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Role of microtubules and centrosomes in the eccentric relocation of the germinal vesicle upon meiosis reinitiation in sea-cucumber oocytes

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Abstract

In the oocytes of many animals, the germinal vesicle (GV) relocates from the center to the periphery of the oocyte upon meiosis reinitiation, which is a prerequisite to the formation of meiotic spindles beneath the cell surface in order for meiosis to succeed. In the present study, we have investigated nuclear positioning using sea-cucumber oocytes. Upon meiosis reinitiation, the GV relocates to the cell periphery beneath a surface protuberance. After GV breakdown, polar bodies were extruded from the top of the protuberance, which we therefore called the animal pole process. The GV relocation was inhibited by nocodazole but not by cytochalasin. Immunofluorescent staining and electron microscopy of microtubular arrays revealed that: (i) in immature oocytes, two centrosomes were situated beneath the animal pole process far apart from the GV, anchoring to the cortex via astral microtubules; (ii) upon meiosis reinitiation, microtubular bundles were newly formed between the centrosomes and the GV; and (iii) the microtubular bundles became short as GV migration proceeded. These observations suggest that microtubules and centrosomes participate in GV relocation. A very large mass of annulate lamellae, having a 20-μm diameter, was found in the vegetal pole of the oocytes.

Keywords: Microtubules; Centrosome; Nuclear positioning; Germinal vesicle relocation; Meiosis; Sea-cucumber oocyte; Annulate lamellae

Introduction

The animal–vegetal axis is a fundamental axis required for an egg to develop. The final step in establishing this axis depends on the success of meiosis because the region from which polar bodies are extruded is finally determined as the animal pole. For the success in extremely unequal divisions to extrude polar bodies, meiotic spindles must be formed just beneath the oocyte cortex. The eccentric positioning of the meiotic spindles has a close relationship with the position of the germinal vesicle (GV) upon the reinitiation of meiosis by oocytes.

In oocytes of some species such as the surf clam, *Spisula solidissima* (Kuriyama et al., 1986; Pielak et al., 2004), mouse (Tombes et al., 1992) and human (Goud et al., 1999), the GV is located in the center of the cells. Upon meiosis reinitiation, the GV breaks down there, and a spindle for meiosis I forms at the site of the GV and migrates toward the oocyte surface. Contrary to this, in many animals, the GV relocates from the center of oocyte to an eccentric position beneath the oocyte surface prior to the breakdown of GV (GVBD). In starfish (Miyazaki et al., 2000) and *Xenopus* (Gard, 1991) oocytes, for example, the GV are located beneath the cortex some time before the

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reinitiation of meiosis. Following GVBD, polar bodies are extruded on the oocyte surface very close to the location of the GV. In other species (Habibi and Lessman, 1984; Maruyama, 1980; Nakashima and Kato, 2001), the GV stays in the center of the immature oocyte. Upon meiosis reinitiation, the GV starts to migrate toward the cell periphery, finally reaching a location just beneath the oocyte surface, which is followed by GVBD. Inhibition of GV relocation or artificial translocation of the GV into the deeper cytoplasm results in the failure of polar body extrusion (Gard, 1993; Maruyama, 1990; Miyazaki et al., 2000). In those oocytes, therefore, the eccentric relocation of the GV beneath the oocyte surface is a prerequisite for successful meiosis.

In many cells, the movement and positioning of nuclei is a microtubule-dependent process (Reinsch and Gönczy, 1998). In starfish, the GV stays in the center of growing oocytes, but in fully-grown oocytes it is located beneath the cortex. The eccentric position of GV is maintained by microtubules nucleated by centrosomes anchored to the cortex (Miyazaki et al., 2000). It is probable that microtubular arrays are also beneficial for GV relocation upon meiosis reinitiation, which is a prerequisite step to anchor the GV to the oocyte surface. We investigated this possibility using oocytes of the sea cucumber, *Holothuria moebi*.

