DNA SYNTHESIS IN THE OOPLASM OF *DROSOPHILA MELANOGASTER*

F. A. MUCKENTHALER and A. P. MAHOWALD

From the Biology Department, The Johns Hopkins University, Baltimore, Maryland, and Woodstock College, Woodstock, Maryland. Dr. Muckenthaler's present address is Department of Biology, State University of New York at Albany

ABSTRACT

Tritiated thymidine was injected into 2-day-old *Drosophila melanogaster* females, and tissue sections were prepared from the ovary for radioautography with both the light and electron microscopes. Besides the expected incorporation of H^3-thymidine into nuclei of nurse cells and follicle cells, there was a relatively high level of incorporation of label into ooplasmic DNA. The highest level of incorporation occurred at stage 12. At the same time, the 15 nurse cell nuclei also incorporate thymidine in spite of the fact that they are breaking down and degenerating. The label in the ooplasm is not removed by extraction with DNase (although this removes nuclear label) unless extraction is preceded by a treatment with protease. Electron microscopic radioautography revealed that 36% of the silver grains resulting from decay of H^3-thymidine are found over mitochondria, with a further 28% being located within 0.25 μ of these organelles. The remaining 36% of the silver grains was not found to be associated with any organelles, and it probably represents synthesis in the cytoplasm by the "storage DNA" characteristic of many eggs. It is suggested that one mechanism acting throughout the egg chamber is responsible for the synchronous synthesis of DNA in the degenerating nurse cells, in the mitochondria of the egg, and in the "storage DNA" of the ooplasm.

INTRODUCTION

The presence of DNA in mitochondria has become widely accepted since the extensive work of Nass and Nass with a wide variety of organisms (19–21) and that of Luck and Reich with *Neurospora* (13). Recently Parsons (25) has been able to show the incorporation of H^3-thymidine into mitochondria of *Tetrahymena*. Stone and Miller (29) have also been able to localize H^3-thymidine incorporation in mitochondria of *Tetrahymena* by means of electron microscope radioautography. Moses (18) previously had noticed that silver grains in the cytoplasm of tissue culture cells grown in the presence of H^3-thymidine were frequently found over mitochondria.

Several studies have been concerned with the difference in bouyant density between nuclear DNA and mitochondrial DNA. Such a difference has been shown in *Neurospora crassa* (13) and embryonic chick heart and liver (27). Dawid (4) found no difference between egg DNA and liver DNA of *Rana pipiens*, but showed that egg DNA of *Xenopus laevis* is slightly more dense than nuclear DNA. He used sedimentation characteristics as an indication of association between egg DNA and mitochondria.

During the course of a study of the fine structure of *Drosophila* embryos (15, 16), it was noticed that many mitochondria evidently had structures resembling those identified to be DNA by Nass and Nass (20). Since there is a proliferation of mito-

chondria during oogenesis, we undertook an investigation of the synthesis of DNA in the mitochondria of *Drosophila* by studying the incorporation of H^3-thymidine during oogenesis. Nigon and coworkers (23, 24) previously studied DNA synthesis by radioautography and they found some ooplasmic incorporation of H^3-thymidine which was resistant to deoxyribonuclease (DNase). We have found that this ooplasmic label is sensitive to hydrolysis with DNase when the hydrolysis is preceded by removal of proteins by proteases. Moreover, by means of electron microscope radioautography, we have been able to determine that much of this cytoplasmic label is localized in the mitochondria.

MATERIALS AND METHODS

Two-day-old virgin females of the Oregon R wild type of *Drosophila melanogaster* were used for all studies. The flies were grown at 25°C prior to and during the experiments.

Light Microscopy

Flies were anesthetized with carbon dioxide and then injected with 0.15 μl (0.15 μc) of a sterile aqueous solution of H^3-thymidine (sp act, 6.7 C/mM, obtained from New England Nuclear Corp., Boston). The microinjection apparatus designed by Dr. H. Ursprung was used for the injection (17). Ovaries were removed from the flies at 5, 10, 30, and 60 min after injection and were fixed for 2 hr in absolute ethanol:glacial acetic acid (3:1) or in absolute ethanol:chloroform:glacial acetic acid (6:3:1). Tissues were dehydrated in ethanol, cleared in benzene, and embedded in paraffin. Sections were cut at 5 μ and mounted as serial sections. Alternate sections were coated with stopcock grease (6) prior to treatment of the slides with different extraction procedures. This technique afforded us the opportunity to compare adjacent extracted and unextracted sections in the radioautographs.

