Electron Microscopic Study on the Zoochlorellae of Adult Green Sponges and Gemmules of *Radiospongilla cerebellata* (BOWERBANK) (Porifera: Spongillidae)

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Abstract

Electron microscopy of green adult [vegetative tissue] and gemmulated *Radiospongilla cerebellata* revealed zoochlorellae to be intracellular and to reside in membrane–bound host vacuoles. In having a pyrenoid and in the mode of reproduction, the zoochlorellae of green adult sponges of this species resembled those reported from *Paramecium bursaria* and *Hydra viridis*. In the gemmules of this species, a few zoochlorellae were observed in some thesocytes. They looked much different from those of adult green sponges. They contained some large polyphosphate granules and many lipid storage bodies, but they had lost the normal chloroplast with arrayed stacks of thyrakoids, starch grains, and a pyrenoid, as seen in the archeocyte of green adult sponges.

Introduction

Zoochlorellae are found as intracellular symbionts in several invertebrate phyla, including the Protozoa, Porifera, Coelenterata and Plathelminthes. Some species of green freshwater sponges contain zoochlorellae [1–3], but there has been only a few reports on the ultrastructure of the zoochlorellae. Williamson [4] investigated the zoochlorellae of green adult sponges and gemmules of *Spongilla lacustris* and reported that zoochlorellae actively reproduced in adult green sponges but not in adult white sponges or gemmules and that pyrenoids were lacking in all of the zoochlorellae.

This paper deals with the ultrastructure of zoochlorellae of adult green sponges and gemmules of *Radiospongilla cerebellata*.

Materials and Methods

The population of *Radiospongilla cerebellata* (BOWERBANK) investigated was located in a small pond in Okayama prefecture (34°30′N, 133°45′E). The green adult sponges and gemmules were collected on 17 August 1983 and 10 October 1983, respectively. Before fixation, a fine slice was made through a portion of the gemmule coat with double–edged razor blade to facilitate infiltration of fixatives and embedding media. The green adult sponges and gemmules were first fixed in a 1:1 mixture of 2%

glutaraldehyde in 0.1M phosphate buffer (pH7.3) and 2% paraformaldehyde in the same buffer and then fixed in 1% osmium tetroxide in 0.1M phosphate buffer. After the fixation, they were dehydrated in an ethanol series and embedded in Epoxy resin. The sections were doubly stained with uranyl acetate and lead citrate solution and examined under a Hitachi HS–9 electron microscope.

Results

Zoochlorellae in adult green sponges

Many zoochlorellae were located intracellularly within archeocytes (Fig. 1 and 2). Each zoochlorella was located in an individual vacuole except during division. The zoochlorellae were characteristically round or oval with a long axis of about 3–6 μm, and possessed a cell wall, nucleus, mitochondria, Golgi complexes, electron dense polyphosphate granules, and a cup–shaped chloroplast. The chlorplast was made up of arrayed stacks of thylakoids, starch grains, and a pyrenoid. A large pyrenoid surrounded by a shell of starch grains was located in the center of the chloroplast (Fig. 2). The matrix of a pyrenoid was dissected by several single thylakoids. Reproducing zoochlorellae were found in some archeocytes. 2–4 autospores were observed in each vacuole from the section (Fig. 3–4).

Zoochlorellae in gemmules

At early stages of gemmule formation after archeocytes, trophocytes, and spongocytes were aggregating, young thesocytes (Fig. 5) and spongocytes contained a few zoochlorellae. These zoochlorellae closely resembled those of adult green sponges (Fig. 6 and 7). Near completion of gemmule coat formation, a few zoochlorellae of the thesocytes had a degenerated chloroplast which lacked the stacks of thylakoids and had a few starch grains (Fig. 9 and 10). The number of lipid storage bodies had increased in the zoochlorellae(Fig. 9). In the thesocytes, when gemmule coat formation was finished, there were a few zoochlorellae having a nucleus, many lipid storage bodies, some large electron dense polyphosphate granules, and a small body which seemed to be a degenerated chloroplast (Fig. 11 and 12).

Discussion

The common algal symbionts of the freshwater sponges and metazoans are unicellular algae of the Order Chlorococcales, generally known as "Zoochlorellae".

Williamson [4] reported that the zoochlorellae of *S.lacustris* lacked a pyrenoid at any stage. The zoochlorellae from some species or strains of green hydra [5] and *Paramecium bursaria* [6] have a pyrenoid in the chloroplast. This paper has shown that the zoochlorellae of *R. cerebellata* have pyrenoids which are a little different from those of the green hydra and *P. bursaria*. That is, pyrenoids of the green hydra and *P. bursaria* are bisected by a stack of 2 thylakoids, whereas those of *R. cerebellata* are dissected by several single thylakoids (Fig. 8).

