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Ultrastructure of carposporogenesis in the red alga *Cryptopleura ruprechtiana* (Delesseriaceae: Ceramiales: Rhodophyta)

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The ultrastructure of carpospore differentiation for the red alga *Cryptopleura ruprechtiana* is described. Carposporogenesis proceeds through three developmental stages. After cleaving from multinucleate gonimoblast initials the terminal gonimoblast cells differentiate to produce carpospores. These young carpospores possess a large nucleus and numerous proplastids with a peripheral thylakoid. During the later stages of young carpospores starch begins to polymerize. Mucilage is formed within dilating concentric membrane bodies, thus forming mucilage sacs. The latter, subsequently, release their contents initiating carpospore wall formation. Intermediate-aged carpospores have more plastids which develop their internal thylakoid system. The endoplasmic reticulum produces granular cored vesicles. Mature carpospores have numerous fully developed plastids, large floridean starch granules and fibrous vacuoles. Curved dictyosomes produce cored vesicles and adhesive vesicles. The nuclear envelope is crenulated and a two-layered wall surrounds the mature carpospore.

INTRODUCTION

Although quite a few studies were done on carposporophyte development and carposporogenesis, most of them have been conducted on members of the Ceramiales which is the largest order of red algae in terms of number of genera (Bold & Wynne, 1985). The early stages of post-fertilization development in this order are simple, whereas the later ones are more complex (Bold & Wynne, 1985). The bulk of the published work on the Ceramiales has concentrated either on early stages (Wetherbee, 1980; Broadwater & Scott, 1982) or on specific events, such as dictyosomal activity associated with carpospore ultrastructure (for references see Delivopoulos & Kugrens, 1984; Tsekos, 1985). Some studies have dealt directly with carpospore development in detail (Kugrens & West, 1973, 1974; Delivopoulos & Diannelidis, 1990, 1991). Moreover, all these studies have focused on members either of the Rhodomelaceae or the Ceramiaceae. One paper (Triemer & Vasconcelos, 1977) deals with a member of the Delesseriaceae but it describes mainly the late stages of

