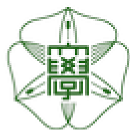
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Title	A fine structure of the outer part of developing fertilized eggs of Fucus evanescens
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Citation	Journal of the Faculty of Science, Hokkaido University. Series 5, Botany, 10(3), 181-188
Issue Date	1976
Doc URL	http://hdl.handle.net/2115/26343
Type	bulletin (article)
File Information	10(3)_P181-187.pdf

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Journ. Fac. Sci., Hokkaido Univ. Ser. V (Botany), **10** (3): 181–188 1976.

A fine structure of the outer part of developing fertilized eggs of *Fucus evanescens*

By

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Ultrastructure of the outer part of *Fucus evanescens* eggs, before and after fertilization, was observed by a transparent type electron microscope. Throughout their development unfertilized as well as fertilized eggs have 1) *gelatinous coat* and 2) *cell membrane*. Before fertilization there exist 3) *egg membrane* under gelatinous coat and 4) *cortical plasm layer* under cell membrane. After fertilization, 5) *cell wall* developes which consists of egg membrane and newly formed *cellulose rich layer*; on the other hand, cortical plasm layer disappears. At the early stage of rhizoidal cell protuberance there occurs a transparent cavity between the growing cell wall and the cytoylasm. Tip growth of rhizoid was discussed in connection with a localization of cellulose synthesis activity in egg cells.

More than hundred works on the developing fertilized eggs of various fucaceous species have been reported in the past several decades by many investigators. Most of them were dealing with cell differentiation, especially in an aspect of polarity, in the course of primary rhizoid formation (or protuberance) at the first cleavage stage of fertilized eggs. A fundamental approach to analyse the problem of polarity in rhizoid formation had been established by WHITAKER 1940. Recently JAFFE 1968, NAKAZAWA 1961, 1969, and others have proposed some prominent general remarks on the mechanism of primary rhizoid differentiation of fucaceous eggs, with special reference to polarity determination.

Fertilized eggs of *Fucus evanescens* are of so-called "apolar" nature; namely, by various environmental factors one polar axis (rhizoid–main body axis) is introduced in eggs, resulting 1) the establishment of some one-directional gradients, then 2) the unequal cell division (the first cleavage) and finally 3) the protuberance toward one-direction from an end side of the smaller cell produced by unequal division. The protuberance and elongation of rhizoidal cell is performed by one-directional growth of cytoplasm accompanying with that of cell wall. In almost all cases of plant cell elongation, *e.g.* in vascular element, root hair, pollen tube, and fern game-

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tophyte rhizoid, it is well known that the elongation growth takes place at a very tip portion being recognized by a localized growth of cell wall at the tip (TAKAHASHI 1961).

NAKAZAWA and TAKAMURA 1968 and NAKAZAWA, TAKAMURA, and ABE 1969 found that the formation of primary rhizoid of *Fucus* eggs was likewise brought about by a typical tip growth at a restricted pole of cells; moreover, they made clear that the tip growth of cell wall was caused by an insertion of new cell wall materials and as well as re-orientaion of cell wall micelles.

In the present paper the writer will report a transparent electron microscope observation of the outer part, especially of cell wall of developing *Fucus* eggs at their rhizoid formation stage.

Material and Method

Receptacles of *Fucus evanescens* Ag. were got at the reef of Chyaratsunai beach (Muroran, Hokkaido). Getting the eggs from receptacles and fixation of eggs for transparent electron microscope specimens were carried out at the Institute of Algological Research, Faculty of Science, Hokkaido University (Muroran).

The surface of receptacles was washed several times with filtered sea water; then they were cut out; eggs and spermatozoids were liberated in a glass vessel with filtered sea water (INOH 1935). Fertilization took place immediately in the course of liberation. Appropriate developing eggs from 15 m to 15 h after fertilization were used for specimens.

Fixation for TEM was made under room temperature with a modified KARNOVSKY's solution (KARNOVSKY 1965): 2.0 g of trioxymethylene (paraformaldehyde) were solved in 25 ml of distilled water at 70°C; drops of 1.0 M NaOH were added until the solution became clear; after cooled, 25 ml of a 0.1 M Na_2–KH_2PO_4 buffer (pH 7.0) containing 13% (v/v) glutaraldehyde were added. Eggs were fixed a few minutes in this solution; they were gathered by a brief gentle centrifugation, and washed 4 times with the same buffer; then they were placed overnight in 1.0% (w/v) OsO_4 in 0.005 M phosphate buffer (pH 7.0) at 4°C.

After fixation with glutaraldehyde and osmiumtetraoxide, the eggs were washed twice with the buffer and then dehydrated through series of acetone solution. For embedding a suspension of fixed eggs in 100% acetone was layered at 4°C on an Epon mixture (LUFT 1961); then acetone was evaporated at 60°C for ca. 1 hour.

