J. Phycol. **9**, 402–407 (1973)

THE FINE STRUCTURE OF THE NUCLEUS AND NUCLEAR ASSOCIATIONS OF DEVELOPING CARPOSPORANGIA IN *POLYSIPHONIA NOVAE-ANGLIAE* (RHODOPHYTA)[1]

Richard Wetherbee[2]

Department of Botany, University of Michigan, Ann Arbor, Michigan 48104
and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

and

Michael J. Wynne

Department of Botany, University of Texas, Austin, Texas 78712
and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

SUMMARY

A high degree of activity of the nuclei in the developing carposporangia of the red alga Polysiphonia novae-angliae *Taylor is described. Profiles of the nucleus are greatly convoluted, resulting in a much increased surface area. Regions where endoplasmic reticulum substitutes for the nuclear envelope are frequently observed. Various cytoplasmic reserves are associated with the nucleus during carposporangium maturation. Lipid bodies, fibrillar bodies, and striated vesicles (or cylindrical bodies) may occur within the nucleoplasm beneath areas of the nuclear envelope substituted by ER. Granules of Floridean starch are observed in proximity to the outer surface of these same areas as well as the nuclear membrane. The homologous nature of the nuclear envelope with the ER is stressed. The role of the nucleus as being actively involved in the synthesis of materials is suggested.*

INTRODUCTION

Our knowledge of red algal ultrastructure has been greatly expanded in recent years with detailed information on many aspects of cell morphology, such as the photosynthetic apparatus (***5,12,13,20,22,25***), cell walls (***2,4,28***), pit connections (***4,5,27,32***), mitosis (***7,23***), cytokinesis (***12***), and the plasmalemma (***9***). Kugrens & West (***18***) and Scott & Dixon (***30***) recently reported on the fine structure of spermatial development, and both pairs of workers have also described (***19,29***) at the EM level the events of tetrasporogenesis, which data are pertinent to the present investigation. Details of synaptonemal complexes were also presented by Kugrens & West (***17***), while Chamberlain & Evans (***6***) reported the results of histochemical and ultrastructural studies of tetraspore development in *Ceramium*. However, relatively few contributions have been published in regard to female sexual reproduction and post-fertilization events. Tripodi (***33***) restricted his examination to the cystocarp of *Polysiphonia sertularioides* and was primarily interested in the possible dictyosomal origin of Floridean starch granules.

Our observations of unique cytological events during various stages of carposporangium morphogenesis in *Polysiphonia novae-angliae* Taylor form the basis of a continuing ultrastructural study. In the first communication we concentrate on features of the distinctive nuclei of the developing carpospores and on the different kinds of reserve products which arise from within the nucleus, or near the nuclear surface, and migrate into the cytoplasm.

MATERIALS AND METHODS

Sexual plants of the red alga *Polysiphonia novae-angliae* Taylor were collected (*Wynne 3558*) on July 7, 1972, at Tarpaulin Cove, Naushon Island, Dukes County, Massachusetts. The specimens were transported to the laboratory for fixation within a few hours of their collection.

Fixation was for 1 hr at room temperature in seawater containing 2% glutaraldehyde, 1% paraformaldehyde, and 1% sucrose. After washing in 0.1 M phosphate buffer, pH 6.8, specimens were placed in 1% OsO_4 in phosphate buffer for 1 hr, washed in cold sodium hydrogen maleate-NaOH buffer, 0.05 M, pH 5.2, and placed in cold 0.5% uranyl acetate dissolved in sodium hydrogen maleate-NaOH buffer (***15***). After 2 hr in the cold and dark they were washed in the maleate buffer and dehydrated in ethanol followed by transfer into absolute acetone. Embedding was in an epon mixture of DDSA, Epon 812, and DMP-30 (25:10:0.8) (***31***). Sections were cut with a diamond knife, stained with lead citrate (***34***), and viewed with a Zeiss 9A electron microscope.

