FINE-STRUCTURAL STUDIES OF THE
GAMETES AND EMBRYO OF
FUCUS VESICULOSUS L. (PHAEOPHYTA)

II. THE CYTOPLASM OF THE EGG AND YOUNG ZYGOTE*

SUSAN H. BRAWLEY
Department of Botany, University of California, Berkeley, California 94720, U.S.A.,

RICHARD WETHERBEE
School of Botany, University of Melbourne, Parkville 3052, Victoria, Australia

AND
RALPH S. QUATRANO
Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331 U.S.A.

AND
The Marine Biological Laboratory, Woods Hole, Massachusetts 02543, U.S.A.

SUMMARY

Following fertilization, there are rapid changes in the appearance of the Fucus egg. Large electron-translucent vesicles (Vj) accumulate fibrillar material, and following pronuclear fusion, they are largely electron-opaque. These vesicles (Vj) are formed originally in unfertilized eggs by smooth endoplasmic reticulum (SER) after release of the eggs from the oogonium. Golgi complex hypertrophy follows fertilization, and this increased activity continues throughout early embryogenesis. Wall formation begins after penetration of the egg by the sperm. Vesicles (Vj) of unknown origin, which have homogeneously fibrillar contents, and Golgi vesicles (Vs) merge with SER-derived vesicles (Vj) after wall formation begins. Osmiophilic bodies are a prominent feature of the egg and embryo. They are penetrated by SER, and subsequently there is a loss of electron-opaque material. Alternatively, they discharge concentrically whorled material into the cytoplasm. The nuclear surface of the egg is convoluted in the period close to fertilization, and electron-opaque material is segregated in the cytoplasmic matrix lying within the nuclear invaginations.

INTRODUCTION

Zygotes of the brown alga, Fucus vesiculosus L., have been used in many biochemical and biophysical investigations related to polarity (Bentrup, Sandan & Jaffe, 1967; Jaffe, 1968, 1969; Quatrano, 1968, 1972; Hogsett & Quatrano, 1973; Nuccitelli & Jaffe, 1975). More recently this system has been used to study cell wall formation (Ley & Quatrano, 1973; Novotny & Forman, 1974, 1975; Quatrano, Stevens & Crayton, 1974; Stevens, 1975). Fucus zygotes are particularly well suited for such

* Portions of these Results have been presented at the Annual Meeting of the Phycological Society of America (1974).
studies, since the unfertilized egg has no wall, and the initiation of wall formation begins immediately following fertilization (Levriny, 1952; Pollock, 1970).

Despite the active interest of physiologists in the *Fucus* embryo, relatively little is known about the cytological features of early embryogenesis. Pollock (1970) investigated the initial stages of fertilization (gamete attraction) at the ultrastructural level. McCully (1968) described the reproductive tissues of *Fucus edentatus* and their maturation by light microscopy and cytochemistry. Quatrano (1972) studied ultrastructural features of the determined site of rhizoid formation in the zygote of *Fucus vesiculosus*. In this paper, we describe the ultrastructure of the egg cytoplasm following release from the oogonial packet through pronuclear fusion.

**MATERIALS AND METHODS**

Plants of the dioecious species, *Fucus vesiculosus* L., were collected at Manomet, Massachusetts, during July, 1973. They were processed as previously described (Brawley, Wetherbee & Quatrano, 1976a).

**RESULTS**

After release from the oogonium, the mature, unfertilized egg is characterized by a highly convoluted nuclear envelope, a cytoplasm filled with numerous vesicles (see Table 1), and the absence of a cell wall.

<table>
<thead>
<tr>
<th>Description</th>
<th>Stage of initial observation</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>V&lt;sub&gt;1&lt;/sub&gt; Contents electron-transparent initially, increasingly fibrillar following fertilization; fibrillar material not tightly packed prior to pronuclear fusion</td>
<td>Mature egg following release from the oogonial reticulum (SER)</td>
<td>Smooth endoplasmic reticulum (SER)</td>
</tr>
<tr>
<td>V&lt;sub&gt;2&lt;/sub&gt; Contents homogeneously fibrillar</td>
<td>Mature egg</td>
<td>Unknown</td>
</tr>
<tr>
<td>V&lt;sub&gt;3&lt;/sub&gt; Contents irregularly distributed in vesicle, composed of radiating fibrils, thicker than those of V&lt;sub&gt;1&lt;/sub&gt; and V&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Following sperm fusion with egg</td>
<td>Golgi complex</td>
</tr>
<tr>
<td>OB Osmiophilic bodies with electron-opaque contents, polarized toward the plasmalemma prior to fertilization, uniformly distributed thereafter</td>
<td>Mature egg, although more common following fertilization</td>
<td>Uncertain (see text)</td>
</tr>
</tbody>
</table>

