Mitochondrial number, cytochrome oxidase and succinic dehydrogenase activity in *Xenopus laevis* oocytes

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SUMMARY

An estimate has been made of the numbers of mitochondria in the mitochondrial cloud (Balbiani body) of *Xenopus laevis* oocytes ranging in size from 50 to 250 μm. The mitochondrial number is expressed in terms of a 'standard' organelle measuring 2 μm in length and 0.2 μm in diameter and is derived by measurements on electron micrographs of sections through the cloud. It is found that the amount of mitochondrial material rises very rapidly as the oocyte grows in size. At the time the cloud disperses, in oocytes of about 300 μm in diameter, it is estimated that there are the equivalent of over 500000 mitochondria in each cell. The rate of increase is very similar to the rate of accumulation of mitochondrial DNA during the same period of growth.

Using a polarographic technique the specific activity of cytochrome oxidase and succinic dehydrogenase was determined in mitochondrial fractions isolated from oocytes over a size range of 80-1200 μm in diameter. Although the specific activity of succinic dehydrogenase remains constant that of cytochrome oxidase falls sharply during the period when the mitochondria are replicating rapidly, i.e. up to about 300 μm diameter. In larger oocytes the specific activity of enzymes appears to remain constant but increasing contamination of the isolated mitochondrial fraction does not allow conclusions to be drawn from the enzyme loading of the mitochondria once they have dispersed from the cloud.

The results are discussed in relation to the possibility that mitochondrial replication preceeds, or at least outpaces, mitochondrial differentiation during the course of oogenesis.

INTRODUCTION

One of the most prominent features of the pre-vitellogenic oocytes of *Xenopus laevis* is the mitochondrial cloud. The cloud contains the equivalent of large numbers of mitochondria and its formation requires the replication of mitochondrial DNA (Al-Mukhtar, 1970, Webb & Smith, 1977) and the transcription of appropriate cytoplasmic and nuclear RNAs necessary for the synthesis of the mitochondrial proteins, including characteristic enzyme assemblies. However, although the development of the mitochondrial cloud in *Xenopus laevis* appears

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to provide a model system for the study of mitochondriogenesis the relationship between DNA replication and the synthesis of the mitochondrial enzymes is not clear. Do the mitochondria differentiate (enzymatically) as each one is formed, or is there a phase of replication in the mitochondrial mass as a whole followed by a phase characterized by enzyme synthesis? The observations reported here seem to provide support for the later alternative in as much as the rapid increase in mitochondrial number is not found to be associated with a proportionate increase in the amount of a key enzyme associated with mitochondrial function. A biochemical approach was used and the results correlate fairly well with those obtained using quantitative cytochemistry (Marinos, 1978a).

As an index of mitochondrial differentiation the activity of cytochrome oxidase and succinic dehydrogenase was measured in oocytes varying in size from 50 to 1200 μm. The enzyme measurements were compared with the increase in the amount of mitochondrial material, determined as a mitochondrial number, as the oocytes increase in diameter. As reported previously (Billett & Adam, 1976) it is difficult to estimate the number of mitochondria in individual oocytes because electron microscope observations reveal extremely long and sometimes branched profiles. This suggests that, at least in some areas, there is a continuous mitochondrial structure rather than discrete particles. In view of the complex nature of the cloud it was suggested that for the purpose of calculating mitochondrial number it was sufficient to regard the cloud as consisting of a number of standard mitochondria of average length of 2.0 μm and with a diameter of 0.2 μm. This concept is used in the present paper to calculate a mitochondrial number which is used to estimate the increase in mitochondrial material as the cloud grows in size. Mitochondrial DNA must of course increase with mitochondrial number and the relevance of previous work in this field to the present study is discussed.