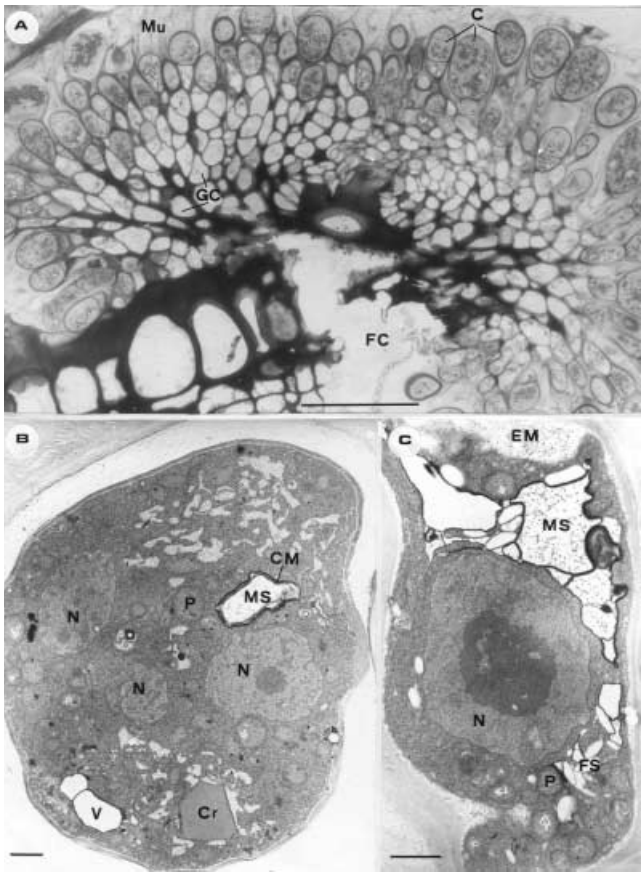
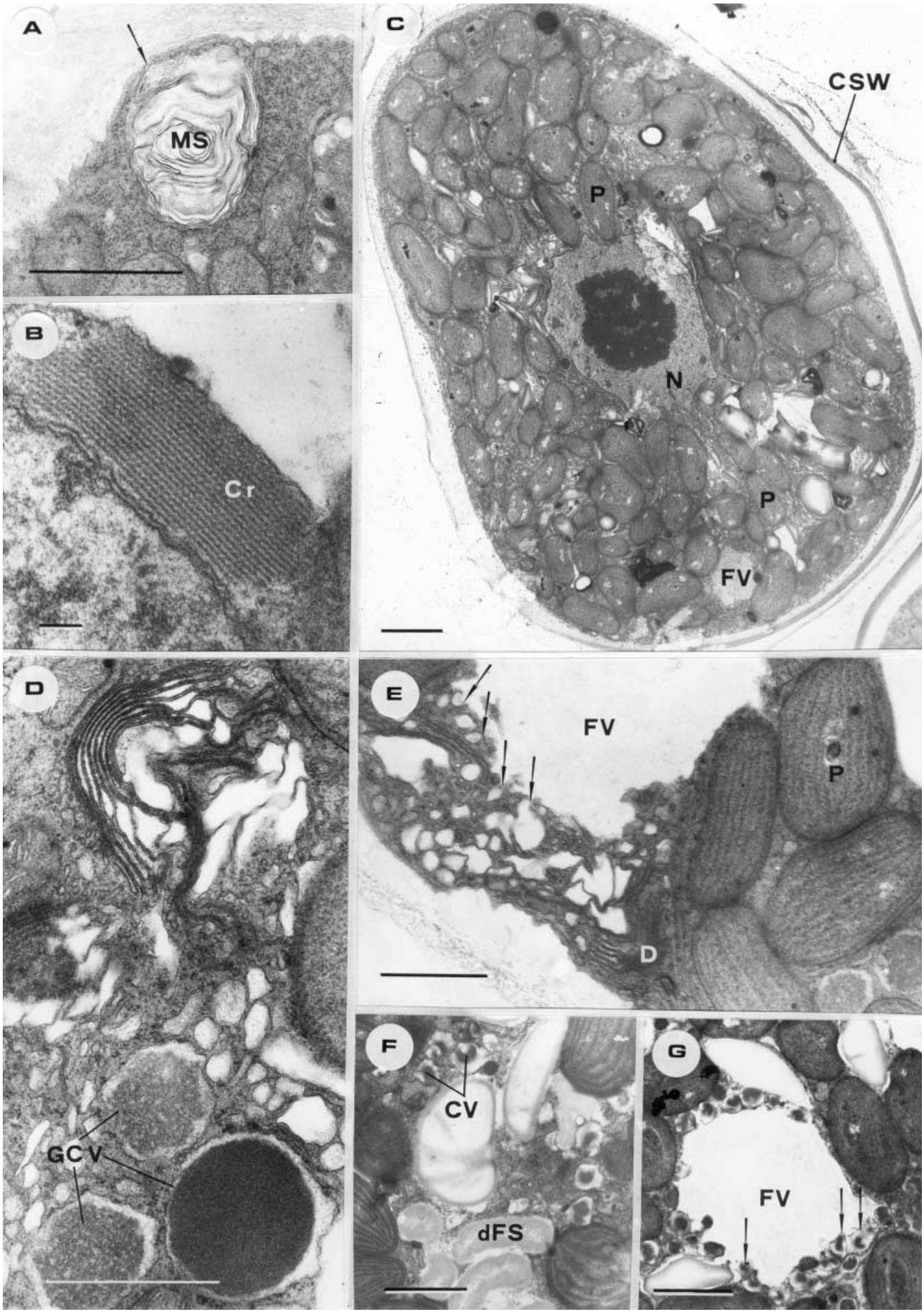


Figure 1. (*Opposite*) (A) Section of a carposporophyte showing a basal fusion cell, many large gonimoblast cells and terminal carpospores; (B) multinucleate gonimoblast initial. Mucilage sac is formed from concentric membranes; (C) differentiating young carpospore with mucilage sacs filled with mucilage which is identical in appearance to the extracellular material in the wall. C, carpospore; CM, concentric membranes, Cr, crystal; EM, extracellular material; FC, fusion cell; FS, floridean starch; GC, gonimoblast cell; MS, mucilage sac; Mu, mucilage; N, nucleus; P, plastid; V, vacuole. Scale bars: A&B, 100 μm; C, 1μm.



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carposporogenesis. A comprehensive study of carposporophyte development and carposporogenesis has not been carried out for a member of Delesseriaceae. Therefore, our recent studies have focused on orders and families from which members have not yet been studied in order to enrich the existing knowledge on the ultrastructure of post-fertilization development on a wider spectrum of representatives for comparative purposes.

After publication of the fine structure of auxiliary and gonimoblast cells during carposporophyte development in the red alga *Cryptopleura ruprechtiana* (C. Agardh) Kylin (Delivopoulos, 2003) of the Delesseriaceae the ultrastructure of carposporogenesis in this species is presented in this study.