**Nuclear envelope and perinuclear region**

The nucleus of the egg is convoluted from the time of release of the eggs from the oogonial packet through the point at which pronuclear fusion occurs, and the nuclear
Cytoplasmic morphogenesis in the Fucus egg

membrane contains distinct pores (Fig. 1). Convolution is particularly marked after fertilization. Just before pronuclear fusion, convolutions of the nuclear envelope are localized toward the advancing sperm pronucleus (Brawley et al. 1976a). Nucleoli are evident, and little heterochromatin is observed in the egg nucleus (Figs. 1, 2). Soon after sperm penetration, the convoluted egg nucleus is surrounded by a few mitochondria and chloroplasts and by vesicles (V1) containing fibrillar material (Fig. 1). In addition, accumulations of electron-opaque material in the cytoplasmic matrix are partially enclosed by the nuclear envelope (Figs. 2, 3).

Vesicle relationships

The egg cytoplasm is characterized by a large number of vesicles (V1) with electron-translucent contents (Fig. 4) and by a smaller number of vesicles (V2) with homogeneously fibrillar contents (Fig. 5). The electron-translucent vesicles (V1) originate from the smooth endoplasmic reticulum (SER) following release of the eggs from the oogonial packets (Fig. 8) and they are produced until fertilization occurs. The Golgi complex is inactive during this pre-fertilization period (Fig. 8). After fertilization, V1s accumulate electron-opaque, fibrillar material (Figs. 5, 6), and the Golgi bodies, which are localized near the plasmalemma, hypertrophy and produce a third type of vesicle (V3) (Figs. 7, 9). The second vesicle type (V2) is present throughout the period in which V1 and V3 are produced (Table 1).

The distribution of mitochondria and osmiophilic bodies is polarized toward the plasmalemma before fertilization and during the initial sperm penetration of the egg (Fig. 4). Following pronuclear fusion, osmiophilic bodies and mitochondria are evenly distributed throughout the cytoplasm, and the contents of V1 become increasingly fibrillar (Figs. 5, 6). Fusion of V2 with identical vesicles (V1) as well as with V3 account in part for the rapid increase in fibrillar content of V1 (Figs. 9, 10). Small, membrane-bound vesicles with electron-translucent contents inside V1 may result from the merging of SER, V1, V2, and V3 with accompanying segregation of material and membrane fragments (Fig. 10).

The osmiophilic bodies stain green to bluish green with toluidine blue O. They are not associated with the chloroplasts during this phase of embryogenesis. Smooth endoplasmic reticulum penetrates the osmiophilic bodies, and it presumably participates in the removal of material from them (Fig. 11). This is particularly common later in embryogenesis when we observed Golgi complexes closely associated with the SER-penetrated bodies. Both are localized near the areas of greatest wall formation (Brawley, Wetherbee & Quatrano, 1974). Osmiophilic bodies also discharge material into the cytoplasm. This material has a crystalline appearance and a regularly spaced, whorled pattern (Figs. 12, 13).

Wall formation

The plasmalemma of the egg is slightly uneven, and it is covered by a small amount of amorphous material in places, but not by wall material prior to fertilization. Some discharge from the unfertilized egg does occur (Fig. 14). Within 20 min following fertilization, fibrillar components are identified within the wall, most of which are
deposited by V_3 (Figs. 15, 16). Vesicles (V_4) containing fibrillar material are also oriented close to the plasmalemma suggesting their role in discharge (Fig. 17). These profiles are less frequent than Golgi vesicle (V_3) discharge during early wall formation. No microtubules are observed near the plasmalemma; however, SER is common (Figs. 16, 17).

