Mitochondrial and other ultrastructural changes in the developing *Habrobracon* embryo

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**SUMMARY**

Cells of the wasp, *Habrobracon juglandis*, were studied by electron microscopy with the view to comparing ultrastructural changes, especially those found in mitochondria, that occur during the first two-thirds of the 29 h embryonic period. In 1- to 2-h embryos (the earliest studied) mitochondria are distributed principally in the periplasm and typically are arranged in clumps with their long axes parallel to each other. Based on a study of profiles occurring in thin sections, most appear to be elongate with poorly developed cristae, have dense matrices and are longer than those of later stages. At 3-4 h of age, in incipient blastoderm cells, the mitochondria are distributed throughout the cytoplasm with 40% located lateral to the nuclei and 42% concentrated in a subnuclear position. Most (81%) exhibit spherical profiles, with well-developed cristae and less dense matrices than those found at earlier ages. In fully formed blastodermal cells (7-8 h), mitochondria are similar morphologically except that a lower percentage (53%) are spherical; almost half (48%) have migrated to a supranuclear location. In early gastrula cells (11-12 h) no significant variations from the blastoderm condition were apparent. Mitochondria in the oldest cells studied (18-19 h) show somewhat greater structural complexity and variability. The number per cell section is drastically reduced compared to earlier ages, but this, at least in part, is related to a reduction in cell size. Changes observed in other cellular constituents are also described. Comparisons are made with similar variations reported in other developing organisms and their possible significance is discussed.

**INTRODUCTION**

The important role that mitochondria play in the general metabolic activities of cells is well known (e.g. see Lehninger, 1965; Green & Baum, 1970). It is possible that they are also involved in functions related to other specialized activities in living systems. For example, evidence has been presented that they may be implicated in morphogenetic processes in developing organisms (Brachet, 1960; Novikoff, 1961; Gustafson, 1965). Furthermore, since mitochondrial function appears to be related to mitochondrial morphology, if the latter changes it can be assumed that there is a concomitant alteration in the former (Hackenbrock, 1966, 1968). Thus more information about morphological alterations may be helpful in assessing or predicting changes in physiological activities associated with development. Many investigators have reported that mito-

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chondrial ultrastructural changes take place during certain phases of late gametogenesis or embryogenesis in a number of animal groups (Okada & Waddington, 1959; Weber, 1962; Mahowald, 1963; Berg & Long, 1964; Yamamoto, 1964; Anderson, Condon & Sharp, 1970; Sathananthan, 1970; Stern, Biggers & Anderson, 1971). In all of the studies just cited, however, a rather limited part of the developmental period was studied. The present paper is an attempt to extend such coverage to include ultrastructural changes occurring in mitochondria and other cellular constituents over a greater proportion of the embryonic period. Accordingly, observations are presented from studies made on five developmental stages (1–2 h, karyokinesis; 3–4 h, preblastoderm; 7–8 h, blastoderm; 11–12 h, early gastrulation; 18–19 h, early organogenesis) covering the initial two-thirds of the embryogeny of the wasp Habrobracon juglandis.

MATERIALS AND METHODS

Eggs from virgin specimens of the ectoparasitic wasp Habrobracon juglandis (= Bracon hebetor) Whiting stock 33 were used exclusively in this investigation. Such eggs, which develop parthenogenetically into males, were deposited by the females on their hosts, the larvae of the Mediterranean flour moth Anagasta kühniella. At timed intervals the eggs were collected so that their age was known within 10 min, oriented on a glass slip and placed in an incubator at 30 ± 0.5 °C (relative humidity 50–60 %). At the desired stage of development, they were fixed, processed and subsequently observed with the aid of a Siemens Elmiskop I.

Eggs of this organism are elongate (ca. 550 μm in length and 160 μm in greatest diameter), slightly curved and covered with a transparent but rather impervious chorion. The presence of the latter necessitated the employment of the following fixation schedule: (a) 3–5 h, 1:7-glutaraldehyde: Sörensen’s 0.2 M phosphate buffer (pH 7-3); (b) 9 h, buffer; (c) 30 min, 1:1, 2 % OsO₄:0.1 M buffer; (d) rinse, buffer; (e) removed posterior or anterior end of egg; (f) 1½ h, 1:1, 2 % OsO₄:0.1 M buffer; and (g) three washes, buffer. Fixation was followed by dehydration in alcohols, 1 h in propylene oxide (two changes), 1 h in 1:1 mixture of propylene oxide and Epon and finally 4 days in Epon at 40 °C. Polymerization was carried out for 12 h at 60 °C.