Williamson [4] observed the zoochlorellae of *S. lacustris* to form only two autospores consistently. The mode of reproduction of zoochlorellae of *R. cerebellata* seems

to be to form four autospores (Fig. 4), as the zoochlorellae of *Hydra viridis* [7] and *P. bursaria* [6].

Williamson [4] observed the zoochlorellae in the gemmules of *S. lacustris* and reported that they contained chloroplasts with a loosely packed thylakoid membrane. The zoochlorellae in the gemmules of *R. cerebellata*, which looked much different from those of adult green sponges, contained degenerated chloroplasts (Fig. 9–10). Osafune et al. [8] reported that the degeneration and regeneration of chloroplasts are induced in *Chlorella protothecoides* by the control of nutritional as well as light conditions. Though there is no evidence that the zoochlorellae of *R. cerebellata* belong to the genus *Chlorella*, I suggest that the degeneration of chloroplasts was caused by changes in the environmental conditions, i.e., from the archeocytes of vegetative tissue to the thesocytes of gemmules.

Therefore, it appears that the zoochlorellae of *R. cerebellata* and *S. lacustris* differ in at least 3 key respects: the presence of a pyrenoid, the mode of reproduction, and chloroplast morphology in the gemmules. Pardy [5] reported that the zoochlorellae from the English strains of green hydra had a great morphological plasticity and the presence of pyrenoids greatly depended upon the host environment. Therefore, though the presence of pyrenoids may not be very important for classification, the zoochlorellae of *R. cerebellata* resemble more closely those of the green hydra and *P. bursaria* than those of *S. lacustris*.

Williamson [4] reported that the zoochlorellae of *S. lacustris* were abundant in green adult sponges and green gemmules but rare in white gemmules having a thick coat. The gemmules of *R. cerebellata* with a thick coat contained much fewer zoochlorellae than the green gemmules of *S. lacustris*, which have a thin coat. Whether the gemmules of other green sponge species that have a thick coat do not contain many zoochlorellae is under investigation.

References

1. Van Tright, H. (1919) Contribution to the physiology of the fresh–water sponges [Spongillidae]. Tijdschr. Ned. Dierk. Ver (2) **17** : 1—220.
2. Gilbert, J. J. and Allen, H. L. (1973) Chlorophyll and primary productivity of some green, freshwater sponges. Int. Rev. Ges. Hydrobiol., **58** : 633—658.
3. Wilkinson , C. R. (1980) Nutrient translocation from green algal symbionts to the freshwater sponge *Ephydatia fluviatilis*. Hydrobiol., **75** : 241—250.
4. Williamson, C. E. (1979) An ultrastructural investigation of algal symbiosis in white and green *Spongilla lacustris* (L.) (Porifera: Spongillidae). Trans. Amer. Micros. Soc., **98** : 59—77.
5. Pardy, R. L. (1976) The morphology of green hydra endo–symbionts as influenced by host strain and host environment. J. Cell Sci., **20** : 655—669.
6. Karakashian, S. J., Karakashian, M. W. and Rudzinska, M. A. (1968) Electron microscopic observation on the symbiosis of *Paramecium bursaria* and its intracellular algae. J. Protozool., **15** : (1) 113—128.

7. Oshman, J. L. (1967) Structure and reproduction of the algal symbionts of *Hydra viridis*. J. Phycol., **3** : 221—228.
8. Osafune, T. and Hase, E. (1975) Some structural characteristics of the chloroplasts in the "Glucose–bleaching" and re–greening cells of *Chlorella protothecoides*. Biochem. Physiol. Pflanzen, **168** : S. 533—542.

Explanation of figures

Fig. 1 Zoochlorellae in the archeocytes of green adult sponges (vegetative tissue). ×3,000.

Fig. 2 An enlarged view of Fig. 1 showing two zoochlorellae. A pyrenoid is seen in the center of the chloroplast of the upper zoochlorella. N, nucleus; S, starch grain. ×7,000.

Fig. 3 Reproducing zoochlorella in the archeocyte of a green adult sponge. Oblique section shows three autospores. N, nucleus; S, starch grain. ×10,000.

Fig. 4 Oblique section through four autospores of a reproducing zoochlorella. ×10,000.

Fig. 5 Two zoochlorellae (arrows) are observed in a young thesocyte containing many immature vitelline platelets. ×2,000.