DISCUSSION

Perinuclear activities

Extrusion of material from the nucleus has been observed in a variety of oocytes (e.g. Raven, 1961; Austin, 1968). Prior to extrusion into the cytoplasmic matrix, nuclear material is often found in the bulges of the nuclear convolutions (e.g. Szollosi, 1965; Longo & Anderson, 1968; Wetherbee & Wynne, 1973; Bell, 1975). In Fucus eggs and young zygotes, we observed electron-opaque material segregated in the perinuclear region; however, it was almost entirely enclosed within nuclear invaginations. Movement of material from the cytoplasmic matrix into the nucleus is thus a possibility and is also suggested by biochemical studies on the migration of proteins into the nucleus (e.g. Bonner, 1975).

Physodes

The profiles of SER-penetrated, osmiophilic bodies observed in this study are similar to ones seen in the adult Fucus thallus by Bouck (fig. 14, 1965), and McCully (fig. 19, 1968), and in Ascophyllum by Rawlence (fig. 1, 1973). The osmiophilic bodies presumably correspond to the physodes of the light microscopist, and they contain a variety of polyphenolic materials (Craigie & McLachlan, 1964; McCully, 1966, 1968). The osmiophilic bodies are probably produced in vesicles during the earliest phase of embryogenesis (Brawley, Wetherbee & Quatrano, unpublished observations), while those observed in older embryos are associated with the chloroplasts (Brawley _et al._ in preparation), as suggested for the physodes of _Dictyota_ by Evans & Holligan (1972b). The possibility that one function of these phenolics is to prevent polyspermy should be evaluated. The effects of the phenolics of _Fucus_ on fertilization in _Arbacia_ have been investigated (e.g. Branham & Metz, 1960).

Vesicles and cell wall formation

The largest vesicle (V_1) arises from the smooth endoplasmic reticulum and lacks fibrillar material in the unfertilized egg. Following sperm penetration, fibrillar material accumulates in V_1. The Golgi complex is increasingly active following fertilization as evidenced by the production of vesicles filled with material which is deposited into the newly formed cell wall. Along with the contents of V_2, a vesicle of unknown origin which also contains fibrillar material, the components of V_3 comprise most of the new cell wall. The wall at this very early time after fertilization consists primarily of alginic acid and cellulose (Ley & Quatrano, 1973). According to work of Novotny & Forman (1975), the fibrillar component which we observed in the wall would be analogous to what they identified as alginate. The vesicles (V_4) containing
Cytoplasmic morphogenesis in the Fucus egg

Homogeneously fibrillar material are ultrastructurally identical to those containing alginic acid in Dictyota (Evans & Holligan, 1972a). Deposition of the sulphated polysaccharide, fucoidan, into the wall begins within 1 h of fertilization (Ley & Quatrano, 1973). Fucoidan is probably transported to the wall via Golgi-derived vesicles (V3) (cf. Evans, Simpson & Callow, 1973).

The appearance of polymers within cytoplasmic membranes and the deposition of them into the wall suggests that intense biosynthetic activity is associated with cell wall biogenesis. This new cell wall can apparently be assembled in the absence of microtubules since we did not observe these cytoplasmic structures associated with the developing wall. Furthermore, Stevens (1975) found that concentrations of colchicine which disrupt the mitotic spindle did not effect wall biogenesis. Immunological techniques used by Vreeland (1972) and fluorescent-labelled lectins that bind to specific polysaccharides in the wall would help correlate structural alterations with the changing chemical components of the vesicles and wall during embryogenesis.

For their stimulating discussions throughout this work as well as for their review of the manuscript, we wish to express our appreciation to Dr Darlene Southworth (Berkeley), Dr John A. West (Berkeley), Dr Helen A. Padykula (Wellesley College) and Eleanor Crump (Berkeley). We thank Dr Mary M. Allen (Wellesley College) and Dr Padykula for the use of their laboratories during a portion of this work and Marea H. Grant (Berkeley) for proofreading the manuscript. Our work was supported by a Sigma Xi Grant-in-Aid-of-Research (to S.H.B.) and by grants (to R.S.Q.) from the NSF (GB37149) and PHS (GM19247). Dr Wetherbee was the recipient of a postdoctoral appointment with Dr West (NSF GB40250) during a portion of this study.