Semi-fine (1 μm) and fine (60–80 nm) sections, either sagittal or transverse, were cut with an LKB ultramicrotome. The former were stained with 1 % toluidine blue and studied under a light microscope for orientation purposes; the latter were stained for 15 min with a 1 % aqueous solution of uranyl acetate, then for 1 min with lead citrate and examined and photographed with the electron microscope.

Measurements of cells and their components of the five embryonic stages studied were made on 20 × 25 cm. enlarged prints of the micrographs at final magnifications of X25200 and X50400.
Mitochondria in Habrobracon

Observations

The embryology of Habrobracon has been described previously (Amy, 1961) but a brief summary of it, emphasizing developmental activities at the particular ages covered in the present study, will aid in understanding the description of results that follows.

The centrolecithal wasp egg has a peripheral layer of cytoplasm (the periplasm) surrounding the yolk. The latter is traversed by fine strands of interconnecting cytoplasm which form a reticulum. At the time of oviposition, the single nucleus is located adjacent to the egg's ventral surface near its anterior end. Within 20 min it migrates to the median longitudinal axis and karyokinesis is initiated. The resulting nuclei become distributed along the longitudinal axis and by 3 h of age have started to move peripherally to populate the periplasm. By 7–8 h of age each nucleus, along with its surrounding cycloplasm, has been or is in the process of being, enclosed in a cell membrane. The resulting cells collectively form the blastoderm. Gastrulation is initiated during the 11–12 h period. Ventral blastodermal cells (the middle plate) begin to invaginate during this time with the subsequent formation of the lateral plates and the dorsal strip from the remaining cells of the original blastoderm. Organogenesis starts within the next 2 h and by 18–19 h, the oldest stage covered in the present investigation, the primordia of most organ systems have been established. Embryonic development is completed and hatching occurs typically at 29 h.

Mitochondria

(a) Karyokinesis (1–2 h of age)

Mitochondria are distributed for the most part in clumps throughout the periplasm. Within each clump the mitochondria are grouped together with those in some aggregates oriented with their long axes parallel with the long axis of the egg (Fig. 1) whilst those in others are oriented perpendicular to the long axis (Fig. 2). Frequently they exhibit an end-to-end arrangement. The structure of the mitochondria appears to be relatively simple with very few, if any, cristae in evidence; matrices are very electron-dense. A few isolated organelles, that are morphologically similar to those of later stages, are randomly distributed throughout the egg cytoplasm. Measurements of profiles indicate that the aggregated mitochondria in these early eggs are approximately twice as long as those of later stages (Table 1).

(b) Preblastoderm (3–4 h of age)

By this stage of development, some of the nuclei that ultimately will become components of the blastodermal cells have started to populate the periplasm. The embryo at this stage is essentially a syncytium, although lateral cell membranes have formed and generally extend below the basal parts of the elongate nuclei. The mitochondria are found largely in the proximal periplasm either
underlying or lateral to the nuclei. They are no longer in clumps and are randomly distributed with respect to the anterior–posterior embryonic axis. Cristae are well formed and the mitochondria exhibit a greater morphological variability than was evident in earlier embryos (Fig. 3). Since the basal cell membranes have not formed, the blastodermal cells are not yet complete so the mitochondrial counts for this age given in Table 1 cannot be compared meaningfully with those at later stages but they do give an indication of mitochondrial distribution with respect to the incipient blastodermal cells. A count of mitochondrial profiles shows that a high percentage (81%) are spherical compared with 50–62% that are so-shaped at later stages (Table 1).

(c) Blastoderm (7–8 h of age)

Blastoderm cells all show lateral membranes; basal membranes have either formed or are in the process of forming and the mitochondria have become redistributed as compared with their positions in the previous stage. By the time the blastoderm has reached its definitive condition large numbers are concentrated in a supranuclear location with a concomitant reduction in numbers in other parts of the cell (Fig. 4). Table 1 shows clearly this shift from approximately 18% at 3–4 h to 48% at 7–8 h.