MATERIALS AND METHODS

Small female *Xenopus laevis* ranging in size from 1 to 6 cm, measured from the top of the head to the cloaca, were used. The animals were killed by immersing them in 0.1 % (w/v) aqueous solution of tricaine methane sulphonate (MS 222). Immediately after death the ovaries were removed and placed in 1 % (w/v) formaldehyde (prepared by the depolymerization of paraformaldehyde) buffered with 0.05 M phosphate buffer at pH 7.4. Whilst in the fixative they were cut up into small 1–2 mm³ blocks and subsequently transferred to fresh fixative and left for one hour at c. 4 °C. After post-osmication the tissue was passed through a graded series of ethanols to remove water before transferring to propylene oxide. This was followed by successive 2:1, 1:1, 1:2 mixtures of propylene oxide:araldite (one hour in each mixture at c. 20 °C) before leaving overnight in araldite alone. The following day the tissue was
transferred to fresh araldite and left for 30 min at 60 °C. At this stage oocytes of known size were selected. The small pieces of ovarian tissue were transferred in a few drops of araldite to a clean microscope slide and viewed under a stereomicroscope equipped with a measuring graticule. Individual oocytes were detached from the tissue using fine tungsten needles and after measurement they were transferred to the bottom of plastic moulds which were then filled with araldite. The araldite was polymerized in the usual way by leaving it for 48 h at 60 °C.

For light microscopy 1 μm sections were used. These were stained with 1 % (w/v) toluidine blue in 1 % (w/v) aqueous sodium borate for 1–2 min at c. 60 °C. For electron microscopy serial sections were cut at 60–90 nm. They were stained with 2 % (w/v) uranyl acetate for 30 min and lead citrate (according to the method of Reynolds, 1963) for 10 min. The ultrathin sections were examined in a Philips EM 300 electron microscope operated at an accelerating voltage of 60 or 80 kV.

For morphometrical studies ribbons containing 4–5 ultrathin sections were taken through the mitochondrial cloud of a given oocyte, these were attached to 200 mesh copper grids. These grids were numbered sequentially and stored in a grid box. When required, grids were selected by systematic random sampling using a random digit table. A good quality section from each grid was then chosen and a proportion of the mitochondrial cloud (not peripheral) was photographed at a magnification of 12500. A print magnification of 32000 was obtained by enlarging the negative. Each print contained about 150 mitochondrial profiles, and the resolution was sufficient to allow the easy measurement of not only the area occupied by the profiles but also the number of cristae in a given profile. To make the sampling easier each print was divided into four parts by a two bar grid.

Profiles/unit area

The number of mitochondrial profiles was counted applying the following convention. Profiles intersecting the top and bottom and left-hand-side bars were counted but not those intersecting the bottom right-hand-side bars. The number of profiles was then counted in each section and converted to the number of profiles/100 μm².

Profile areas

Three out of ten electron micrographs obtained for each oocyte were selected at random and the areas of about 50 profiles were measured on each print by using a polar planimeter. From these measurements the mean profile area was calculated.
Size of oocytes and mitochondrial clouds

These measurements were made on individual oocytes stained with diamino-benzidine following the procedure described by Marinos (1978a). Diamino-benzidine stains the clouds intensely and this facilitates measurement of their diameter. Using whole mounts cloud diameters were measured in approximately 60 oocytes ranging in diameter from 50–300 \( \mu \)m. These measurements were carried out using a light microscope equipped with \( \times 40 \) objective and an ocular graticule where one division corresponded to 2.3 \( \mu \)m on the specimen plane. The diameters of both oocyte and mitochondrial cloud were measured on three different axis on sections which were considered to pass approximately through the centre of the cells. During these measurements it becomes clear that the shape of both oocytes and mitochondrial cloud can be considered as spherical. It was not possible to make accurate measurements in oocytes greater than 300 \( \mu \)m in diameter because at this stage the oocytes become opaque with the commencement of vitellogenesis.

Estimation of mitochondrial number

The calculation of mitochondrial number was based on the Delesse principle that the real density of the profiles in a section is, on average, equal to the volume of the density of the structures under examination (Weibel, 1969). If we consider that the mean volume of a mitochondrion in the cloud of a particular oocyte is \( V_m \) and the total volume occupied by all the mitochondria within the cloud is \( V \), then the total number of mitochondria in the cloud will be:

\[
N_u = \frac{V}{V_m}
\]  

(1)

On the other hand, according to the Delesse principles, the ratio of the area \( S \) occupied by the mitochondrial profiles to the total area \( S_t \) within which they will occur will be equal to the ratio of the total volume \( V \) occupied by the mitochondria in a cloud volume of \( V_c \) to the volume \( V_c \):

\[
\frac{S}{S_t} = \frac{V}{V_c} \quad \text{or} \quad V = \frac{S}{S_t} \times V_c
\]  

