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Response of the Cortex to the Mitotic Apparatus during Polar Body Formation in the Starfish Oocyte of *Asterina pectinifera*

Yukihisa Hamaguchi*, Setsuko K. Satoh, Taketoshi Numata, and Miyako S. Hamaguchi

Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152-8551, Japan

ABSTRACT. **In order to understand the mechanism of unequal division, polar body formation was investigated using the oocytes of the starfish, *Asterina pectinifera*. Cortical actin filaments were quantitatively measured after staining the maturing oocytes with fluorescently labeled phalloidin using a computer and image-processing software. Before polar body formation, at first the actin filaments at the animal pole decreased and subsequently the animal pole bulged. On the other hand, actin filaments surrounding the animal pole increased gradually and made a cleavage furrow around the animal pole as the bulge grew. Then the furrow ingressed and finally a polar body formed. When the surface force was calculated according to the cell shape, the surface force decreased at the animal pole but the force at the contractile ring increased. When by micromanipulation the mitotic apparatus was detached and translocated to the cortex other than the animal pole, polar body formation occurred all over the cortex of the oocyte, which indicates that the response of the whole cortex to the mitotic apparatus is equal. These results indicate that the decrease in the actin filaments and surface force near the centrosome of the mitotic apparatus as well as the increase in the actin filaments and surface force at some distance of the centrosome is important for cytokinesis.**

Key words: actin filament/mitotic apparatus/starfish oocyte/polar body formation/unequal division

The position where the contractile ring forms depends on the position of the mitotic apparatus (MA) (Inoue, 1990; Rappaport, 1971, 1996; Schaerer-Brodbeck and Riezman, 2000). In equal division, MA is at the center of the cell or away from the center of the cell but its axis is parallel to the cortex (Fig. 1e). In this case the contractile ring forms on the cortex at the same distance from the two centrosomes (Fig. 1f). On the other hand, in unequal division, MA is shifted away from the center of the cell and its axis is tangential to the cortex (Fig. 1a). In this case, however, the contractile ring forms on the cortex at the different distances from the two centrosomes (Fig. 1b).

In oogenesis of multicellular animals, an egg is formed through two serial unequal divisions (meiosis I and II). In starfish oocytes, MA for the first maturation division is organized shortly after germinal vesicle breakdown (Schroeder, 1985; Satoh *et al*., 1994, 1996). Polar body formation is an extreme case of unequal division because the diameter of the polar bodies is less than 1/10th that of the oocyte.

In the present study, the distribution of actin filaments in the cortex has been investigated quantitatively during the first polar body formation in the oocytes of the starfish. The surface force was also estimated. Further the cortical response of the oocyte was investigated after detaching MA away from the cortex of the animal pole.

Materials and Methods

Oocytes were obtained from ovaries of the starfish, *Asterina pectinifera* and treated with 1-methyladenine to induce meiotic division. Maturing oocytes were inseminated or treated with the ionophore, A23187, shortly after germinal vesicle breakdown to elevate the fertilization envelope.

The fertilized eggs at appropriate time after nuclear envelope breakdown were fixed for 30 min with glucose F-buffer supplemented with 3.0% formaldehyde according to Mabuchi (1994) with a slight modification. Glucose F-buffer contained 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, 10 mM MOPS (pH 7.4), and 0.5 M glucose. They were stained for 30 min with rhodamine-phalloidin or BODIPY FL-phallacidin for actin filament observation and the

*To whom correspondence should be addressed: Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152-8551, Japan.
Tel: +81–3–5734–2244, Fax: +81–3–5734–2946
E-mail: yhamaguc@bio.titech.ac.jp

