns in disc and follicle cell nuclei were also examined, but the low copy number and small size of these nuclei precluded an extensive analysis.

28S + 18S ribosomal DNA. The rDNA sequences are distributed throughout nurse cell nuclei (Fig. 4a). Many of the grains fall over areas that stain only lightly with Giemsa; presumably they correspond to the dispersed nurse cell nucleolus described by Dapples and King (1970). In the smallest nurse cells examined, hybridization is sometimes limited to part of the nucleus (Fig. 4b). Only the nucleolus in dispersed salivary gland nuclei is a target for the rDNA probe (Fig. 4c). A single distinct site of hybridization corresponding to the nucleolus is also clear in follicle cells (Fig. 4a) and disc cells (Fig. 4d).

Histone genes. In stage 9/10A nurse cell nuclei, histone genes are tightly clustered in a small number of sites (Fig. 5a). The number of distinct sites varies: 750C stage 9/10A nurse cell nuclei have 2–10 while 1500C nurse cell nuclei have 4–13 (Fig. 6). Some of this variation may reflect superimposition of distinct sites, or fragmentation of a sin-

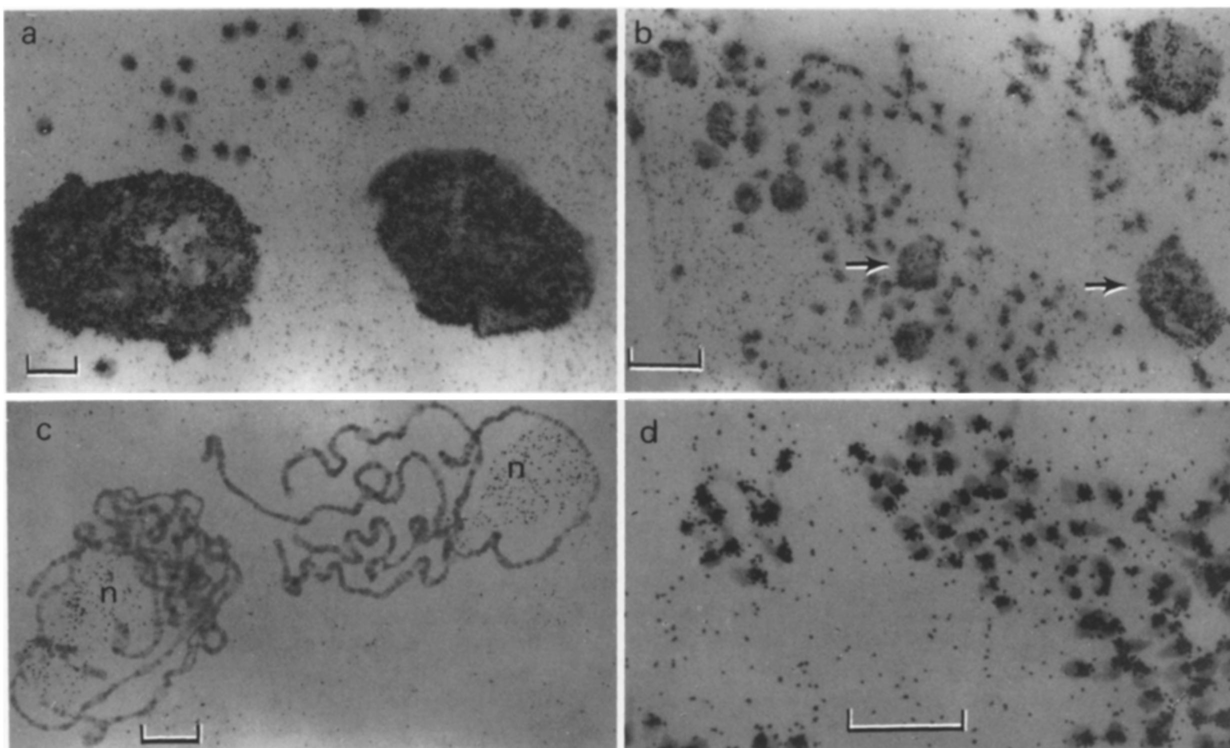

Fig. 4a–d. Localization of 28S + 18S ribosomal RNA genes. Nuclei were hybridized with a ^{3}H-cRNA probe made from plasmid pDmrY22. **a** Two nurse cell nuclei and several follicle cell nuclei from a stage 9 egg chamber. **b** Nurse cell (*arrows*) and follicle cell nuclei from younger egg chambers. **c** Spread polytene chromosomes from salivary gland nuclei. *n* nucleolus. **d** Disc cell nuclei. Bars represent 20 µm

