DIMORPHIC PIT CONNECTIONS IN THE RED ALGA *PSEUDOGLOIOPHLOEA*

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INTRODUCTION

Pit connections are unusual structures found almost exclusively in the septa of "higher" red algae (*Florideophycidae*). Their structure (Myers et al., 1959; Bouck, 1962; Peyrière, 1963; Bishoff, 1965; Bisalputra et al., 1967) and formation (Ramus, 1969 *b*) have been described with the aid of the electron microscope. Pit connections, at maturity, consist of two structural components: a circular septal aperture and a lenticular plug fitted into the aperture. The plug completely disrupts the cytoplasmic continuity which once existed between adjacent cells (Ramus, 1969 *b*).

Previously, the mature pit connections of an individual were assumed to be structurally identical. This paper will describe some interesting structural differences found in the red alga *Pseudogloiophloea*, with the aid of both the light and electron microscopes, and will consider the significance of these differences.

MATERIALS AND METHODS

Laboratory-grown (Ramus, 1969 *a*) filmaentous and fleshy phases of the red alga *Pseudogloiophloea* were fixed for 2 hr at 1°C in 4% neutralized glutaraldehyde in a 0.1 M, pH 6.8 phosphate buffer, and rinsed for 1 hr in several changes of buffer.

Material to be examined with a light microscope was stained for several minutes in acetocarmine (Jensen, 1962) plus a trace of ferric citrate, and then excess stain was removed by rinsing in 45% acetic acid. Fleshy stages were squashed before mounting in glycerine, while filamentous phases were mounted directly in glycerine.

Material to be examined with an electron microscope was postfixed and stained for 3 hr at 20°C in 2% OsO_4 in a 0.1 M, pH 6.8 phosphate buffer, thoroughly rinsed in buffer, and dehydrated in a graded series of ethanol followed by several changes of anhydrous propylene oxide. Dehydrated material was perfused with Epon 812 (1 part A:2 parts B), first for 6 hr in a graded series of propylene oxide–Epon mixtures, then in each of several changes of pure Epon, and finally polymerized for 48 hr at 60°C. Embedded material was sectioned longitudinally (with respect to filament axes) with a Ge-Fe-Ri diamond knife and a Sorvall Porter-Blum MT-2 ultramicrotome, and the sections were mounted on copper grids without support film.

Sections were poststained for less than 1 min in Reynold's alkaline lead citrate and examined with a Zeiss EM 9A or a Siemens 1A electron microscope.

OBSERVATIONS

The developmental sequence in laboratory culture of the marine red alga *Pseudogloiophloea* (Nemaliales, Chaetangiaceae) was recently described (Ramus, 1969 *a*). The sequence includes two morphologically similar filamentous phases, one gametophytic (haploid) (Fig. 1) and the other sporophytic (diploid) (not shown here), and an erect fleshy phase which differentiates directly from the gametophytic filamentous phase (Fig. 1). The gametophytic fleshy phase (which ultimately bears sex organs) is constructed of an axial core of parallel filaments whose branches radiate outwards. All these filaments cohere by means of dense mucilage and thus form a fleshy pseudoparenchymatous structure which grows apically.

The structure of mature pit connections in both filamentous phases was found to differ from that o the highly differentiated fleshy phase. Consequently, two distinct types of pit connections can be observed in the same gametophyte (Fig. 1), one in the filamentous phase and another in the fleshy phase.

Light Microscope

Mature pit connections in the filaments of the gametophytic fleshy phase are of the type usually seen in red algae, i.e. a simple septum with a central, circular aperture closed by a lenticular plug (Fig. 2). The aperture may vary in diameter (0.5–2.0 μ), and the plug may vary in thickness (0.5–1.0 μ), but the basic design of the pit connection remains the same.

Mature pit connections in both filamentous phases are quite different from those just described for the fleshy phase. The septum is greatly thickened around the aperture, forming a distinct ringlike structure subtending the aperture and plug (Figs. 3 and 4). The ring is synthesized sometime after plug formation and appears in cells

