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Original article

Homology between mitochondriogenesis in the avian and amphibian oocyte

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Summary — Cytochrome oxidase cytochemistry was used to unequivocally identify the spread of mitochondria during oogenesis in the adult Japanese quail. This enabled us to compare their distribution with the distribution in the *Xenopus laevis* oocyte (Tourte *et al,* 1984). In the quail the paranuclear mitochondrial cloud initially disperses homogeneously but afterwards segregates into 2 populations: (i) a population localized in the basophilic cortical layer (surrounding the vegetal pole); and (ii) clusters of mitochondria distributed geometrically around the germinal vesicle in the animal pole. The mitochondria in these clusters have a high cytochrome oxidase activity, which reflects their functionality. This perinuclear crown of mitochondrial clusters actively replicates mtDNA in both animal species and builds up most of the stock of the mitochondria in the full-grown oocyte. Our study suggests that the perinuclear group of mitochondria will segregate in the somatic cells of the future embryo, whilst the original subcortical group will become localized in the primordial germ cells.

mitochondria / oogenesis / Japanese quail / *Xenopus laevis* / cytochrome oxidase

Résumé — Homologie entre la genèse mitochondriale dans l'ovocyte d'Oiseau et d'Amphibien. *La distribution des mitochondries dans l'ovocyte de la caille japonaise adulte a été étudiée par une méthode cytochimique démontrant l'activité de la cytochrome oxydase. Elle a été comparée avec la distribution des mitochondries dans l'ovocyte de* Xenopus laevis *(Tourte* et al, *1984). Dans l'ovocyte de caille la masse mitochondriale paranucléaire, après s'être dispersée dans tout l'ovoplasme, se divise en 2 populations. La première population se localise dans la couche basophile corticale, tout autour du pôle végétatif. La deuxième population est formée de groupements de mitochondries présentant une symétrie radiale autour de la vésicule germinative dans le pôle animal. L'activité de la cytochrome oxydase dans ces derniers amas est élevée. Dans les 2 espèces animales étudiées une synthèse d'ADN mitochondrial a été décelée dans la population paranucléaire qui fournit la plus grande partie du stock*

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mitochondrial de l'ovocyte mûr. Notre étude suggère que cette dernière population mitochondriale sera finalement incorporée dans les cellules somatiques du futur embryon, tandis que la première population (originellement corticale) se retrouvera dans les cellules germinales primordiales.

mitochondrie / ovogenèse / caille japonaise /* Xenopus laevis *I cytochrome oxydase

INTRODUCTION

Mitochondrial RNA-DNA hybridization experiments first demonstrated that the oocyte mitochondria in *Xenopus* populate the soma of the off-spring (Dawid and Blackler, 1972). Since then it has been shown that the mitochondria in adult cells of many different species are of maternal origin. More precisely, this has been demonstrated in the Japanese quail by Watanabe *et al* (1985). Cytoplasmic determinants have sometimes been identified as the mitochondrion itself, for instance, the mitochondrial DNA of the 'petite' mutants of yeast is physically altered (Mounolou *et al,* 1966). Moreover, it has been shown that the early embryonic development in amphibians (Gurdon, 1985) and quail (Callebaut, 1987) is largely dependent on the regional organization of the ooplasm (more particularly the local distribution and type of mitochondria; Mignotte *et al,* 1987). In previous studies we examined [^{3}H]thymidine incorporation in quail postlampbrush oocytes and found very RNA-rich subcortical cytoplasmic organelles containing mitochondria (Callebaut, 1973, 1983a), which we therefore called Ticos ([^{3}H]thymidine-incorporating cytoplasmic organelles). [^{3}]Thymidine incorporation also takes place in the paranuclear mitochondrial cloud during the prelampbrush stage (Callebaut, 1973), which suggests mitochondrial DNA synthesis. In oocytes of *Xenopus laevis* a heterogeneous distribution and replication activity of mitochondria have also been described (Tourte *et al,* 1984), which strongly resemble the mitochondrial accumulations seen in quail oocytes of corresponding stages (*ie* first a paranuclear mitochondrial cloud and later a perinuclear 'crown' of groups of mitochondria, clearly separated from the cortical mitochondria). Since cytochrome oxidase (one of the 3 large enzyme complexes of the respiratory chain; Alberts *et al,* 1989) can be demonstrated histochemically at the inner mitochondrial membrane (Seligman *et al,* 1968; Roels, 1970, 1974), we used cytochrome oxidase activity to study the mitochondrial distributions in sections under an optical microscope.

