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Appearance of heterogeneity in morphology and nucleoid distribution among chloroplasts in germling cell of *Bryopsis plumosa* (Hudson) C. Ag. (Chlorophyceae)*

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The appearance of heterogeneity in the morphology and nucleoid distribution among chloroplasts in the germling cell of the green alga *Bryopsis plumosa* (Hudson) C. Ag. was investigated. About two weeks after germination chloroplasts which were morphologically distinguishable from many other chloroplasts appeared near the giant primary nucleus. They were small and generally poor in starch. At this stage, remarkably extended chloroplasts were often observed around the primary nucleus as well. As compared with the usual spindle-shaped chloroplast, most of the perinuclear chloroplasts had nucleoids densely. Several days later some of the perinuclear chloroplasts bore one or a few larger nucleoids, sometimes reaching 3.0 μm in diameter. The number of small chloroplasts with large nucleoids gradually increased, but, inversely, the frequency of occurrence of conspicuously extended chloroplasts decreased. Based on the present observation, the process of the preferential appearance of small chloroplasts with large nucleoids in the perinuclear region is discussed.

Key Index Words: Bryopsis—*Chlorophyceae—chloroplast heterogeneity—chloroplast nucleoid—germling cell.*

In the green algal genus *Bryopsis* (Codiales, Chlorophyceae) the zygote resulting from the union of biflagellate anisogametes germinates into a single branched cell, or germling cell. In contrast with the coenocytic gametophyte cell, the germling cell is uninucleate, and it contains a large primary nucleus at maturity (Neumann 1969, Burr and West 1971, Rietema 1971). Saito *et al.* (1989) found that in *B. plumosa* (Hudson) C. Ag. the giant primary nucleus was always associated with small lenticular chloroplasts which were generally poor in starch and often lacked pyrenoids. These chloroplasts were distinguishable from the majority of chloroplasts in the same germling cell in respect of DNA distribution as well, suggesting that the mature germling cell of *Bryopsis* is one of the remarkable cells in which marked variations in morphology and DNA distribution can be seen in the chloroplast population. The process of the occurrence of these heterogeneous chloroplasts and the cause of their exclusive perinuclear distribution, however, remained unknown (Saito *et al.* 1989). The present investigation was carried out to clarify the initial stage of the development of these heterogeneous chloroplasts in conjunction with their preferential localization near the primary nucleus.

Materials and Methods

The plumose gametophyte plants of *Bryopsis plumosa* (Hudson) C. Ag. were collected from Murohama beach, Miyatojima Islands,

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Miyagi Prefecture, Japan. Freshly liberated male and female anisogametes were mixed together, and the resultant zygotes were allowed to germinate and grow in Petri dishes, each of which were half-filled with an enriched seawater medium (Provasoli 1968), under 12 : 12 LD cycle (white fluorescent lamps, ca. 2,000 lux) at 25°C.

For fluorescence microscopy, some of the germling cells were fixed in 2.5% glutaraldehyde dissolved in seawater for 30 min at 20°C. After slight rinse in seawater, they were then stained with 4′,6-diamidino-2-phenylindole (DAPI), 0.1 μg/m*l* in buffer S (Nishibayashi and Kuroiwa 1982), for 1 hr at 20°C. They were then examined with a Nikon XF-EFD fluorescence microscope, using a Nikon UV excitation filter set (UV330-380, DM400, 420).

