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In situ study of chorion gene amplification in ovarian follicle cells of *Drosophila nasuta*

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Abstract. The temporal and spatial pattern of replication of chorion gene clusters in follicle cells during oogenesis in *Drosophila melanogaster* and *Drosophila nasuta* was examined by [^{3}H] thymidine autoradiography and by *in situ* hybridization with chorion gene probes. When pulse labelled with [^{3}H] thymidine, the follicle cells from stage 10-12 ovarian follicles of both *Drosophila melanogaster* and, *Drosophila nasuta* often showed intense labelling at only one or two sites per nucleus. *In situ* hybridization of chorion gene probes derived from *Drosophila melanogaster* with follicle cell nuclei of *Drosophila melanogaster* and *Drosophila nasuta* revealed these discrete [^{3}H] thymidine labelled sites to correspond to the two amplifying chorion gene clusters. It appears, therefore, that in spite of evolutionary divergence, the organization and programme of selective amplification of chorion genes in ovarian follicle cells have remained generally similar in these two species. The endoreplicated and amplified copies of each chorion gene cluster remain closely associated but the two clusters occupy separate sites in follicle cell nucleus.

Keywords. Chorion genes; amplification; endoreplication; *Drosophila; in situ* hybridization.

Introduction

Unequal replication of different DNA sequences in endoreplicating cells appears to be a characteristic feature of *Drosophila* ontogeny (for recent reviews, see Spradling and Orr-Weaver, 1987; Raman and Lakhotia, 1990). While in most cases certain sequences are under-replicated, at least one example is known where specific gene sequences, the chorion genes, are amplified in a developmental stage and cell type specific manner: the genome of *D. melanogaster* contains two clusters of chorion genes, one on X-chromosome and one on chromosome 3, which are selectively amplified in follicle cells between stages 8-14 of ovarian follicle growth (Spradling and Mahowald, 1980; Spradling *et al.,* 1980; Spradling, 1981). Studies on molecular organization of chorion genes in a number of *Drosophila* species (Martinez-Cruzado *et al.,* 1988) have shown that there are two clusters of chorion genes which show follicle-cell specific amplification in different species. In the present study, we have used a simple strategy to cytologically identify and compare the location and time course of amplification of chorion genes in ovarian follicle cells in *D. melanogaster* and *D. nasuta.* Phylogenetically, *D. nasuta,* belonging to the *immigrans* species group (Throckmorton, 1975), is more distantly related to *D. melanogaster* than those belonging to the *virilis/repleta* or the Hawaiian group of species examined by Martinez-Cruzado *et al.,* (1988). Our present studies provide useful information on the cytology of amplifying chorion genes and also show that

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the organization of chorion genes in *D. nasuta* is generally similar to that in *D. melanogaster*.

Materials and methods

[^3H] *Thymidine labelling of ovarioles*

Ovaries from 3-4 days old healthy females of wild type *D. melanogaster* and *D. nasuta* were dissected out in Poels' salt solution (Lakhotia and Mukherjee, 1980). Follicles between stages 9 and 12 were identified following the procedure described by King (1970), separated and transferred to fresh Poels' salt solution containing [^{3}H] thymidine (100 μCi/ml; sp. act. 66 Ci/mM, Amersham) for 20 min at 24°C. The labelled follicles were washed with cold isotope-free salt solution, briefly fixed in 1:3 aceto-methanol and squashed in 45% acetic acid. After removal of the coverslips, the preparations were autoradiographed using Kodak NTB-2 nuclear emulsion. After 3-4 days of exposure, the autoradiograms were developed, fixed, stained with Giemsa and examined for labelling of the follicle cells in follicles of different stages.