MATERIALS AND METHODS

After decapitation and opening of the abdomen of regularly laying Japanese quails, parts of the ovary and pediculated oocytes from different stages were fixed. The samples were fixed in a 2% glutaraldehyde solution (0.1 M Na-cacodylate buffer, pH 7.5–7.6 with 0.1% calcium chloride) for 2–3 h on ice. After thorough rinsing in cacodylate buffer containing 7.5% sucrose, chopper sections of 60 μm thickness were made. They remained overnight in the buffer solution and were then incubated at 37°C for 1 h in an incubation medium for cytochrome oxidase activity at pH 6, containing DAB, cytochrome C, manganese chloride as an activator and catalase in order to destroy any hydrogen peroxide (Cornelis *et al,* 1985). After enzyme staining the chopper sections were thoroughly rinsed 3 times with a freshly prepared 10% sucrose solution in water.

Osmication was performed at 0°C for 5 h in 2% osmiumtetroxide in Na-cacodylate buffer (0.1 M). The controls were sections treated with the incubation medium containing 0.001 M KCN, a selective inhibitor of cytochrome oxidase. The toxicity of cyanide is due to its ability to bind tightly to the cytochrome oxidase complex and thereby block all electron transport. After dehydration, the samples were embedded in paraffin or LX-112

(Ladd, Burlington, USA) epoxy resin. Roels (1974) showed that cytochrome oxidase activity can be demonstrated with this technique, whilst the DAB reaction product localizes cytochrome C. Epon-embedded material was sectioned at 2 μm thickness and paraffin embedded material at 7 μm. The follicles and included oocytes were classified in 1 of 3 stages according to Callebaut (1973): prelampbrush, lampbrush (phase I with homogeneous ooplasm and phase II presenting a cortical differentiation) and postlampbrush.

RESULTS

Controls

No staining was observed after addition of cyanide to the incubation medium.

Prelampbrush stage oocytes

The paranuclear mitochondrial cloud was stained in the sections through prelampbrush oocytes (fig 1).

Electron microscopy of the sections through this mitochondrial cloud clearly showed that the technique used only gives a precipitate on the inner membrane of the mitochondria folded into numerous cristae (fig 2). This indicates that the technique is specific to the studied mitochondria and permitted their visualisation as long intermingled rods under the light microscope. A concentration of mitochondria was sometimes already found at the periphery of the ooplasm at this stage.

Lampbrush chromosome stage

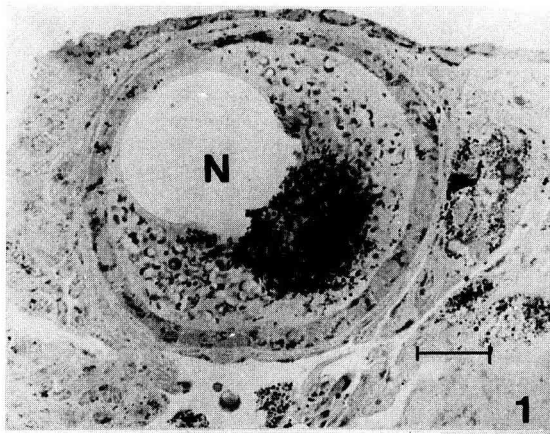
During the transition from prelampbrush to lampbrush stage, we observed no double Balbiani complex formation as described by Guraya (1976) in the ovary of the domestic fowl *(Gallus domesticus)*. With the increase in volume of the oocyte, the mitochondria spread into the surrounding ooplasm and its periphery. Finally, the original paranuclear cloud completely disappeared during phase I of the lampbrush stage (fig 3). Indeed a paranuclear concentration of mitochondria was no longer seen in a series of 7 μm thick sections through the whole germinal vesicle area. The mitochondria spread nearly homogeneously into the ooplasm. During phase II of the lampbrush stage (Callebaut, 1973) the homogeneous distribution of the mitochondria disappeared and an oocytal cortex appeared, which was clearly distinct from the central ooplasmic mass. This was accompanied by a cortical concentration of mitochondria.

