Ultrastructures of the Golgi Body and Cell Surface in *Cyanidioschyzon merolae*

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We are deeply interested in determining the minimum ultrastructural constituents of plant eukaryotic cells. *Cyanidioschyzon merolae* and *Cyanidium caldarium* are very small unicellular eukaryotes which range from 2 μ m to 6 μ m in diameter (De Luca *et al.* 1978, Merola *et al.* 1981). Each alga contains one cell nucleus, one mitochondrion and one chloroplast (De Luca *et al.* 1978, Merola *et al.* 1981). Merola *et al.* (1981) reported that *C. merolae* multiplied by binary division, while *C. caldarium* had 4 endospores, which indicated that *C. caldarium* multiplied by two consecutive binary divisions. Suzuki *et al.* (1992) reported that the cell nuclei in *C. merolae* and *C. caldarium* forma A had DNA contents of 8 Mbp and 18 Mbp, respectively. We examined both *C. merolae* and *C. caldarium* using a serial thin-section electron microscopic technique after rapid freezing in liquid propane and a substitution fixation (Kuroiwa *et al.* 1994). The results showed that many basic organelles could be observed in both algae. However, while a Golgi body and a cell wall could be observed in *C. caldarium* cells, these could not be observed in *C. merolae* cells.

The aim of this study was to re-examine the existence of the Golgi body and cell surface in *C. merolae* using rapid freezing in liquid propane and a substitution fixation, and silver methenamine staining.

Materials and methods

C. merolae and *C. caldarium* RK-1 were generous gifts from Prof. Pinto (Napoli University, Italy) and Prof. Nagashima (Science University of Tokyo, Japan), respectively. They were cultured in Allen's medium (Allen 1959) under CO₂ aeration at 40°C with a 12-hr light/dark cycle at a light intensity of about 3,000 lux. Division of *C. merolae* cells was synchronized by the method of Suzuki *et al.* (1994). *C. merolae* cells were collected in test tubes after the cells were slightly swelled in distilled water. Sample pellets were sandwiched between copper plates (1 mm $\times$ 4 mm $\times$ 0.2 mm), and put into cooled liquid propane at -190°C , which was replaced by acetone containing 1% osmium at -85°C . For conventional chemical fixation, the cells were fixed in 2% glutaraldehyde and 2% OsO₄ solution according to the standard method. Finally, all of the samples were embedded in Spurr resin. Thin sections were cut on an ultramicrotome with either a glass or diamond knife (Eiko En. Co., Kawasaki). The ultrathin serial sections were double-stained with 3% uranyl acetate and 1% lead citrate and observed under an electron microscope (JEOL 1200CX, Tokyo). In addition, the thin sections were stained with silver methenamine for electron microscopic demonstration of polysaccharide-like structures (Rambourg and Leblond 1967, Kuroiwa 1989).

Results

Golgi Apparatus

In a previous report, we could not identify a typical Golgi body in *C. merolae* cells which were fixed under conventional chemical fixation or rapid freeze substitution fixation (Kuroiwa *et al.* 1994). In this study, we re-examined the fine structure of the cytoplasm in *C. merolae* cells fixed by rapid freezing. In *C. merolae* cells, chloroplast division, mitochondrial division and cell-nuclear division occur in that order during cell division (Kuroiwa *et al.* 1995). Fig. 1A shows a cell during the early stage of cell division. The dense material of the plastid-dividing ring can be seen around the chloroplast (arrowhead in Fig. 1A). Golgi bodies appeared in the cytoplasm close to both poles of the cell nucleus (Fig. 1A). One Golgi body was cut at its periphery. The Golgi body was composed of transfer vesicles (small arrow in Fig. 1A), two Golgi cisternae (mid-size arrow in Fig. 1A) and secretory vesicles (large arrow in Fig. 1A). An association between the transfer vesicles and the nuclear membrane could often be seen (Fig. 1A). The secretory vesicles were distributed throughout the entire cytoplasm (large arrows in Fig. 1A).

Fig. 1B–D shows two serial thin-sections of a cell containing a dividing chloroplast. As chloroplast division progressed, the dense materials of the plastid-dividing ring and microbodies appeared at the bridge of the dividing chloroplast (Fig. 1B, C). Golgi bodies remained near both poles of the cell nucleus. At a higher magnification, the Golgi body was composed of transfer vesicles (small arrow in Fig. 1D), a Golgi cisterna (mid-size arrow in Fig. 1D) and secretory vesicles (large arrows in Fig. 1D). After cytokinesis, the Golgi bodies were separated into the daughter cells (data not shown). Based on these results, we concluded that *C. merolae* cells contain one Golgi body.

Cell surface

Since *C. merolae* cells maintain regular shapes, such as football-, peanut- and delta-shaped structures, it was expected that there was a special structure on the cell surface. However, when *C. merolae* and *C. caldarium* RK-1 were mixed, fixed and stained under the same conditions to avoid obtaining faulty results due to technical differences, a typical cell wall was seen in *C. caldarium* RK-1, but not in *C. merolae* as described previously (Fig. 2A, Kuroiwa *et al.* 1994). When we re-examined the fine structures of these cells, we noticed that there were many filamentous structures on the exterior surface of the cell membrane (large arrow in Fig. 2A). The cell membrane was coated with an electron-dense thin layer from which the filaments approximately 100 nm in length and 8 nm in diameter protruded. Such filamentous structures did not appear on the surface of a cell wall of *C. caldarium* RK-1 (small arrows in Fig. 2A).