In situ hybridization of chorion gene probes with follicle cell nuclei

Unlabelled squash preparations of ovarian follicles (stages 10-12 of *D. melanogaster* and stage 13 of *D. nasuta*) were hybridized *in situ* with two chorion gene probes derived from the X-chromosomal (pl03) and autosomal (p302) gene clusters of *D. melanogaster,* respectively: the X-chromosomal probe includes the entire s38 gene with its flanking 5' and 3' regions while the autosomal probe spans the stretch encompassing sl8, sl5 and sl6 genes together with upstream region of the sl8 gene (see Spradling, 1981 for details). The probe DNAs were nick-translated using [^{3}H] dNTPs (Amersham) to obtain specific activities ranging between 1-2 × 10^7cpm/μg DNA. The procedure for *in situ* hybridization to nuclear DNA was as described by Pardue (1986). While the ovarian follicle preparations of *D. melanogaster* were hybridized singly with either of the probes, those of the stage 13 follicles of *D. nasuta* were hybridized using both the labelled probes together. Hybridization was detected by autoradiography using Kodak NTB-2 emulsion. The slides were exposed for autoradiography for 2 weeks.

Results

Data on [^{3}H] thymidine labelling of follicle cells are presented in table 1 and some examples are illustrated in figure 1. The frequency of labelled follicle cell nuclei remained high in ovarian follicles of stage 9 through 12; however, the pattern of nuclear labelling changed remarkably in later stages. Up to stage 10A, majority of follicle cells of both the species showed a uniform nuclear labelling (figure 1a); in some nuclei discrete clusters of labelling were also seen (figure 1b). In stage 10B of *D. nasuta,* the frequency of nuclei with clustered labelling was very high (data for 10B of *D. melanogaster* could not be obtained). Since the numbers of grain clusters in this category of labelled nuclei from stages 9 to 10B varied and since individual clusters were often not distinct (see figure 1b), no attempt was made to sub-group

Table 1. [^{3}H] Thymidine labelling of ovarian follicle cells of *D. melanogaster* and *D. nasuta* at different stages of ovarian follicle growth.

				Labelling patterns (% among labelled)			
				Uniform	Clustered		
Stage	Species	Total nuclei	Nuclei unlabelled (%)		1–5*	1	2
9–10A	*melanogaster*	151	2·0	81·1	18·9	*	*
	nasuta	355	7·6	74·6	15·2	*	*
10B	*nasuta*	110	13·6	8·4	91·6	*	*
11–12	*melanogaster*	632	13·8	1·6	4·0	70·6	10·1
	nasuta	301	19·6	0·7	2·0	34·5	43·2

Clustered labelling = labelling restricted to one or more discrete region(s) of the nucleus.

* Individual clusters of labelling not distinct.

these nuclei on the basis of the numbers of labelled cluster(s) seen per follicle cell nucleus: it may, however, be noted that in several of them only one or two distinct clusters of grains were seen. [^{3}H] Thymidine incorporation in follicle cells from stages 11 and 12 was found to be restricted, in most cases, to only one or two discrete sites (figure 1 c, d). In nuclei with two labelled clusters, one was less labelled than the other. The chromocentre formed by fusion of pericentromeric heterochromatin of different chromosomes remained distinctly visible as a compact mass in nuclei of *D. nasuta* and this was always unlabelled.

Chorion gene probes were hybridized *in situ* to know location of the chorion gene clusters in follicle cell nuclei of the two species. When either of the two probes (X-chromosomal or autosomal, see 'materials and methods') were hybridized *in situ* with stage 10–13 follicle cells of *D. melanogaster,* a single site of dense labelling was seen in each follicle cell nucleus, although a few scattered silver grains were also occasionally present elsewhere in the nucleus. Figure 2a and b illustrate the patterns of hybridization seen with the X-chromosomal probe. The hybridization patterns with the autosomal probe were similar and therefore, not shown. When both the labelled probes were simultaneously hybridized *in situ* with stage 13 follicle cell nuclei of *D. nasuta,* two distinct sites of hybridization were seen in each nucleus. It is notable that the degree of hybridization of either of the *D. melanogaster* derived chorion gene probes was markedly less with *D. nasuta* than with *D. melanogaster* follicle cells.