The basic extraction techniques employed were as follows:

1. 1 N HCl at 60°C for 6 min.
2. 10% perchloric acid at 2–4°C for 16 hr.
3. 10% perchloric acid at 70°C for 20 min.
4. Pepsin (2× crystallized, Worthington, Freehold, New Jersey) (1 mg/ml) in 0.02 N HCl at pH 1.8 for 10 min at 37°C.
5. Trypsin (1× crystallized, Worthington) (0.05 mg/ml) in 0.05 M phosphate buffer at pH 7.6 for 10 min at 37°C.
6. Alpha-chymotrypsin (3× crystallized, Worthington) (0.1 mg/ml) in 0.05 M phosphate buffer at pH 7.6 for 10 min at 37°C.
7. Ribonuclease (RNase) (3× crystallized, beef pancreatic, Worthington) (0.2 mg/ml) in 0.05 M veronal-acetate buffer at pH 6.5 for 2 hr at 37°C.
8. Deoxyribonuclease I (DNase) (1× crystallized, pancreatic, Worthington) (0.1 mg/ml) in 0.05 M veronal-acetate buffer, at pH 6.5, containing 0.003 M $MgSO_4$, for 3 hr at 37°C.

After the extraction procedure, stopcock grease was removed from the slides with xylene. Slides were then taken to water and passed through 5% trichloroacetic acid (TCA) at 2–4°C for 5 min to remove acid-soluble materials, washed again in water, and air dried. Slides were dipped in Kodak NTB-2 emulsion diluted 1:1 with 0.05% sodium lauryl sulfate in distilled water. The slides were exposed for 1 to 3 wk, developed in Kodak D 19, and stained with methyl green–pyronin.

Electron Microscopy

Flies were injected with H^3-thymidine as above, and after 1 hr were anesthetized with carbon dioxide. The ovaries were removed in saline at 2–4°C and fixed for 1 to 2 hr in 1% OsO_4 buffered with veronal-acetate at pH 7.8–8.0 with Zetterqvist's salt solution added. After fixation, the ovaries were dissected apart into single ovarioles in cold buffered saline and then placed in 5% TCA solution at 2–4°C for 30 min. Subsequently, they were washed twice with cold saline and then dehydrated in ethanol and embedded in Araldite 502 (Hysol Corp., Olean, New York) according to the technique of Luft (14). Grids with thin sections were coated with Ilford L4 nuclear track emulsion according to Caro and van Tubergen (3). The grids were stored at 4°C in a box with Drierite for periods of 2, 3, or 4 months and then developed in dilute Microdol-X for 4 min at 20°C. After being dried, the grids were stained for 10 min in a 1:50 dilution of Karnovsky's lead hydroxide stain (10), or with alcoholic uranyl acetate. An RCA EMU-3F electron microscope was used.

In order to determine which oocytes had the highest activity before thin sections were made, 1-μ sections were cut with a Porter-Blum microtome MT-1, transferred to a slide, and coated with Kodak NTB-2 emulsion. These slides were developed 1 and 2 wk later. The highest activity was found in oocytes of stages 12 and 13. These oocytes were selected for thin sectioning and for electron microscope radioautography. To obtain preservation of fine structure in oocytes is difficult in late stage 13 and stage 14, most probably because of contraction of the newly formed vitelline membrane. Unfortunately, the vitelline membrane cannot be dissected off until after the egg has been laid. Consequently, most of the electron microscope observations were made on oocytes of stage 12.

RESULTS

Light Microscopy

The incorporation of H^3-thymidine was studied in *Drosophila* ovaries fixed from 5 to 60 min after injection of the isotope. Besides the expected dense labeling of nuclei of nurse and follicle cells (but never of the oocyte nucleus), a definite ooplasmic label was detected in eggs of stages 10 to 13 inclusive. A relatively heavy label was found in the ooplasm of eggs of stages 12 and 13 which had been exposed to the isotope for 60 min (Figs. 1 *a*, 2 *a*). Distribution of label is uniform throughout the ooplasm for all periods of incorporation, the basic difference being an increase of label density with the longer periods of incorporation.

By the use of different incorporation periods, an indication can be obtained of the time of greatest ooplasmic incorporation of thymidine. When flies were killed 5 or 10 min after injection of the isotope, the greatest amount of ooplasmic labeling occurs in stage 12, indicating that most of the synthesis takes place at this time. At 60 min after injection, the highest activity is found in late stage 13. This indicates that the differentiation of the oocyte from early stage 12 to stage 13 requires approximately 1 hr. During this brief period, nearly all the detectable synthesis of cytoplasmic DNA occurs.

At stage 11, the nurse cells break down and a large cytoplasmic stream is visible extending from the ring canal of the nurse cells into the ooplasm. At this same stage all 15 nurse cell nuclei, even though they are becoming pycnotic, as is typical for degenerating cells, are found to incorporate H^3-thymidine even after a 5-min exposure to the isotope. This late nuclear synthesis corresponds to the beginning of the period when the highest detectable ooplasmic incorporation of thymidine occurs. At first, it was thought possible that this label might be transferred to the ooplasm along with the other products from the nurse cells. However, there is never a more intense label in the cytoplasmic stream coming from the nurse cells as they break down than in other regions of the ooplasm. The gradient of label observed after the incorporation of RNA precursors in the housefly ovary (1) indicates that the radioautographic technique is sufficiently sensitive for detection of a gradient in this type of material. Vanderberg (30) has found evidence for a transfer of DNA from the trophic tissues to the growing oocytes in *Rhodnius*, and Durand (5) has demonstrated a transfer of material from the follicle cells to the ooplasm after thymidine incorporation in *Gryllus*. But in *Drosophila*, because of the even distribution of label throughout the ooplasm without any concentration in the "nutrient stream" or adjacent to the follicle