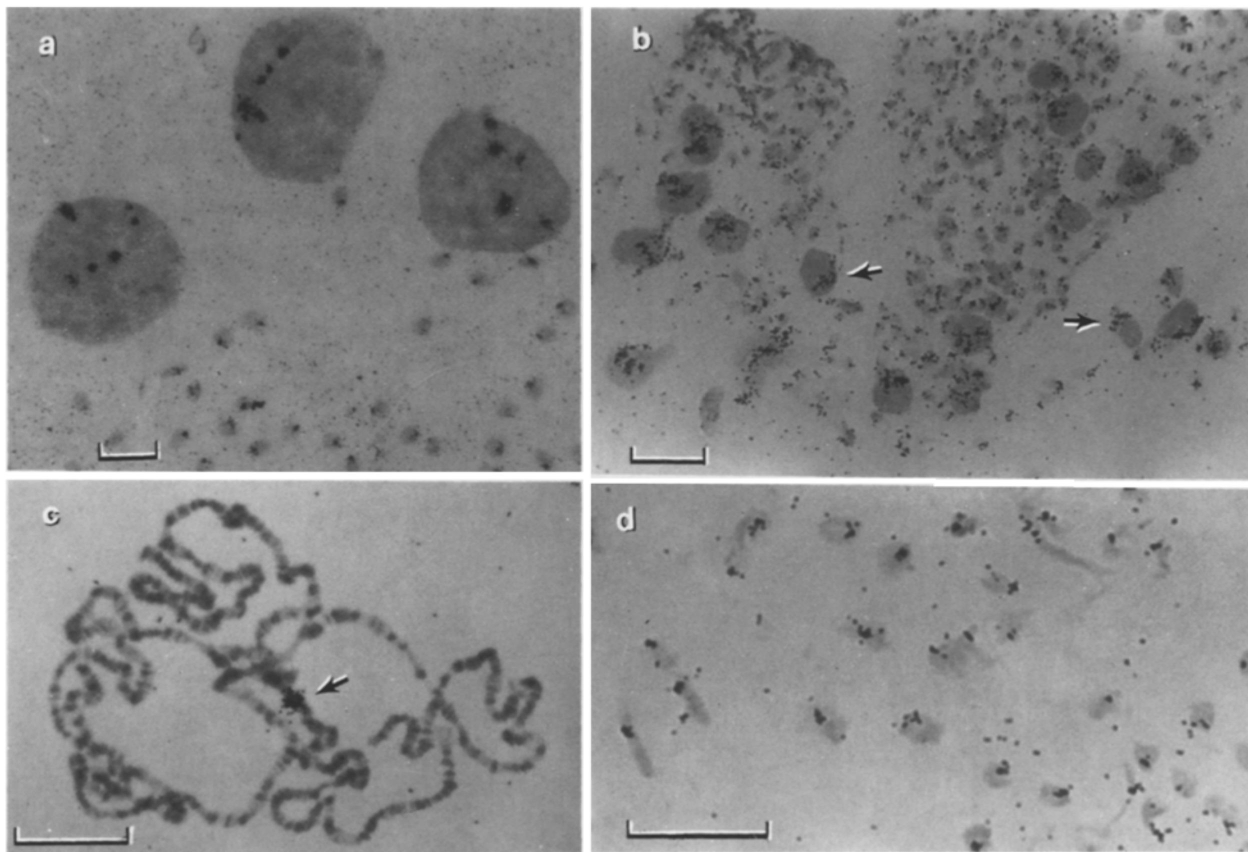

Fig. 5a–d. Localization of histone genes. Nuclei were hybridized with ^{3}H-cRNA made from plasmid aDm-3000-1. **a** Three nurse cell nuclei and several follicle cell nuclei from a stage 9 egg chamber. **b** Younger nurse cell (*arrows*) and follicle cell nuclei. **c** Spread polytene chromosomes from a salivary nucleus. *Arrow* indicates site of hybridzation at base of chromosome *2L*. **d** Disc cell nuclei. Bars represent 20 µm