(d) Beginning of gastrulation (11–12 h of age)

Dorsal and lateral blastodermal cells that remain at the embryo’s surface are the largest observed in this study but the number of mitochondria they contain is not significantly greater than that found at 7–8 h nor is their distribution significantly different (Table 1). Invaginated cells (the former middle plate)
Table 1. Sizes and numbers of cells and cellular components in Habrobracon embryos of different ages (based on profiles in electron micrographs of thin sections)

<table>
<thead>
<tr>
<th>Age (h) and developmental stage</th>
<th>Cells</th>
<th>Nuclei</th>
<th>Mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Mean size, μm (± S.D.)</td>
<td>Mean size, μm (± S.D.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2, karyokinesis</td>
<td>7</td>
<td>6.7 ± 4.2* (± 1.1)</td>
<td>3.7 ± 3.1 (± 0.5)</td>
</tr>
<tr>
<td>3–4, preblastoderm</td>
<td>23</td>
<td>8.7 ± 3.7 (± 1.6)</td>
<td>4.7 ± 2.5 (± 0.9)</td>
</tr>
<tr>
<td>7–8, blastoderm</td>
<td>7</td>
<td>10.4 ± 4.5 (± 2.4)</td>
<td>4.3 ± 2.7 (± 1.0)</td>
</tr>
<tr>
<td>11–12, early gastrulation</td>
<td>13</td>
<td>8.4 ± 4.8 (± 1.3)</td>
<td>3.7 ± 2.9 (± 0.5)</td>
</tr>
<tr>
<td>(surface cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(invaginated cells)</td>
<td>7</td>
<td>6.1 ± 3.6 (± 1.3)</td>
<td>3.4 ± 2.4 (± 0.4)</td>
</tr>
<tr>
<td>18–19, early organogenesis</td>
<td>7</td>
<td>5.5 ± 3.7 (± 0.5)</td>
<td>3.1 ± 2.5 (± 0.6)</td>
</tr>
</tbody>
</table>

S.D. = standard deviation.

* At this age the figures given do not represent actual cell size, since basal cell membranes have not formed, but rather they show the length of the partially formed lateral cell membranes.
Fig. 3. A portion of the proximal periplasm underlying the nuclei of a late 3–4 h embryo showing the typical arrangement and structure of mitochondria at the preblastoderm stage of development. × 30500.

Fig. 4. A sagittal section of a blastoderm cell from a late 7–8 h embryo. The distal pole of the cell shows processes (C); a limited amount of endoplasmic reticulum (arrow), glycogen granules (G), lateral cell membrane (L), nucleoli (NU) and mitochondria (M) are visible in other parts of the cell. × 13500.
Mitochondria in Habrobracon

Fig. 5. A sagittal section of a ventral ectodermal cell of an 18–19 h embryo. Endoplasmic reticulum (ER) with attached ribosomes is well developed as compared with early cells while mitochondria (M) are less numerous. × 13500.

Fig. 6. A portion of a ventral ectodermal cell of an 18–19 h embryo showing structural details. Mitochondrion (M), endoplasmic reticulum (ER), nucleus (N), glycogen (G). × 30600.

are generally about the same size as 7–8 h blastodermal cells but often have a somewhat higher percentage of spherical rather than elongate nuclei and the average number of mitochondria per section is somewhat smaller.

(e) Early organogenesis (18–19 h of age)

Fig. 5 is an electron micrograph of a ventral ectodermal cell. The number of mitochondria in single ultrathin sections of embryos of this age seemed considerably reduced when compared with those seen in sections of younger embryos and no discernible pattern of distribution was observed. In general, mitochondria
exhibit somewhat greater morphological complexity and variability when compared with those at earlier stages studied (Fig. 6). The number of mitochondria per section is drastically reduced when compared with 7–8 and 11–12 h embryos although this is probably related, at least in part, to a reduction in cell size (Table 1).

Other cellular constituents

Free ribosomes, a limited amount of smooth endoplasmic reticulum (ER) and small vacuoles are present largely in the peripheral cytoplasm of the earliest eggs examined in the present study. Yolk spheres, lipid droplets and large deposits of glycogen are abundant in the central region between the cytoplasmic reticulum strands. By 3–4 h of age, cytoplasmic extensions of the periplasm are common, lateral cellular membranes have begun to form between the nuclei of the incipient blastoderm and small quantities of rough ER have appeared. Nucleoli are first visible in cells of the 7–8 h embryo, lateral membranes have elongated and by the end of this period basal cell membranes have formed. Most of the structures just described can be seen in Figs. 3 and 4.

During the remainder of the embryonic period studied, rough ER gradually increases in quantity until it is relatively very abundant by 18–19 h (Figs. 5, 6) and Golgi bodies appear. At the same time glycogen and other nutrients decrease in amount and cytoplasmic processes are lost.