MATERIALS AND METHODS

Thalli of *Cryptopleura ruprechtiana* with cystocarps of various sizes were collected during low tides from rocks at Campus Point on the Santa Barbara campus of the University of California. Small pieces of the thalli were immediately fixed for light and electron microscopy as previously described by Delivopoulos (2003). Thin sections cut with a Diatome diamond knife on a Reichert–Jung E ultramicrotome were post-stained for 45 min with 1% aqueous uranyl acetate and 15 min with lead citrate and examined with a Zeiss 9S-2 electron microscope.

RESULTS

Carposporophytes of *Cryptopleura ruprechtiana* (Figure 1A) comprised a basal fusion cell and many large, multinucleate gonimoblast cells which cleave to form carpospores. The carposporophyte is surrounded by extensive mucilage and pericarp cells. Only terminal gonimoblast cells differentiate to form carpospores (Figure 1A).

Gonimoblast initials are large cells with many nuclei (Figure 1B). The cytoplasm contains proplastids, while vacuoles, mucilage sacs and crystals are also present (Figure 1B). The multinucleate gonimoblast initials divide to form clusters of gonimoblast cells. The terminal ones of these become meristematic and produce carpospores.

Young carpospores are embedded within mucilage produced by mucilage sacs of the gonimoblast cells (Figure 1B). Cytoplasmically, young carpospores are simple, possessing a large nucleus with a prominent nucleolus and numerous proplastids with the peripheral thylakoid. Incipient formation of starch granules begins with most of the formation occurring near the nucleus (Figure 1C). The most striking feature denoting carpospore differentiation is mucilage sac formation. Mucilage sacs are derived from the concentric membrane bodies which dilate during mucilage formation within the membranes (Figure 1C). The membranes of the mucilage sacs finally fuse with the plasma membrane and discharge their contents, thus initiating deposition of the first carpospore wall layer inside the compressed fibrils of the carposporangial mucilage (Figure 2A). In addition, crystalline inclusions occasionally occur in the cytoplasm (Figure 2B).

Carpospores continue to increase in size, probably due to the increase in cytoplasmic components. Intermediate-aged carpospores (Figure 2C) are characterized by a significant increase in the number of plastids resulting from division of developed plastids. This stage is characterized by additional wall deposition and formation of fibrous vacuoles (Figure 2C). A thickened electron translucent carpospore wall layer surrounds the differentiating carpospore at this stage and fibrous vacuoles are present in the peripheral regions of the cytoplasm (Figure 2C). During this stage curved dictyosomes produce vesicles with fibrillar content (Figure 2D) which contribute to the enlargement of fibrous vacuoles (Figure 2E). Meanwhile, the endoplasmic reticulum produces granular cored vesicles, which exhibit varying degrees of condensation (Figure 2D).

Mature carpospores have numerous fully developed plastids and starch granules (Figure 3A). The final stage of carpospore differentiation is characterized by intense dictyosomal activity in addition to having the features of the intermediate-aged carpospores. As differentiation proceeds dictyosomes produce cored vesicles (Figure 2F) which also contribute to the fibrous vacuole enlargement (Figure 2G). Some starch granules exhibit a degenerate appearance (Figure 2F). Nuclei of the mature carpospores exhibit an irregular profile having a highly crenulated nuclear envelope (Figure 3B). The final product of the dictyosomal activity is dark-staining, granular-appearing vesicles (Figure 3C) called adhesive vesicles. Crystalline inclusions (Figure 3D) are also among the contents of the mature carpospores.

DISCUSSION

This study represents the first step-by-step description of carposporogenesis in a member of the Delesseriaceae. After light microscopic examination of post-fertilization development in *Cryptopleura ruprechtiana*, Kylin (1956) stated that most of the gonimoblast cells form carpospores. However, according to the present study only the terminal groups of gonimoblast cells differentiate to form carpospores as in *Caloglossa leprieurii* (Triemer & Vasconcelos,

Figure 2. (*Opposite*) (A) Mucilage sac ready to release its contents (arrow); (B) crystalline inclusion in the cytoplasm of a young carpospore; (C) low magnification of intermediate-aged carpospore containing few starch granules, a fibrous vacuole and numerous well developed plastids; (D) curved dictyosome producing vesicles which will form the fibrous vacuoles. Granular cored vesicles derived from the endoplasmic reticulum; (E) dictyosome-derived vesicles contribute (arrows) to the formation of fibrous vacuoles; (F) cored vesicles and degenerating starch granules in the cytoplasm of a mature carpospore; (G) cored vesicles contributing (arrows) to the enlargement of the fibrous vacuoles. Cr, crystal; CSW, compressed mucilage of carposporangial wall; CV, cored vesicle; D, dictyosome; dFS, degeneraring floridean starch; FV, fibrous vacuole; GCV, granular cored vesicle; MS, mucilage sac; N, nucleus; P, plastid. Scale bars: A, B, D–G, 1 μm; C, 2 μm.