Fig. 6 An enlarged view of Fig. 5 shows a zoochlorella possessing a cell wall, a nucleus, and a chloroplast. The zoochlorella resembles those of Fig. 2. L, lipid droplet; S, starch grain; V, vitelline platelet. ×10,000.

Fig. 7 Another zoochlorella in the thesocyte at the same stage of Fig. 5. A conspicious pyrenoid located in the center of a chloroplast. Four neighbouring starch grains surround the pyrenoid. L, lipid droplet. ×10,000.

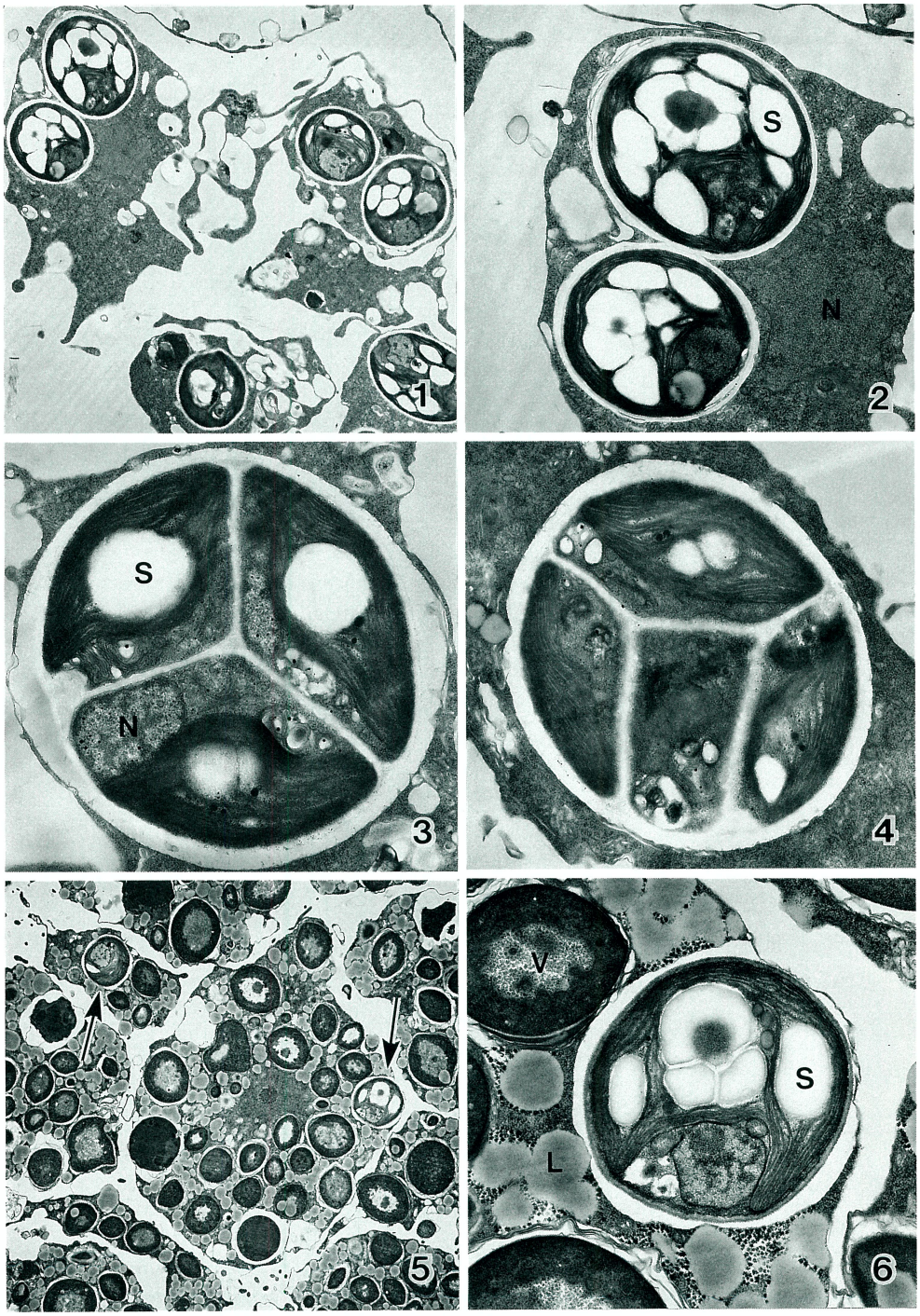
Fig. 8 An enlarged view of Fig. 7 showing that several single thylakoid membranes enter the pyrenoid. S, starch grain. ×21,000.

Fig. 9 Zoochlorella in the thesocyte as the gemmule coat formation nears completion. The degenerating chloroplast have a small amount of starch grains. L, lipid droplet; N, nucleus. ×10,000.