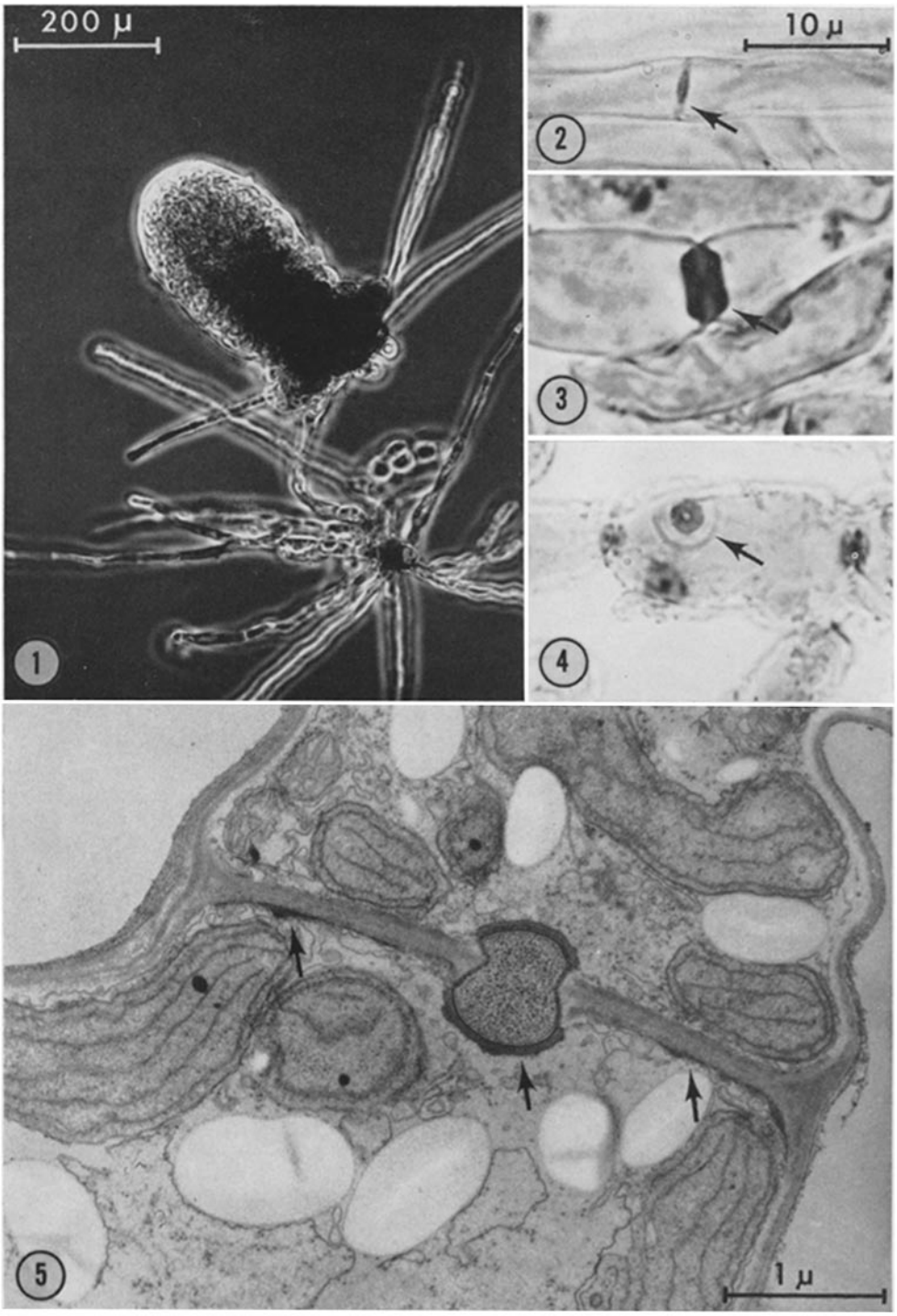

FIGURE 1 Young gametophyte of *Pseudogloiophloea*. Phase-contrast photomicrograph of fleshy phase differentiated from filamentous juvenile. × 100.

FIGURE 2 Side view of pit connection in gametophytic fleshy phase. Note plug (arrow) in septal aperture. × 2000.

FIGURE 3 Side view of pit connection in gametophytic filamentous phase. Note stained ring around aperture (arrow). × 2000.

FIGURE 4 End view of pit connection in gametophytic filamentous phase. Note stained ring around aperture (arrow). × 2000.

FIGURE 5 Longitudinal section through a young pit connection in the gametophytic filamentous phase. Note dense material condensing on septum (arrows) and face of plug × 23,000.

FIGURE 6 Longitudinal section through a pit connection of gametophytic filamentous phase. Note granular material condensing on septum (arrows) to form ring around aperture. *l*, lipid body; *m*, mitochondrion; *p*, plastid; *s*, floridean starch granule; *v*, vacuole. × 23,000.