(2)

In the material used in this paper \( S_t \) represents the area of the \( \times 32000 \) print of the electron microscope negative. From equations (1) and (2) the number of mitochondria in a particular cloud is given by:

\[
N = \frac{S}{S_t} \times \frac{V_c}{V_m}
\]  

(3)

The calculation of the mean volume \( V_m \) was based on the equation (Weibel & Gomez, 1962):

\[
V = BS^{-3/2}
\]
This relates the volume \( V \), of a structure to its mean cross sectional area, \( S \), when the structure is sectioned at random in any direction. \( B \) is a co-efficient depending on the shape of the structure. In the present study \( V \) corresponds to the mean volume \( V_m \) and \( S \) to the mean profile area \( S_m \). Thus:

\[
V_m = B \cdot S_m^{1/2}
\]  

(4)

The mitochondria were considered as cylindrical structures with a diameter of \( D = 0.2 \mu m \) and a length of \( L = 2 \mu m \). \( B \) is a function of \( D/L \) alone. When \( D/L = 0.1 \) (the present case) then \( B = 4 \).

Biochemical Assays

*Xenopus laevis* females were fatally anaesthetized and the ovaries removed immediately, as described in the previous section. The tissue was then transferred to an isolation medium made up as follows (Paul, 1970):

- Sodium chloride, 8.0 g;
- Potassium chloride, 0.2 g;
- Disodium hydrogen orthophosphate, \( 12H_2O \), 1.15 g;
- Potassium dihydro orthophosphate, 0.2 g;
- Disodium EDTA \( \times 2H_2O \), 0.2 g;
- Glucose, 0.2 g;
- Distilled water to give a total volume of 1000 ml.

The pH was adjusted to 7.4 with sodium bicarbonate.

The ovaries were cut into small (2–3 mm³) pieces and transferred to 50 ml of the medium containing 0.1 % protease (Type V, Sigma Chemicals). The tissue was incubated for 15–20 min at 20 °C with frequent agitation. At the end of this time most of the oocytes had been released from their follicle cells. The oocytes were collected by means of a Pasteur pipette and washed thoroughly by transferring them through at least five changes of chilled 0.03 M-Tris-HCl buffer at pH 7.4. Mature animals were used to isolate oocytes over 500 \( \mu m \) in diameter whereas young toadlets, ranging from 4–6 cm in length were used to isolate oocytes smaller than 400 \( \mu m \).

After isolation and washing the oocytes were sorted out into groups according to size and a known number (varying from 300–600) was homogenized in a Dounce homogenizer with 4 ml of 0.03 M Tris-HCl containing 1 M EDTA and 0.25 M sucrose. Homogenization and all subsequent stages took place at 0–2 °C. The oocytes were broken manually with six passes of the teflon pestle with a diameter 0.2–0.25 mm less than the internal diameter of the mortar. The homogenate was diluted to 16 ml with buffer and then placed in two 10 ml centrifuge tubes (8 ml in each). Centrifugation took place in the 10 × 10 ml 20° angled rotor of an MSE Superspeed 50 centrifuge. The homogenate was centrifuged twice for 15 min at 1000 \( g \) approximately to remove cell debris and nuclei. The 1000 \( g \) supernatant was then transferred to clean
centrifuge tubes and centrifuged for 20 min at approximately 10000 g. This produced the first mitochondrial pellet which was resuspended in buffer and again centrifuged as before to produce the washed mitochondrial pellet. Finally the two washed pellets were resuspended and combined in 1 ml of 0.05 M phosphate buffer at pH 7.4. 0.1 ml aliquots of this preparation were used for the enzyme and protein determinations described below. In all cases duplicate or triplicate measurements were made.

Both cytochrome oxidase and succinic dehydrogenase activity was determined at 25 ± 0.5 °C using a ‘Rank’ oxygen electrode. Instead of activating the en-
zymes by using detergents or ultrasonics, as it has been suggested by several workers, the method of production of swollen mitochondria was preferred (Schnaitman & Greenwalt, 1968). Detergents or ultrasonics usually reveal maximum enzymic activity (sometimes conceivably high) and require more elaborate procedures. In this work we simply wanted to compare activities of various stages of oocyte development. We were not interested about maximal activities of enzymes. The swollen mitochondria proved to be highly permeable to the reagents used as judged by the almost immediate response of the oxygen electrodes. In addition all reagents were used in excess so that the rate-limiting factor was the enzyme itself.