Fig. 10 A part of a zoochlorella in the thesocyte at the same stage as Fig. 9. The degenerating chloroplast does not show arrayed stacks of thylakoids as shown in Fig. 8. S, starch grain. ×21,000.

Fig. 11 Thesocytes at the finish of gemmule coat formation contain three zoochlorellae. The zoochlorellae in which large polyphosphate granules are visible look much different from those of Fig. 1 and 5. ×3,000.

Fig. 12 An enlarged view of Fig. 11 showing a zoochlorella. The zoochlorella possesses many lipid storage bodies, two polyphosphate granules, and a small body that seems to be a degenerating chloroplast. L, lipid droplet; N, nucleus. ×10,000.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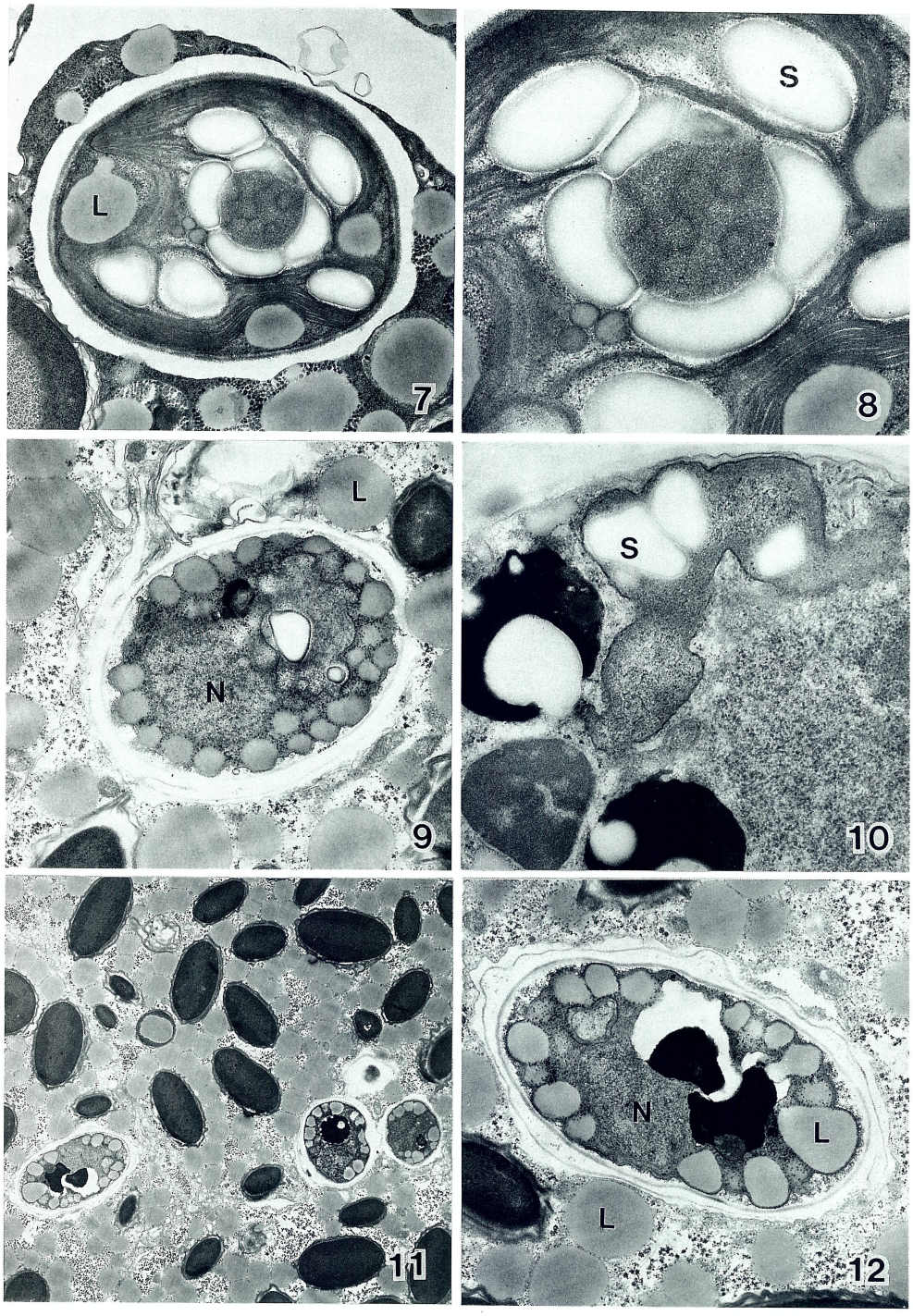
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