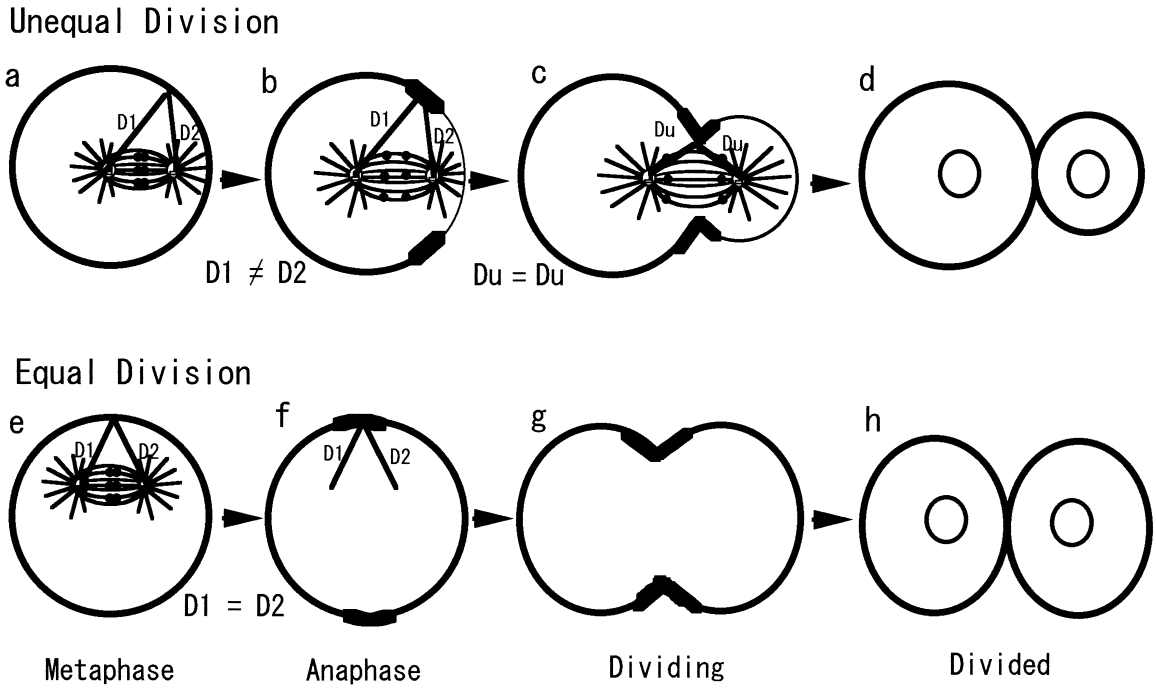

Fig. 1. Schematic representation of the relationship between the centrosome and the contractile ring in the cases of equal and unequal divisions. The upper row shows from metaphase to divided stage at unequal division (a–d) and the lower row shows from metaphase to divided stage at equal division (e–h). D1, D2, and Du are distances between the centrosome and the contractile ring. Thickness of the outline of the cells indicates the extent of actin accumulation in the cortex.

DAPI for chromosome observation. The living oocytes were observed mainly with a differential interference contrast (DIC) and polarization microscope.

The surface force was calculated according to the method of Hiramoto (1968), and Timoshenko and Woinowsky-Krieger (1959). Two principal curvatures R1 and R2 of the surface of the oocyte are calculated from the shape of the oocyte, which is assumed to be the shape of revolution along the axis of the animal and vegetal poles. The surface force along this shape, T2, and the surface force along the cross section tangential to this shape, T1, are calculated from these equations as follows. P is internal pressure. At the animal and vegetal poles T1 is equal to T2.

$$T1 = P \times R2 / 2,\ T2 = [P \times R2\ (2 - R2 / R1)] / 2$$

Displacement of MA was carried out according to Lutz *et al*. (1988) with modifications (Hamaguchi, 2001). The tip of a micropipette was inserted into the oocyte through the fertilization envelope and the cortex, and then inserted into the spindle of the meiotic MA. Moving the pipette in the direction of MA axis, MA was detached from the animal pole and translocated elsewhere in the oocyte.

Results and Discussion

1. Distribution of actin filaments in the cortex during the first polar body formation

In order to measure the actin distribution at the cortical region of the animal pole during polar body formation precisely, the oocytes were stained fluorescently and observed with a confocal microscope in accordance with Satoh and Hamaguchi (2000). The cortex of the animal pole region was dissected from the image and the mean values of the intensity in the cortex were calculated along the animal-vegetal axis and perpendicular to the axis. In the measurement along the animal-vegetal axis, one peak was obtained at the furrow. In the measurement perpendicular to the animal-vegetal axis, two peaks were obtained and the distance between the peaks was designated as the furrow diameter.