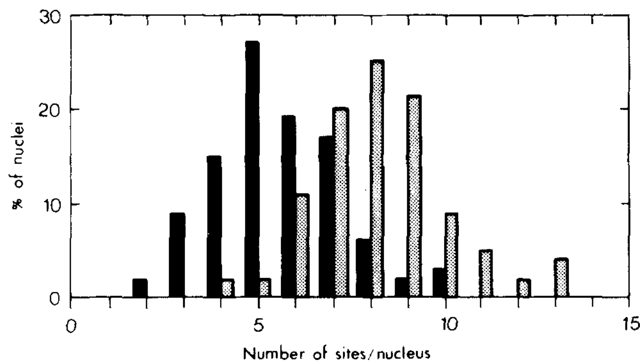

Fig. 6. Number of sites of hybridization of histone sequences in nurse cells. Nurse cells were hybridized in situ to a tritiated probe for histone gene sequences. Autoradiography was used to determine the number of sites within the nucleus to which the histone probe bound. A site was defined as a continuous region of fused silver grains. The emulsion was greatly overexposed (exposure times 20–30 days). The number of sites in 750C (*black bars*) and 1500C nuclei (*shaded bars*) are shown separately

gle site, during squashing. However, the variation is unchanged if egg chambers are fixed before squashing, and it is thus likely that the number of sites is variable, even within intact nuclei of the same size. Sites within a nucleus differ from one another in grain number, presumably reflecting differences in the number of homologous copies of the histone locus at each site. In more than half the nuclei all the sites are restricted to less than a third of the nuclear area. Younger nurse cell nuclei also have multiple histone clusters (Fig. 5b). In the youngest nurse cells, the size of the target nuclei is too small to permit us to distinguish multiple sites from a single site, but it is clear that the histone genes are restricted to part of the nucleus.

In contrast, histone loci in follicle and disc cells appear to form a single tight cluster (Fig. 5a, d). In spread preparations of salivary gland chromosomes, more than 99% of hybridization is near the base of the left arm of chromosome *2* (Fig. 5c), as expected if the probe hybridizes only to histone genes (Pardue et al. 1977; Lifton et al. 1978).

5S genes. The fully replicated 5S loci are also nonrandomly distributed in nurse cells. As with the histone probe, there are clusters of grains, and these clusters are restricted to part of the nucleus in both stage 9 and younger nurse cells (Fig. 7a, b). However, the number of 5S gene clusters in each nucleus is higher than the number of histone clusters. The 750C stage 9/10A nuclei have 10–23 sites while the 1500C nuclei have 15–38 sites. Prefixed nuclei have the same pattern. Sites vary in grain number, and it is possible that additional sites with small numbers of 5S genes also exist. There is a tendency for the 5S sites to be clustered along the edge of the nucleus (e.g. Fig. 7a).