Cytochrome oxidase was assayed according to the method of Schnaitman Erwin & Greenwalt (1967). The assay system contained 0.05 M phosphate buffer at pH 7.4, 3 × 10^{-5} M cytochrome C (horse heart type III, Sigma Chemicals) and 3 × 10^{-5} M, N, N, N', N tetramethyl-p-phenylene diamine dihydrochloride. The reaction was started by adding 0.1 ml of 0.02 M sodium ascorbate and the reaction recorded for 3–5 min. Controls were prepared by omitting cytochrome C or by inhibiting the reaction with 0.01 M KCN. Succinic dehydrogenase was assayed according to the method of Schnaitman and Greenwalt (1967). Samples of 0.1 ml mitochondrial suspension were added to 2 ml of a medium containing 0.05 M phosphate buffer at pH 7.4, 25 mM succinate and 0.6 mM KCN. The reaction was initiated by the addition of 0.1 ml 20 mM phenazine methosulphate and recorded for 5–10 min. Controls were prepared by omitting the substrate from the medium. Figure 1 illustrates typical measurements obtained by the polarographic method.

The protein content of the mitochondrial pellet suspensions was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The concentration of protein was found with the aid of a standard plot prepared by using crystalline bovine serum albumin at concentrations ranging from 5–500 g/ml.

Fig. 1. Upper left: SDH activity test recording. After the addition of PMS a lag period of 30–60 secs is observed where no decrease in the oxygen is detectable. After this period a slow linear decrease in the oxygen content is recorded for a period of 5–6 min. By the end of this period the rate of the reaction decreases non-linearly until eventually becomes zero. Upper right: Control recording. By omitting the substrate from the medium no activity can be recorded after the addition of PMS. However addition of substrate (succinate) restores activity indicating that the observed decrease in oxygen is solely due to the oxidation of succinate by SDH. Lower left: Cytochrome oxidase activity recording. The reaction is initiated by the addition of the substrate (ascorbate). A linear decrease is observed for a period of 2–5 min. This is followed by a non-linear decrease until, finally, becomes zero. Lower right: Control recording of Cytochrome oxidase activity. The control was prepared by omitting the substrate or by the addition of KCN to a final concentration of 0.01 M (present case). It is evident that the addition of KCN in to the incubation medium completely inhibits the reaction. Furthermore, no activity is observed in substrate depleted media. It is therefore concluded that the observed activity is due to Cytochrome oxidase activity.
The mitochondrial content of the pellet preparations was determined by using electron microscopy. Mitochondrial pellets were prepared from oocytes over the size range used in the biochemical assays. The washed pellets were fixed at the bottom of the centrifuge tubes with a few drops of 1 % (w/v) formaldehyde in 0.05 M phosphate buffer at pH 7.4. After one hour at 4 °C the formaldehyde was removed and replaced by buffered 2 % osmium tetroxide for a further hour at 4 °C. Dehydration of the fixed pellet and embedding were carried out in the way described for the oocyte preparations.

RESULTS

Mitochondrial number

Using the methods described in the previous section an estimate was made of the increase of the amount of mitochondrial material as the oocyte grows in size. The measurements were restricted to oocytes measuring from 50 μm to 300 μm in diameter. At this stage the clouds are compact and often occupy a juxtanuclear position (Figs 2, 3). Below about 50 μm the oocytes do not possess a cloud. Above 300 μm diameter the cloud begins to disperse. The relation between the diameter of the oocyte and that of the mitochondrial cloud is illustrated in Fig. 7. These measurements were made on diamino-benzidine-stained material (Fig. 2). A better indication of the growth of the cloud in relation to oocyte size is obtained by calculating relative volumes (Table 1). This shows, for example, that as the oocyte increases in diameter from 125 μm to 250 μm there is a 12-fold increase in mitochondrial volume.

