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[依頼論文]

Immunolocalization of Chloroplast Nucleoids of Synchronized *Chlamydomonas reinhardtii* by Use of a Monoclonal DNA Antibody

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Cells of *Chlamydomonas reinhardtii* were synchronized under a 12 : 12 h light : dark regimen. Immunogold anti-DNA labeling clearly occurred over nuclei, chloroplasts and mitochondria in synchronized cells. Computeraided reconstruction of three-dimensional model of the cells revealed the spacial distribution of these DNAs in synchronized cells. Chloroplast-nucleoids temporarily migrated to near the pyrenoid forming the large clusters (5 to 6) of gold particles during the first few hours and also at the end of light period during the cell cycle. In any particular section, deposition of gold particles was seen simultaneously over the pyrenoid region, starch grains and the thylakoid membranes in a chloroplast.

Key words: *Chlamydomonas reinhardtii,* chloroplast-nucleoid, anti-DNA monoclonal antibody, pyrenoid.

Introduction

Chloroplast nucleoids were first described in *Chlamydomonas moewusii* by Ris and Plaut[1] using electron micorscopy and with light microscope using acridine and Feulgen staining. They reported one or more irregularly shaped DNA-containing bodies near the pyrenoid in *C. moewusii.* Goodenough[2] also observed chloroplast-nucleoids near the pyrenoid in *C. reinhardtii* by electron microscopy. The gathering of cp-nucleoids around the pyrenoid has been observed in *Chlamydomonas* and various other algae using a DNA fluorochrome 4′,6-diamidino-2-phenylindole (DAPI)[3-5]. Miyamura and Hori[6] repored the specific localization and unique shape of cp-nucleoid in the pyrenoid of *Caulerpa okamurae.* Ehara *et al.*[7] have shown using DAPI that changes in profile and distribution of cp-nucleoids occur in synchronized *C. reinhardtii*: the formation of the compact aggregate of cp-nucleoids around the pyrenoid occurred twice during the cell cycle. They also suggested that different morphologies of cp-nucleoids are due to various configurations of protein components[7]. Recently, Zhang and Wu[8] demonstrated that the concomitant changes of the abundance and the distribution of frx B protein with the behavior of cp-nucleoid in *C. renhardtii.*

In the present report we describe the three-dimensional immunolocalization of cp-nucleoids in the vicinity of the pyrenoid in synchronized *C. reinhardtii* using mouse anti-DNA monoclonal antibody (MAB 030) followed by 15 nm gold-conjugated secondary goat anti-mouse IgG antibody.

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Materials and Methods

The algal strain used was *Chlamydomonas reinhardtii* (Chlorophyta) obtained from the Algal Culture Collection of the University to Tokyo (IAM C-9).

Synchronization of cells

Methods of culture and synchronization of algal cells (under a 12 hr light-12 hr dark regimen) have been described previously by Osafune *et al.*[9].

Immunoelectron microscopy

For electron microscopy, cells were fixed with 4% paraformaldehyde (16% solution: EM Sci., USA) and 0.1% glutaraldehyde (v/v) with cacodylate buffer (pH 7.2) for 45–60 min at 4℃. The cells were then washed 3 times with cacodylate buffer alone by centrifugation and the pellet was embedded in 2% agarose which was then cut into small cubes. The cubes were passed through an ethanol series (50% to 9% v/v) and were then placed in acetone. The cubes were then embedded in L. R. White resin medium grade (The London Resin Co., Ltd., Hampshir, England). Rolymerization of the resin was carried out at 50℃ for 3 days. Thin sections, cut with a diamond knife on a