Relation between the ratio of cortical fluorescence at the animal pole and the height of the animal pole was investigated. The fluorescence intensity of the cortex at the animal pole decreased with the increase of the height of the animal pole and finally the intensity at the cortex of the polar body became 0.5. Relation between the peak fluorescence intensity at the furrow and the diameter of the furrow was also investigated. Before the bulge formation for the first polar body, in some oocytes an actin accumulation was observed and the peak fluorescence ratio was about 1.1. After bulge formation the ratio gradually increased to twofold compared to that of the control cortex. Actin accumulation in the contractile ring is well-known (Mabuchi, 1994; Schroeder, 1968, 1970, 1972; Yonemura and Kinoshita, 1986). Actin distribution during polar body formation similar to this study was reported by Shimizu (1990) using *Tubifex* oo-

cytes. Actin decrease in the polar region may induce weakening of the region, and, therefore, polar relaxation was suggested according to polar weakening (Hamaguchi and Hiramoto, 1978; Wolpert, 1960), although Rappaport and Rappaport (1985) suggested that the bulge at polar body formation may not result from local surface weakening.

2. Estimation of the surface force around the animal pole

Surface forces were calculated at polar body formation. The surface forces at the animal pole decreased gradually. On the other hand, T2, the surface force along the presumptive contractile ring increased greatly and, however, T1, the surface force tangential to the contractile ring increased slightly.

Surface force to time relation was also investigated. The surface force at the animal pole decreased during polar body formation but the surface force at the vegetal pole did not change. The surface force at the contractile ring increased greatly and then decreased.

The surface force near the animal pole was measured by Ohtsubo and Hiramoto (1985), who reported that the force surrounding the animal pole increased during polar body formation, although they did not measure the force at the animal pole.

3. Cortical response of the oocyte after detaching MA away from the cortex of the animal pole

A polar body forms naturally at the animal pole; after germinal vesicle breakdown, MA at meiosis I forms and takes up an asymmetric position, being oriented perpendicular to the cell cortex and attached at the embryonic animal pole. By DIC microscopy this region was transparent without yolk granules (Fig. 2), although by polarization microscopy the birefringent spindle was clearly observed (Satoh *et al*., 1994).

MA at meiosis was displaced from the animal pole to various regions in the oocyte with a micropipette. When MA was released near the cortex or pushed against the cortex away from the animal pole, the polar body extruded at this site (Fig. 2), which indicates that MA reattached to the cortex of this site. As shown in Fig. 2, when the manipulation was carried out during meiosis I, the first and second polar bodies were extruded at the region where MA had been displaced, but not at the original animal pole. Consequently, as shown in Fig. 3, polar body formation occurred everywhere on the cortex of the oocytes, although it was reported that cortical polarity existed along the animal-vegetal axis (Schroeder, 1985). When the displacement of MA was carried out at meiosis II, the second polar body also extruded. In previous reports, displacement of the nucleus or nuclear materials was carried out before MA formation and resulted in ectopic polar body formation as described below. Chambers (1917) reported that the second polar body was produced some distance away from the first one in the oocyte of a nemertine, *Cerebratulus*, when the daughter nucleus of the first meiotic division was pushed about elsewhere. Shirai and Kanatani (1980) reported that the first polar body formed at the vegetal pole of the oocytes of *A. pectinifera* when the germinal vesicle was translocated from the animal pole to the vegetal pole by centrifugation. Gard (1993) reported that ectopic polar body formation occurred except for the cortex around the vegetal pole of the oocytes of *Xenopus* when the germinal vesicle was translocated by cooling and inverting. In this study, however, it was found that, even after the formation of the meiotic MA, its displacement resulted in ectopic polar body formation, which indicates that the site of polar body formation is determined after the formation of MA (Hamaguchi, 2001).