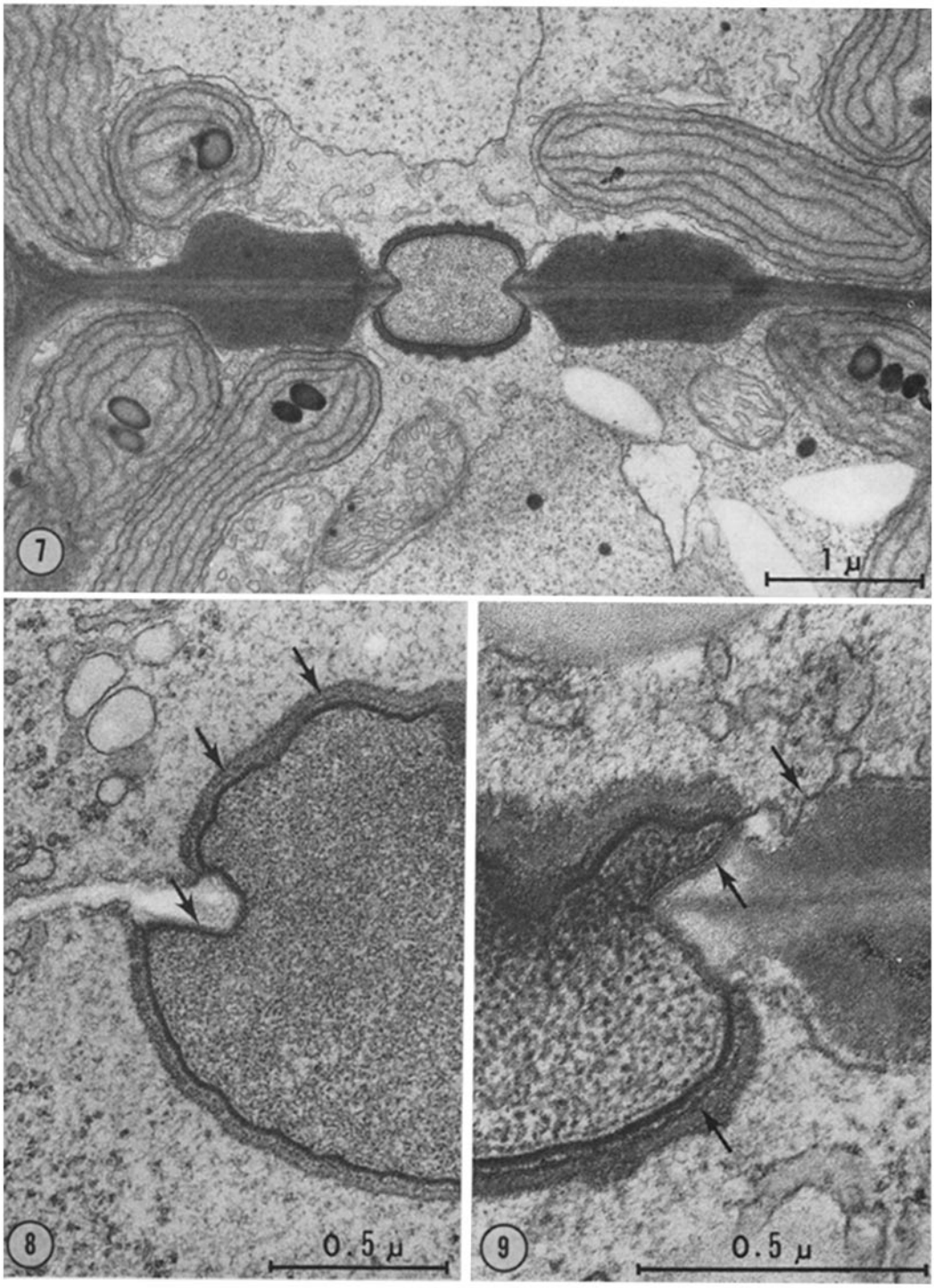

FIGURE 7 Longitudinal section through a mature pit connection of the gametophytic filamentous phase. Note ring formed as an elaboration of septum around aperture. × 23,000.

FIGURE 8 Longitudinal section through a mature pit connection (micrograph cut vertically through center) of the gametophytic filamentous phase. Note plasmalemma around septal aperture (lower arrow) and membrane bounding the plug (upper arrows) somewhat obscured by condensed amorphous material. × 54,000.

FIGURE 9 Longitudinal section through mature pit connection (micrograph cut vertically through center) of gametophytic fleshy phase. Note membrane around plug and through septal aperture (arrows). × 90,000.

some distance behind the apex of a growing filament, i.e. maturing cells. The ring can reach a thickness of several microns.