Profiles of cells and nuclei, measured at all the stages studied, reveal that considerable change occurs in their mean size and shape during development (Table 1). At the end of the blastema divisions, the profiles of the spherical nuclei average over 3 \( \mu \text{m} \) in diameter. During the formation of the blastoderm they elongate and enlarge slightly, averaging 4.7 \( \mu \text{m} \) in length and 2.5 \( \mu \text{m} \) in width. During early gastrulation the nuclei of the surface cells are about the same size (4.3 \( \times \) 2.7 \( \mu \text{m} \)) as they were in the blastula while those of invaginated cells are somewhat smaller (3.7 \( \times \) 2.9 \( \mu \text{m} \)). Ectodermal cells at 18–19 h have nuclei that are slightly smaller (3.4 \( \times \) 2.4 \( \mu \text{m} \)) than those of the gastrula while those of mesodermal cells are the smallest of all (3.1 \( \times \) 2.5 \( \mu \text{m} \)). Nuclear envelopes possess typical annuli at all stages studied. Centrioles are common and typically occupy notch-like indentations of the nuclear envelope. Desmosomes are also evident in completely formed cellular membranes. Changes in overall cell size parallel those just described for the nuclei (Table 1).

DISCUSSION

Mitochondria

The observations presented here, based on profiles in electron micrographs, indicate that during embryonic development in Habrobracon the mitochondria are pleomorphic. Variability involves changes in shape, size and structure of cristae as well as differences in number and distribution. Similar differences have been reported in a wide variety of other developing organisms.
Mitochondria in Habrobracon

The mature ovum of the slug contains spherical or oval mitochondria with poorly defined internal structure while 8-cell stages have highly polymorphic forms, two or three times larger than those in the egg and relatively very abundant, with an elaborate system of well-defined cristae (Sathananthan, 1970). Mahowald (1963) found few oblong or elongate mitochondrial profiles in Drosophila embryos undergoing blastoderm formation but, after the blastoderm had formed, long profiles and branched forms were numerous. Berg & Long (1964) reported that the mitochondria of the 16-cell sea-urchin embryos were of uniform size but in the early gastrula there was an indication of an increase in size, in number and in the number of cristae. Yamamoto (1964) working with fish embryos found small, spherical mitochondria with two or three cristae in the early blastula; the gastrula, on the other hand, had organelles that were ovoid or rod-shaped with numerous cristae and showed up to a twofold enlargement.

Among a wide variety of mammalian embryos that have been studied, mitochondrial changes in the pre- and post-fertilization stages are essentially similar to those for lower forms just described. The rabbit (Anderson, Condon & Sharp, 1970) and the mouse (Stern et al. 1971) will serve to illustrate the typical situation. Mitochondria, spherical or spheroid in shape and generally with a few circularly arranged cristae, are typical of those found in mature ova. In later developmental stages the cristae are more diverse in form and in the morula and blastocyst they are generally transversely oriented and much more numerous. In the rabbit, there is also a gradual increase in mitochondrial size and in the number of cristae present during these last two stages.

In the cases just cited, although the data given for the different organisms do not always cover the same developmental stages, there is a general tendency for mitochondrial morphology to change as development progresses. In the earliest stages mitochondria are typically spherical, relatively small and scarce, with few cristae; in later stages they are frequently elongate, larger, more numerous and contain greater numbers and often more complicated cristae. The situation in Habrobracon with respect to the last named structures is about the same but it differs greatly in so far as mitochondrial size and shape are concerned. In 1–2 h embryos the mitochondria are evidently predominantly elongate and much longer than those of that shape found in later stages. As development proceeds, however, they seem to undergo much change since relatively more spherical profiles are evident at later stages particularly at 3–4 h of age.

Mitochondria of an Habrobracon oocyte, as shown in an electron micrograph (Cassidy & King, 1972), resemble in structure and size those seen in the present study in the 3–4 h embryo. Presumably they are in this condition when they move into the oocyte from the nurse cells, then assume the simpler morphological condition seen in the 1–2 h embryo. Since mitochondrial metabolic activity is directly related to the number and structural complexity of cristae
(Linnane, Vitols & Nowland, 1962; Lehninger, 1965), it is assumed that these organelles must be relatively quiescent at this stage in embryogenesis. Furthermore, since reversible ultrastructural changes associated with respiratory-related transformations have been reported for isolated liver mitochondria (Hackenbrock, 1966, 1968), it is possible that the structural changes in cristae reported in the present paper reflect such physiological variation in *Habrobracon*. If these inferences are valid, very rapid changes in activity could accompany the observed morphological alterations of the organelle between the time the oocyte is formed and the early embryonic condition is reached.