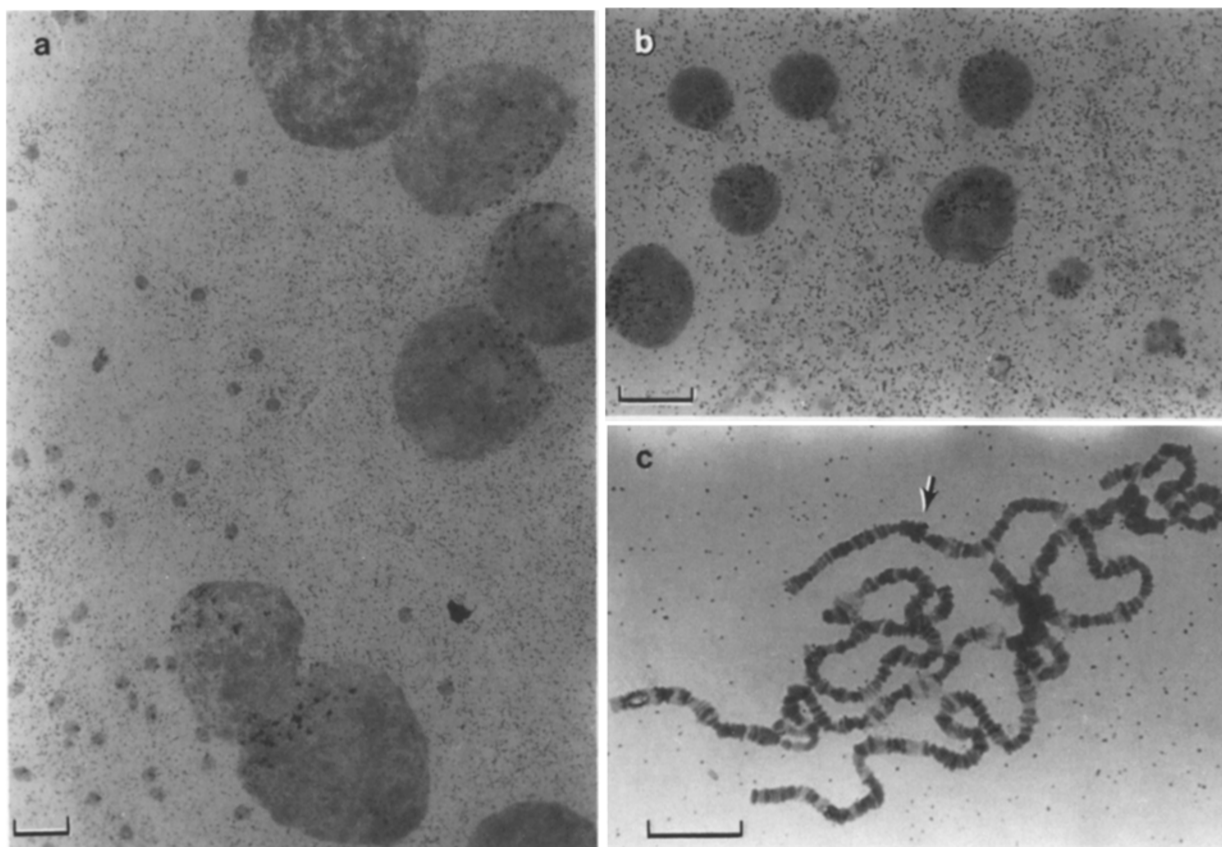

Fig. 7a–c. Localization of 5S genes. Nuclei were hybridized with a ^{3}H-cRNA probe made from plasmid 12D1. **a** Nurse cell and follicle cell nuclei from a stage 9 egg chamber. **b** Younger nurse cell nuclei. **c** Polytene chromosomes from a salivary gland nucleus. *Arrow* indicates site of hybridzation on chromosome *2R*. Bars represent 20 μm

In spread salivary gland nuclei, the single site of hybridization (containing more than 95% of the grains over the chromosomes) is near the end of *2R* (Fig. 7c), which is the cytological location previously reported for 5S genes (Wimber and Steffenson 1970).

Telomere sequences. Telomere-specific sequences in nurse cell nuclei do not appear to be restricted to a part of the nucleus. However, grains are distributed in a 'patchy' fashion rather than being evenly dispersed (Fig. 8a). When salivary gland chromosomes are spread out, a hybridization site at the tip of each chromosome can be seen (Rubin et al. 1978; our Fig. 8b).

Copia sequences. Copia sequences appear to be randomly distributed within nurse cell nuclei (Fig. 9a). In salivary gland nuclei multiple distinct sites of hybridization are seen (Fig. 9b), as expected from previous work (Finnegan et al. 1978).

Satellite sequences. The degree of localization of the 1.705 g/cm^3 satellite sequences varies from nucleus to nucleus. At one extreme are nuclei with 90% of the grains within 10% of the nuclear area; at the other extreme are nuclei with grains patchily distributed throughout the nucleus (Fig. 10a, b). This variable localization is in marked contrast to salivary gland, follicle cell, and disc nuclei, which have a single tight cluster of grains (Fig. 10a–d). In salivary gland preparations 90% of hybridization is to the chromocenter and hybridization to *21D* was not observed (compare Peacock et al. 1978).

Discussion

DNA contents of nurse cell nuclei are not doublings of the diploid value

The total DNA contents of nurse cell nuclei increase dramatically as the nurse cells grown, reaching 1,500 times the haploid amount of DNA in the largest cells. During the approximately 60 h of nurse cell growth (King 1970), most sequences must double eight or nine times beyond their copy number in a 4C nucleus. The nuclei within an egg chamber do not replicate in synchrony, as is shown by the labeling of only some of the nuclei after a short pulse of ^{3}H-thymidine (King and Burnett 1959; our observations). We find that this asynchrony produces nuclei within a single egg chamber that differ two-fold in DNA content as early as stage 4.

The maximal nurse cell DNA contents reported in previous studies are 1024C (Jacob and Sirlin 1959; King 1970) or 2048C (Schultz 1956). The inferred number of doubling cycles is in agreement with our results; however these previous studies did not describe a systematic deviation from