Postlampbrush chromosome stage

During the early postlampbrush stage many mitochondria were seen to concentrate in the cortex, where they formed part of the basophilic cortical layer localized between the surface ooplasm (yolk precursor transition zone) and the deeper intermediary (primordial) yolk (Callebaut, 1974, 1975).

Cytochrome oxidase staining in 2 μm sections permitted us to histochemically identify the presence of mitochrondria in subcortical cytoplasmic aggregates, Ticos (Callebaut, 1973). These are visible in the germinal disc of postlampbrush oocytes with a diameter of 3.5 mm or more (fig 4). Electron microscopy (Callebaut, 1983a) demonstrated that they contain large numbers of mitochondria which have a somewhat different aspect from the mitochondria found in the granulosa cells and are also different from mitochondria observed during other stages of oogenesis.

At low magnification with the electron microscope, the localization of the Ticos was much more obvious after cytochrome oxidase staining (fig 5) than on unstained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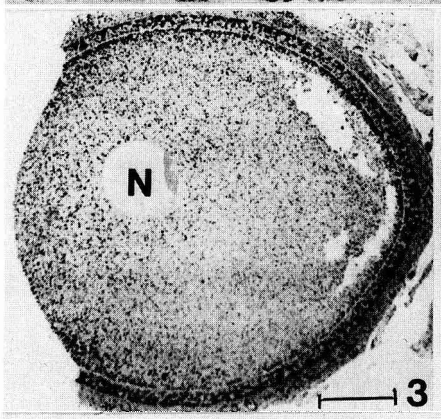
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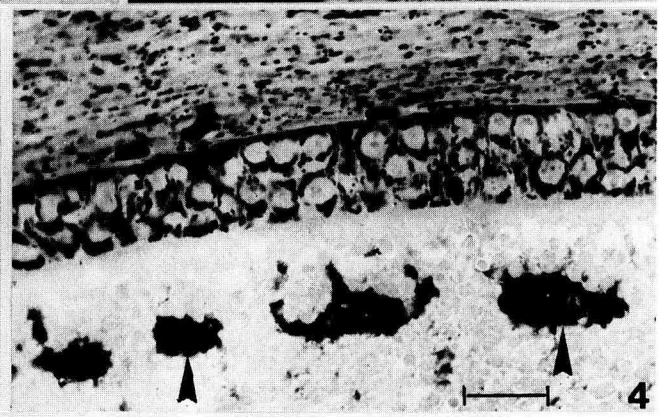
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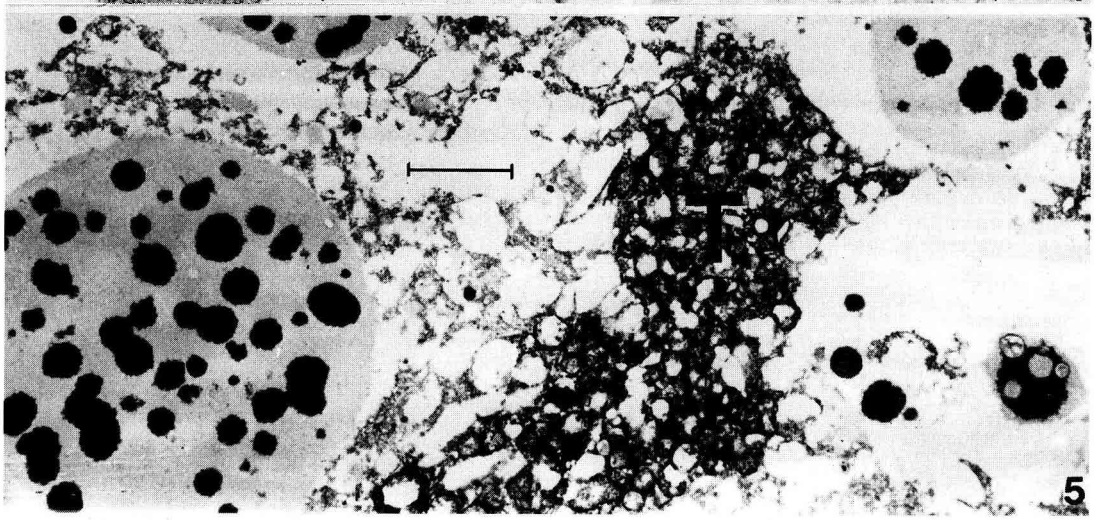
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sections. Indeed, in unstained sections the Ticos were only recognizable by their localization and by the presence of mitochondria in large numbers, pressed in between the yolk vacuoles. After cytochrome oxidase staining the reaction product precipitated on the cristae and inner envelope in the prelampbrush stage mitochondria. In the Ticos the same reaction product was seen to leak into the immediate neighbourhood outside the mitochondria. The intense staining of the Ticos after cytochrome oxidase cytochemistry can probably be explained by the diffusion and selective adsorption of high levels of oxidized DAB on the polyribosomal RNA (Roels and Goldfischer, 1971; Novikoff *et al,* 1972; Böck, 1973) lying in vicinity of the Tico mitochondria (Callebaut, 1983a). Thus, dark areas were seen even at low magnification in the places where Ticos were present (fig 5), in contrast to the aspect of some of the mitochondrial matrix and the more empty aspect of the surrounding yolk material.