Discussion

Extensive studies have been made at molecular level to analyze the process of selective amplification of chorion genes in ovarian cells of *D. melanogaster*. the follicle cells undergo a few cycles of endoreplication up to stage 9; in addition, the chorion gene clusters are selectively amplified beginning at stage 8 so that by stage 13-14, the X-chromosomal chorion genes are amplified by about 20x and those on the chromosome 3 by about 60-80x of the haploid copy number (Spradling and Mahowald, 1980; Spradling *et al.,* 1980; Spradling, 1981; Spradling and Orr-Weaver, 1987). The cytological organization of the two chorion clusters and their

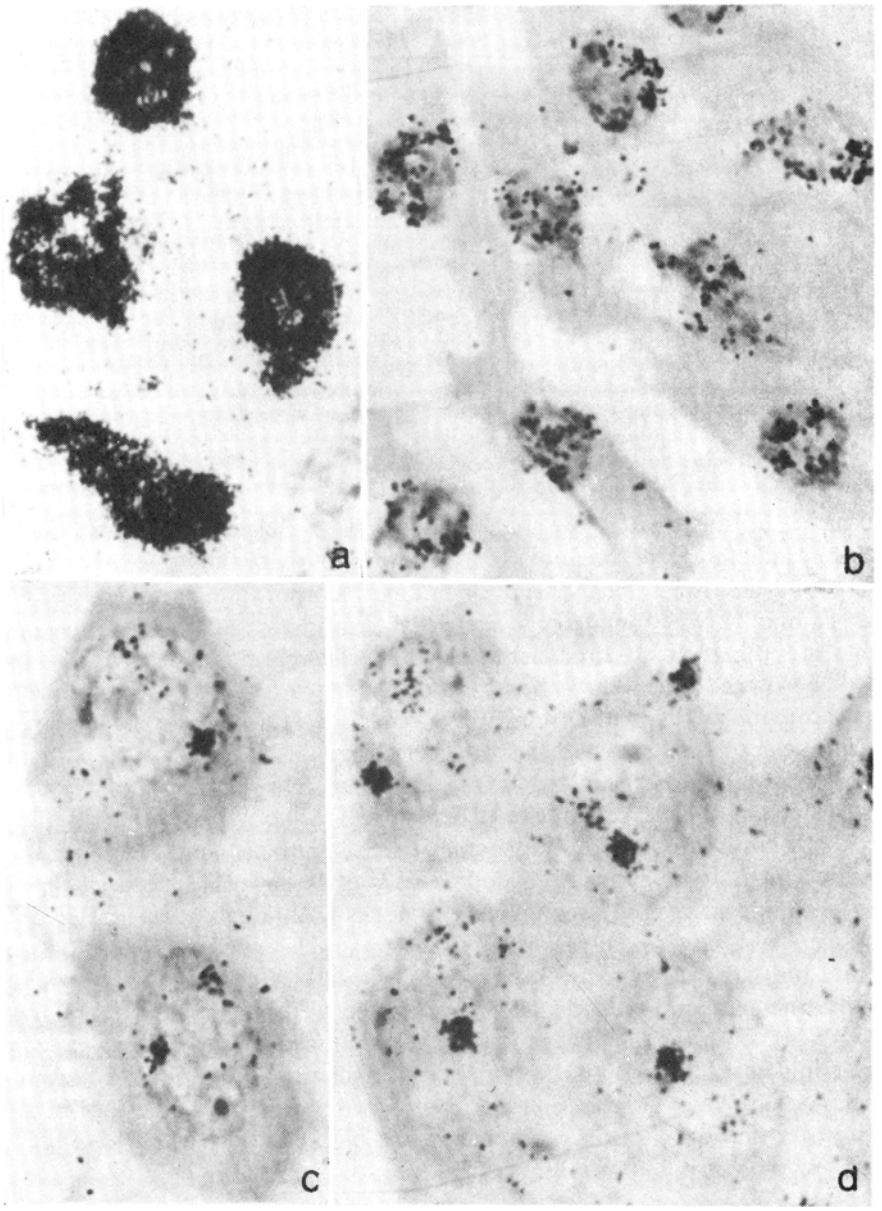

Figure 1. [^{3}H] Thymidine labelled autoradiograms of follicle cells of *D. nasuta* from ovarian follicles of stages 9 **(a),** 10B **(b),** 11 **(c)** and 12 **(d).** Note the labelling restricted to only one or two sites in nuclei from stage 11 **(c)** and 12 **(d)** with one site often more labelled than other.

amplified copies in follicle cello nuclei of *D melanogaster* is, however, not known. Our present study provides some information on this aspect in *D. melanogaster* and *D. nasuta.*