TABLE I

Ratio of Grain Counts in Extracted and Unextracted Sections of Labeled Drosophila Ooplasm after Treatment of Adjacent Sections with Different Extraction Procedures

Extraction procedure	No. of samples	Extracted / Unextracted
A. 1 N HCl, 60°C	8	0.87 ± 0.11*
B. PCA, 2°C	4	0.99 ± 0.06
C. PCA, 70°C	4	0.32 ± 0.10
D. Pepsin	10	1.16 ± 0.13
E. Trypsin	10	1.68 ± 0.18
F. DNase (3 hr)	8	0.98 ± 0.22
G. RNase	8	0.93 ± 0.08
H. 1 N HCl, 60°C-DNase	8	0.74 ± 0.11
I. PCA, 2°C-DNase	4	0.93 ± 0.19
J. Pepsin-DNase	7	0.26 ± 0.07
K. Trypsin-DNase	7	0.11 ± 0.06
L. Pepsin-RNase	8	1.43 ± 0.15
M. Trypsin-RNase	6	1.27 ± 0.21
N. RNase-DNase	7	0.97 ± 0.10

*Standard Error.
PCA = 10% perchloric acid.

cells, even after brief (5-min) periods of incorporation, we conclude that the ooplasm is the site of DNA synthesis. Moreover, this is supported by the fact mentioned earlier that there is no change in pattern of distribution of label during longer periods of incorporation but only an increase in density of the label. This does not exclude the possibility that the template for such synthesis is supplied by the nurse cells (*see* Discussion).

Standard extraction techniques with DNase remove the label from nuclei of nurse and follicle cells, but they leave the ooplasmic label intact. Hence, a series of extractions was performed to find out whether the ooplasmic label might be due to something other than DNA. Grain counts over the ooplasm were carried out at a magnification of 500 with a net reticule. Each sample indicated in Table I is based on the total of 20 counts, each covering an area of 290 μ^2, with 10 counts being done on extracted and 10 on adjacent unextracted sections of egg chambers. Thus, e.g., the 8 samples of procedure A, Table I, consist of 160 individual counts. The ratios were obtained by computing the ratio of the number of grains in extracted:unextracted sections for each 20-count sample, and then a mean and standard error were determined for the samples for each extraction procedure in Table I.

Several conclusions about the ooplasmic label are clear from the data presented in Table I:

1. Procedures which are effective in extracting RNA from tissue sections have no appreciable effect on the ooplasmic label observed (procedures A, B, G).
2. The ooplasmic label is not subject to the action of DNase alone during 3 hr, confirming the work of Nigon and Gillot (23), or to action of RNase and DNase used consecutively (procedures F, N; and Figs. 1 *a* and *b*).
3. When treatment with a proteolytic enzyme precedes treatment with DNase, most of the ooplasmic label is removed (procedures J, K; and Figs. 2 *a* and *b*); and when a different treatment (procedure C), which would be expected to affect protein as well as DNA is used (26), a major portion of the label is removed.

Trypsin is more effective than pepsin in rendering the material subject to action of DNase. Chymotrypsin was similar to trypsin in its action. At the concentrations used (one-half of those usually recommended for histochemical tests), the treatment with trypsin-DNase leaves only a small residual label in the ooplasm whereas pepsin-DNase leaves a slightly higher level of label. A concentration of pepsin twice that indicated in procedure 4 above was slightly more effective but still not so effective as trypsin, whereas a concentration of trypsin one-fifth that indicated was nearly as effective as the one used. We presume also that the extraction of proteins produced by the treatment with 10% perchloric acid (PCA) at 70°C is necessary for the removal of ooplasmic DNA in this procedure.

The increase of label indicated for the sections which had been treated with pepsin and trypsin alone could be explained by the contraction of tissues produced by these enzymes (11). Another factor contributing to the increase of label could be the closer apposition of isotope and emulsion after removal of the overlying protein. Trypsin, when used alone, produced a greater increase of label than pepsin. There may be a correlation between this and the observation that trypsin is more effective in rendering the labeled material subject to the action of DNase, since both cases could involve the ability of the protease to remove protein associated with DNA. When higher concentrations of pepsin and trypsin were used, the ratios of the number of grains in extracted:unextracted sections were smaller than those shown in Table I. This indicates that the action of the enzyme is responsible for the increase of label present after such extractions.

Electron Microscopy

The presence in mitochondria of *Drosophila* embryos of DNA fibrils similar to those described by Nass and Nass (19) can be seen in Fig. 3. These are very common in the mitochondria of the embryo, but are not clearly found in the oocyte. The reason for the difference is not certain, but possibly it is due to the compression of egg chambers caused by the vitelline membrane during dehydration and embedding of the ovaries. This membrane cannot be removed during oogenesis because the cortex of the egg adheres tightly to it until after the egg is laid. The vitelline membrane had been removed from embryos in which mitochondrial fibers were seen.