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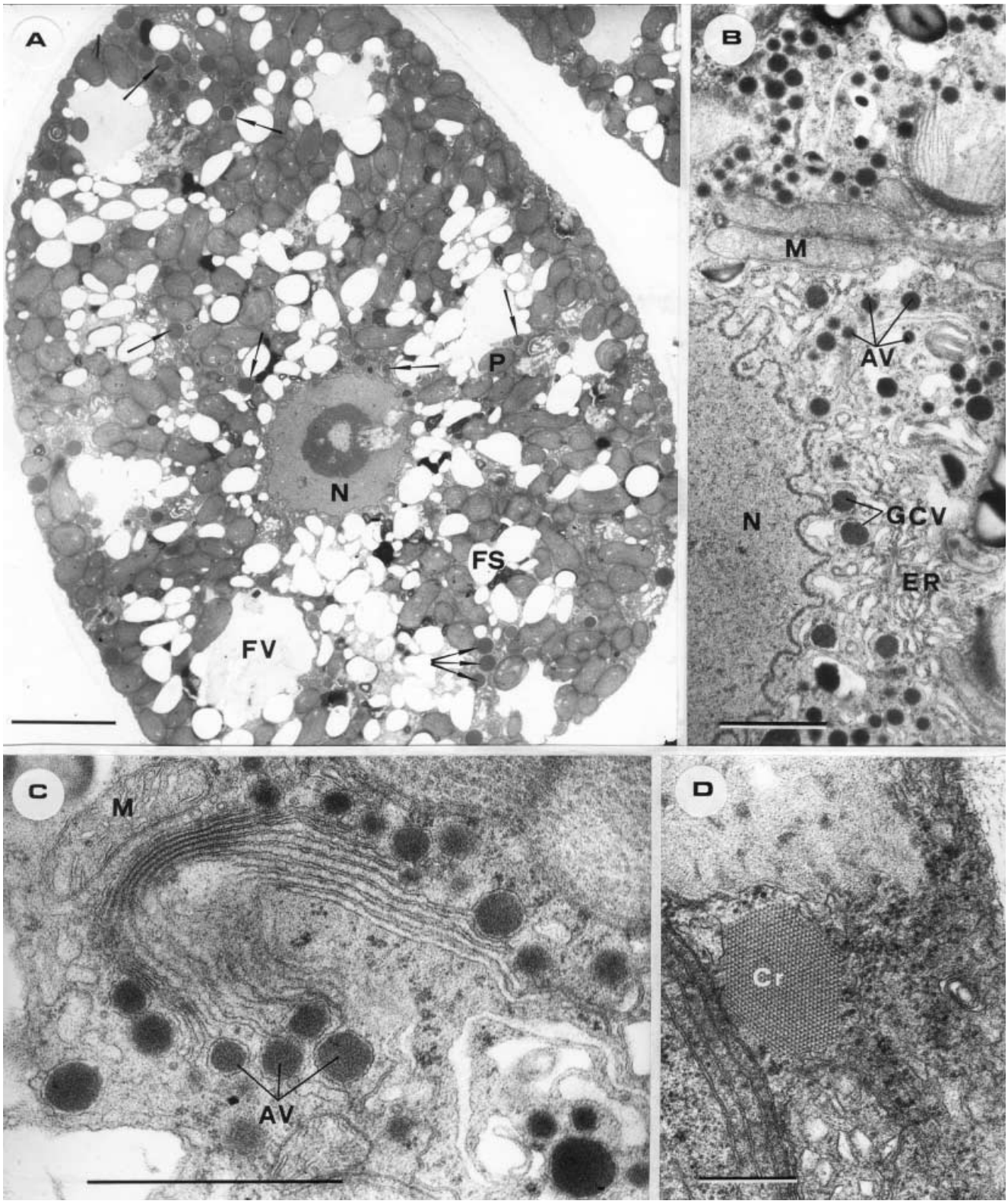

Figure 3. (A) Low magnification of a typical mature carpospore. Large fibrous vacuoles, numerous starch granules, plastids and granular cored vesicles (arrows) are present; (B) crenulated nuclear envelope surrounded by granular cored vesicles and adhesive vesicles; (C) curved dictyosome producing adhesive vesicles; (D) crystalline inclusion in the cytoplasm of a mature carpospore. AV, adhesive vesicle; Cr, crystal; ER, endoplasmic reticulum; FS, floridean starch; FV, fibrous vacuole; GCV, granular cored vesicle; M, mitochondrion; N, nucleus; P, plastid. Scale bars: A, 5 μm; B, C, 1 μm; D, 0.5 μm.