The mean number of mitochondrial profiles/100 μm² for oocytes of the chosen size range is given in Table 2. There appears to be no significant change over this range and an average figure of 330/100 μm² was used to calculate mitochondrial numbers. Figure 4 is typical of the electron microscope prints on which these measurements were made. Using the basic assumptions described in the previous section (Materials and Methods) the mitochondrial

Fig. 2. Intact oocyte (c. 200 μm diam.) of Xenopus laevis treated with diamino-benzidine. Mitochondrial cloud (m.c.) stains intensely (x 300).

Fig. 3. 1 μm section through centre of Xenopus laevis oocyte (c. 200 μm diam.) Mitochondrial cloud is (m.c.) compact and adjacent to germinal vesicle (x 250).

Fig. 4. Electron micrograph of part of mitochondrial cloud of oocyte. Formaldehyde-osmium tetroxide fixation. Stained with uranyl acetate and lead citrate. (x 250000).

Fig. 5. Electron microscope preparation of mitochondrial fraction from Xenopus laevis oocytes c. 80–130 μm diam. Fraction consists of large numbers of mitochondrial profiles (m.p.) (x 10000).

Fig. 6. Electron microscope preparation comparable to that shown in figure 4 but prepared from 500–600 μm oocytes. Only about half the profiles (m.p.) appear to be derived from mitochondria. (x 10000).
Fig. 7. Regression line showing relation of mitochondrial cloud diameter and oocyte diameter in small (up to 6 cm) *Xenopus laevis* females.

Table 1. *Relation between oocyte and mitochondrial cloud volume in Xenopus laevis.* Measurements derived from data shown in Fig. 6

<table>
<thead>
<tr>
<th>Oocyte diameter (μm)</th>
<th>Cloud diameter (μm³ × 10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.2</td>
</tr>
<tr>
<td>75</td>
<td>17.5</td>
</tr>
<tr>
<td>100</td>
<td>59.2</td>
</tr>
<tr>
<td>125</td>
<td>140.4</td>
</tr>
<tr>
<td>150</td>
<td>274.2</td>
</tr>
<tr>
<td>175</td>
<td>473.8</td>
</tr>
<tr>
<td>200</td>
<td>752.4</td>
</tr>
<tr>
<td>225</td>
<td>1123.0</td>
</tr>
<tr>
<td>250</td>
<td>1783.0</td>
</tr>
</tbody>
</table>

Numbers for oocytes over the size range from 50 to 250 μm diameter are given in Table 3. Using oocyte size as an index of growth it is obvious that the number of mitochondria increase very rapidly in oocytes above 100 μm in diameter.
Table 2. *Mitochondrial profile count from electron micrographs of central sections of mitochondrial cloud of Xenopus laevis*

<table>
<thead>
<tr>
<th>Oocyte size (μm)</th>
<th>Mean number of mitochondrial profiles/100 μm²</th>
<th>Oocytes compared</th>
<th>Difference of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>324 ± 11</td>
<td>50-100</td>
<td>-11</td>
</tr>
<tr>
<td>100</td>
<td>335 ± 11</td>
<td>100-200</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>335 ± 10</td>
<td>200-300</td>
<td>5</td>
</tr>
<tr>
<td>300</td>
<td>330 ± 11</td>
<td>300-400</td>
<td>11</td>
</tr>
<tr>
<td>400</td>
<td>319 ± 8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Student's t test indicates that there is no significant difference in the density (number/unit area) of profiles in oocytes over the size range measured.

**Cytochrome oxidase and succinic dehydrogenase activity**

Oocytes of various sizes from 80 to 1200 μm diameter were collected from 25 different animals. The assays were carried out on aliquots of the 10000 g mitochondrial fractions prepared as described above. The enzyme activities were expressed as moles O₂ mg of pellet protein/minute. The results indicate that the specific activity of the cytochrome oxidase declines as the oocytes increase in size whereas that of the succinic dehydrogenase remains essentially the same. The ratio between cytochrome oxidase activity and succinic dehydrogenase activity was calculated for each determination. This ratio decreases in a non-linear fashion; the rate of decrease being faster in oocytes up to approximately 200 μm in diameter. These results are summarized in Table 4.