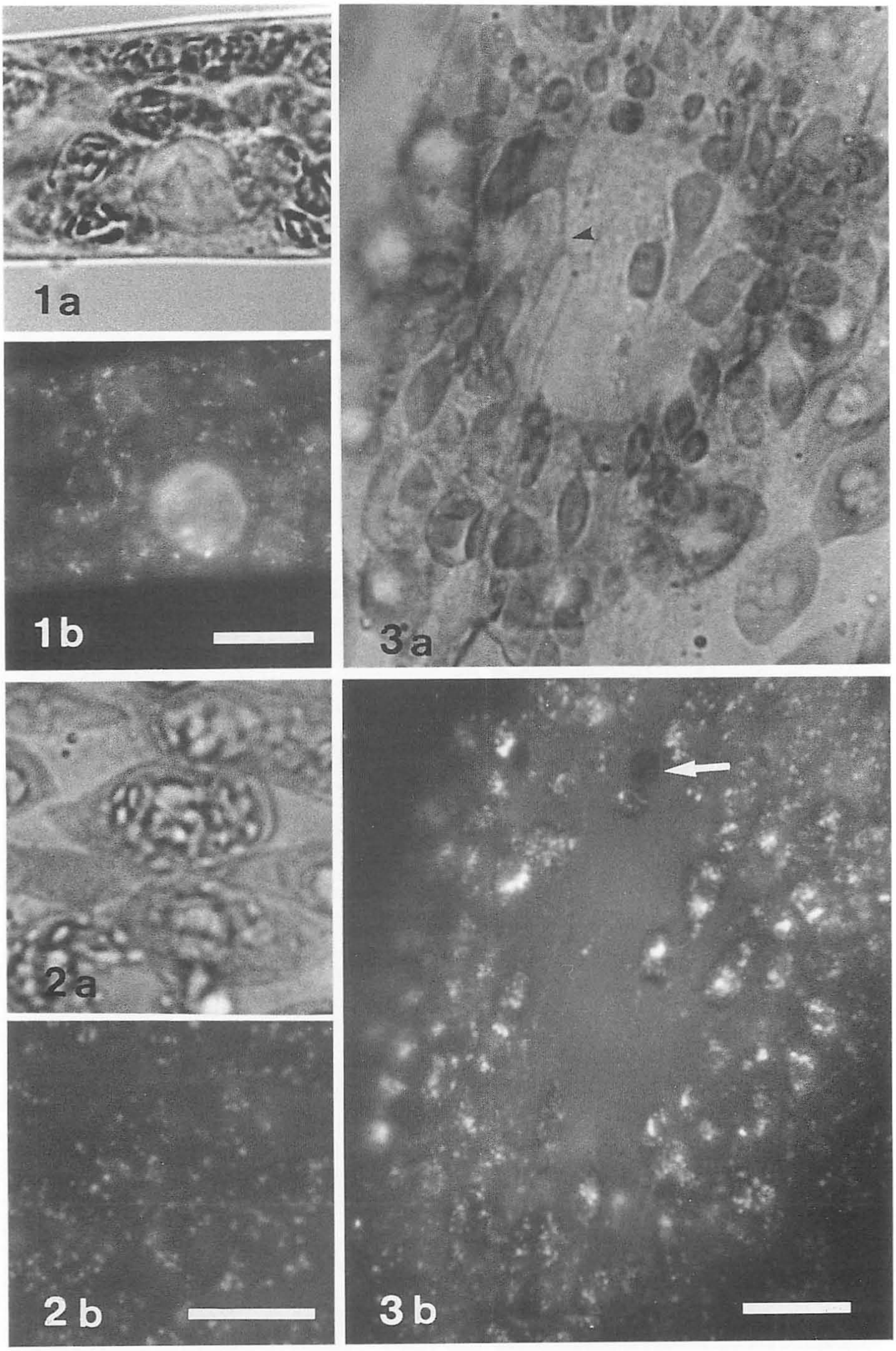
Results

Immediately after plasmogamy the male and female nuclei united together into a single zygote nucleus of 2–3 μm in diameter. The zygote germinated into a filamentous cell, which later branched at several sites. The nucleus gradually swelled and reached about 10 μm in diameter at seven days after germination. The number of chloroplast increased with time. The chloroplast contained one or, sometimes, two pyrenoids, each attending abundant starch (Fig. 1a). When germling cells were stained with DAPI, the nucleus emitted blue-white fluorescence of the DNA-DAPI complex and numerous, bright fluorescent nucleoids were distributed within the chloroplast exhibiting red autofluorescence of chlorophyll (Fig. 1b).