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1977). A phylogenetic sequence is evident (Hommersand, 1963) according to which one type of gonimoblast is completely transformed into carpospores, an intermediate situation being the differentiation of about one-half of the gonimoblast cells into carpospores and the third type comprising the gonimoblast having only terminal carpospores. *Cryptopleura* gonimoblast belongs to the third type.

Cytoplasmic differentiation of carpospores in *Cryptopleura* proceeds through three developmental stages as in *Caloglossa leprieurii* (Triemer & Vasconcelos, 1977). Significant cytological changes have allowed us to recognize similarities and differences with the respective process in other species. For instance, there are no reports of mucilage sacs in the carpospores of other Ceramialean species (for references see Delivopoulos & Kugrens, 1984) with one exception (Tsekos, 1985). Mucilage sacs come from the concentric membrane structures and have been reported in many red algal species (Tsekos, 1981; Delivopoulos & Kugrens, 1984; Delivopoulos, 2003). In gonimoblast cells and young carpospores mucilage sacs contribute to carpospore wall initiation inside the carposporangial mucilage. These sacs discharging their contents disappear gradually during carpospore differentiation and are completely used up by carpospore maturation (Tsekos, 1981, 1985). Therefore, the mucilage sacs are the main source of mucilage surrounding the carposporophyte. However, in *Caloglossa leprieurii* (Triemer & Vasconcelos, 1977) the mucilage is produced from the dictyosomes of the pericarp cells.

A notable feature of the nuclei of *Cryptopleura* mature carpospores is their highly crenulated nuclear envelope. This profile considerably increases the surface of the nuclear envelope and consequently facilitates the flow of information from the nucleus to the cytoplasm in metabolically active cells with intense transcriptional activity as is the case of developing carpospores (cf. also Tsekos, 1985).

It is known that during carposporogenesis in *Chondrea tenuissima* starch grains are digested in dictyosomes (Tsekos, 1985). In the case of the red alga *Cryptopleura ruprechtiana* there seem to be starch granules that are digested within lytic compartments.

The production of different types of vesicles is a common feature in red algal carposporogenesis. *Cryptopleura* dictyosomes are responsible for the production of three types of vesicles. First, dictyosome vesicles with fibrous contents fuse with each other to form large, fibrous vacuoles. Fibrous vacuoles are a distinct feature of the mature carpospores in various red algal species (for references see Pueschel, 1990). However, fibrous vacuoles are absent from mature carpospores of some but occur in the other Ceramialean species studied (Triemer & Vasconcelos, 1977). The second product of the dictyosomes in *Cryptopleura* is the commonly reported cored vesicles (for references see Pueschel, 1990). The third product of the dictyosomes is the adhesive vesicles, and their origin was determined to be the dictyosomes (Pueschel, 1990). Studies in *Faucheocolax attenuata* Setch. (Delivopoulos & Kugrens, 1984) and in *Plocamiocolax pulvinata* Setch. (Kugrens & Delivopoulos, 1986) revealed that the adhesive vesicles are dictyosome-derived and represent a condensed stage of the commonly reported cored vesicles. It is clear that in the case of *Cryptopleura* the adhesive vesicles are directly dictyosome-derived.

In addition to the above mentioned dictyosomal activity in *Cryptopleura* the endoplasmic reticulum produces granular cored vesicles similarly to *Plocamiocolax* (Kugrens & Delivopoulos, 1986). Adhesive vesicles and granular cored vesicles were not reported in *Caloglossa* of the Delesseriaceae (Triemer & Vasconcelos, 1977).

Proteinaceous crystalline inclusions have been observed in other red algal species and are regarded to represent storage of protein or nitrogen for future utilization (Pueschel, 1990). The occurrence of crystals in developing carpospores reinforces the idea that crystals might be mobilized and utilized during the developmental stages of sporogenesis (Wetherbee et al., 1984).

The wall of the mature carpospore in *Cryptopleura* consists of two layers similar to *Caloglossa* (Triemer & Vasconcelos, 1977). However, the outer layer appears as a distinct layer upon compression of diffuse mucilage fibrils during carpospore enlargement. Further ultrastructural studies are needed in the Delesseriaceae in order to clarify the boundary between the Delesseriaceae and Rhodomelaceae which has been a controversial issue (Wynne, 1983).

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