Examination of the mitochondrial fractions by electron microscopy revealed that preparations from oocytes up to about 400 μm consisted largely of mitochondria (Fig. 5). In contrast fractions prepared from oocytes greater than 400 μm diameter were obviously contaminated by numerous vesicles, granular aggregates and round electron dense bodies, (Fig. 6). Thus it may be concluded

Table 3. *Mitochondrial number in Xenopus laevis oocytes*

<table>
<thead>
<tr>
<th>Oocyte size (μm)</th>
<th>Mean number of mitochondria cloud x 10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.48 ± 0.1</td>
</tr>
<tr>
<td>75</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td>100</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>125</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>150</td>
<td>60 ± 14</td>
</tr>
<tr>
<td>175</td>
<td>103 ± 24</td>
</tr>
<tr>
<td>200</td>
<td>164 ± 38</td>
</tr>
<tr>
<td>225</td>
<td>238 ± 56</td>
</tr>
<tr>
<td>250</td>
<td>390 ± 89</td>
</tr>
</tbody>
</table>
Table 4

<table>
<thead>
<tr>
<th>Mean oocyte diameter (m)</th>
<th>Mean specific activities of mitochondrial pellet moles</th>
<th>Mean ratio of specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDH</td>
<td>Cyt. Ox.</td>
</tr>
<tr>
<td>95 ± 11</td>
<td>0.30 ± 0.02</td>
<td>19.3 ± 4.9</td>
</tr>
<tr>
<td>204 ± 21</td>
<td>0.26 ± 0.05</td>
<td>11.8 ± 1.7</td>
</tr>
<tr>
<td>384 ± 22</td>
<td>0.03 ± 0.02</td>
<td>8.4 ± 1.3</td>
</tr>
<tr>
<td>557 ± 29</td>
<td>0.43 ± 0.02</td>
<td>6.3 ± 2.1</td>
</tr>
<tr>
<td>1204 ± 63</td>
<td>0.19 ± 0.03</td>
<td>2.1 ± 0.4</td>
</tr>
</tbody>
</table>

The mean oocyte size is calculated from five different animals; the oocyte diameters of 50–60 oocytes were measured in each case.

The differences between the cytochrome oxidase activities are significant ($P < 0.05$), those between the succinic dehydrogenase activities are not.

that the estimated specific activities for oocytes up to 400 μm relate to a fraction which may properly be called mitochondrial. In fractions prepared from 400–1200 μm oocytes the degree of contamination is marked and increases with oocyte size. Thus the calculated activities of the enzymes must be underestimated.

DISCUSSION

The increase in mitochondrial number reported in this paper will only reflect a true increase in mitochondrial material if there is no significant variation in mitochondrial morphology over the size range studied and if the basic assumption of a random distribution of profiles is correct. Measurements of the mean number of cristae per unit length of profile and of mitochondrial volume indicate that the structure is fairly uniform over the size range investigated (Marinos, 1978b). The examination of large numbers of electron micrographs (Billett & Adam, 1976, Marinos, 1978b) suggest that an effectively random distribution of profiles prevails, although there are a few areas in which profiles are clumped together, and others in which either circular or longitudinal profiles are dominant. However, these apparently non-random arrangements are not sufficiently numerous to alter our calculations which indicate an 800-fold increase in mitochondrial material as the *Xenopus* oocyte enlarges from 50 to 250 μm in diameter.

It is of interest to compare the rate of increase in mitochondrial number with the rate at which mitochondrial DNA accumulates in the oocytes of *Xenopus* (Webb & Smith, 1977). Although there is only a limited overlap of the two measurements (c. 100 μm to 300 μm diameter oocytes) it can be seen that the increased mitochondrial number corresponds to the phase of mito-
chondrial DNA synthesis. However the rate at which the mitochondrial material increases (as measured by profile counts) greatly exceed the accumulation of DNA; the two events although obviously related appear to be out of step. In this connexion it is interesting to note that the amount of mitochondrial DNA in a 250 μm oocyte suggests an abnormally high DNA content for individual mitochondria and that it may be misleading to imagine that mitochondrial number can be strictly related to DNA content (Billett, 1979). Rapid mitochondrial DNA synthesis continues until the oocytes reach 400–500 μm in diameter. In oocytes larger than 500 μm DNA synthesis appears to be much reduced (Webb & Smith, 1977).