Electron micrographs of the thin sections prepared for radioautography were taken at × 12,000 to 18,000 initial magnification, and the correlation

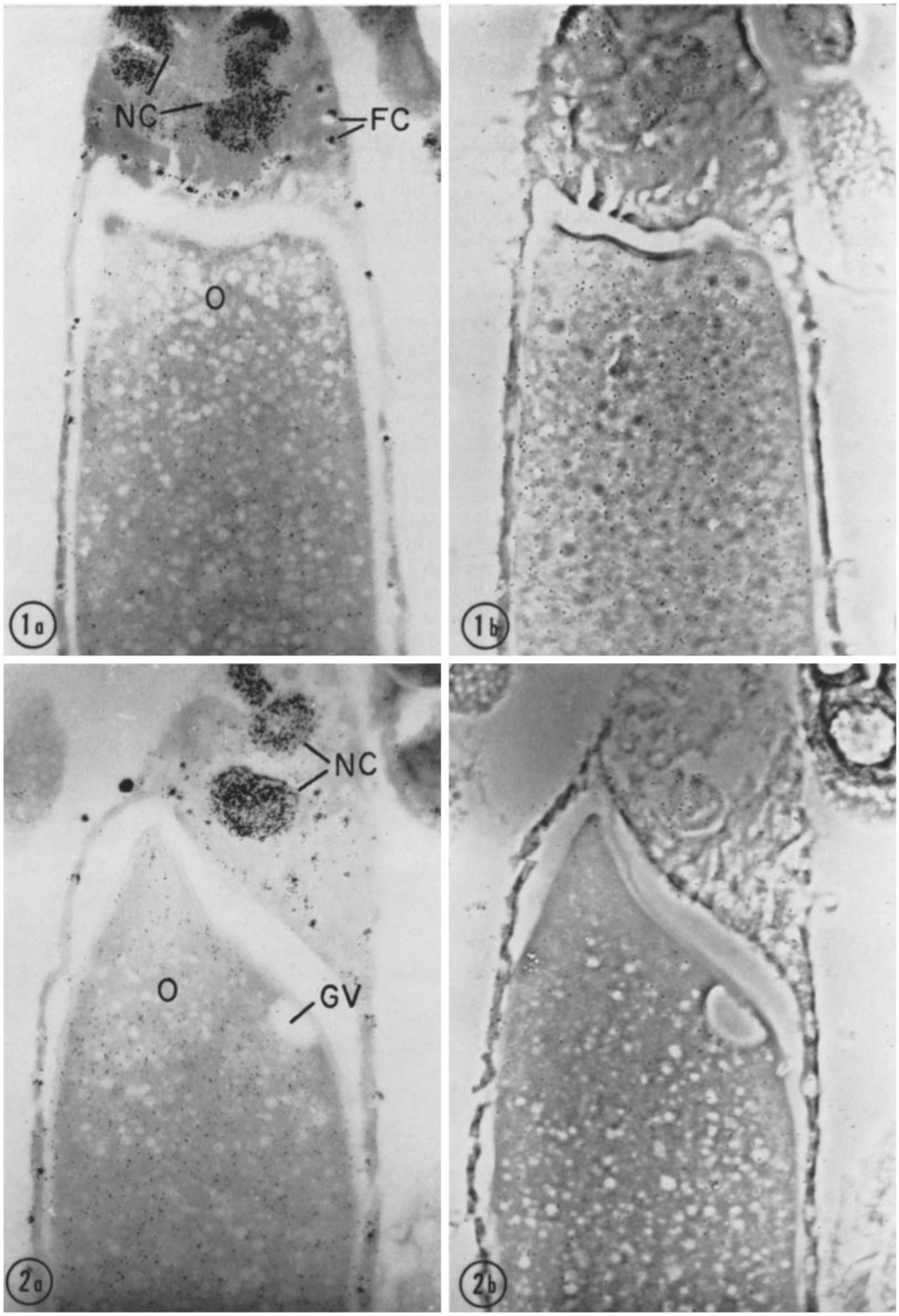


FIGURE 1 *a*, Radioautograph of unextracted egg chamber. *b*, Radioautograph of adjacent section of egg chamber extracted with RNase and DNase, consecutively. In Fig. 1 *b*, the label in nurse cell (*NC*) and follicle cell (*FC*) nuclei is almost completely removed, whereas the label in the ooplasm (*O*) is unaffected by the extraction. Ovary was fixed 1 hr after injection of H^3 thymidine. × 400.