To elucidate whether or not the filaments in *C. merolae* cells contained polysaccharide, as do those in other plants, we stained *C. merolae* cells with silver methenamine for comparison with *C. caldarium* RK-1. Silver methenamine accumulated in a thin layer on the exterior surface of *C. merolae* cells (large arrow in Fig. 2B) and on the cell wall of *C. caldarium* RK-1 (small arrows in Fig. 2B). In addition, these dense materials appeared on starch grains in

Fig. 1. Electron micrographs of *Cyanidioschyzon merolae* cells which contain a cell nucleus (n), chloroplasts (c), mitochondria (m), a microbody (mb) and Golgi bodies (asterisks) during the early stage of cell division (A) and the middle stage of cell division (B–D). Golgi bodies were composed of transfer vesicles (small arrows in A and D), one to two cisternae (mid-size arrow in A and D) and secretory vesicles (large arrows in A and D). B and C were serial thin sections. (D) is high-magnification image of the area in (C) labeled with asterisks. The appearance of the dense material of a plastid-dividing ring (arrowheads in A and B) suggests cell division. Scale bars represent 1 μm (A–C) and 0.1 μm (D).

Fig. 1

Fig. 2. Electron micrographs of cells which contained chloroplasts (c) and starch grains (s) in A and B) during interphase cells in *Cyanidioschyzon merolae* and *Cyanidium caldarium* RK-1 before (A) and after treatment with silver methenamine (B). *C. merolae* cells were covered with a filamentous layer which protruded from an electron-dense layer (large arrow in A), while the cell wall of a broken *C. caldarium* cell was observed (small arrows in A). After staining with silver methenamine, dense materials appeared on the exterior surface of the cell membrane in *C. merolae*, the cell wall in *C. caldarium* RK-1 and starch grains in both cells. Scale bars represent 1 μ m (A and B).

both cells (s in Fig. 2B). These results suggest that the cell surface of *C. merolae* is different from the cell wall of general algae and plants and that *C. merolae* cells maintain their shape using a specific filamentous layer.

Discussion

Golgi Apparatus

Broadwater and Scott (1994) summarized the characteristics of cells in various unicellular red algae. Golgi bodies are observed in many red algae, as well as in other eukaryotes. Red algae such as *Porphyridium purpureum* and *Glaucosphaera vacuolata* have typical Golgi bodies. Each cell contains several Golgi bodies which have more than 8 Golgi cisternae, while the primitive red algae, *Galdieria sulphuraria* (Ueda and Yokochi 1981) and *C. caldarium* forma A contain a few Golgi bodies (Kuroiwa *et al.* 1994). Golgi cisternae in *G. sulphuraria* and *C. caldarium* contain approximately 5 and 3 Golgi cisternae, respectively. In this study, *C. merolae* cells had a single Golgi body which was composed of one or two Golgi cisternae, secretory vesicles and transfer vesicles. However, a trans-Golgi network was not observed. The results suggest that Golgi bodies in *C. merolae* have a simpler architecture than those in other eukaryotes. The Golgi apparatus in *C. merolae* is arranged around the periphery of the cell nucleus. Since the transfer vesicles could be seen between the cell nucleus and Golgi cisternae, the Golgi bodies in *C. merolae* might differ from those in *G. sulphuraria* (Ueda and Yokochi 1981) and be similar to those in *Dixoniella grisea*, which present a third type of Golgi association (Broadwater and Scott 1994).

Since *C. merolae* cells in this study contained only one Golgi body, suplication of the Golgi body can be studied in detail during the mitotic cycle.

Cell surface

All non-controversial unicellular members of the *Porphyridiales* (Chapman 1974) and *G. vacuolata* (Richardson and Brown 1970) lack a cell wall, and instead have a mucilaginous sheath, the thickness of which varies with the culture conditions. In a previous paper, we reported that *C. caldarium* cells have a cell wall, while *C. merolae* cells do not. The present experiments confirmed these results. The surface of the cell membrane in *C. merolae* had a filamentous layer with a structure similar to those in the red alga, *R. violacea* and the giant amoeba, *Peromyxa carolinensis* (Giese 1979), which may move using these filamentous structures. These filaments may contain actin. However, although *C. merolae* contains an actin gene (Takahashi *et al.* 1995), the surface of the cells was not stained with phalloidin (Suzuki *et al.* 1995). These results suggest that the filaments at the exterior surface of the cell membrane do not contain actin.

After staining with silver methenamine, the electron-dense materials were seen on the cell wall of *C. caldarium* cells and on starch grains in both *C. caldarium* and *C. merolae* cells. These results suggest that the components of the cell wall in *C. caldarium* are similar to those of higher organisms.

Although *C. merolae* and *C. caldarium* live together in acidic hot springs, *C. merolae* does not have a cell wall while *C. caldarium* have a hard cell wall. Adaptation may play a role in this difference.

Summary

The presence of a Golgi body and a cell wall in *Cyanidioschyzon merolae* was re-examined by electron microscopy after rapid-freeze substitution fixation to understand the minimum

ultrastructural characteristics of eukaryotes. *C. merolae* cells contained one Golgi body which was composed of one to two cisternae, transfer vesicles and secretory vesicles. A trans-Golgi network was not seen, but association between the transfer vesicles and the nuclear membrane was often observed. The cells did not have a cell wall, but were covered by many filaments which were protruded from a thin electron-dense layer on the exterior surface of the cell membrane.

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