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An ultrastructural study of oocyte atresia in the starfish *Pisaster ochraceus*

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ABSTRACT

The alterations involved in oocyte atresia of the starfish *Pisaster ochraceus* were investigated using both light and electron microscopy. It was shown that atresia may be defined by three patterns of cell destruction. Initially, the small electron-lucent vesicles produced by the Golgi complex underwent amalgamation into groups. This was followed by loss of vesicle membranes and consequent formation of transparent necrotic zones in the cytoplasm. The second pattern, ultrastructurally comparable with autophagic cell death, was marked by apparent amalgamation of the morphologically similar electron-lucent vesicles into growing vacuoles, giving rise to a multibranched autophagic vacuole. This vacuole engulfed the cytosol granules and ultimately came to occupy the entire space within the oocyte. In addition, the cytosol insulation inside of the 'apoptotic body-like spheres' was regularly observed. Thus, it is supposed that oocyte destruction may occur by a complex mechanism that includes elements of necrosis, autophagic cell death and apoptosis.

1. Introduction

Programmed cell death connected with oocyte resorption or oocyte atresia is a physiological process that occurs in the gonads of both invertebrates and vertebrates. Atresia occurs as a mechanism to provide space for the growth of eggs (Mermillod et al., 1999; Devine et al., 2000), as a consequence of egg competition for blood supply (Boyle and Chevis, 1992), as a reaction to environmental stress (McCormic et al., 1989) and infection (Avarre et al., 2003), as a factor of fecundity regulation (Kurita et al., 2003), or as a method of destruction of unspawned mature oocytes in post-spawning ovaries (Tyler et al., 1982; Lango-Reynoso et al., 2000). The most accepted pattern of ovarian atresia is apoptosis (Hsueh et al., 1996; Tilly, 1998). However, in some cases the ultrastructural and molecular mechanisms of oocyte destruction are reported as either distinct from apoptosis (Devine et al., 2000) or as a combination of apoptosis with either necrosis (Saraste and Pulkki, 2000) or autophagic cell death (Velentzas et al., 2007).

The present study examines the details of oocyte atresia in the starfish *Pisaster ochraceus*, which appears to display ultrastructural characteristics of three patterns of cell death, namely necrosis, autophagic cell death and apoptosis.

2. Materials and methods

According to Fraser et al. (1981), spawning of *P. ochraceus* occurs in the months of May through August with a peak during May and June. In populations of *P. ochraceus* residing in the Deep Cove on Vancouver Island, spawning occurs in July and August (Crawford, unpublished data). Based on this data, specimens of the sea star *P. ochraceus* were collected at Deep Cove, Vancouver Island in July of 2008. The animals were housed in sea water tanks in the Aquatic Centre at the University of Victoria. To inhibit spawning and provoke egg destruction, animals were kept in darkened tanks until the end of September and then were investigated in regard to the condition of the reproductive cells. The gonads of three females were surgically removed from one arm of each sample. The dissected animals were immediately placed in sea water tanks for regeneration. The ovary pieces were taken for the microscopy study.

Small pieces of the ovaries were fixed in 2.5% glutaraldehyde buffered with cacodylate buffer (pH 7.4). The osmolarity of the fixative was adjusted to 1250 mOsm by adding NaCl. The materials were then postfixed for 2 h in 1% OsO_4 in sea water. After dehydration in increasing concentrations of ethanol, the fixed eggs were transferred to 100% acetone and embedded in Epon 812. The eggs were then sectioned on an ultramicrotome (Sorvall Porter-Blum MT-1; Thermo Fisher Scientific, Inc., Waltham, MA, USA) using a diamond knife.

The eggs were identified and staged in terms of atresia on a light microscope (Zeiss III RS; Carl Zeiss AG, Oberkochen, Germany) using 1 μm thick sections stained with Richardson's stain (Richardson et al., 1960). For transmission electron microscopy, thin sections were

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mounted on Formvar-coated metal slot grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and photographed using a transmission electron microscope (Hitachi H-7000; Hitachi Ltd. Corp, Tokyo, Japan).

3. Results

Two types of eggs were found in the ovaries of *P. ochraceus*. Smaller numbers of oocytes seemed normal and appeared dark in colour (Fig. 1A). From an ultrastructural perspective, these cells were peculiar in having peripheral low-density vacuoles, cortical granules and mitochondria (Fig. 1B). The cytoplasm was filled with yolk granules (Fig. 1C), which had variable electron densities and existed in three differently coloured patterns (Fig. 1D). The cytoplasm was full of small electron-lucent vesicles (Fig. 1D), which seem to originate from Golgi complexes (Fig. 1D, insert).

A large proportion of the oocytes were atretic and had electron-lucent regions within them (Fig. 1A). The oocytes marked by the initial stages of atresia were filled with small electron-lucent vesicles (Fig. 2A). The aggregated vesicles were frequently seen to amalgamate, followed by the loss of their membranes and the appearance of 'empty zones' in the cytoplasm (Fig. 2B). In addition, some of the mitochondria located near these empty zones had lost their membranes (Fig. 2C).