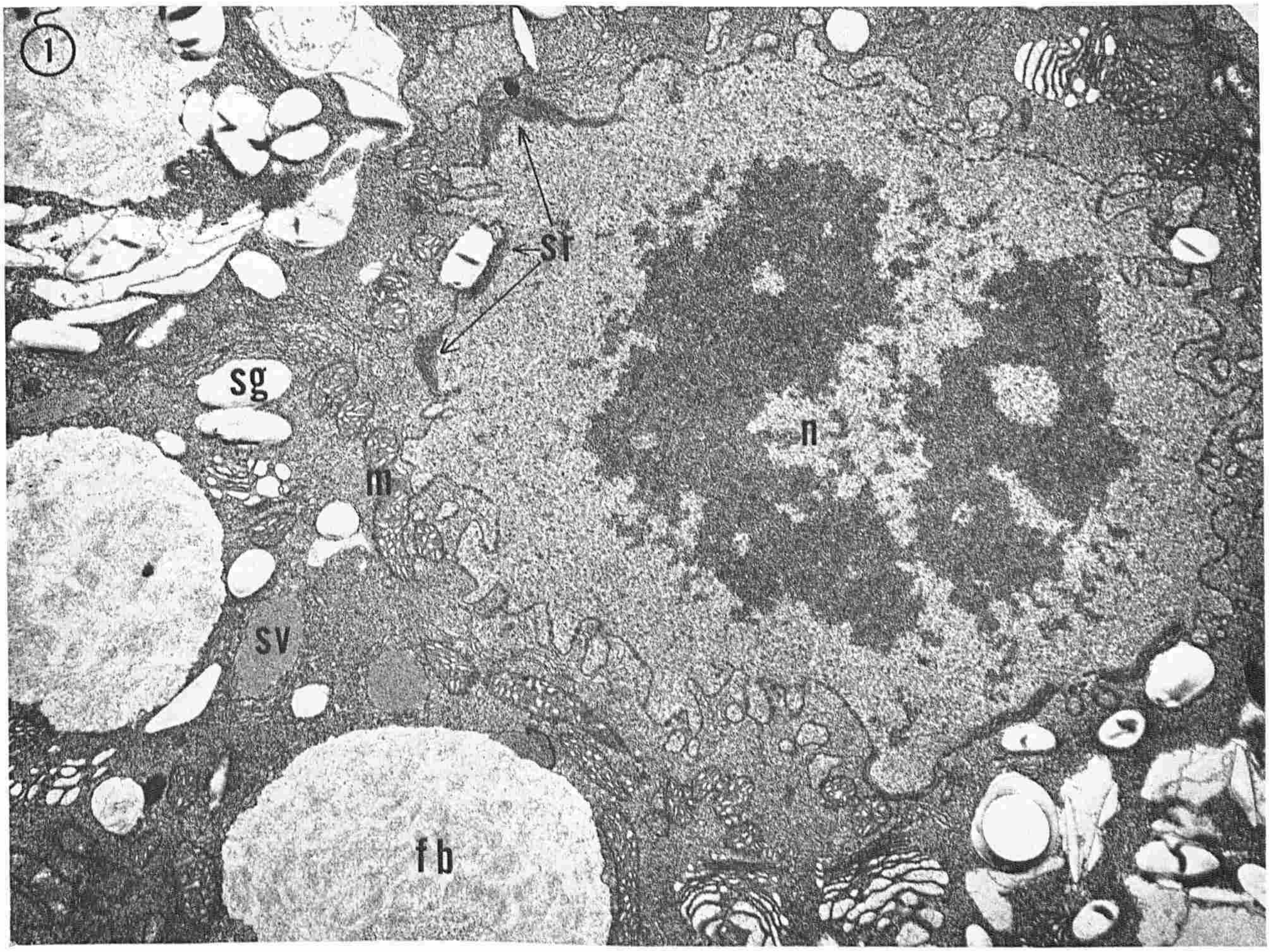
RESULTS

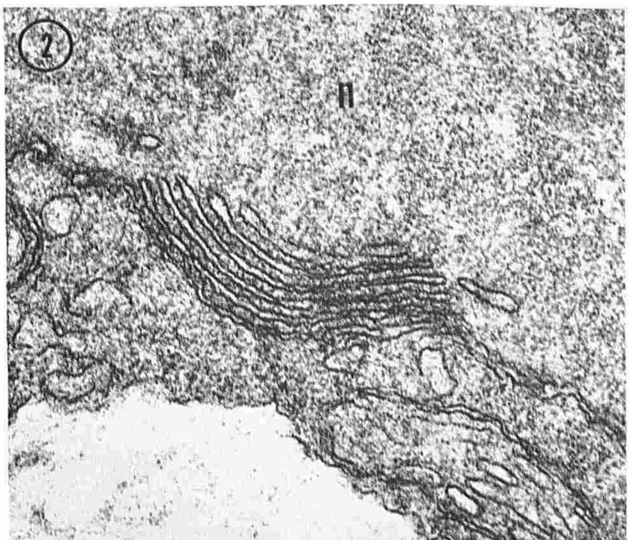
Observations will be presented in 2 categories: features of the nucleus and reserve materials associated with the nucleus.

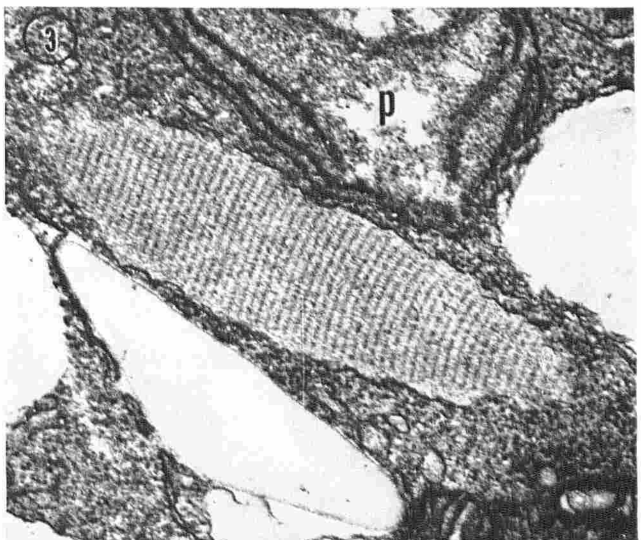
The nucleus. In developing carpospores which have been cut off from the large fusion cell, which is the product of sexual reproduction, the nuclei (Fig. 1) are observed to have very irregular, highly

[1] *Received March 5, 1973; revised July 3, 1973.*

[2] Present address: Department of Botany, University of California, Berkeley, California.