[^{3}H] Thymidine labelling restricted to one or two discrete region(s) in a majority of follicle cell nuclei from stages 10B to 12 of both species is indicative of the

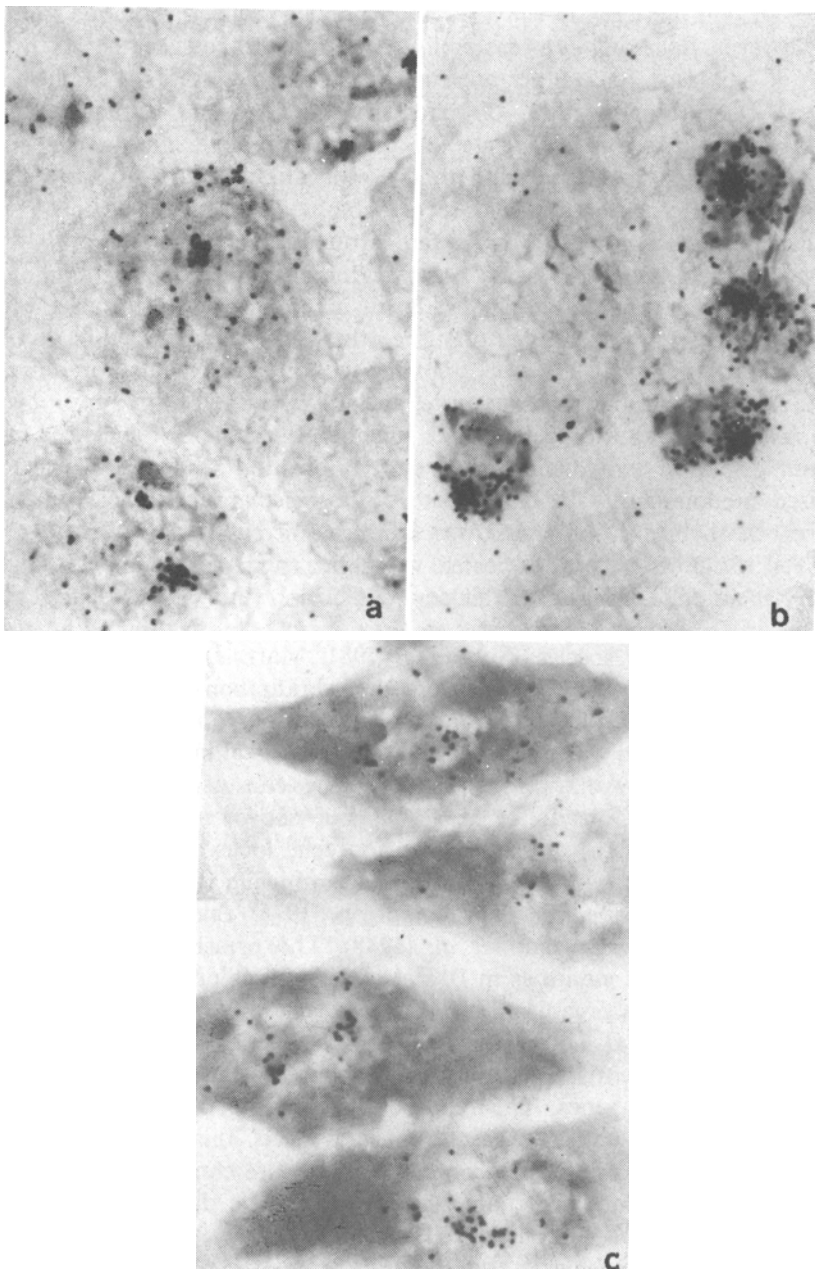

Figure 2. *In situ* hybridization of *D. melanogaster* chorion gene probes with follicle cells: **(a)** stage 10 and **(b)** stage 13 follicle cells of *D. melanogaster* hybridized with pl03.47; **(c)** stage 13 follicle cells of *D. nasuta* hybridized with mixed pl03.47 and p302.76 probes.

ongoing replication associated with amplification of chorion gene clusters. Some of the nuclei with labelling restricted to a few regions in stages 9 to 10B may represent nuclei in late S phase of endoreplication cycles, perhaps analogous to the 2D and