Electron Microscope

The electron microscope confirms the initial observations made with the light microscope, i.e. there is a distinct dimorphism in the structure of the pit connections.

In mature pit connections of the gametophytic fleshy phase the septum remains simple (Ramus, 1969 *b*). The plasmalemma is continuous around the rim of the aperture (Fig. 8). The plug itself is composed of particles, 50–100 A in diameter, bounded by a dense layer approximately 200 A thick. A membrane appears to surround the plug but is obscured by a thick layer of amorphous material. (Fig. 8).

Pit connections in the filamentous phases, immediately after aperture and plug formation, are similar to those in the gametophytic fleshy phase (Fig. 5). Then, however, dense material begins to condense on either side of the septum (Figs. 5 and 6). This material is differentially deposited in the form of large granules, the septal area adjacent to the aperture receiving the greatest quantity (Fig. 6). In this manner, a distinct ring is formed on the septum around the aperture and plug (Fig. 7). Unfortunately, the origin and composition of this material are unknown.

The plug itself does not differ in structure from that of the fleshy phase. It is composed of particles, 50–100 A in diameter, bounded by a dense layer 200 A thick (Fig. 9). Quite visible in the amorphous material, which appears on the surface of the plug, is a tripartite membrane about 80 A thick (Fig. 9). The plasmalemma is continuous around the rim of the aperture and also makes contact with the membrane which bounds the plug (Fig. 9).

Although the plugs are usually biconvex in both filamentous and fleshy phases, occasionally one face may be concave (Fig. 9). The concave face, when present, is always directed towards the apex of the filament.

DISCUSSION

Previously, there were no reports that the structure of mature pit connections differed in the same red alga. However, it was found that pit connections in both gametophytic and sporophytic filamentous phases of *Pseudogloiophloea* differ from those in the gametophytic fleshy phase. The difference in structure lies only in the elaboration of the septum in the filamentous phases. Here, material is condensed differentially on the septum to form a discrete ring around the aperture. The septum remains simple in the fleshy phase.

In the case of the gametophyte, the simple filamentous phase differentiates a highly structured fleshy phase. Both phases are of the same genotype and chromosome number but are very different in structure. It is conceivable, then, that the structure of pit connections in these two phases could also be different.

Since little is known of the function of pit connections, the function of the ring around the aperture is impossible to assess at this time.

The heterotrichous habit (see Fritsch, 1935, for review) is recognizable in the filamentous phases of *Pseudogloiophloea*. Fritsch maintains that the elaboration of the erect phase of heterotrichous filaments is an advanced feature, from the standpoint of evolution. If the fleshy phase is to be considered derived, then so should the simpler pit connection of the fleshy phase.

In a previous paper (Ramus, 1969 *b*), similarities between septum formation (centripetal accretion of wall material, associated lomasomes) and structure (central aperture, brief appearance of parallel flattened vesicles) between *Pseudogloiphloea* and the Ascomycete *Ascodesmis* were mentioned. Structural similarities also exist between the elaborated septum of *Pseudogloiophloea* and the dolipore septum (see Bracker, 1967, for review) of the Homobasidiomycetidae, both possessing ringlike elaborations around the septal aperture.

Plugs of the filamentous phases and the fleshy phase are bounded by an 80 A tripartite membrane. This limiting membrane is often obscured by amorphous material which collects at the faces of the plugs, as was seen in *Lomentaria* (Bouck, 1962) and *Laurencia* (Bisalputra et al., 1967). The plugs of *Pseudogloiophloea* are composed of 50–100 A granules, as are those of *Laurencia*, and are surrounded by a dense 200 A layer as are those of *Lomentaria* and *Laurencia*. In no place do the plugs come into direct contact with the cytoplasm, nor is there cytoplasmic continuity between adjacent cells.

SUMMARY

Pit connections of the marine red alga *Pseudogloiophloea* (Nemaliales, Chaetangiaceae) were studied with both the light and electron microscopes, and an interesting structural dimorphism was found. In the filamentous phases, the septum is elaborated to form a distinct ring around the aperture and plug, whereas this septal ring is not present in the fleshy phase. The ring is formed by a differential deposition of granules on the septa of maturing cells.

The plugs themselves do not differ in structure in the various phases of the developmental sequence of *Pseudogloiophloea*. They consist of 50–100-A particles surrounded by a dense 200 A layer. The plugs are bounded by a 80 A tripartite membrane which joins the plasmalemma.

In no place do the plugs come into direct contact with the cytoplasm, nor is there cytoplasmic continuity between adjacent cells.

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