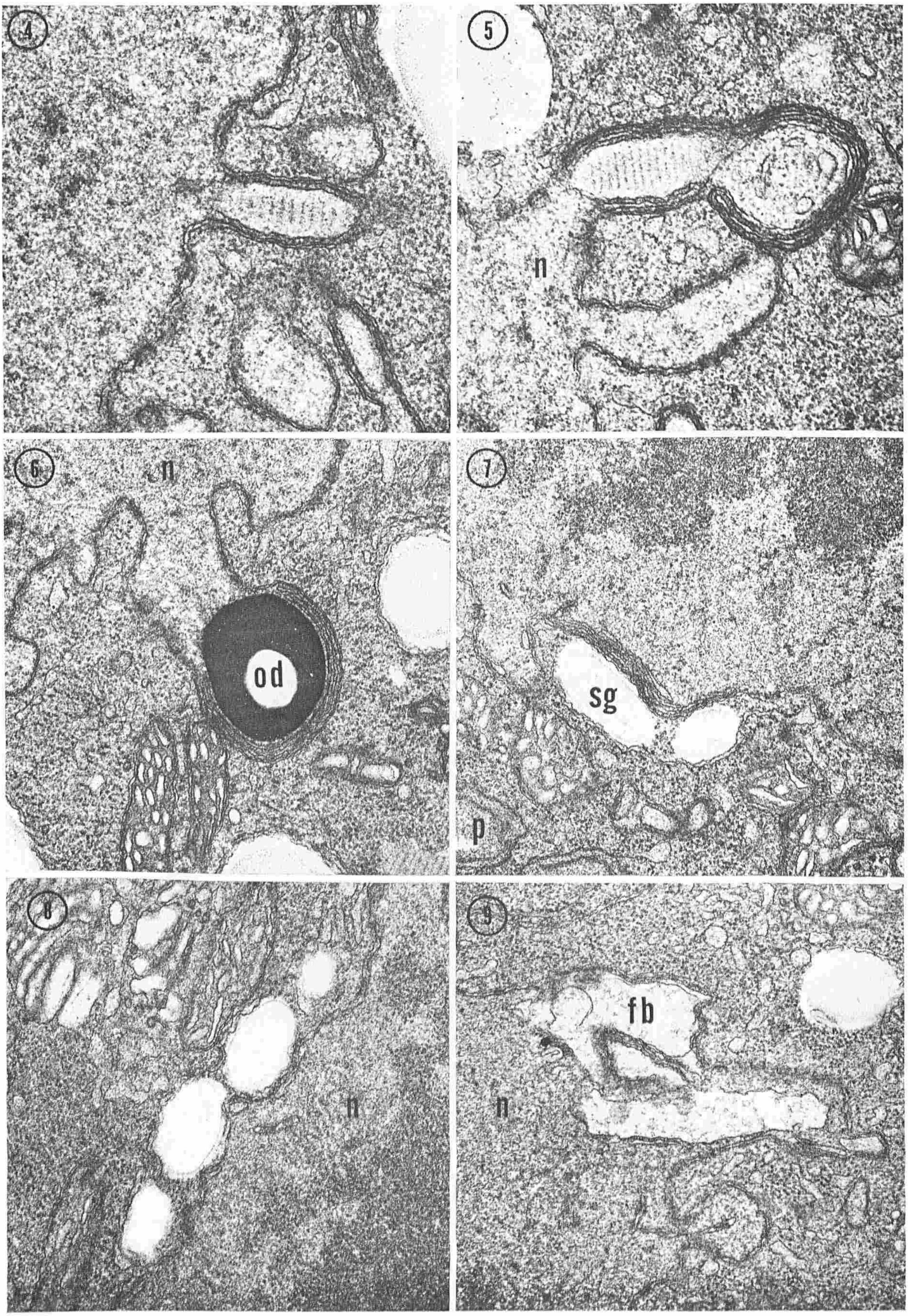






NOTE: Abbreviations used in figures: *fb*, fibrillar body; g, Golgi body; *m*, mitochondrion; *n*, nucleus; *od*, osmiophilic droplet; *p*, proplastid; *sr*, stacked endoplasmic reticulum; *sg*, starch granule; *sv*, striated vesicle.

FIG. 1–3. *Polysiphonia novae-angliae*. FIG. 1. Portion of developing carposporangium with convoluted nucleus (*n*) and regions of stacked ER (*sr*, *arrows*) locally replacing the nuclear envelope. Mitochondria (*m*) surround the nucleus, and dilated Golgi bodies (*g*) are conspicuous. Large fibrillar bodies (*fb*), striated vesicles (*sv*), and starch granules (*sg*) are also present. × 9900. FIG. 2. Detail of stacked ER replacing the nuclear envelope. × 65,600. FIG. 3. Striated vesicle bound by a unit membrane, lying in the cytoplasm. × 31,500.


4
5
n
6
n
od
7
sg
p
8
n
9
fb
n

lobed profiles. They appear to have undergone extensive membrane proliferation and evagination, the latter significantly increasing the total surface area of the nuclear membrane. Numerous nuclear pores are revealed by sections tangential to the surface of the nuclear envelope. A frequently observed characteristic of the nucleus in developing carposporangia is that restricted portions of the envelope are replaced by what is interpreted to be endoplasmic reticulum (Fig. 1, 2). This intervening of ER may consist of a stack of 2–6 or more lamellae, the outermost being without annuli and continuous with the nuclear envelope and the others being located within the nucleoplasm.