In some cases, the vesicles did not lose their membranes but rather amalgamated with the growing autophagic vacuoles (Fig. 2D). These growing vacuoles appeared to have fused with the peripheral low-density vacuoles (Fig. 2E) and what appeared to be yolk granules were often seen inside of growing autophagic vacuoles (Fig. 2F). Cortical granules were also frequently observed to be taken up by the amalgamating vacuoles (Fig. 2G). After being engulfed, each granule usually joined with the growing autophagic vacuole (Fig. 2H). Indeed, it appeared that the cortical granules might have been selectively absorbed into the growing autophagic vacuole (Fig. 2I).

In some atretic oocytes, the developing autophagic vacuole was observed to form branches that engulfed fragments of cytoplasm (Fig. 3A). These fragments seem to have detached from the main

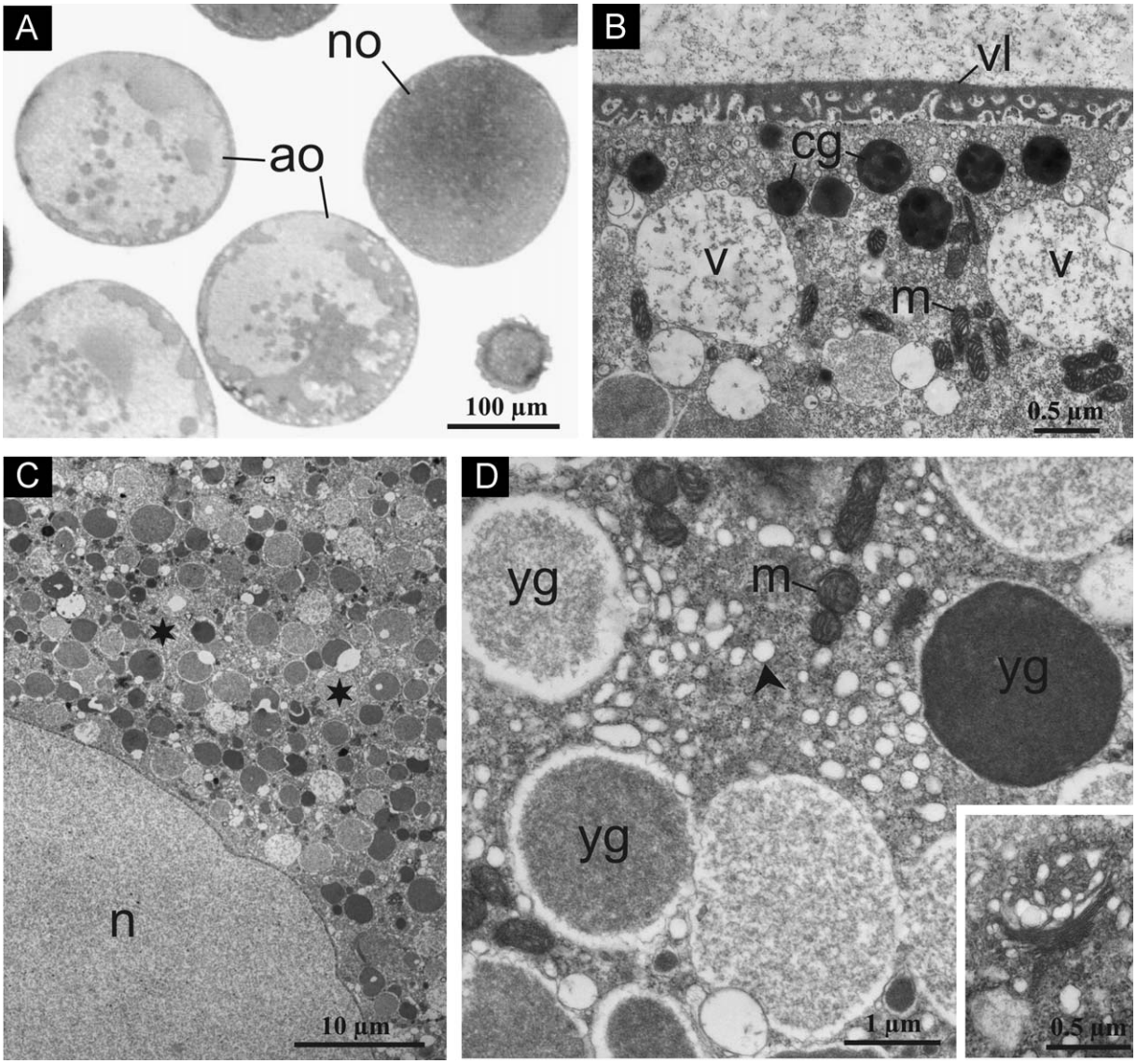

Fig. 1. Oocytes of the starfish *Pisaster ochraceus*. (A) Normal (no) and atretic (ao) oocytes classified by light microscopy. Note that normal oocytes are darkly coloured while atretic oocytes have electron-lucent areas in their content. (B) The normal oocyte periphery by transmission electron microscopy (TEM). cg, cortical granules; m, mitochondria; v, low density vacuoles; vl, vitelline layer. (C) The central area of a normal oocyte by TEM; the asterisks show the abundant yolk granules. n, nucleus. (D) Electron-lucent vesicles (arrowhead) and yolk granules (yg) in the oocyte cytoplasm by TEM. m, mitochondrion. Note that there are three types of differently coloured yolk granules. Insert: A Golgi complex producing the electron-lucent vesicles.