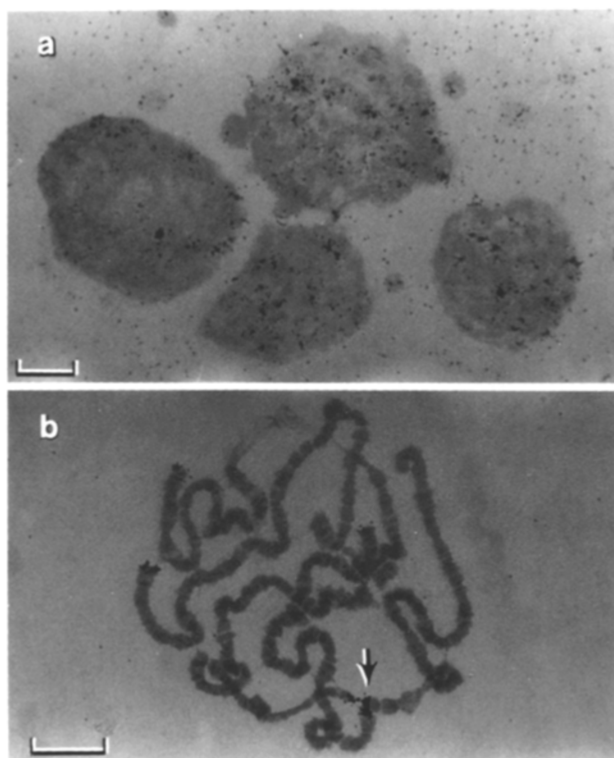

Fig. 8a, b. Localization of a telomere sequence. Nuclei were hybridized with ^{3}H-cRNA made from a plasmid containing a 3 kb telomere-specific sequence. **a** Stage 9 nurse and follicle cell nuclei. **b** Salivary gland chromosomes. *Arrow* indicates three fused telomeres. Bars represent 20 μm

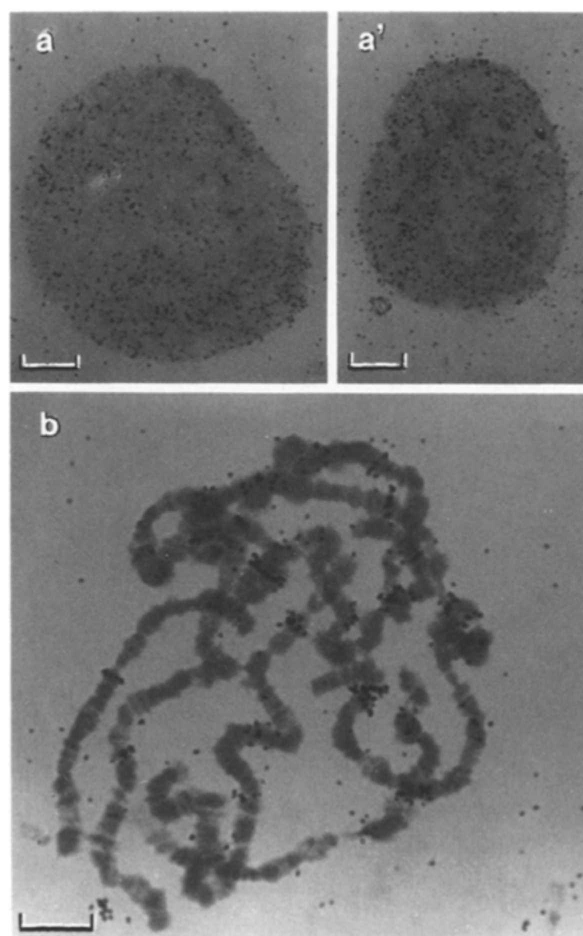

Fig. 9a, b. Localization of copia sequences. Nuclei were hybridized with a ^{3}H-cRNA probe made from plasmid cDm 351. **a** Stage 9 nurse cell nuclei. **b** Salivary gland chromosomes. Bars represent 20 μm

full replication. In contrast, we find that the DNA contents of nurse cell nuclei that are between S-phases are significantly less than exact doublings of the DNA content of diploid cells (Table 2). A possible explanation is that some sequences replicate little, or not at all, during nurse cell growth. We therefore tested whether specific sequences were underrepresented by hybridizing labeled probes for satellite, 28S+18S rDNA, 5S, histone, and telomere sequences to large nurse cell and disc cell nuclei in situ.

Replication of specific sequences in nurse and follicle cells

We find that 1.705 g/cm^{3} satellite sequences are greatly underrepresented in stage 9 nurse cells (Table 4), being present at levels representing only three or foor doublings of the satellite content of 4C mitotic cells. Satellite sequences constitute about 20% of the diploid *D. melanogaster* genome (Brutlag et al. 1977). Hence most of the difference between the total DNA contents observed and those predicted if all sequences replicate equally during nurse cell growth could be due to satellite underreplication, if all satellite sequences underreplicate to the same extent.

We have also measured the amount of satellite sequences in ovarian follicle cells. The amount in follicle cells is close to that in 2C diploid cells (Table 4). This observation implies that once follicle cells have stopped proliferating, satellite replication may be blocked, even at the 2C to 4C transition.

