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Is there a germ plasm in mouse oocytes?

Arkadiy Reunov
Institute of Marine Biology, Vladivostok, Russia

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Summary

It was found that in the Graafian oocytes of laboratory mice *Mus musculus* the population of electron-dense bodies contains two patterns of structures. One of these, designated as cortical granules, originated from the Golgi complex and was surrounded by a membrane. The other was discovered as cristae-containing mitochondrial derivatives lacked an outer membrane. It was found that the mitochondrial derivatives underwent progressive condensation and transformed into electron-dense bodies similar to germinal bodies of metazoan animals. Based on examination of Graafian follicle oocytes from 5 female individuals, about 15% of electron-dense bodies were cortical granules. However, about 85% of electron-dense bodies were condensing mitochondrial derivatives transforming into electron-dense bodies.

Keywords: *Germinal bodies, Mitochondria, Mouse, Oocytes, Ultrastructure*

Introduction

It is known that germ line polar plasm is maternally accumulated in eggs of *Caenorhabditis*, *Drosophila* and *Xenopus* (Mahowald, 1977; Strome & Wood, 1982; Ikenishi, 1998) and then shows continuity throughout the life cycle of these metazoan representatives. However, mouse eggs and early embryonic cells have been reported (Williamson & Lehmann, 1996; Snow & Monk, 1983; Saffman & Lasko, 1999; Matova & Cooley, 2001; Yoshimizu *et al*., 2001) as lacking inherited germ line determinants and it seems intriguing. Since the ability of mouse stem cells to develop into oogonia *in vitro* was recently reported (Hübner *et al*., 2003) it seems reasonable that some determinative factor must exist in these cells.

This paper presents data from an ultrastructural study of laboratory mouse *Mus musculus* oocytes focused on a search for structures that could be morphologically compared with germinal bodies.

Materials and methods

To prevent the effect of chemical substances on sex cell ultrastructure 5 female adult (2 months old) laboratory mice *Mus musculus* were killed without any sort of chemical influence by very fast decapitation. The peritoneum was opened and the ovaries dissected out. The tissues were prepared for electron microscopy by fixation in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and in 2% osmium tetroxide buffered with the same buffer. Following dehydration in a graded series of ethanol and acetone, the material was embedded in Epon-Araldite. Sections were cut on an Ultracut-E (Reichert) ultramicrotome using a diamond knife, stained with uranyl acetate and lead citrate, and examined with a JEM 100 B transmission electron microscope.

The Graafian follicle oocytes of 5 female individuals were studied. One block from each female was prepared for sectioning. Three technically perfect sections from each block were examined by transmission electron microscopy. Thus a total of 15 sections of Graafian follicles from 5 individuals were studied. For each oocyte section the electron-dense bodies were calculated and examined.

Results

The Graafian follicle oocyte cytoplasm contained electron-dense bodies (Fig. 1A). Fifteen per cent of these structures (Fig. 2B) are electron-dense vesicles surrounded by a membrane (Fig. 1B). As transmission electron microscopic observation shows, these vesicles originate from the Golgi complex. Another pattern

All correspondence to: Dr A. Reunov, Institute of Marine Biology, Far East Branch of the Russian Academy of Sciences, 17 Palchevsky St, 690041 Vladivostok, Russia. e-mail: arkadiy_reunov@hotmail.com