DISCUSSION

Marinos (1978) demonstrated that cytochrome oxidase activity has a maximum in the mitochondria of the 200 μm diameter mitochondrial cloud *X laevis* oocytes. Since the mitochondrial mass increases in size during the previtellogenic period and because it contains mainly long intermingled mitochondria, it is generally assumed that this is the cytological manifestation of intense and localized mitochondrial biogenesis.

Our present study demonstrates that the original paranuclear mitochondrial cloud present in the prelampbrush stage completely spreads over the ooplasm during the ensuing phase I of the lampbrush stage.

The strongly basophilic Ticos are organized geometrically (in rays and concentric circles) in the germinal disc, spread over an area of 300–400 μm around the germinal vesicle; they are clearly separated from the cortical layer. Our present study seems to indicate that, despite their unusual morphology, the Ticos seem to contain functionally active mitochondria. Indeed the mere presence of mitochondrial DNA and RNA does not garantee functional mitochondria, as shown by 'petite' mutants, which have no cytochrome oxidase activity and lack respiring mitochondria (Alberts *et al,* 1989).

In actively growing vitellogenic oocytes of *X laevis*, mitochondria segregate into 2 populations, one of which remains around the germinal vesicle and forms a 'crown' around it (Tourte *et al,* 1984). Here mtDNA is also actively replicated and builds up most of the stock of the mitochondria in the full-grown oocyte. The other population group moves

Fig 1. Cytochrome oxidase reaction in 2 μm thick epon section through a quail prelampbrush oocyte; N: germinal vesicle. Note the intensely stained paranuclear mitochondrial cloud; bar: 20 μm.
Fig 2. Electron micrograph of a 60 nm thick epon section through mitochondria of a prelampbrush oocyte after cytochrome oxidase staining. Note the precipitation of reaction product on the inner mitochondrial membrane folded into numerous cristae; bar: 500 nm.
Fig 3. Cytochrome oxidase staining in 2 μm thick epon section through a larger quail lampbrush oocyte (phase I) with an approximately homogeneous spread of mitochondria in the ooplasm; around the germinal vesicle (N) there is no perinuclear accumulation of mitochondria; bar: 100 μm.
Fig 4. Cytochrome oxidase staining in 2 μm thick epon section through the animal pole of a quail postlampbrush oocyte (4 mm diameter). Note the intense staining of the Ticos (arrowheads); bar: 20 μm.
Fig 5. Electron micrograph of a section through Ticos material (T) after cytochrome oxidase staining; leaking cytochrome oxidase activity is seen between the mitochondria. Note the high contrast between Ticos material and surrounding cellular constituents; bar: 2 μm.