Reserves associated with the nucleus. Simultaneous with the appearance of the convoluted nuclear surface, various cytoplasmic reserves arise in abundance. These deposits are often observed within or adjacent to the nucleus and frequently in proximity to the discontinuous regions of the nuclear envelope, which have been substituted by the ER lamellae. At this same developmental stage, large numbers of mitochondria often are observed around the periphery of the nucleus lying close to apparently active Golgi bodies.

The cytoplasmic reserves fall into 4 categories: striated vesicles (of probable proteinaceous nature; cf. *33*), osmiophilic droplets (of probable lipoidal nature), Floridean starch granules, and the large fibrillar bodies (of uncertain composition). These structures are described separately.

1. Striated vesicles. The cytoplasm of maturing carpospores typically contains scattered, elongate, membrane-bound, cylindrical structures, which will herein be referred to as striated vesicles in conformity with the terminology of Kugrens & West (*19*). The periodicity of the striations (Fig. 3) is approximately 320 Å. Smaller vesicles have also been observed within protrusions (Fig. 4, 5) of the nuclear envelope, surrounded by 1 or more cisternae and containing elements of the same periodicity as the larger striated vesicles scattered throughout the cytoplasm. The location and appearance of these smaller striated vesicles would suggest that they represent primordia arising from the nuclear surface.

2. Osmiophilic droplets. Spherical structures of electron opacity are present in the cytoplasm of the maturing carpospores. It is difficult to determine whether this material is bound by a unit membrane. The electron density of the inclusions would strongly suggest a lipoidal composition. These same stained deposits have been observed within nuclear evaginations (Fig. 6), which often appear constricted at their bases, again suggestive of a possible means of transport of the enclosed deposits from the interior of the nucleus.

3. Floridean starch granules. Granules of Floridean starch of variable size are abundantly distributed throughout the cytoplasm. They are apparently not membrane bound. These deposits show no particular spatial relationship to the proplastids, which are situated at the periphery of the cell. The starch granules are closely associated with the highly dilated Golgi bodies as well as with the nuclear envelope (Fig. 7, 8). They are more frequently located in proximity to the stacked cisternae within the nucleus (Fig. 7) than to the nuclear envelope proper (Fig. 8).

4. Large fibrillar bodies. Distinctive cytoplasmic inclusions of irregular shape composed of unevenly fibrillar material are a conspicuous feature of the maturing carpospores (Fig. 1). They are bound by a unit membrane. Few of these vesicles are apparent in the mature carpospore. Early in carpospore development these cytoplasmic organelles are observed to occur with the nucleus (Fig. 9) and in association with the envelope substituted by ER.

DISCUSSION

The occurrence of irregularly shaped, highly convoluted nuclei has been reported in the carpospore of a different species of *Polysiphonia* (*33*), as well as in the tetraspores of the genera *Levringiella* (*19*), *Ceramium* (*6*), and *Ptilota* (*29*). Comparable nuclear profiles exist in other plant material such as ferns (*3,24*) and pollen grains (*14*). Investigators have referred to the cells containing such nuclei as undergoing periods of active synthesis, while Kugrens & West (*19*) expressed the opinion that the irregular outline of the nucleus may be due to the pressure exerted by the crowded cytoplasmic structures. The significance of our examination of the carpospores of *Polysiphonia novae-angliae* is that a variety of cytoplasmic structures can be observed in association with the highly evaginated nucleus during this critical phase of carpospore morphogenesis. Some of these so-called reserve products, namely, the striated vesicles, the fibrillar bodies, and the osmiophilic droplets, are observed within the nucleoplasm (*ie*, within outpocketings of the nuclear envelope or near regions locally substituted by ER). Lipid bodies have also been previously reported (*1*) to occur within

