DISTRIBUTION OF INTRACELLULAR CALCIUM DURING OVARIAN DEVELOPMENT OF ZEBRAFISH, DANIO RERIO

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Abstract: The aim of this study was to assess distribution of intracellular calcium during stages of oogenesis in zebrafish using a combined oxalate-pyroantimonate technique. The oocyte development of zebrafish was categorized into four stages: primary growth, cortical-alveolus, vitellogenic, and maturation, based on morphological criteria. Calcium in the primary growth stage were detected in the cytoplasm, mitochondria, nucleus, and follicular cells. At the cortical-alveolus stage, calcium particles were found in the cortical alveoli. Large amounts of intracellular calcium was accumulated in follicular cells, zona radiata and inside the cortical alveoli. In the maturation stage, calcium wan only visible in mitochondria. Basic information about calcium distribution during zebrafish oogenesis may contribute to better understanding of its role in oogenesis.

Keywords: Follicular cell, Oocyte, Ovary,Oxalate–pyroantimonate, Zebrafish.

I. INTRODUCTION

During oogenesis, the oocyte undergoes developmental changes including four principal stages of oocyte growth [1].The basic pattern of oogenesis and its regulation is similar in all teleosts [2]. The morphological characteristics of oocytes provide information on the dynamics of oogenesis, including final maturation and ovulation [3]. The ultrastructure of ovarian tissue has been used to clarify the reproductive cycle of teleosts [4], since it provides reliable and accurate insight into the reproductive status of the female. Calcium plays a pivotal role in diverse biological processes including meiosis resumption [5], cortical reaction [6], and embryo cell division [7]. Numerous studies have demonstrated differences in the distribution of intracellular calcium deposits in oocytes depending on their stage of meiotic maturation. These oocytes differ in the ability to deliver calcium from intracellular deposits to the cytoplasm [8] 2006).Despite the important role of calcium in the growth and differentiation of germ cell types in the ovary, data on the distribution of intracellular calcium deposits at different stages of oocyte development in zebrafish is lacking. In addition, location of the intracellular calcium storage sites in different ovarian cell types is not known. The aim of this study was to describe and compare the distribution of intracellular calcium deposits at different stages of oogenesis in zebrafish.

DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Six female zebrafish were anaesthetized in ice water and killed, and the gonads were dissected. Then ovaries were removed and immediately immersed in a fixative of 2% glutaraldehyde, 2% formaldehyde, 90 mM potassium oxalate, and 1.4% sucrose, pH 7.4, at 4 ° C. The tissues were washed three times, 15 min each, in distilled water and post-fixed in 1% osmium tetroxide and 2% potassium pyroantimonate for 3 h at 4 °C. Samples were rinsed thoroughly 3 times for 15 min each in cold distilled water with pH raised to 10 using KOH [9]. Samples were dehydrated through an acetone series, and embedded in resin (EPON). A series of ultrathin sections were cut using a UCT Ultramicrotome (Leica Microsystems, Wetzlar, Germany) mounted on copper grids, double-stained with uranyl acetate and lead citrate, and examined with a 1010 transmission electron microscope (TEM) (JEOL Ltd., Tokyo, Japan) operating at 80 kV. For better detection of calcium deposits, samples were examined without contrast prior to staining by uranyl acetate and lead citrate. After treatment with oxalate-pyroantimonate, calcium deposits in the ovary are visible in TEM micrographs as electron-dense precipitates.

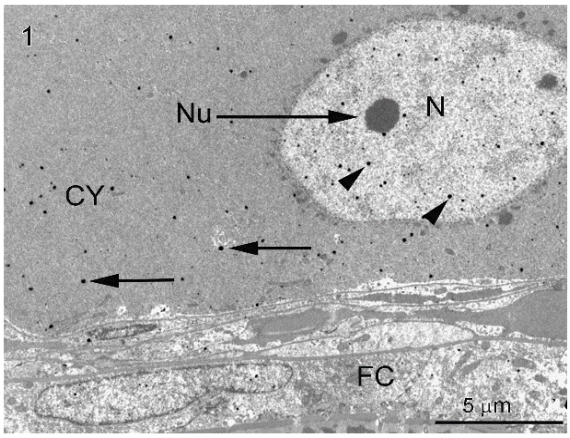


Fig. 1. Intracellular calcium in the follicular cells, cytoplasm (arrow), and nucleus (arrowheads).CY: cytoplasm, FC: follicular cells, N: nucleus, Nu: nucleoli.

II. RESULTS AND DISCUSSION

3.1. Primary growth

A typical characteristic of first stage of oogenesis is a large nucleus relative to the volume of cytoplasm. Calcium deposits were particularly apparent in the cytoplasm and especially in the nucleus (Fig. 1). The synchronous fluctuation of the level of calcium deposits in the cytoplasm of the oocyte and follicular cells is not surprising considering their proximity and wide variety of biologically active molecules that are regularly exchanged between them [8].