REFERENCES


S. H. Brawley and others


(Received 15 April 1975)
Fig. 1. The nucleus of a fertilized egg before pronuclear fusion. The surface of the nucleus is convoluted at this stage. The cytoplasmic matrix contains large vesicles (v₁) whose contents are electron-translucent prior to fertilization, as well as vesicles (v₂) with homogeneously-fibrillar contents, and osmiophilic bodies (ob). The nucleus is surrounded by a few mitochondria (m) and chloroplasts (c). × 17 500.
Fig. 2. Electron-opaque material in the perinuclear region (arrows). In mature eggs and young zygotes, these profiles are common in the perinuclear region. Nucleoli (nl) remain present throughout this stage. × 36000.

Fig. 3. Electron-opaque material in the nucleus (x) and in the perinuclear area (arrow). The nucleoplasm is largely euchromatin. ob, osmiophilic bodies. × 36000.
Cytoplasmic morphogenesis in the Fucus egg
Fig. 4. The cytoplasm of an unfertilized egg. Fibrillar material is not present in the vesicles (v₁) at this stage. Osmiophilic bodies (ob) and mitochondria (m) are more common near the plasmalemma than in the interior of the cell. × 13500.

Fig. 5. The cytoplasm of a fertilized egg prior to pronuclear fusion. Wall formation is evident (arrow). The vesicles (v₂) whose contents were electron-translucent are becoming fibrillar and the vesicles (v₃) with homogeneously fibrillar contents are also evident. The post-fertilization hypertrophy of the Golgi complex (g) is observed. The localization of mitochondria (m) and osmiophilic bodies (ob) near the plasmalemma is less pronounced than in the unfertilized egg. × 4600.

Fig. 6. The cytoplasm of the fertilized egg following pronuclear fusion. Osmiophilic bodies (ob) are common throughout the cytoplasm. The vesicles (v₄) have contents which are heterogeneously fibrillar with little electron translucency remaining. c, chloroplasts. × 3500.
Cytoplasmic morphogenesis in the Fucus egg
Fig. 7. Cytoplasm of an egg immediately after fertilization showing vesicles (v,) with a characteristic content (arrow) produced by the Golgi complex (g). m, mitochondria. \( \times 45,000 \).

Fig. 8. Electron-translucent vesicles (v,) appear to be derived from the smooth endoplasmic reticulum (ser) in eggs prior to fertilization (paired arrows). The SER elements (single arrow) near the plasmalemma are not distended. The Golgi complex (g) appears inactive at this stage. Mitochondria (m) are localized near the plasmalemma in the unfertilized egg. \( \times 40,500 \).
Cytoplasmic morphogenesis in the Fucus egg
Fig. 9. Cytoplasm of a fertilized egg before pronuclear fusion. Fibrillar material (arrow) forms and becomes membrane-bound within the vesicles ($v_1$). Golgi vesicles ($v_3$). $\times 21500$.

Fig. 10. Cytoplasm of a fertilized egg before pronuclear fusion. Golgi vesicles ($v_2$) and fibrillar vesicles ($v_1$) are shown merging (arrow). The merging of vesicles of different types may produce segregated material and membrane fragments ($x$). $\times 35000$. 
Cytoplasmic morphogenesis in the Fucus egg

Fig. 11. Osmiophilic bodies (ob) are permeated with smooth endoplasmic reticulum in the fertilized egg (arrows). \(v_1\), fibrillar vesicles; \(v_3\), Golgi vesicles. \(\times 30000\).

Fig. 12. Osmiophilic bodies (ob) also discharge material possessing regularly spaced, concentric whorls (arrow). These bodies appear within multivesicular bodies (x) in the cytoplasm of a fertilized egg. \(\times 51000\).

Fig. 13. Ordered spacing of material discharged from osmiophilic bodies. \(\times 102000\).
Fig. 14. The surface of the unfertilized egg. Some discharge of material occurs (arrow) during the period prior to fertilization but no wall is present. m, mitochondria. × 31000.

Fig. 15. Golgi vesicles (v₃) discharging their contents into the wall of an egg 20 min after fertilization. × 70000.
Fig. 16. Amorphous and fibrillar material of the cell wall 20 min after fertilization. Profiles of smooth endoplasmic reticulum (ser) are near the plasmalemma. × 176,000.

Fig. 17. A vesicle (v₁) with homogeneously fibrillar contents prior to discharge into the wall (x) 20 min after fertilization. Note fibrillar vesicles (v₂), Golgi vesicles (v₃), and smooth endoplasmic reticulum (arrow). × 54,000.