FIGURE 2 *a*, Radioautograph of unextracted egg chamber. *b*, Radioautograph of adjacent section of egg chamber extracted with pepsin and DNase. In Fig. 2 *b*, the label is removed from nurse cell nuclei (*NC*) *and* oooplasm (*O*). Ovary fixed 1 hr after injection. Germinal vesicle (*GV*). × 400.

between grain localization and the underlying structure was determined from the negatives. Definite localization of some grains over mitochondria is apparent in the radioautographs (Figs. 4, 5). In some preparations, the localization of the grains was clear on the fluorescent screen of the electron microscope. In these instances, electron micrographs were taken of the doubtful cases only. The results of all counts taken are tabulated in Table II. A grain was considered to be over a mitochondrion if at least one-half of it was over the organelle. Similarly, at least one-half of the grain must be within 0.25 or 0.5 μ of the organelles to be included in the columns of Table II. Although only 36% of the silver grains was located over mitochondria, yet another 28% was located within 0.25 μ of the organelle. No other obvious localizations for the remaining 36% of the silver grains could be found. Extraneous silver grains due to background were found to be negligible in this material.

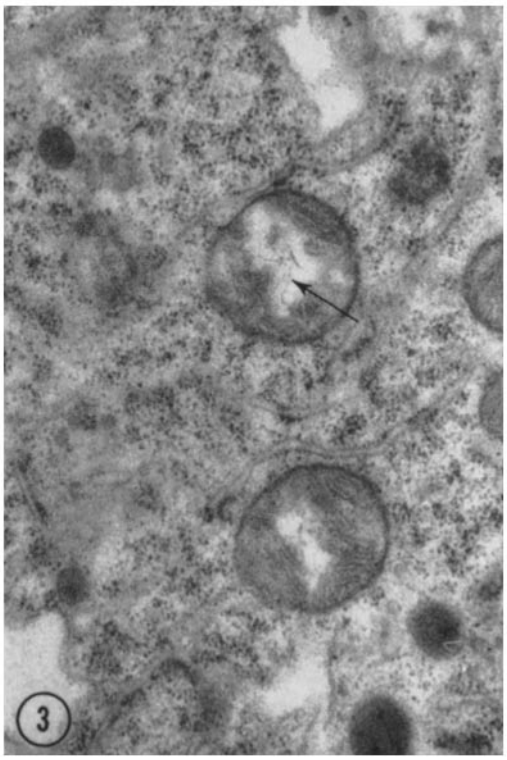
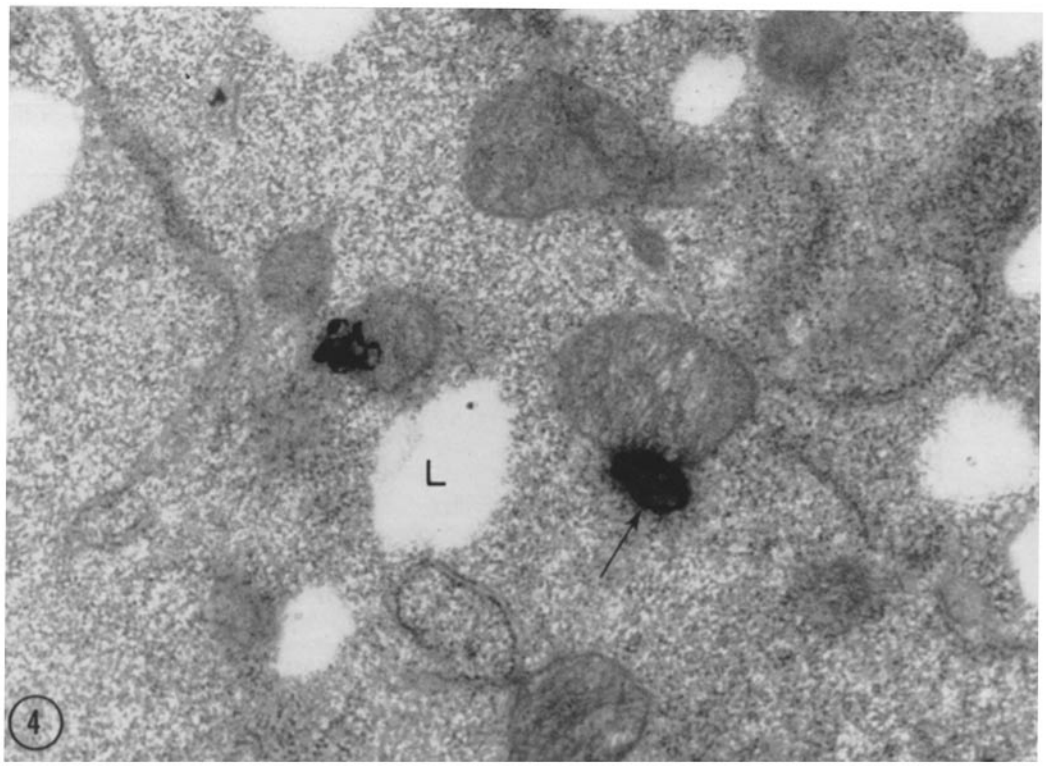