There have been several other studies of satellite levels in *Drosophila* ovaries. Endow and Gall (1975) quantitated DNA sequences on cesium chloride gradients, and concluded that satellite sequences were overreplicated in pupal ovaries of *D. virilis*, and present in ovaries of newly eclosed adults in the same proportion as in diploid cells. Although the DNA in these tissues was from cells at earlier stages than we used, it is surprising, in the light of our results, that underreplication was not already obvious. Renkawitz-Pohl and Kunz (1975) found that satellite sequences constitute 44% of the DNA in *D. virilis* diploid cells, but only 16% of the DNA in ovarian DNA from flies 2 d after eclosion. In *D. hydei*, satellites constitute 13% of diploid DNA, but only 2%–3% of the DNA in nurse cells and follicle cells (Renkawitz and Kunz 1975). Our results with *D. melanogaster*, which distinguish between follicle cells and nurse cells and between nurse cells with different degrees of endoreplication, demonstrate a more extreme underreplication of satellite in both nurse and follicle cells than has been reported before.

Histone genes are also underrepresented in both nurse and follicle cells, although they replicate several times more than satellite sequences. In contrst, another tendemly repeated gene family, that encoding 5S RNA, appears to replicate fully in both nurse and follicle cells (Table 4; see also Renkawitz-Pohl 1978; Jacobs-Lorena 1980).

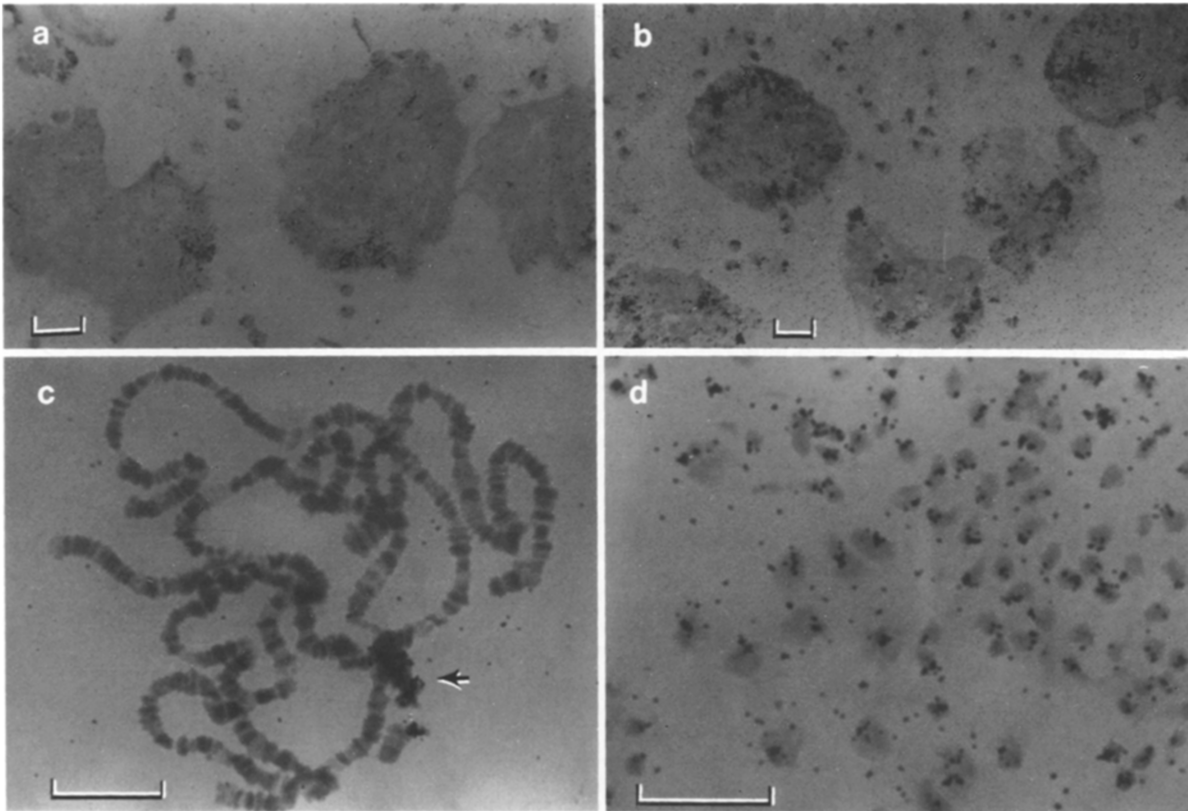

Fig. 10a–d. Localization of satellite sequences. Nuclei were hybridized with ^{3}H-cRNA made from a plasmid containing 1.705 g/cm^3 satellite sequences. **a** and **b** Nurse and follicle cell nuclei from stage 9 egg chambers. **c** Salivary gland chromosomes. *Arrow* indicates the site of hybridization at the chromocenter. **d** Disc cell nuclei. Bars represent 20 μm