In immature oocytes of this species, the GV rests in the center (Fig. 1a). The oocytes bear a cytoplasmic protuberance such as that seen in other holothurians (Maruyama, 1980; Ohshima, 1925; Smiley and Cloney, 1985). Upon meiosis reinitiation, the GV starts to migrate toward and finally reaches the area beneath the protuberance, which is followed by GVBD. The protuberance is, therefore, a good landmark to mark the point of the oocyte surface where the GV is to migrate and then anchor. These features are useful in analyzing the mechanism of GV relocation. Results of the present study have revealed that the centrosomes are situated beneath the oocyte cortex some distance from the GV in fully-grown immature oocytes, and that microtubular arrays formed by the centrosomes participate in the eccentric relocation of the GV upon meiosis reinitiation. In both the animal and vegetal pole regions of the oocytes, prominent annulate lamellae were observed. In the animal pole region, they were found associated closely with the centrosomes and along the microtubules. At the vegetal pole, a huge mass of annulate lamellae was present, which was defined as the substrate of the ‘clear spot’ that had been observed by light microscopy as a vegetal pole-specific structure in sea-cucumber oocytes (Maruyama, 1981).

Materials and methods

Preparation of oocytes

Specimens of the aspidochirote holothurian, *H. moebi*, were collected in the breeding season (July) from the intertidal zone of the coast near the Tateyama Marine Laboratory, Marine and Coastal Research Center of Ochanomizu University, Chiba, Japan and maintained in running seawater. Immature oocytes excised from the

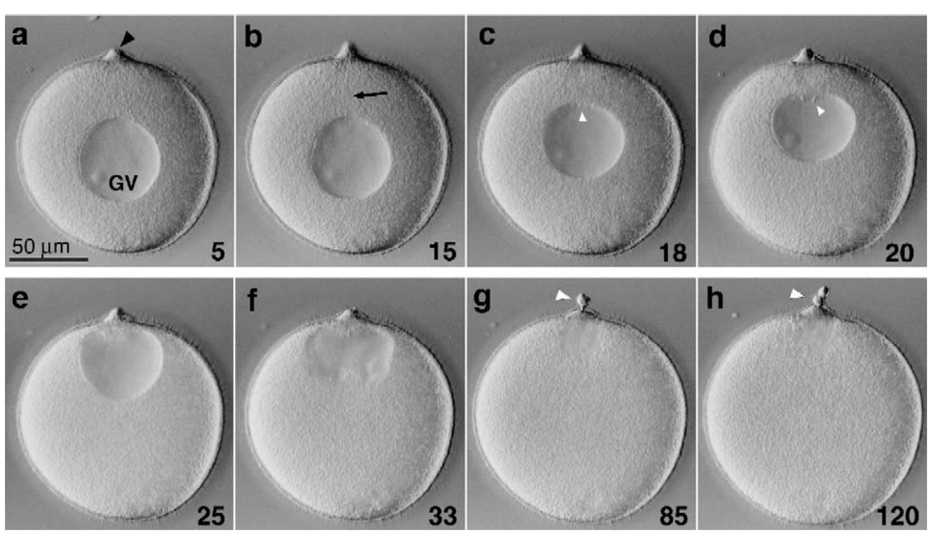

Fig. 1. Process of oocyte maturation. Difference interference-contrast (DIC) microscopy. Numerals at the lower right corner indicate the time (min) after meiosis reinitiation with a starfish-nerve extract. This is applied to other figures. (a) A GV bearing a nucleolus is situated at the center of immature oocytes. The oocyte bears a prominent protuberance that we term the animal pole process (arrowhead). (b) After oocytes were treated with the nerve extract, the GV slightly elongated, then started to migrate toward the animal pole process. At this time, fibrous structures appeared between the animal pole process and the GV (arrow). (c) During the migration, the GV became flattened in an area facing the process, and some indentations formed (arrowhead). (d) The flattened area became broader and the indentations grew larger (arrowhead). (e) When reached beneath the process, the GV became hemispheric. (f) GVBD. (g) Extrusion of the first polar body (arrowhead) at the top of the process. (h) Second polar body extrusion (arrowhead).