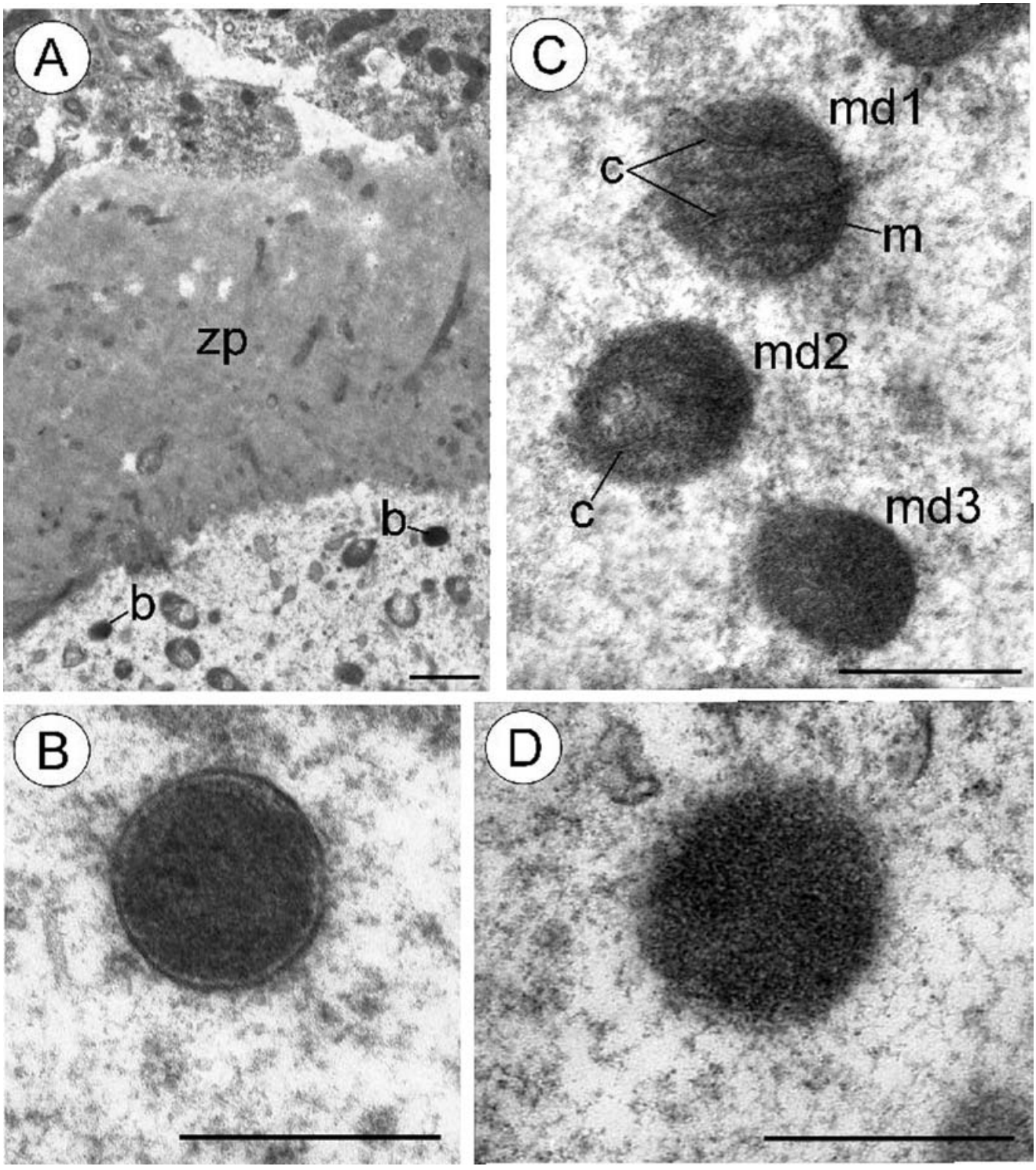

Figure 1 The electron-dense bodies in mouse Graafian oocytes. (*A*) Electron-dense bodies (b) in the peripheral area of the oocyte. zp, zona pellucida. (*B*) Electron-dense body (vesicle) surrounded by a membrane that presumably is a cortical granule. (*C*) Mitochondrial derivatives: md1, a mitochondrial derivative in which the cristae (c) and part of an outer membrane (m) can be discerned; md2, a mitochondrial derivative in which an outer membrane is no longer seen but the cristae (c) still can be found; md3, a mitochondrial derivative in which the cristae as well as the outer membrane are already absent. (*D*) A finally condensed mitochondrial derivative (md3) that can be structurally compared with a germinal body. Scale bars represent: (*A*), 1 μm; (*B*)–(*D*), 0.5 μm.

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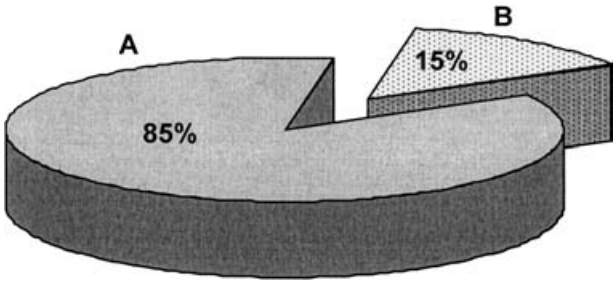