←

FIG. 4–9. *Polysiphonia novae-angliae.* FIG. 4. Evagination of nucleus containing a small striated vesicle with a periodic arrangement of elements. ×52,800. FIG. 5. Evagination of the nucleus (*n*) with one digitation containing a striated vesicle. ×42,000.
FIG. 6. Osmiophilic droplet (*od*), whose central core has been leached away, within an evagination of the nucleus (*n*). ×37,400.
FIG. 7. Starch granules (*sg*) lying outside the nucleus close to a region of stacked ER. Proplastids (*p*) are also present. ×37,000.
FIG. 8. Starch granules aligned along the outer nuclear envelope without the stacked ER. ×21,900.
FIG. 9. Bodies containing fibrillar contents (*fb*) and lying within the nucleoplasm. ×25,300.

nuclei. Another reserve product, the Floridean starch granules, is often in close association with the nucleus along the outer surface of the nuclear envelope.

Fibrillar bodies have been seen both in the monospores of the red alga *Smithora* (*21*) and in the tetraspores of *Levringiella* (*19*), *Ceramium* (*6*), and *Ptilota* (*29*). Different functions for these bodies have been suggested. In *Smithora* (*21*) it was hypothesized that these vesicles release a mucilaginous secretion to aid spore liberation. In *Ceramium* (*6*) the fibrillar material was regarded as being contained in vacuoles derived from Golgi vesicles and passed out into the walls. Following spore release this fibrillar material was thought to function as a protective mucilaginous layer. For *Ptilota* the fibrous vacuoles in late stages of tetraspore development were indicated (*29*) to be reservoirs of complex polysaccharides, which are involved in the secretion of an outer coating required for spore attachment. On the other hand, in *Levringiella* (*19*) it was suggested that the fibrillar bodies contribute to wall synthesis since they are used up during spore development. Cytoplasmic formations comparable to the large fibrillar bodies were also shown in carpospores in *Polysiphonia* by Tripodi (*33*) but without comment.

The fibrillar bodies bear a resemblance to the secretory vesicles occurring in the digestive cells of the sundew *Drosera*, which arise from Golgi bodies and fuse into larger secondary vesicles before being expelled from the cytoplasm (*10*). A similarity also exists in regard to the cytoplasmic inclusions of the desmid *Cosmarium*, in which 2 categories of large vesicles with fibrillar contents of a polysaccharide nature occur (*8*). In both *Drosera* and *Cosmarium*, the fibrillar bodies are associated with the function of mucilage production. A comparable role is likely in the carpospores of *Polysiphonia*, since the attachment of spores is "in all probability due to the production of mucilage" (*11*, p. 607).

The appearance of ER lamellae substituting for portions of the nuclear envelope and arranged in parallel stacks shows some resemblance to annulate lamellae (*16*). However, the configuration of stacked lamellae as seen in Fig. 2 does not conform to that of annulate lamellae in that the former lamellae lack pores, or annular structures, and they are located within the nucleoplasm rather than lying externally. Where intranuclear annulate lamellae have been reported (*16,26*) to occur, they appear as single cisternae with straight or curving courses but not in stacked arrangements. Typical annulate lamellae have been observed to occur during the postfertilization events of *Polysiphonia novae-angliae* but at a time in development earlier than the stage reported here (unpublished observations).

"Concentric membrane bodies" were described by Kugrens & West (*19*) to reside both within and outside the nucleus during prophase of tetrasporogenesis. "CMB" indicated in their fig. 4 may be the equivalent of the ER lamellae substituting for nuclear envelope discussed in this paper; however, the "CMB" in their fig. 3 is definitely not the same.

Tripodi (*33*) observed striated vesicles in the carpospores of *Polysiphonia*, which he designated as "crystal-like bodies." He suggested these structures were proteinaceous in composition and possess a periodicity of about 250 Å, which is somewhat at variance with our calculation of 320 Å. Kugrens & West (*19*) demonstrated the same structure which they termed striated vesicle in developing tetraspores of a related genus. They discussed the origin of such vesicles from rough ER on the basis of observations made on carposporogenesis rather than on tetrasporogenesis and consequently did not include any visual evidence of their formation. We cannot confirm the gradual degeneration of these striated vesicles during the course of carpospore development as was described for tetraspore development (*19*), nor have we detected the presence of cored vesicles which were pictured by Kugrens & West. Similar cored vesicles were observed (*6*) in the tetraspores of *Ceramium*, and they were afforded a probable adhesive function.