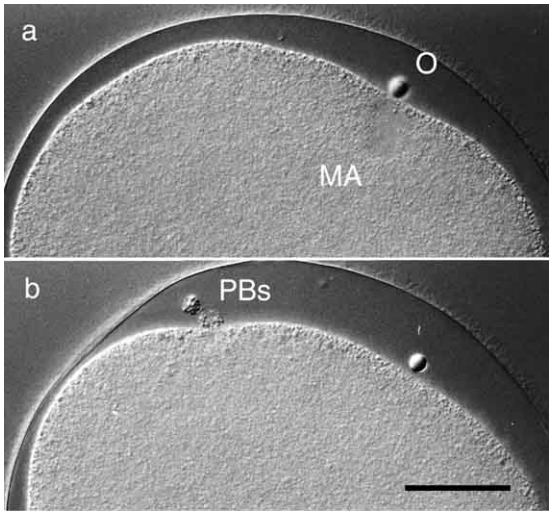

Fig. 2. Release of MA at meiosis I at the cortical sites away from the animal pole resulted in ectopic polar body formation. (a) Before manipulation in order to mark the animal pole at meiosis I, a small paraffin oil drop (O), formed at the tip of the micropipette was attached to the pole with a micropipette. Beneath the drop, MA was in the transparent cytoplasmic region. (b) After MA was displaced from the animal pole with a micropipette and released near the cortex away from the animal pole, the polar bodies extruded at this site (PBs). Scale bar is 50 μm.

4. Conclusions

In this study, the conclusions are as follows.

1. The actin filaments at the animal pole decreased during polar body formation, whereas the actin filaments surrounding the animal pole increased.

2. The surface force at the animal pole decreased, whereas the surface force at the presumptive contractile ring increased.

3. When the detached MA was released near the cortex, ectopic polar body formation occurred everywhere on the cortex of the oocytes.

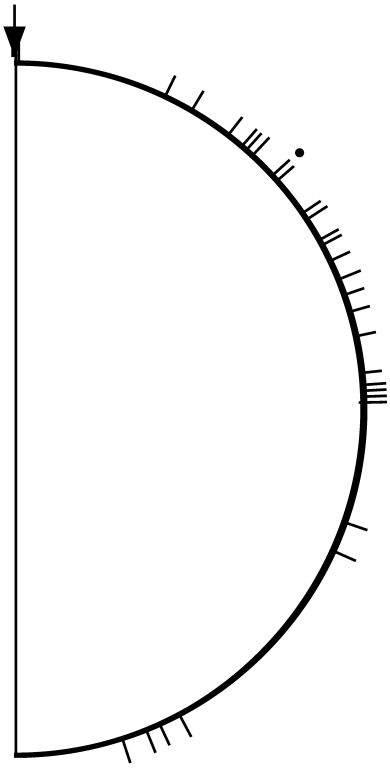

Fig. 3. Summary of the site at polar body extrusion after MA displacement near the cortex. Half of the circle shows the experiments in which MA at meiosis I was displaced to other cortical sites. The pins on the circle were the samples in which the polar body was extruded. The dotted pin indicates two samples in which MA was displaced at the same angle. The latitudinal angle at the polar body extrusion was measured from the animal pole up to 180 degree because the left or right half of the oocyte could not be discriminated at this stage. The arrow shows the animal pole.

Therefore, during unequal division these results indicate that the actin filaments and the resultant generated forces decrease near the centrosome of MA, but increase at some distance from the centrosome. Thus, the different distances from the two centrosome to the contractile ring become equal after relaxation of the polar cortex owing to the contraction of the cortex other than the animal pole (Fig. 1c–d). Moreover, the cortical relaxation occured wherever MA was displaced near the cortex of the oocytes. Wolpert (1969) explained polar body formation by the local relaxation of the surface at the animal pole, and Hamaguchi and Hiramoto (1978) and Ohtsubo and Hiramoto (1985) suggested the local relaxation of the cortex at the animal pole. This study provides evidence of the decrease in both actin and surface force at the animal pole for their explanation. Furthermore, the increase in both actin and surface force at the region adjacent to the animal pole, which results in contractile ring formation, is also evidence essential to cytokinesis.

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