In the present study, furthermore, it is necessary to check with accu-

racy whether a sectioned tiny portion of eggs would represent the rhizoid forming site or not. To dissolve this problem the author designed a method as follows: eggs were settled for 8 hours in darkness on a layer of Epon; then they were illuminated from one side; the rhizoid forming site was at the shaded side, as already well known; at 10 to 14 h after fertilization, warm 3.0% agar dissolved in sea water was poured; thus the orientation of eggs was fixed by hardening of agar; also in agar layer the development of rhizoid does proceed normally; finally, the cytological fixation and following procedures for TEM were carried out as above mentioned.

Specimens made by ultra thin sectioning were observed with Hitachi's TEM in the Biological Laboratory, Department of General Education, Hokkaido University.

Result and Discussion

Spermatozoids of *F. evanescens* are surrounded by a thin gelatinous coat containing polysaccharide sulphates, probably fucoidin (NOVOTNY and FORMAN 1974, and TAKAMURA, unpublished); this substance seems to be produced in spermatangia and to coat spermatozoids, and to dissolve partly by sea water.

In the outer part of mature, but unfertilized eggs of *F. evanescens* the following layers are able to recognized by TEM observation (Fig. 1. A):—

1) A gelatinous coat (gc). Obviously eggs do adhere to any object by it and it may have a role at fertilization, at least for catching spermatozoids. Its chemical nature seems also like as that of spermatozoids (TAKAMURA, unpublished). And as time proceeds it dissolves by sea water. By TEM observation it shows several tangential stratification.

2) An egg membrane (em). This is a very thin layer; its role is not at all clear, but it may have a meaning for fertilization.

3) A lipo-protein plasm membrane (lp). A typical cell membrane common to all organisms.

4) A cortical layer (cl). This layer is a part of cytoplasm lying outermost; it seems like as the same object named as ectoplasm. It contains several cytoplasmic minute bodies more than the inner ordinary cytoplasm part, and seems to have a very important role for morphogenesis or differentiation of the cell, *e. g.* in the present case for cell wall formation after fertilization.

On the other hand, soon after fertilization, the outer part of eggs alters its layering, especially accompanying a cell wall formation (Fig. 1. B).

1) A gelatinous coat (gc). After fertilization its viscosity becomes

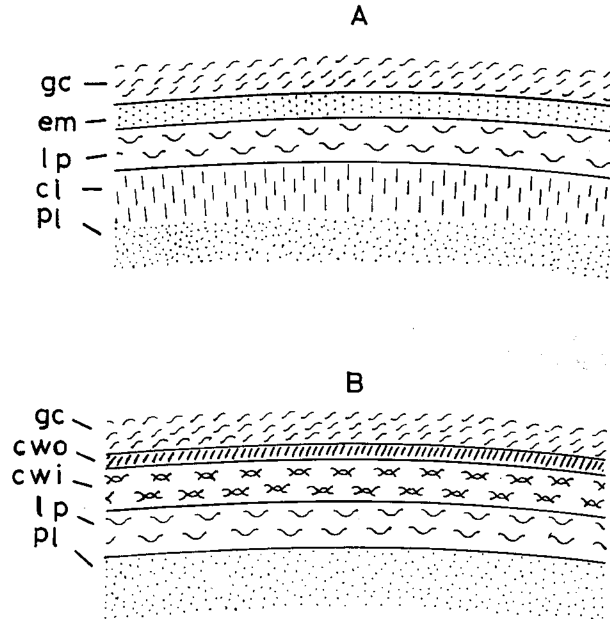

Fig. 1. Electron micrographic diagram of the outer part of *Fucus evanescens* eggs. A. Mature, but unfertilized egg. B. Fertilized egg. gc: gelatinous coat; em: egg membrane; lp: lipo-protein cell membrane; cl: cortical plasmlayer; pl: endo-cytoplasm; cwo: outer part of cell wall (same as em of A); cwi: inner part of cell wall, newly devloped and rich in cellulose.

higher than before fertilization.

2) Cell walls. (a) Pre-existed egg membrane (Fig. 1. A, em) comes to be a component of cell wall as the outer one (cwo). (b) The inner cell wall (cwi) is newly formed after fertilization; although chemical nature of its components could not be detected by the EM observation method here employed, there is no doubt about that it contains cellulose substance which was revealed by calcofluol white fluorescence staining and by birefringent observation (NAKAZAWA and TAKAMURA 1968; NAKAZAWA, TAKAMURA and ABE 1969), and further it seems to contain polysaccharide sulphates, probably fucoidin, detected by various histochemical staining (NOVOTNY and FORMAN 1974; TAKAMURA, unpublished). One may also expect an existence of alginic acid.