The role of the Golgi body in spore development has been emphasized by the studies of McBride & Cole (*21*) and Kugrens & West (*19*). Tripodi (*32*) observed structural associations between Golgi cisternae and starch granules in the red alga *Pterocladia* and suggested a dictyosomal origin of these cytoplasmic deposits. On the other hand, starch granules have been observed (*23*) juxtaposed to the outer nuclear membrane in vegetative cells of the red alga *Membranoptera* without any apparent association with Golgi bodies. The hyperactive appearance of the Golgi bodies in our material and the peripheral aggregation of mitochondria around the nucleus would suggest their participation in these processes occurring during the maturation of the carpospores.

In summation, the results of the present study would stress a more active involvement of the nucleus than has been previously reported and perhaps even the active synthesis of storage materials by the nucleus. Various reserve bodies are observed within the nucleus or immediately adjacent to the nuclear envelope or portions substituted by apparent ER. Carpospores of red algae are known to undergo immediate germination following their release from the cystocarp, and the abundance of these cytoplasmic reserves would suggest the anticipation of the germination process. Another noteworthy observation arising from this study is the fact that layered lamellae of ER appear to locally replace portions of the nuclear envelope. The interconvertibility of the nuclear envelope and the ER would emphasize their homology.

ACKNOWLEDGMENT

We wish to express our gratitude to Dr. Everett Anderson (Department of Anatomy, Harvard Medical School, Boston, Massachusetts) for suggesting the above procedure used in preparing our material for electron microscopy. We are also indebted to Dr. Garry T. Cole of the University of Texas at Austin for critical reading of the manuscript and his suggestions offered.