1D type labelling patterns in salivary gland polytene nuclei (Lakhotia and Sinha, 1983). However, following considerations show that the persistent [^{3}H] thymidine labelling at only 1 or 2 site(s) per nucleus in majority of stage 11—12 follicle cells is associated with amplification of chorion gene clusters rather than with [^{3}H] thymidine incorporation related to general endoreplication cycles: (i) these labelled region(s) do not correspond to late-replicating heterochromatin since the chromocentric heterochromatin was seen to be actually unlabelled in all cells as would be expected from the fact that heterochromatin regions remain grossly under-replicated in these cell types as in other endoreplicating nuclei (Hammond and Laird, 1985; Tiwari and Lakhotia, 1990); (ii) in both species, in nuclei with two labelled sites, one was less labelled than the other—this agrees with the fact that of the two chorion gene clusters, one amplifies to a greater extent (Spradling, 1981) and is thus expected to incorporate more label; (iii) when hybridized *in situ* with the two *D. melanogaster* chorion gene probes together, the stage 13 follicle cell nuclei of *D. nasuta* displayed two distinct sites of hybridization while each probe singly hybridized predominantly to only 1 site of *D. melanogaster* follicle cell nucleus; moreover, the hybridization signal was found to increase from stage 10 to 12/13 follicle cell nuclei as may be expected with increasing copy numbers of chorion genes due to amplification during this period. Chorion gene sequences are known to be unique in *Drosophila* genome and to show cross-hybridization with analogous sequences in different species (Spradling, 1981; Martinez-Cruzado *et al.,* 1988; Spradling and Orr-Weaver, 1988). Therefore, the hybridization patterns leave no doubt that the [^{3}H] thymidine incorporation at 1 or 2 regions only in most of the stage 11–12 follicle cell nuclei was related to ongoing amplification of chorion genes.

Earlier studies on evolution of chorion gene clusters in several species of *Drosophila* (Martinez-Cruzado *et al.,* 1988) had shown a high conservation of overall organization of the autosomal chorion gene locus. *D. nasuta* belonging to the *immigrans* species group of the sub-genus *Drosophila* is phylogenetically more distant from *D. melanogaster* (Throckmorton, 1975) than any of the species examined by Martinez-Cruzado *et al.,* (1988). The presence of two amplifying chorion gene loci in *D. nasuta* as in *D. melanogaster* thus provides further evidence for conservation of the organization of chorion gene loci in the genus *Drosophila.* Martinez-Cruzado *et al.,* (1988) noted a high degree of sequence divergence in chorion genes in different species but a strong conservation of proximal 5' flanking and 5' region of the genes. The reduced hybridization of the *D. melanogaster* chorion gene probes with *D. nasuta* nuclei reflects this sequence divergence. However, since in our study, the probes spanned entire chorion genes, the regions of conservation or divergence cannot be identified. The difference in the relative frequencies of stage 11-12 follicle cell nuclei with one or two [^{3}H] thymidine labelled sites in *D. melanogaster* and D. *nasuta* seems to reflect small differences in the temporal programmes of amplification of the two chorion gene clusters in these two species.

The localization of replicating regions or specific gene sequences in intact nuclei in cytological preparations provides very useful information on the 3-dimensional organization of nucleus since the preparatory steps do not distort the general *in situ* structure. Using *in situ* hybridization to localize specific gene sequences in endoreplicated ovarian nurse and follicle cell nuclei of *D. melanogaster,* Hammond and Laird (1985) concluded that in these nuclei, the copies on endoreplicated

homologous strands remain associated to a varying degree of closeness depending upon the nature of the sequence examined. In this context, the present observations show that the endoreplicated and amplified copies of chorion genes maintain a fairly close association within each of the two clusters but the two clusters themselves appear to remain spatially distinct. In relation to the 3-dimensional organization of different gene sequences in nuclei, Hammond and Laird (1985) also considered the possibility if there was a functional compartmentalization such that similarly transcribed sequences stay together. Our observations suggest that such spatially distinct compartments may not be necessary since although the two chorion gene clusters are programmed to selectively amplify and transcribe in follicle cell nuclei within a limited time span, they are located in distinctly separate nuclear areas: if compartmentalization was required, one would expect to find the two chorion gene clusters in close vicinity rather than separate as seen in this study.

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