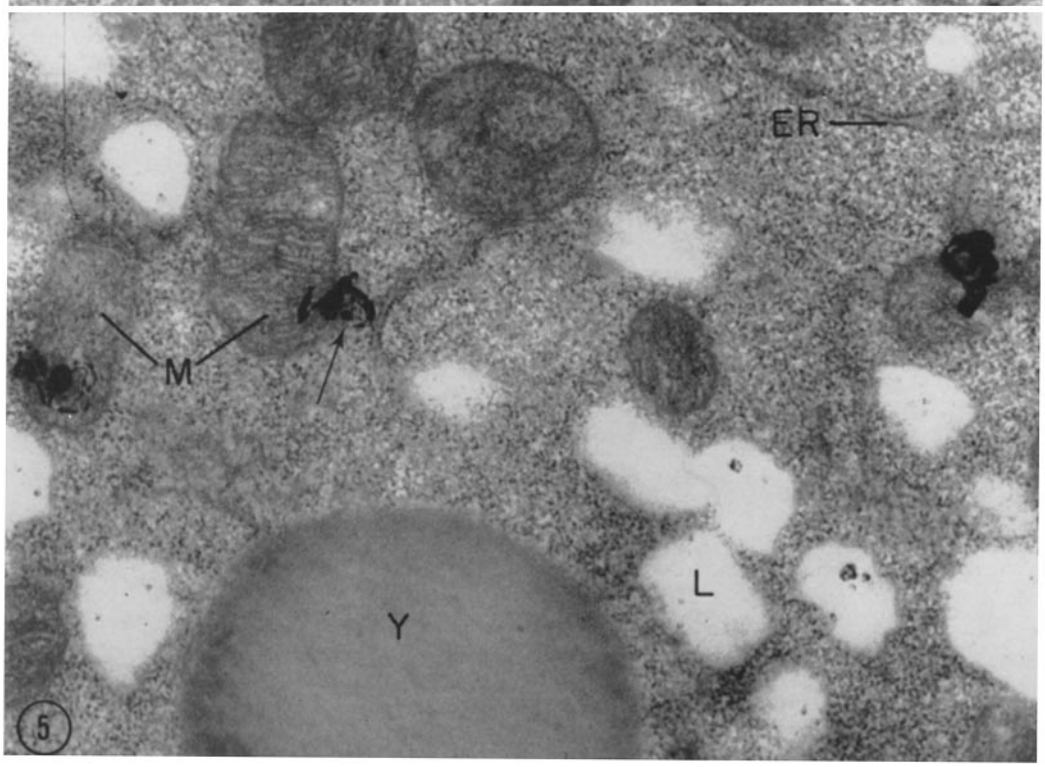
FIGURE 3 Electron micrograph of mitochondria from a *Drosophila melanogaster* embryo 3 hr old. DNA-fibrils are clearly present in one mitochondrion (arrow) and possibly in the other. Fixation for 1 to 2 hr in 1% OsO_4 buffered with veronal-acetate at pH 7.8–8.0 with Zetterqvist's salt solution added. × 32,000.

In order to determine the significance of the frequency of silver grains over the various regions of the oocyte, the relative area occupied by each region was determined by the following method: electron micrographs were printed at random and the various regions cut out with a razor blade. Each region was weighed and the percentage of the total weight of the print determined. Approximately 1,200 μ^2 of tissue were recorded in this manner. We find (*see* Table II) that the mitochondria cover 6% of the area of the section while having 36% of the grains located over them.

Several conclusions can be drawn from the data of Table II. The amount of label present in areas not associated with mitochondria is significant. The ratio of A/B for background, if the values for A (% of total grains) and B (% of total area of section) could be measured, would be nearly zero rather than the ratio of 0.43 indicated for the ooplasmic regions more than 0.5 μ away from mitochondria. The second point is that for the region within 0.25 μ of mitochondria the ratio of the percentage of total grains to the percentage of the total area is four times higher than for regions not near mitochondria. If we assume that the label not due to β-emissions from the DNA of mitochondria is randomly distributed, this ratio of four shows that the majority of the grains in this region is actually due to β-emissions from incorporated H^3-thymidine in mitochondria. The results are not clear for the region 0.25 to 0.5 μ away from mitochondria, but the difference between the ratio 0.57 and 0.43 may be an indication of the amount of label in this region that is also due to β-emissions in mitochondria.

It is difficult to determine how close to a mitochondrion a silver grain must be in order to permit the observer to attribute the activated grain to a β-emission in the mitochondrion. Caro (2) has determined the limit of resolution for the Ilford L-4 emulsion to be 0.1 μ. In the case of the complex grains produced by Microdol-X development, Moses (18) has approximated the maximum distance between the center of the grain and the β-particle source which activated that grain to be 0.3 μ. This value also corresponds to the observations of Hay and Revel (8) who found that some silver grains presumably produced by β-particles from H^3-thymidine incorporated into nuclear DNA





FIGURES 4 and 5 Electron micrographs of thin sections of ooplasm prepared for radioautography and exposed for 4 months before development. Besides the localization of the silver grains (arrow), the density of the mitochondria (*M*) should be noted as well as the typical accumulation of unattached ribosome particles in the cytoplasm of the egg. The endoplasmic reticulum (*ER*) is present only in the granular form and occurs as large flattened citernae. *Y*, proteinaceous yolk. *L*, extracted lipid droplets. × 39,000.