Distribution of mitochondria in developing organisms is another parameter that has been considered by a number of authors. King (1960) reported that the mitochondria of the mature *Drosophila* egg were scattered throughout the ooplasm, in *Habrobracon* they are much more numerous in the periplasm with a smaller number occupying the cytoplasmic reticulum. The grouping of mitochondria observed in the periplasm of *Habrobracon* in the 1–2 h embryo is reminiscent of mitochondrial 'clouds' observed in *Xenopus* oogonia and oocytes (Al-Mukhtar & Webb, 1971), in the chicken oocyte (Romanoff, 1960) and in hamster oocytes and zygotes (Hadek, 1969). Lehninger (1965) has described a similar situation in the sea urchin and has correlated it with a physiological change in the early embryo. He reported that in some species the unfertilized eggs exhibit a very low level of respiration. At that time the mitochondria form aggregates of up to 60 individual organelles. After fertilization they disaggregate and the respiratory rate increases. With respect to the morphological aspects of aggregation this behavior conforms closely to that described in the present study in early embryos. Perhaps physiological changes of this sort occur in *Habrobracon* as well although confirmatory tests were not made. One additional point should be mentioned, however: haploid *Habrobracon* eggs develop parthenogenetically so the activating mechanism is presumably different from that operating under the more usual sperm-egg interactions and is apparently initiated during the laying process. In the present study the fact that clumped mitochondria are present 1–2 h after oviposition may mean that for some reason disaggregation is considerably delayed and, if the analogy can be extended still further, respiratory rate increase is similarly postponed.

During blastoderm formation in *Habrobracon* the mitochondria move to a supranuclear position in the blastodermal cells just as they do at the same stage in *Drosophila* (Mahowald, 1963). I was unable to demonstrate a greater concentration of them in the ventral mid-region cells of the blastoderm (as compared with the dorsal mid-region cells) as reported by that investigator. As for the distribution of mitochondria into presumptive germ layer cells during gastrulation, my data are too meagre and equivocal to demonstrate support for either the differential distribution of larger numbers into the mesoderm and endoderm of the slug gastrula (Sathananthan, 1970) or the equal distribution
Mitochondria into all presumptive germ layers of the early sea-urchin gastrula (Berg, Taylor & Humphreys, 1962).

It is recognized that serious limitations are encountered in attempting to use data from mitochondrial profiles seen in ultrathin sections to deduce information about the size, shape and numbers of such three-dimensional structures. Whether the observed variations in profiles are a reflection of true changes in mitochondrial morphology associated with developmentally related interactions or whether they involve merely differences in orientation and arrangement with respect to individual organelles, changes in shape, length or similar variable of these notoriously plastic organelles must await further study.

Other cellular constituents

Free ribosomes are abundant in 1–2 h eggs of *Habrobracon* resembling those reported in oocytes of the same species (Cassidy & King, 1972) and persist during later stages; they are not, however, as markedly clustered as they appear to be during blastoderm formation in *Drosophila* (Mahowald, 1963). Smooth ER is relatively poorly developed in early eggs and oocytes of both (*Habrobracon*: Cassidy & King, 1972; *Drosophila*: Okada & Waddington, 1959; the gradual appearance of rough ER in *Habrobracon* as development proceeds follows a different pattern in *Drosophila* to the extent that, in the latter, it develops much more rapidly during blastoderm formation (Mahowald, 1963). The general concept that ER increases in quantity and structural complexity in the course of differentiation (Fawcett, 1959) is certainly upheld by results of the present study. Lipid droplets, yolk spheres and glycogen deposits are similar to those reported in *Habrobracon* oocytes by Cassidy and King (1972). They report also that *Habrobracon* oocyte nuclei contain one or two nucleoli. Such structures were not observed in the present study up to 3–4 h of age but they were present in 7–8 h embryos. According to Okada & Waddington (1959) and Mahowald (1963), these organelles appear in *Drosophila* at the time cellular membranes start to form. Such membranes develop in *Habrobracon* at 3–4 h, so nucleoli appear somewhat later in this form than they do in *Drosophila*. Yamamoto (1964) has shown that they appear during the middle blastula stage in *Oryzias* which probably coincides better with their time of appearance in *Habrobracon*.

The author is grateful to Dr M. Bessis and his colleagues of the Institut de Pathologie Cellulaire for their hospitality and assistance. Particular thanks are due Mlle Vinzens, Mme Le Coq and Mme Prenant for invaluable technical assistance.

Supported in part by a United States Public Health Service Fellowship (1 F03 HD50748-01) from the National Institute of Child Health and Human Development.
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(Received 3 January 1975, revised 17 April 1975)