Cell plate orientation in the first cleavage of Fucus eggs

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Fertilized eggs of *Fucus evanescens* were cultured in the state of being attached to glass slides kept in natural sea water, and in experimental media: (a) sea water containing 0.005 M sodium nitroprusside, (b) sea water containing 75 μ g/ml chloramphenicol, (c) sea water containing 0.205 M gossypitrin, (d) sea water containing 0.1 M KCl, and (e) sea water containing 0.2 M KI. As a result, the eggs developed ordinarily in natural sea water, while in media (a) to (e) the first division occurred without bulging of the rhizoid, and the cell plate always formed perpendicular to the glass surface to which the egg was attached. This was independent of gravity. A kind of contact stimulation may take part in the orientation of the cell plate.

Key Index Words: cell plate; Fucus eggs; spindle axis.

In development of Fucus eggs (zygotes), ordinarily, first occurs bulging of the rhizoid pole, then the egg divides into two daughter cells. At this time, if the egg is cultured under uniform illumination or in the dark, the cell plate is formed perpendicular to the longitudinal axis of the elongated egg and perpendicular to the surface of the substratum to which the egg is attached. This may be explained that the bulging of the rhizoid pole took place parallel with the surface of the substratum, so the egg elongated in that direction, and the spindle developed along the longitudinal axis of the egg. If this is true, how would it be if the cleavage took place before bulging of the rhizoid pole as in cases reported by INOH (1935) and NAkazawa (1977a). For this question, the present experiments were carried out.

Material and Method

Receptacles of *Fucus evanescens* were collected at Charatsunai, Muroran, Japan. Fertilized eggs were obtained according to ABE's method (ABE 1970). One hour after being fertilized, the eggs were transferred to experimental media contained in petri dishes 9 cm in diameter, and were cultured under diffuse light of the laboratory at about 18° C. The control medium was natural sea water. The experimental media were (a) sea water containing 0.005 M sodium nitroprusside, (b) sea water containing $75 \,\mu g/ml$ chloramphenicol, (c) sea water containing 0.005 M gossypitrin, (d) sea water containing 0.1 M KCl, and (e) sea water containing 0.2 M KI. The concentration of the experimental agents was designed by reference to preliminary experiments and former reports (NAKAzAWA 1977b, 1978). In the petri dish was laid a glass slide horizontally, on which were sown the eggs. In 5 hours, the eggs were attached to the slide with their own mucilage, then some of the slides were vertically transferred to deeper vessels for testing the influence of gravity on the egg development. 60 to 80 eggs were cultured on the same slide. 24 hours after fertilization, the culture was examined under the microscope. 200 to 300 eggs were observed in each of the culture media. Experiments were repeated twice, and almost the same results were obtained.

Results

Cultured in natural sea water, more than 90% eggs formed rhizoid in 24 hours, and divided into 2 cells (Fig. 1A). Their rhizoids were all bulged out and elongated almost parallel with the plane of the glass slide irrespective of whether the slide was set horizontally or vertically. The cell division took place forming a cell plate perpendicular to the longitudinal axis of the egg, that is, perpendicular to the plane of the glass slide irrespective of gravity.

In the experimental media (a), (b), (c) and (d), most of the eggs did not form rhizoids, but cell division took place (Fig. 1B).

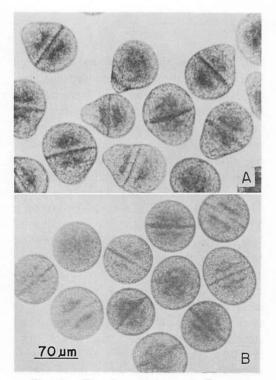


Fig. 1. The first division of *Fucus* eggs forming cell plate perpendicular to the glass surface to which the egg is attached. A. Division after bulging of the rhizoid in natural sea water; B. Division before bulging of rhizoid cultured in sea water containing $75 \,\mu\text{g/ml}$ chloramphenicol.

The spindle was formed just parallel with the plane of the glass slide. As a result, the cell plate was perpendicular to it, independent of gravity. In (e) medium, nuclear division did occur, but further processes did not proceed. In this case, the spindle remained for a long time, and all of the spindles were oriented parallel with the slide, suggesting the cell plate would be formed at right angles to it.

Discussion

In ordinary mitosis, the cell plate is formed perpendicular to the spindle axis. As to the orientation of the spindle, there is the classical hyopthesis presented by HERTWIG and HERTWIG (1884). According to it, the spindle axis develops in the direction of the longitudinal axis of the cell. This is certain in case of division after elongation of the Fucus egg, as seen in ordinary development. But the same cannot be applied to the case of division before elongation as in the above (a) to (e) media, because the division took place in the spherical egg. According to COOK and PAOLILLO (1980), the cell plate develops in the plane so as to make it be the minimal area. In the present case, however, the cell plate develops through the center of the spherical egg, to which the minimal area hypothesis cannot be applied. In addition, the cell plate always develops perpendicular to the glass slide surface to which the egg is attached, while the direction toward which the cell plate is faced is random. In the shake-cultured cells of Sequoia, the single cell is divided by the plane parallel with the glass surface if the cell is placed on a glass slide (BALL 1963).

In the state of being attached to a substratum, some contact stimulations are supposed. Moreover, there will develop some intracellular gradients between the attached side and the free side. For instance, metabolites will diffuse out of the cell surface on the free side, while it is difficult on the attached side, so that, concentration gradients of some metabolites will be set up in a direction perpendicular to the surface of the substratum. And it seems that in some cases the spindle axis develops in the direction of such gradients like in cultured cell of *Sequoia* (BALL 1963), but in some other cases, in a direction perpendicular to it, as in the present experiments. What determines which, however, is yet unknown.

The present experiments were carried out at the Institute of Algological Research, Hokkaido University, Muroran.

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中沢信午:ヒバマタ卵の第1分裂における細胞板の方向決定

ヒバマタ (Fucus evanescens) の受精卵をスライドガラス面に付着させ、これを自然海水および (a) 0.005 M のニトロプルシドナトリウムを含む海水, (b) 75 μg/ml のクロラムフェニコールを含む海水, (c) 0.005 M の ゴシピトリンを含む海水, (d) 0.1 M の KCl を含む海水,および (e) 0.2 M Kl を含む海水で培養した。その 結果,自然海水では正常に発生したが,(a)~(e)の液では仮根が生ずることなく、すべてガラス面に直角な細 胞板を形成して分裂がおこった。これは重力の方向とは関係ない, 細胞板の方向決定には一種の接触刺激が作用 するかもしれない。(990 山形市小白川町 1-4-12 山形大学理学部生物学教室)