We find that the genes for 28S+18S ribosomal RNA are only slightly, if at all, underrepresented in large nurse cell nuclei (Table 4). Jacobs-Lorena (1980) fractionated egg chambers by size, and found, by filter hybridization, that stage 9 egg chambers had 85% of the diploid rDNA level. Studies on *D. virilis* ovaries found rDNA to be at about 50% of the diploid level (Renkawitz and Kunz 1975; Endow and Gall 1975), while isolated nurse cells and follicle cells of unspecified size had 45% and 70% of diploid levels, respectively (Renkawitz and Kunz 1975). Our results suggest, on the contrary, that the extent of rDNA underreplication in *D. melanogaster* is greater in follicle cells than in nurse cells (Table 4). Since the synthesis of rRNA is one of the major transcriptional functions of nurse cell nuclei (see King 1970), it would have been surprising if rDNA were greatly underreplicated in nurse cells. Ribosomal DNA clearly replicates more during endoreplication in nurse cells than it does in salivary gland cells (see Hammond and Laird 1985). Hence satellite underreplication does not necessarily lead to the underreplication of rDNA.

It has been suggested that the extreme ends of polytene chromosomes are underreplicated in salivary gland nuclei (Laird et al. 1974; Roberts 1979). Our results show that the telomere-specific sequence that we have studied is not greatly underreplicated in nurse cells, nor in salivary gland cells (Hammond and Laird 1985). Hence either the suggestion that telomeres are greatly underreplicated is incorrect, or the sequence we have studied is not within the underreplicated region.

Satellite sequences replicate in large nurse cells

During the eight or nine rounds of replication in nurse cell growth, satellite sequences appear to double only three or four times. However, during the last round of nurse cell replication, the 1.705 g/cm^3 satellite sequences almost double (Fig. 2). Hence most of the replication of satellite sequences probably takes place late in nurse cell growth, inplying that replication is primarily blocked early, perhaps when the multiple copies of each chromosome are held more tightly together (Brun and Chevassu 1958). Other underreplicated sequences also appear to be doubling during the last round of replication (Fig. 2).

Some polytene cells have fully replicated satellite sequences (see Schmidt 1980). Ribbert (personal communication) finds that satellite sequences in nurse cells of *Calliphora* replicate fully whether or not the nurse cells have polytene chromosomes, although in the polytene trichogen cells satellite sequences are underreplicated. Zacharias (1979) also found heterochromatic sequences to be fully replicated in nurse cells, but not in salivary gland cells, of *Prodiamesa*. These apparent exemptions of the germ-line derived nurse cells from satellite underreplication are especially interesting in connection with the suggestion that satellite sequences, being frequently lost from somatic tissues, but maintained in the germ-line, have a germ-line specific function (John and Miklos 1979). However, our results for *Drosophila*, and results for *Anopheles* (Redfern 1981) and *Hyalophora* (Crippa and Telfer 1971), demonstrate con-

siderable underreplication of satellite sequences in nurse cells. Therefore it appears that the extent of satellite replication, and the cell types in which it occurs, depend on the species, and cannot yet be correlated reliably with particular cell types or particular chromosome structures.

Some sequences are restricted to part of the nurse cell nucleus

We used two probes that hybridize to single loci on different chromosomes: histone and 5S sequences. Both loci consist of tandemly repeated copies of a gene that is actively transcribed during oogenesis (Mermod et al. 1977; Anderson and Lengyel 1980). Most, if not all, of the several hundred histone loci in large nurse cell nuclei appear to be restricted to no more than a third of the nuclear volume. We observe a similar limited distribution for the 5S loci, confirming an earlier report by Szabo (1974).

In contrast, we find that telomere sequences are always dispersed, albeit patchily, in nurse cell nuclei (Fig. 8a). If the telomeres of each chromosome arm were tightly bound to one another, six distinct sites would be seen. Even if the patchy distribution observed does reflect multiple sites rather than complete dispersion, there are clearly considerably more than six sites.

Satellite sequences, which mark centromeres, also in some cases appear to be distributed throughout nurse cell nuclei (Fig. 9b), despite being extensively underreplicated. In other nuclei, however, most of the satellite sequences are restricted to a relatively small area (Fig. 10a). Evidently there is some tendency for centromeric regions to aggregate, including centromeres of different chromosomes, but the tendency is much less than in polytene cells (where centromeres are fused into a chromocenter), or in diploid interphase cells (Fig. 10d). We cannot tell if the aggregation is a feature of the satellite sequences themselves, or is caused by their proximity to centromeres. The latter conclusion was reached by Lifschytz and Hareven (1982) for interphase diploid nuclei of *Drosophila*, based on experiments showing that blocks of heterochromatin are clustered only because they are linked to interacting centromeres.