TABLE II

Localization of Silver Grains in Labeled Drosophila Ooplasm

	Mitochondria	0.25 μ around mitochondria	0.5 μ around mitochondria	Other areas
A. % of total grains over	36.1	27.9	11.7	24.3
B. % of total area of section	5.9	16.5	20.7	56.9
Ratio of A/B	6.1	1.7	0.57	0.43

In line A are recorded the percentages of the 1,072 grains counted which were localized over mitochondria, within 0.25 or 0.5 μ of mitochondria, and which were not localized in these areas. In line B, the relative areas occupied by the regions for which grains were counted are listed as percentage of total area. The method used for determining these values is described in the text. The weight of these regions is taken as an indication of the area of the structure in thin sections. The ratio in line C expresses the amount of label present in relation to the area.

were located outside but adjacent to the nuclear envelope. Moreover, as we have shown in Table II, the frequency of grains within the area 0.25 μ around the mitochondria is four times higher than that found over other areas of ooplasm. Consequently, we consider the silver grains in the region 0.25 μ around mitochondria as principally due to label incorporated into the DNA of mitochondria.

DISCUSSION

A number of reviews (e.g. 7) have appeared on the subject of cytoplasmic DNA which discuss both cytoplasmic inheritance and the possible function of cytoplasmic DNA. However, a number of points related to our findings must be considered: first, the relationship between the cytoplasmic DNA and the protein which prevents the extraction of this DNA; secondly, the significance of the subcellular localization as we have found it; and finally, some possible explanations for the pulse of ooplasmic DNA synthesis during *Drosophila* oogenesis.

1. *Significance of Pretreatment with Proteases*

Nigon and Gillot (23) have previously studied the incorporation of thymidine during oogenesis of *Drosophila*, and our results correspond to theirs in in the major details. They detected a significant incorporation of thymidine in the ooplasm, but they were unable to extract this label with DNase or with reagents specific for RNA. No hypothesis was presented concerning the nature of this cytoplasmic material. Nass et al. (22) have found that the DNA of isolated adult rat liver mitochondria is also resistant to DNase. In their study, only 20 to 35% of the DNA was digested after 8 hr in the presence of DNase and the percentage removed by DNase was not increased even if the acid-soluble, lipid, and RNA fractions were extracted first. They did not try prior protease treatment.

Our data demonstrate that the extraction of ooplasmic DNA by a standard DNase extraction technique is possible only after the removal of protein from the sections. Nass et al. (22) have observed that the DNA fibrils of mitochondria and the nucleoplasms of bacteria react similarly to preparatory techniques for electron microscopy, and they interpret this to mean that mitochondrial DNA is like bacterial DNA in not being present as a nucleohistone. A similar picture in *Drosophila* (Fig. 3) makes it unnecessary to postulate a special DNA-protein complex for the mitochondria of this species. A possible explanation for the necessity of proteolytic enzyme pretreatments prior to DNA extraction can be found in the observation with the electron microscope that the mitochondria of the egg are extremely dense after fixation with both glutaraldehyde and osmium tetroxide. There is probably a higher concentration of protein within the organelle due to the retention of protein by the fixative. The acetic-alcohol fixation and the ethanol dehydration used for the light microscopy and for the extraction studies would certainly disrupt mitochondria, and this would result in the precipitation of much protein around the DNA of the organelle. This protein, then, would merely be "protecting" the DNA from the action of DNase and would not be bound to the DNA in a DNA-protein complex. A similar DNA-protein association could also explain why the nonmitochondrial DNA is not extracted by the usual DNase techniques.

2. *Ultrastructural Localization of Silver Grains*

We have already considered the question of resolution of radioautography in thin sections. Approximately 64% of the silver grains is found over mitochondria or within the expected range of grains for the L-4 emulsion. In a similar study, Stone and Miller (29) found that 73% of the silver grains was localized over mitochondria and that another 20% was within 0.5 μ of the organelles. An explanation for this divergence in results may be found in the large amounts of cytoplasmic DNA and polydeoxyribonucleotides characteristic of eggs of many species, including *Drosophila* (28). Because of the techniques used in our work, we are able to detect only that ooplasmic DNA which incorporates precursors during oogenesis. A large proportion of this DNA is associated with mitochondria, but a significant amount is extramitochondrial. The nonmitochondrial DNA may be a "storage" DNA necessary to provide precursors for the rapid nuclear proliferation of early embryogenesis. Dawid (4), however, does not think that the amphibian egg DNA which he has studied has a storage function. Our experiments indicate that a considerable amount of this extramitochondrial DNA is synthesized during the later stages of oogenesis. Jacob and Sirlin (9) have observed that during stage 10 the nurse cell nuclei lose some of their DNA. It is possible that this is the source of the ooplasmic DNA that incorporates thymidine.

3. *Synchronous Synthesis of DNA*

In previous studies on the synthesis of cytoplasmic DNA, no evidence has been reported for a pulse of DNA synthesis. The cytoplasmic replication of DNA in the *Drosophila* oocyte appears to be different in this regard. Our data indicate that there is a rather high degree of synchrony of cytoplasmic replication (the period of synthesis being less than 1 hr), and that this synthesis also corresponds to a DNA synthetic period in the nurse cells.