When the mitochondrial cloud disperses our calculations suggest that there are the equivalent of well over 500000 mitochondria in the Xenopus oocyte. Although this number is large it would, if it corresponded to fully functional mitochondria, only be sufficient to populate the cells of a Xenopus embryo at the end of gastrulation (Bristow & Deuchar, 1964). There is, however, convincing evidence that mitochondriogenesis, as measured by renewed mitochondrial DNA synthesis and increased cytochrome oxidase activity, does not occur until a relatively late, hatching, stage of development (Chase & Dawid, 1972). This suggests that there may be a further, and substantial, increase in the number of mitochondria after the cloud disperses.

The enzyme measurements reported in this paper also indicate a lack of correspondence between mitochondrial number and enzyme activity during the previtellogenic phase of oogenesis. Our evidence for this depends on the accuracy of the enzyme assays which have been used. Cytochrome oxidase and succinic dehydrogenase activity was measured by a polarographic technique (Lassler & Brearley, 1969). However, although the method itself is accurate and reliable we are well aware that the calculation of the specific activity of the mitochondrial fraction depends on the purity of the sample obtained by centrifugation. If the protein content does not relate solely to mitochondria the results are misleading. Examination of the 10000 g mitochondrial fractions by electron microscopy suggests that those prepared from 50–400 μm oocytes are rich in mitochondria whereas those from larger oocytes are clearly contaminated. The presence of apparently continuous regions of mitochondrial structure in the intact cloud (Billett & Adam, 1976), is obviously a potential complicating factor. If such regions remained intact they would fractionate at lower centrifugal forces than 10000 g. However, examination of low-speed fractions (1000 g) using phase-contrast microscopy showed only debris and yolk platelets, nothing could be identified as part of a mitochondrial cloud. In addition neither cytochrome oxidase nor succinic dehydrogenase could be detected in significant amounts in the low-speed fraction. We are therefore confident that the enzyme activities calculated relate specifically to mitochondria for oocytes in the size range 50–400 μm.

Mitochondrial preparations from oocytes above 400 μm diameter are
obviously contaminated with material which fractionates with the mitochondria and no reliance can be placed either on the measurements of specific activity of the two enzymes or on the continuing decline of the amount of cytochrome oxidase relative to succinic dehydrogenase. The measurements of enzyme activity in oocytes below 400 μm are considered more reliable and are in any case more relevant to the period of mitochondriogenesis characterized by the growth of the cloud and the commencement of its dispersal. Estimates of the size of oocyte in which the cloud begins to disperse vary from about 250 μm (Billett & Adam, 1976) to 400 μm (Webb & Smith, 1977; Marinos, 1978). The discrepancy can partly be explained by differences in methods of preparation of the oocytes and in methods of measurement. There also appears to be a significant difference between female Xenopus of different ages (Sharif-Zandhieh and Billett, unpublished observations). The present series of observations covers the range of oocytes sizes during which the cloud grows rapidly in size and then begins to disperse. The results suggest that before the cloud disperses the rapid increase in mitochondrial number is not accompanied by a similar rise in cytochrome oxidase activity. As the specific activity of succinic dehydrogenase remains constant during this period there is a significant alteration in the ratio of the activity of the two enzymes as the oocytes increase in size from approximately 100 μm to 400 μm in diameter.

Although the cytochrome oxidase result in somewhat surprising it is confirmed by other observations using a completely different technique of measuring the enzyme activity of the cloud in situ using the diamino-benzidine reaction as a basis for quantitative cytochemistry (Marinos, 1978a). However it should be remembered that both the cytochemical and biochemical methods of assay may not give a true measure of the in vivo activity of the cytochrome oxidase in the mitochondria. The presence of some compensating factor in the intact oocyte could account for the lack of correlation between the increased mitochondrial number and the activity of the enzyme. Bearing this reservation in mind however it still appears possible that the mitochondria only become fully differentiated after the cloud disperses and that, as in other cells, the synthesis of enzymes, such as cytochrome oxidase, is repressed during mitochondrial (DNA) replication (see Barath & Kuntzel, 1972, for discussion). In the case of Xenopus oocytes this may be confined, to a large extent to the period during which the cloud forms and grows to its maximum size.

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Oocyte mitochondria in *Xenopus laevis*


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