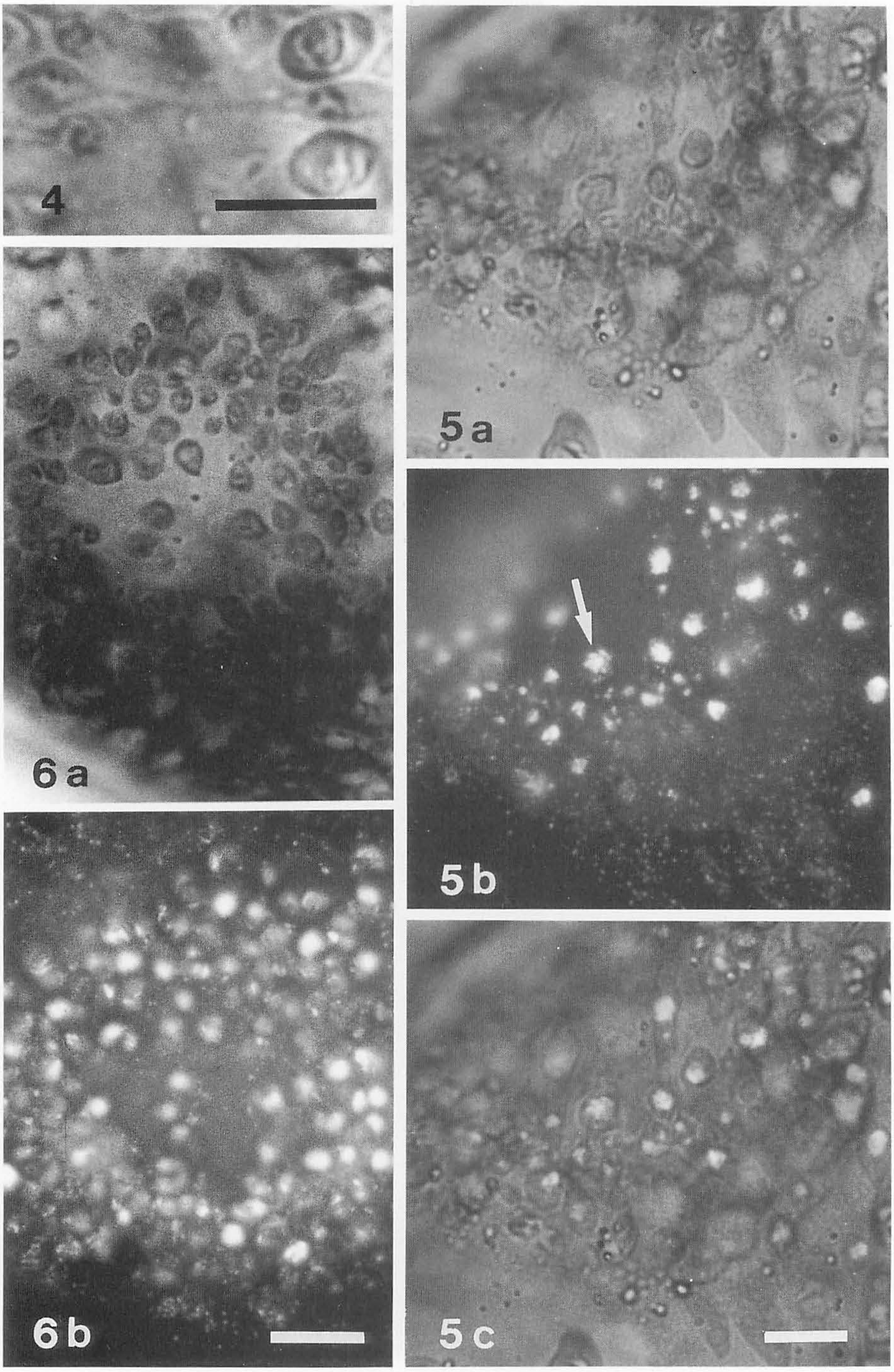
At about two weeks after germination the nucleus measured approximately 25 μm in diameter, and it was enveloped in a thick layer of cytoplasm. Most conspicuous in the perinuclear cytoplasm was the presence of chloroplasts which were different from many other chloroplasts by size and shape. As compared with the usual spindle-shaped chloroplasts (Fig. 2a), they were generally small and meager in starch, and they took various shapes, spherical, oblong, and so on (Fig. 3a). Their sizes also varied conspicuously. Some of the perinuclear chloroplasts remarkably extended in one direction and the extending parts sometimes branched irregularly (Fig. 3a, arrowhead). In some cases, the extending chloroplast assumed a dumbbell-shape (Fig. 4). The usual spindle-shaped chloroplast contained 40–60 nucleoids, which were ca. 0.2 μm in diameter and scattered uniformly within the chloroplast except in pyrenoids (Fig. 2b). By contrast, most of the perinuclear chloroplasts included nucleoids densely (Fig. 3b). A remarkable feature of this stage is the presence of chloroplast with few nucleoids (Fig. 3b, arrow). Several days later some of the perinuclear chloroplasts attended one or a few large nucleoids (Figs. 5a–c), which sometimes attained 3.0 μm in diameter. Some of these large nucleoids seemed to consist of several bright regions (Fig. 5b, arrow). The number of chloroplast bearing these relatively large, bright nucleoids gradually increased. In 20-day-old germling cells the majority of the perinuclear chloroplasts included one or a few large bright nucleoids (Figs. 6a, b). Remarkably extending chloroplasts were rarely observed at this stage.