3.2. Cortical alveolus

Commencement of the cortical alveolus stage was indicated by the appearance of cortical alveoli within the oocyte. Calcium particles appeared to be transported from follicular cells into the cytoplasmic space via zona radiata pores to aggregate inside the cortical alveoli (Fig 2). A study of sea urchin egg clearly showed the cortical granules to contain a higher level of calcium than did other large vesicular compartments, implying that the egg periphery contains more calcium than the interior [10]. Studies have shown that the cortical layer of sea urchin eggs sequesters 14% of the total egg calcium [10].

3.3. Vitellogenic stage

Large amounts of calcium continued to be transported from follicular cells into the oocyte via the zona radiata and was accumulated inside the cortical alveoli (Fig. 3).Calcium stores in cortical granules are thought to stabilize the structure of the membrane or to possibly be the source of the intracellular calcium that is released at fertilization to initiate the cortical reaction and activate development [11]. The universal trigger of this activation process occurs with gamete fusion and a series of changes leading to cortical granule content discharge and embryogenesis [12].

3.4. Maturation

The concentration of calcium deposits decreased in areas of follicular cells and oocytes. Calcium deposits were still visible in oocyte mitochondria (Fig. 4).Calcium deposits were also observed in the mitochondria, which can be explained by the large, internally negative, membrane potential across the inner membrane of the mitochondria resulting in intake of calcium across the inner membrane [13]. Calcium released from mitochondria of the mouse oocyte has been postulated to play a significant part in the regulation of meiosis resumption [14].

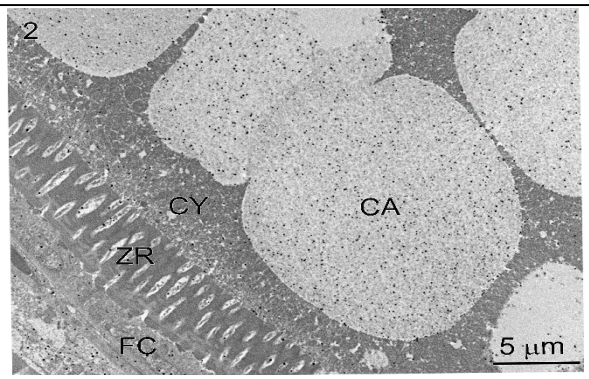


Fig.2. Cortical alveoli containing numerous aggregated calcium deposits apparently transported from follicular cells into the cytoplasmic space via zona radiata pores.CA: cortical alveoli, CY: cytoplasm, FC: follicular cells.

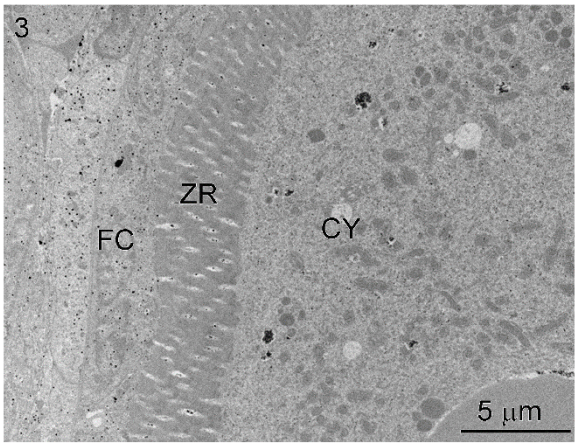


Fig. 3. Transmission electron micrograph shows transport of calcium deposits from extracellular space into cytoplasmic space via zona radiata pores (arrowheads).CY: cytoplasm, FC: follicular cells, ZR: zona radiata.

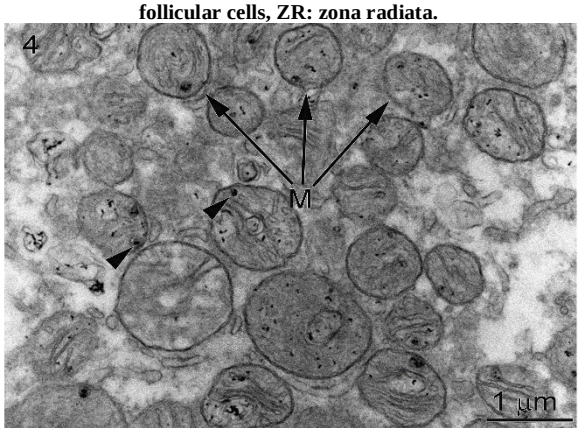


Fig.3. Micrograph shows intracellular calcium inside mitochondria (arrowheads). M: mitochondria.

CONCLUSIONS

The present study has provided basic information on the presence of calcium in several organelles that likely serves as a source for calcium-dependent events in zebrafish oogenesis. The distribution of calcium deposits probably reflects the physiological status of the oocyte and may be involved in the regulation of cellular processes during oocyte maturation.

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