3) A lipo-protein plasm membrane (lp). Any changes can not be at all observed, compared with before-fertilization.

4) In the cytoplasm (pl). A cortical layer (cl) which was character-

istically marked in unfertilized egg cells strikingly disappears.

Except for cellulose wall formation, there are many similar developmental events between *Fucus* eggs and sea urchin ones. We will here refer some analogical matters: egg membrane of *Fucus* and vitelline membrane of sea urchin; cortical layer of *Fucus* and layer of cortical granules of sea urchin; various physiological traits, *e.g.* increasement of respiration (RUNNSTRÖM 1949).

At 12 to 16 h after fertilization a restricted portion of the fertilized egg, *i.e.* a tip part of the established rhizoid cell, protuberates toward one direction, resulting to form a primary rhizoid. Electron micrograph of this stage (the beginning of rhizoid protuberance) is shown in Fig. 2. Main features of layering pattern are same as those in fertilized eggs (Fig. 1. B). Between cell wall and cytoplasm there is a transparent vacant space; at present it is not decisive whether this space would be produced by a differential growth between cell wall and cytoplasm, or by an artifact.

As already mentioned (see introduction), when a cell tip does elongate the very tip portion of cell wall grows prominently by a new cell wall formation accompanying mainly an insertion of cellulose substances supplied from cytoplasm. This phenomenon should be brought about by a series of subcellular and biochemical events as follows: the migration of some cell constituents, which would be considered concerning to cellulose synthesis, to the tip part (*i.e.* to a side presumptive for rhizoid formation); or the activation of cellulose synthesis at that portion; a number of new born

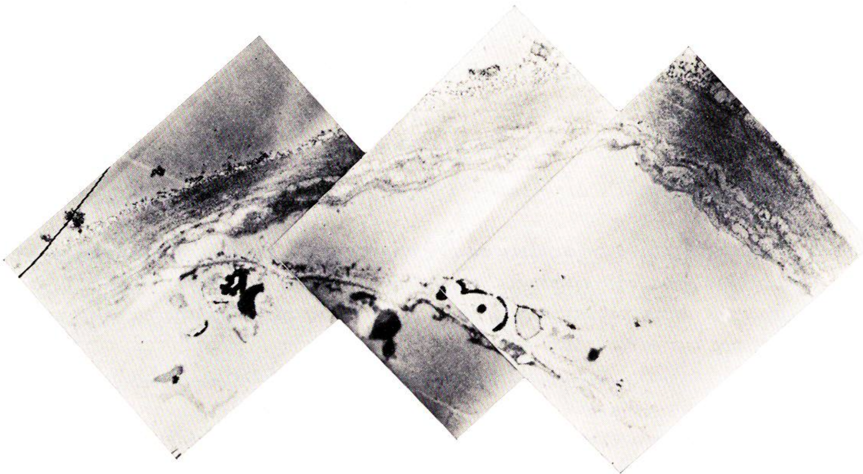

Fig. 2. An electron micrograph of the rhizoid protuberance of *Fucus evanescens* fertilized eggs.

cellulose molecules as well as of another wall substances will be integrated into the pre-existed cell wall whose micellar texture already became loose by certain agents (*e. g.* by concentrated auxin-like substances at the rhizoid pole side as one of intracellular gradients derived from "polarity inducing factor"). In the growth of cotton seed hairs FARR 1948 had once proposed the existence of a particular cell organella concerning to cellulose synthesis; in the case of *Fucus* eggs we could not find such one. JAFFE 1968 had referred a personal communication by Dr. B. BOUCK, according to whom abundant Golgi bodies and vescicles derived from them were observed by EM in the elongating rhizoid cell wall of *Fucus vesiculosus* embryo; although it becomes increasingly interesting that the Golgi complex does an essential role for cell wall biology (including geotropism), the auther could not observed a special localization of Golgi complex at the early stage of rhizoid formation in *F. evanescens* eggs. A polar localization of RNA particles at the rhizoid formation stage of fucaceous eggs has been reported by some investigators (NAKAZAWA 1966; NAKAZAWA and TAKAMURA 1967; QUATRANO 1972; TAKAMURA and MAEDA 1970). Apparently RNA particles are migrating from the periphery of nucleus to the rhizoid presumptive portion of eggs; if they were polysomes, one may imply that the localized activity of cellulose synthesis at the rhizoid pole of eggs would be explainable.

Acknowledgement

The author wishes to express his cordial thanks to Prof. Dr. I. HARADA of Hokkaido University and Prof. S. NAKAZAWA of Yamagata University for their generous suggestions in the course of this study.

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