As we indicated earlier, all 15 nurse cell nuclei at stage 11, even though they are becoming pycnotic, are found to incorporate H^3-thymidine following a 5- to 10-min exposure to the label. This late nuclear synthesis corresponds to the beginning of the period when the highest detectable ooplasmic incorporation of H^3-thymidine occurs. It is possible that the same mechanism which stimulates all of the nurse cell nuclei to start synthesis simultaneously is also responsible for initiating the ooplasmic synthesis. The extramitochondrial DNA would also be stimulated to incorporate DNA precursors by a mechanism that affects the whole egg chamber. Because of the nature of this material, it will be difficult to clarify the actual mechanism operative in initiating DNA synthesis, but at least similar phenomena may be sought in other organisms.

Nass and his coworkers (21) have found that in embryos and in rapidly proliferating tissues the mitochondria have larger DNA fibrils. They were able to find these fibrils in every branch of the polymorphic mitochondria present in the embryonic chick heart. In mature tissues, however, the DNA fibrils are smaller and there is usually only one per mitochondrial profile in thin section. From these observations, there does not appear to be an immediate relationship between the synthesis of more mitochondrial DNA and the division of the organelle. Hence the DNA synthesis which occurs during oogenesis in *Drosophila* may be a preparation for an increase in number of mitochondria during embryonic development rather than for an increase in number during oogenesis.

This work was supported in part by an AEC contract, AT-(30-1)-1695, supervised by Dr. C. P. Swanson, by National Science Foundation Grant No. GB-2232 to Woodstock College, and by a United States Public Health Service Post-doctoral Fellowship No. 1-F2-GM-25,689-01 to Dr. Muckenthaler from the National Institute of General Medical Sciences.

Received for publication 16 August 1965.

REFERENCES

1. Bier, K., *J. Cell Biol.*, 1963, **16**, 436.
2. Caro, L. G., *J. Cell Biol.*, 1962, **15**, 189.
3. Caro, L. G., and Van Tubergen, R. P., *J. Cell Biol.*, 1962, **15**, 173.
4. Dawid, I. B., *J. Mol. Biol.*, 1965, **12**, 581.
5. Durand, M., *Compt. rend. Acad. sc., Paris*, 1955, **241**, 1340.
6. Feder, N., and Sidman, R. L., *Stain Technol.*, 1957, **32**, 271.
7. Gibor, A., and Granick, S., *Science*, 1964, **145**, 890.
8. Hay, E. D., and Revel, J. P., *J. Cell Biol.*, 1963, **16**, 29.

9. JACOB, J., and SIRLIN, J. L., *Chromosoma*, 1959, **10**, 210.
10. KARNOVSKY, M. S., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 729.
11. KAUFMANN, B. P., GAY, H., and MCDONALD, M., *Cold Spring Harbor Symp. Quant. Biol.*, 1950, **14**, 85.
12. KING, R. C., RUBINSON, A. C., and SMITH, R. F., *Growth*, 1956, **20**, 121.
13. LUCK, D. J. L., and REICH, E., *Proc. Nat. Acad. Sc.*, 1964, **52**, 931.
14. Luft, J. H., *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
15. MAHOWALD, A. P., *Exp. Cell Research*, 1963, **32**, 457.
16. MAHOWALD, A. P., *Develop. Biol.*, 1963, **8**, 186.
17. MELTON, C. G., Ph.D. Thesis, Johns Hopkins University, 1964.
18. MOSES, M. J., *J. Histochem. and Cytochem.*, 1964, **12**, 115.
19. NASS, M. K., and NASS, S., *J. Cell Biol.*, 1963, **19**, 593.
20. NASS, S., and NASS, M. K., *J. Cell Biol.*, 1963, **19**, 613.
21. NASS, M. K., NASS, S., and AFZELIUS, B., *Exp. Cell Research*, 1965, **37**, 516.
22. NASS, S., NASS, M. K., and HENNIX, U., *Biochim. et Biophysica Acta*, 1965, **95**, 426.
23. NIGON, V., and GILLOT, S., *Exp. Cell Research*, 1964, **33**, 29.
24. NIGON, V., and NONNENMACHER, J., *Develop. Biol.*, 1961, **3**, 210.
25. PARSONS, J. A., *J. Cell Biol.*, 1965, **25**, 641.
26. PEARSE, A. G. E., Histochemistry, 1960, London, J. and A. Churchill, 213.
27. RABINOWITZ, M., SINCLAIR, J., DESALLE, L., HASELKORN, R., and SWIFT, H., *Proc. Nat. Acad. Sc.*, 1965, **53**, 1126.
28. SCHULTZ, J., *Cold Spring Harbor Symp. Quant. Biol.*, 1956, **21**, 307.
29. STONE, G. E., and MILLER, C. L., JR., *J. Exp. Zool.*, 1965, **159**, 33.
30. VANDERBERG, J. B., *Biol. Bull.*, 1963, **125**, 556.