The genes for 28S + 18S ribosomal RNA are widely dispersed in large nurse cells (Fig. 4). Since other single-site sequences are restricted in their distributions, it would be of interest to determine whether or not extrachromosomal copies of rDNA sequences exist in nurse cell nuclei.

What restricts the distribution of sequences?

The observed restriction of histone and 5S sequences to part of the nucleus could be effected by a tight binding of all the copies of each chromosome at a small number of points, or by a more general weak attraction between homologous chromosomes. Alternatively, it could simply reflect limited dispersal of homologous chromosomes following duplication.

The sequences that we have studied differ markedly in their pairing properties, suggesting that pairing depends on particular loci. Histone loci, for example, are tightly clustered into a small number of sites. A close association of the two homologous histone loci in brain cells has been reported previously by Lifschytz and Hareven (1982). 5S genes are also clustered in nurse cells, but into a larger number of sites. If there are several loci along each chromosome that form a small number of tight clusters, then this clustering could be sufficient to hold the multiple copies of each chromosome together, as illustrated in Figure 11.

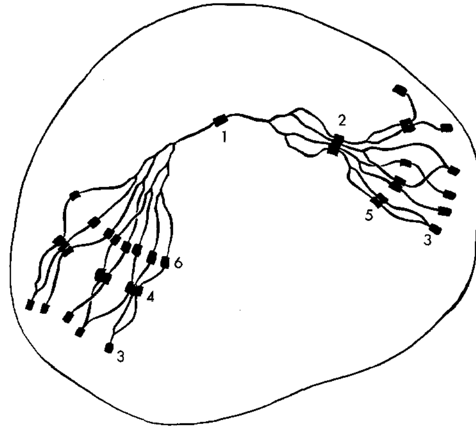

Fig. 11. Drawing of the proposed chromosome structure in nurse cells. For clarity, a single chromosome is drawn as if it had only eight copies of a fully replicated sequence – hence the full extent of underreplication is not shown. Numbered regions represent sequences with the following properties: *1* highly underreplicated (e.g. satellite sequences); *2* underreplicated, strong pairing (e.g. histone sequences); *3* a sequence near the telomere; *4, 5* fully replicated, moderate pairing (e.g. 5S DNA), *6* fully replicated, weak pairing

We suggest that nurse cell chromosomes have a structure resembling a polytene chromosome partially separated into oligotene fibrils, as seen, for example, in some Cecidomyiidae (Ashburner 1980). What determines which loci are clustered in a few sites, and which fall apart more readily? One possibility is that the determinant is chromosome location; another is that it is autonomous to a sequence. These alternatives could be distinguished by examining the clustering of, for example, histone genes in nurse cell nuclei of stocks with inversions or translocations that move the histone locus, or by seeing if cloned histone sequences inserted elsewhere in the genome by P-element mediated transformation induce clustering. It would also be interesting to see if the sequences present at the ectopic pairing sites of polytene chromosomes (of which the histone locus is one: see Pardue et al. 1977) are always tightly clustered. In the long term, it may be possible to produce nurse cells with analyzable polytene chromosomes by manipulating the number of pairing sites, and the strength of interactions between the copies of a chromosome. Preliminary attempts to increase pairing in nurse cells by raising *Drosophila* at 18° C, a protocol effective in *Calliphora*, (Ribbert 1979) produced no change in the clustering of histone or 5S genes, but had variable effects on the clustering of satellite and telomere sequences.

The nurse cell nucleus as a model interphase nucleus

In nurse cell nuclei, neither centromeres nor telomeres are tightly clustered, suggesting that such tight associations are specific features of polytene salivary nuclei rather than general features of transcriptionally active *Drosophila* nuclei. Heterochromatic sequences are often associated with the nuclear membrane of interphase nuclei (Comings 1980; Hancock and Boulitas 1982).

We have studied sequences that are transcribed in nurse cell nuclei by RNA polymerase I (28S+18S ribosomal RNA genes), II (histone genes) and III (5S genes). Although 28S+18S rRNA genes are dispersed in large nurse cell nuclei, they appear to occupy a distinct nucleolar compartment, albeit of unusually convoluted form (see King 1970). Are there separate compartments for RNA polymerase II and III transcription, and are nontranscribed sequences excluded from such compartments? This question could be addressed by analyzing the distribution, within a single nurse cell nucleus, of two sequences transcribed by the same RNA polymerase, or of two sequences expected to be in different compartments (histone and satellite sequences, for example).

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