P26 - MITOCHONDRIAL MASS, DISTRIBUTION AND ACTIVITY DURING SEA URCHIN OOGENESIS

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The sea urchin egg is a favourite model for studies of the molecular biology and physiology of fertilization and early development, yet we know sparingly little of its oocytes and of mitochondria behaviour during oogenesis.
The process of oogenesis in most echinoderms is asynchronous so each ovary lobe has hundreds of oocytes at all stages of development. At the beginning of oogenesis, the oocyte is about 10 µm in diameter. During the vitellogenic phase of oogenesis, the oocyte accumulates yolk proteins and grows to ten times their original size to 80 to 100 µm in sea urchins. The oocyte, arrested at the prophase of the first meiotic division, is apparent with its large nucleus, the germinal vesicle (GV), containing a prominent nucleolus. Echinoid (such as sea urchin) and Holothurian oocytes complete meiotic maturation prior to fertilization, distinct from other echinoderms and almost all others animals. As maturation progresses, it occurs the GV breaks down (GVBD). These eggs may then be stored for weeks to months within the female before they are spawned, and the proportion of eggs in the ovary increases from early to late season, as the numbers of oocytes decline [1].
Mitochondria, generally known as the powerhouses of eukaryotic cells, play a primary role in cellular energetic metabolism, homeostasis and death. These organelles, with their multicopy genome maternally inherited, are directly involved at several levels in the reproductive process since their functional status influences the quality of oocytes and contributes to the process of fertilization and embryonic development.
It has been demonstrated that the number of maternal mitochondria is sufficient to support development until late stages without new synthesis of mitochondrial DNA or production of new organelles [2]. During embryogenesis mitochondrial mass does not change, whereas mitochondrial respiration increases [3]. The behaviour of these organelles during oogenesis remains at moment unclear.
In the present paper we studied, by Confocal Laser Scanning Microscopy tecnologies (CLSM), the mass and distribution, the activity and the DNA content of sea urchin *Paracentrotus lividus* mitochondria during oogenesis, by *in vivo* incubating oocytes of different size with cell-permeant probes specific for mitochondria and for DNA and by immunodetection of hsp60 chaperonine, a well known mitochondrial marker.
In particular the oocytes were grouped in six classes: < 10, 20/30, 40/50, 60/70, 80/90 µm, and 90 µm ovulated egg, on the base of diameters. Microscopic observations were performed capturing 2 µm thick layers of oocytes. Of the several thousands oocytes we observed, 20 for each different oogenesis stage were analyzed and processed. In order to interpret results and to draw unequivocal conclusions, we measured by IMAGE J software analysis the intensity values of fluorescent signals, as suggested in Agnello et al 2008 [4].
The mitochondria of oocytes with a diameter between 20 and 70 µm, appeared to give rise to clusters that disappear in that of 80 µm. In the oocytes between 60 and 90 µm the red fluorescence seems to be more evident around the germinal vesicle (the merge tends to red), suggesting an increasing oxidative phosphorylation activity.
In the ovulated eggs, red and green fluorescence are uniformly distributed suggesting that mitochondria are dispersed in the cytoplasm. In addition the merge of green and red colours

shows that the whole mitochondrial population is consuming oxygen at the same level (the resulting colours tends to yellow), figure 1.
In order to calculate the total mitochondrial mass and activity we integrated the values of pixel intensities for all captured sections and used the arithmetic means to draw a statistical analysis. Results suggest a parallel rise of mitochondrial mass and activity, suggesting that the amount and activity of organelles change remarkably during oogenesis.

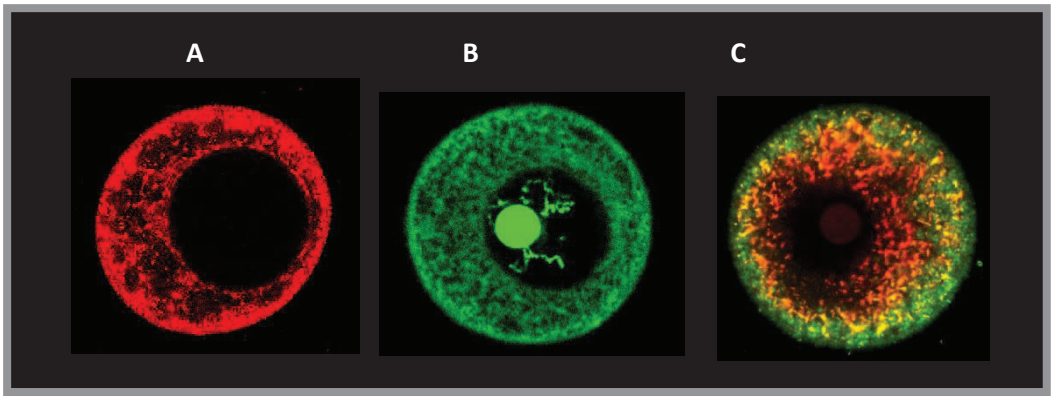

Figure 1 shows the distribution of hsp60 protein, detected by immunofluorescence analysis (A), the mitochondrial and genomic DNA, after in vivo incubation with PicoGreen probe (B) and the merge of green and red fluorescence signal, respectively due to mitochondrial mass and activity, after in vivo incubation with Mitotraker Green and Orange (C). The size of the oocytes reported is 80 μm.

Results suggest that mitochondria are actively duplicating and that mitochondrial DNA is replicating during the different oogenesis phases. It is noteworthy that around the germinal vesicle, especially in the larger oocytes, next to the germinal vesicle breakdown, the organelles are more active in oxygen consumption, probably due to the major energetic needing in this key moment of gametogenesis.

[1] Wessel G.M., Voronina E., and Brooks J.M. (2004) Obtaining and handling echinoderm oocytes. In "Methods in Cell Biology", Elsevier. Vol.74, Chapter 5, pp. 87-114.
[2] Matsumoto L., Kasamatsu H., Pik´o L. and Vinograd J. (1974) Mitochondrial DNA replication in sea urchin oocytes. J. Cell Biol. 63: 146–159.
[3] Morici G., Agnello M., Spagnolo F., Roccheri M.C., Di Liegro C.M. and Rinaldi A.M. (2007) Confocal microscopy study of the distribution, content and activity of mitochondria during *Paracentrotus lividus* development. Journal of Microscopy. 228: 165-173.
[4] Agnello M., Morici G., Rinaldi A.M. (2008) A method for measuring mitochondrial mass and activity . Cytotechnology. 56: 145-149.