Figure 2 Diagram of the distribution of electron-dense bodies in mouse Graafian oocyte. (*A*) The population of condensing (md1, md2, md3) and condensed mitochondrial derivatives that presumably are developing and formed germinal bodies. (*B*) The population of membrane-bounded vesicles that presumably are cortical granules.

of structures can be distinguished as transforming mitochondria. Some of the mitochondria observed in oocytes were found with a ruptured outer membrane and appeared as cristae-containing mitochondrial derivatives of the first type (md1) (Fig. 1C). During maturation these structures differentiate into mitochondrial derivatives of the second type (md2), lacking an outer membrane but still containing some cristae (Fig. 1C). In mitochondrial derivatives of third type (md3) both the outer membrane and the cristae were no longer discernible (Fig. 1C). As differentiation continued the mitochondrial derivatives underwent progressive condensation (Fig. 1C). When finally condensed the md3 mitochondrial derivatives appeared as round bodies containing an electron-dense friable substance (Fig. 1D). The condensation of mitochondrial derivatives could occur in any part of the cytoplasm but the finally formed electron-dense bodies tend to localize in the peripheral area of the oocyte. The average amount of condensing and finally condensed mitochondrial derivatives per oocytes considered was about 85% (Fig. 2A).

Discussion

In oocytes of mice and other mammals, the bodies located along oolemma are usually described as cortical granules originating from the Golgi complex and are surrounded by a membrane (Siracusa *et al*., 1985; Kress *et al*., 2001). This pattern of vesicles was found in oocytes of *M. musculus* during present study. However, it is obvious from some previous data (Eddy, 1975) and our observations that in mouse oocytes there is a distinct type of electron-dense bodies that structurally coincide with the germinal bodies (= germinal granules or polar granules) of other metazoan representatives.

Despite the fact that *Vasa*, *Oskar* and *Tudor* proteins as well as mitochondrial ribosomal RNA have been shown to be components of the polar granules in *Drosophila* (Iida & Kobayashi, 1998; Kashikawa *et al*., 1999) and *Xenopus* (Kobayashi *et al*., 1998), the exact structure of such bodies in these and other Metazoa remains unknown. Both (large and small) the mitochondrially encoded ribosomal RNAs are reported to be exported from mitochondria to polar granules (Matova & Cooley, 2001), but the mechanism of this transport is not clear.

In the present study the mitochondria in mouse oocytes are designated as basic structural components of germinal bodies and this probably would highlight why the mitochondrial ribosomal RNA finds itself in structures of this type (polar granules) in *Drosophila* and *Xenopus* oocytes (Iida & Kobayashi, 1998; Kobayashi *et al*., 1998; Kashikawa *et al*., 1999). Indeed, the mitochondrial derivatives of mouse were typically observed condensing and transforming to germinal bodies ultrastructurally comparable with analogous structures found in other metazoan animals (Eddy, 1975; Williams, 1989; Klag & Bilinski, 1993; Saffman & Lasko, 1999; Matova & Cooley, 2001; Isaeva & Reunov, 2001). It should be stressed that the significance of mitochondria in the origin of the polar granules was pointed out in an earlier study of *Drosophila* oogenesis (Mahowald, 1962) though these organelles were not suspected to be precursors of polar granules. However, figure 7 of Mahowald's paper (1962) clearly shows the mitochondrial derivatives obviously participating in polar granule formation. The formation of yolk distinct 'lamellar bodies' from mitochondria was described for oocytes of the teleost fish *Noemacheilus barbatulus* (Riehl, 1977). It seems possible that the mitochondria may be a precursor of germinal bodies (= germinal granules or polar granules) in oocytes of various multicellular animals, but this needs to be investigated by detailed ultrastructural study in a wide range of organisms.

Obviously, germ line determinants might have not been discovered in mouse oocytes and embryonic cells for the reason that these determinants are small, scattered germinal bodies, analogous to those observed in present study. As our ultrastructural observations indicate, the germinal bodies in *M. musculus* do not form the large aggregations comparable with those in *Caenorhabditis*, *Drosophila* and *Xenopus* (Matova & Cooley, 2001) and, hence, they are difficult to find. It seems possible that germinal bodies formed in late oocytes may be segregated by some mouse blastomeres from the first divisions to pass to primordial germ cells, where such structures have been found by ultrastructural analysis (Spiegelmann & Bennett, 1973).

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