REFERENCES

1. Altmann, H. W. & Pfeifer, U. 1969. Beitrag zur Kenntniss der Karyosphäridien. Fetthaltige Sphäridien nach partieller Hepatekomie. *Virchows Arch. B. Zellpath.* **2**: 220–8.
2. Bailey, A. & Bisalputra, T. 1970. A preliminary account of the application of thin-sectioning, freeze-etching, and scanning electron microscopy to the study of coralline algae. *Phycologia* **9**:83–101.
3. Bell, P. R. 1972. Nucleocytoplasmic interaction in the eggs of *Pteridium aquilinum* maturing in the presence of thiouracil. *J. Cell Sci.* **11**:739–55.
4. Bisalputra, T., Rusanowsky, P. C., & Walker, W. S. 1967. Surface activity, cell wall, and fine structure of pit-connection in the red alga *Laurencia spectabilis*. *J. Ultrastr. Res.* **20**:277–89.
5. Brown, D. L. & Weier, T. E. 1970. Ultrastructure of the freshwater alga *Batrachospermum*. I. Thin-section and freeze-etch analysis of juvenile and photosynthetic filament vegetative cells. *Phycologia* **9**:217–35.
6. Chamberlain, A. H. L. & Evans, L. V. 1973. Aspects of spore production in the red alga *Ceramium*. *Protoplasma* **76**:139–59.
7. Chapman, R. L., Chapman, M. R., & Lang, N. J. 1971. An ultrastructural study of the nucleus in *Porphyridium*. *J. Phycol.* **7**(Suppl.):3.
8. Chardard, R. 1972. Etude ultrastructurale et cytochimique des inclusions cytoplasmiques de *Cosmarium lundellii* Delp. *Comp. Rend. Acad. Sci.* **275D**:1367–70.
9. Cottler, M. H. 1971. Plasmalemmal extensions in *Chondrus crispus* (L.) Stackh. *J. Ultrastr. Res.* **37**:31–6.
10. Dexheimer, J. 1972. Quelques aspects ultrastructuraux de la sécrétion de mucilage par les glandes digestives de *Drosera rotundifolia* L. *Comp. Rend. Acad. Sci.* **275D**: 1983–6.
11. Fritsch, F. E. 1945. *Structure and Reproduction of the Algae. Vol. II.* Cambridge University Press. 939 pp.
12. Gantt, E. & Conti, S. F. 1965. The ultrastructure of *Porphyridium cruentum*. *J. Cell Biol.* **26**:365–81.
13. ——— 1966. Granules associated with the chloroplast lamellae of *Porphyridium cruentum*. *J. Cell Biol.* **29**: 423–34.
14. Heslop-Harrison, J. 1963. Ultrastructural aspects of differentiation in sporogenous tissue. *Symp. Soc. Exp. Biol.* **17**:315–40.
15. Karnovsky, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. *J. Cell Biol.* **35**:213–36.
16. Kessel, R. G. 1968. Annulate lamellae. *J. Ultrastr. Res.* Suppl. **10**:82 pp.
17 Kugrens, P. & West, J. A. 1972. Synaptonemal complexes in red algae. *J. Phycol.* **8**:187–91.
18. ——— 1972. Ultrastructure of spermatial development in the parasitic red algae *Levringiella gardneri* and *Erythrocystis saccata*. *J. Phycol.* **8**:331–43.
19. ——— 1972. Ultrastructure of tetrasporogenesis in the parasitic red alga *Levringiella gardneri* (Setchell) Kylin. *J. Phycol.* **8**:370–83.
20. Lichtle, C. & Giraud, G. 1970. Aspects ultrastructuraux particuliers au plaste du *Batrachospermum virgatum* (Sirdt)–Rhodophycée–Nemalionale. *J. Phycol.* **6**:281–9.
21. McBride, D. L. & Cole, K. 1971. Electron microscopic observations on the differentiation and release of monospores in the marine red alga *Smithora naiadum*. *Phycologia* **10**:49–61.
22. ——— 1972. Ultrastructural observations on germinating monospores in *Smithora naiadum* (Rhodophyceae, Bangiophycidae). *Phycologia* **11**:181–91.
23. McDonald, K. 1972. The ultrastructure of mitosis in the marine red alga *Membranoptera platyphylla*. *J. Phycol.* **8**:156–66.
24. Mühlethaler, K. & Bell, P. R. 1962. Untersuchungen über die Kontinuität in der Eizelle von *Pteridium aquilinum* (L.) Kuhn. *Naturwissensch.* **49**:63–4.
25. Neushul, M. 1970. A freeze-etching study of the red alga *Porphyridium*. *Am. J. Bot.* **57**:1231–9.
26. Ollerich, D. A. & Carlson, E. C. 1970. Ultrastructure on intranuclear annulate lamellae in giant cells of rat placenta. *J. Ultrastr. Res.* **30**:411–22.
27. Ramus, J. 1969. Pit connection formation in the red alga *Pseudogloiophloea*. *J. Phycol.* **5**:57–63.
28. ——— 1972. The production of extracellular polysaccharide by the unicellular red alga *Porphyridium aerugineum*. *J. Phycol.* **8**:97–111.
29. Scott, J. L. & Dixon, P. S. 1973. Ultrastructure of tetrasporogenesis in the marine red alga *Ptilota hypnoides*. *J. Phycol.* **9**:28–46.
30. ——— 1973. Ultrastructure of spermatium liberation in the marine red alga *Ptilota densa*. *J. Phycol.* **9**:85–91.
31. Sporn, M. B., Wanko, T., & Dingman, W. 1962. The isolation of cell nuclei from rat brain. *J. Cell Biol.* **15**: 109–20.
32. Tripodi, G. 1971. Some observations on the ultrastructure of the red alga *Pterocladia capillacea* (Gmel.) Born. et Thur. *J. Submicr. Cytol.* **3**:63–70.
33. ——— 1971. The fine structure of the cystocarp in the red alga *Polysiphonia sertularioides* (Grat.) J. Ag. *J. Submicr. Cytol.* **3**:71–9.
34. Venable, J. H. & Coggeshall, R. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407–8.