towards the vegetal pole, where it becomes localized in a subcortical layer and stops replicating mtDNA early in vitellogenesis. Organelles of this population are components of the germ plasm (Mignotte *et al,* 1987). A similar phenomenon seems to occur in the quail. Indeed one of us (Callebaut, 1983b, 1984, 1987) has shown that the germinal yolk ooplasm of the primordial germ cells in the quail contains yolk that has penetrated through the basophilic cortical layer during oogenesis and become localized in the deeper paraxial δ ooplasm containing part of the germ disc, forming part of the nucleus of Pander (fig 6). That the primordial germ cells are mainly derived from the deep central layer (endophyll) of the blastoderm (Callebaut, 1983b, 1987) or from the upper layer (Eyal-Giladi *et al,* 1981; Cuminge and Dubois, 1989, 1992) is still a matter of dispute, but does not interfere with our conclusions. The mitochondrial crown appears in *X laevis* oocytes of 600 μm diameter (*ie* a 6 times smaller diameter than the quail oocyte). This crown is formed by groups of mitochondria, which are also radially and concentrically disposed around the germinal vesicle and are also well isolated from the cortical layer. Despite the large differences in diameter with the quail oocyte (a factor of 6–10), the surface area occupied by the crown in the *X laevis* oocyte is approximately as large as the surface area containing Ticos in the postlampbrush quail oocyte. In the same way as in the quail oocyte at the end of oogenesis, in which the germinal vesicle moves near the animal pole, the crown becomes less visible. The mitochondria are however still found in clusters around the germinal vesicle (Tourte *et al,* 1984).

At certain developmental stages in both *X laevis* and quail oocytes, the mitochondria and [^{3}H]thymidine incorporation are similarly distributed in the animal pole. This seems to indicate that this pattern is not an exception in vertebrates. The aspect and distribution of mitochondria found in the Ticos laden area (γ ooplasm, fig 6) are distinctly different from the mitochondria in other regions of the oocyte (Callebaut, 1983a; Syens and Callebaut, 1986). Mitochondria are absent or rare in the α ooplasm. The δ ooplasm contains mitochondria which are derived from the basophilic cortical layer after passage in

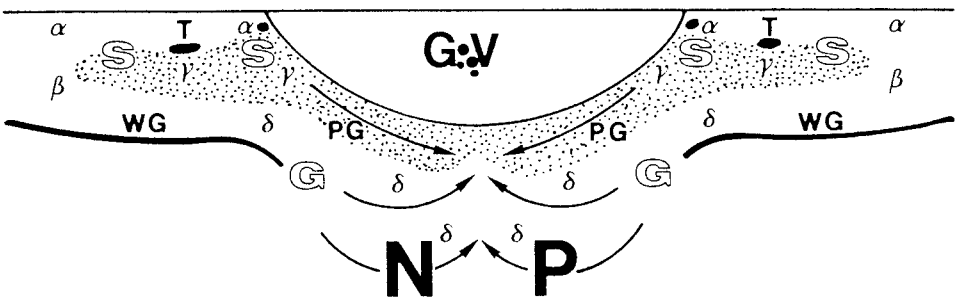

Fig 6. Schematic drawing of a quail germinal disc (end of postlampbrush stage) representing the localization of the 2 populations of mitochondria. In G: localization of germinal yolk plasm (δ ooplasm; Callebaut 1983b, 1984, 1987), which has passed in and through the basophilic cortical layer during the middle postlampbrush stage and which will settle in the primordial germ cells. In S: the more superficial Ticos-laden γ ooplasm (stippled area), which has penetrated between the Ticos (T) and eroded them, will become incorporated into the somatic cells of the future embryo; arrows below the germinal vesicle (GV) indicate the convergence of the ooplasmic layers sliding below it; NP: nucleus of Pander; PG: polar granules; WG: wedge granules; α, β, γ, δ indicate the approximate localization of the corresponding ooplasms.

and through this layer. At the end of the oogenesis, Ticos material is distributed in the γ ooplasm (fig 6), which becomes incorporated into the somatic cells of the blastoderm (Callebaut, 1987). This suggests that part of the inherited mitochondria in the embryonic somatic cells is derived from Ticos mitochondria.

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