Discussion

The present investigation revealed that small chloroplasts with poor starch, or heterogeneous chloroplasts, appeared near the primary nucleus at about two weeks after germination (Fig. 3a). In *Bryopsis* the male gametangial chloroplasts degenerate markedly during gametogenesis, becoming small and yellowish (Burr and West 1970, Ogawa 1988). These degenerate chloroplasts lacked DNA discernible with DAPI staining (Kuroiwa and Hori 1986, Ogawa 1988), and eventually they were destroyed after copulation. Accordingly, the chloroplast of the female gamete origin is the parent of the perinuclear heterogeneous chloroplasts. The giant primary nucleus of 14-day-old germling



Figs. 1–3. Light micrographs of germling cells of *Bryopsis plumosa*. a, ordinary transmitted light; b, fluorescence. 1a and 1b. The same field of part of a seven-day-old cell. Each chloroplast contains numerous nucleoids. Bar=10 μm. 2a and 2b. The same view of part of a 14-day-old cell. Nucleoids are uniformly distributed throughout the chloroplasts except in pyrenoids. Bar=10 μm. 3a and 3b. The same field of perinuclear part of a cell of 14 days old. Some of the perinuclear chloroplasts extend, and the extending part of a chloroplast (arrowhead) branches irregularly. Perinuclear chloroplasts contain nucleoids densely. A small chloroplast with few nucleoids (arrow). Bar=10 μm.



Figs. 4–6. Light micrographs of germling cells of *Bryopsis plumosa*. a, ordinary transmitted light; b, fluorescence; c, superimposition of a and b. 4. Perinuclear part of a 14-day-old cell. An extending chloroplast takes a dumbbell-shape. Bar=10 μm. 5a, 5b and 5c. The same view of perinuclear part of a 16-day-old cell. Perinuclear chloroplasts often contain large nucleoids, some of which seem to consist of smaller ones (arrow). Bar=10 μm. 6a and 6b. The same view of perinuclear part of a cell of 20 days old. In most of perinuclear chloroplasts large nucleoids are present. Conspicuously extending chloroplasts cannot be seen. Bar=10 μm.

cell often attended chloroplasts which extended unidirectionally (Fig. 3a) and sometimes took a dumbbell-shape (Fig. 4). The frequent appearance of these chloroplasts was typical of this stage. The frequency of occurrence of them decreased with time, whereas, inversely, the number of small lenticular chloroplast increased (Fig. 6a). Fluorescence microscopy revealed that nucleoids were distributed less dense in stretching parts than in pyrenoidal peripheries of the extending chloroplast and that small lenticular chloroplasts with few nucleoids were often present near the primary nucleus of 14-day-old cells (Fig. 3b, arrow). Based on these observations the initial process of occurrence of perinuclear small chloroplasts may reasonably be explained as follows: Some of the chloroplasts situated near the primary nucleus first extend remarkably and, then, they are torn off or divide into smaller ones.

Most of the perinuclear chloroplasts first contained small nucleoids densely (Fig. 3b), but later some of them included one or a few large nucleoids (Fig. 5b). The large nucleoid sometimes seemed to consist of several smaller fluorescent regions (Fig. 5b, arrow). These results suggest that the large nucleoid might result from the accumulation of smaller ones. It is unclear why the perinuclear chloroplasts of 14-day-old germling cells contain nucleoids densely. DNA may be highly replicated in these chloroplasts.

The vegetative cell of the Dasycladalean alga *Acetabularia* is known to include chloroplasts both with and without DNA (Woodcock and Bogorad 1970, Coleman 1979, etc.). Although the intracellular distribution of the DNA-deficient chloroplast is heterogeneous, it exists throughout the vegetative cell (Lüttke 1981). In *Bryopsis*, however, the heterogeneous chloroplasts usually showed the exclusive perinuclear distribution (Saito *et al.* 1989). Our preliminary observation using 11-day-old germling cells revealed that the primary nucleus and the chloroplasts situated near the nucleus moved together slowly within the cell at least for several hours, while the other chloroplasts seemed to migrate randomly. The close association of chloroplasts and the giant primary nucleus may be established at this early stage.

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小川　茂*・浜田和行**・和田俊司**：**緑藻ハネモ発芽細胞における葉緑体の形態および核様体の分布における不均質性の出現**

緑藻ハネモ (*Bryopsis plumosa*) の発芽細胞において，他の葉緑体と形態および DNA の分布で異なる葉緑体の出現する過程を調べた。発芽後約 2 週間すると，小型で，澱粉粒をあまり含まない葉緑体が発芽細胞の巨大 1 次核周辺に現れた。核周辺には，著しく伸長した葉緑体もしばしばみられた。これら核周辺葉緑体には核様体が密に分布していた。数日後，核周辺葉緑体には大きい核様体がみられた。大きい核様体を有する葉緑体の数は徐々に増加したが，著しく伸長した葉緑体の出現頻度は低下した。今回の観察にもとずき，大きい核様体を有し，小型の葉緑体が巨大 1 次核周辺に選択的に出現する過程について考察した。（*943 新潟県上越市山屋敷町　上越教育大学自然系生物，** 980 仙台市青葉区荒巻字青葉　東北大学理学部生物学教室）