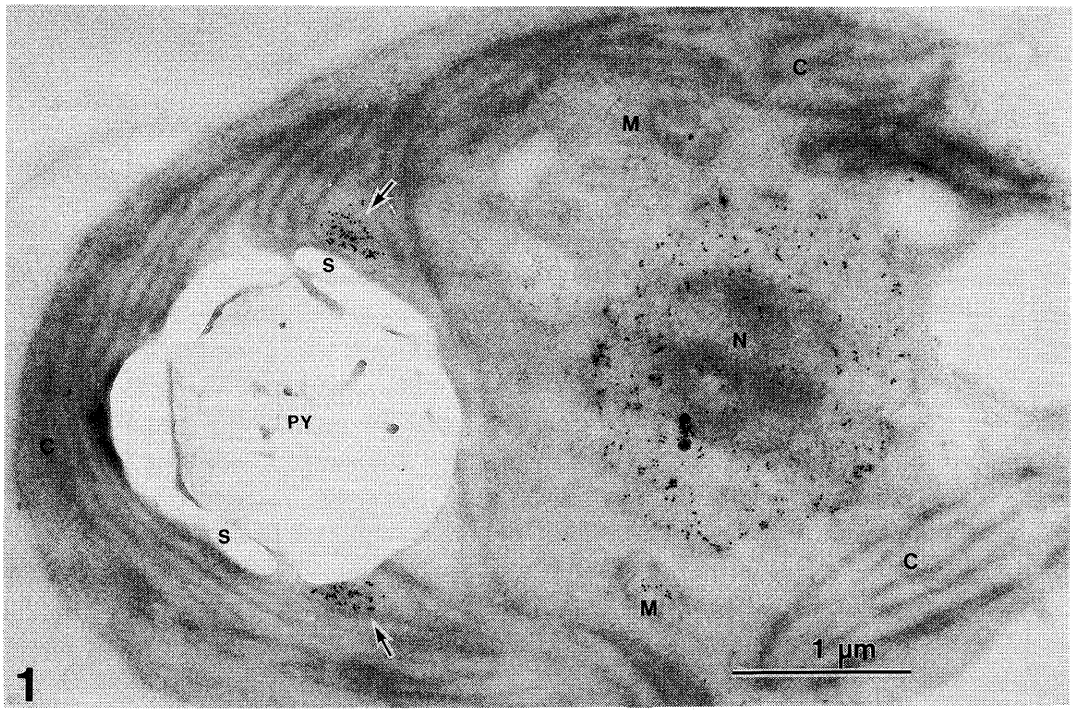

Fig. 1. Immunogold anti-DNA labeling of a section through a cell of *Chlamydomonas reinhardtii* 4 hrs into the light period. The nucleus, chloroplast and mitochondria are clearly labeled with gold. The arrows show the regions of chloroplast-nucleoids and the arrowheads show mitochondrion. ×28,000.
Abbreviations used here and on subsequent pictures: C, chloroplast; M, mitochondrion; N, nucleus; PY, pyrenoid; S, starch.

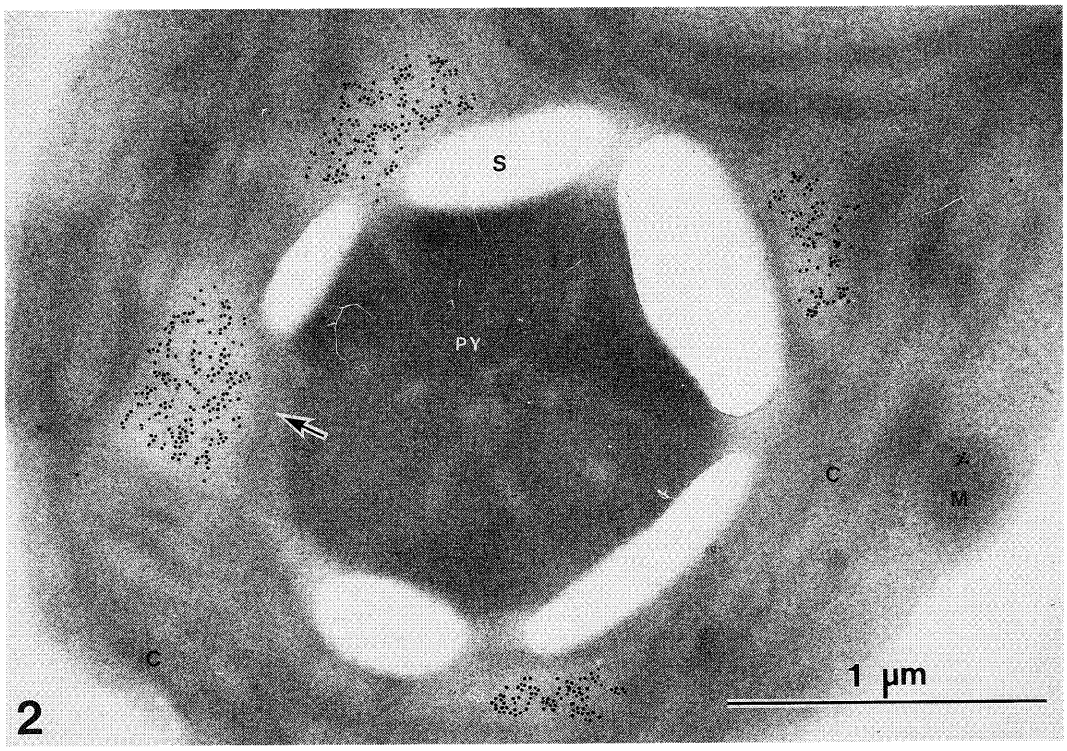

Fig. 2. A high magnification of a section of the pyrenoid region in a cell 4 hrs into the light period. Note that cluster (at the arrow) of gold particles is present over stroma in chloroplast section, but not over the pyrenoid or starch grains. A small amount of gold label is seen over mitochondrion (M). ×57,000.

Sorvall MT-1 microtome, were placed on nickel slit grids which were floated section side down on drops of 0.01 M potassium phosphate buffer (pH 7.4) containing 0.85% (w/v) sodium chloride (phosphate-buffered saline, PBS) and additionally, 0.5% (w/v) bovine serum albumin, for 30 min at room temperature. The grids were then floated on PBS containing the mouse anti-DNA monoclonal antibody MAB030 (Chemicon, CA, USA) at 20–40 fold dilution for overnight at 4℃[10, 11]. The grids were then washed twice in PBS puls 0.05 Tween 20 (Sigma) by flotation, and were then floated on PBS containing a 20 fold dilution of goat anti-mouse IgG 15 nm gold conjugate (Zymed, USA) for 20 min. The grids were subsequently floated on 3% (w/v) uranyl acetate for 30 min and after drying were viewed in a JEOL 100B or a JEOL 100CX electron microscope at 80 kV[12, 13].

Computer-aided three-dimensional distribution of DNA

Using Nikon's Cosmozone programmer, these data were entered on a digitizing table[14, 15]. The three-dimensional data in the computer were viewed on a color display (NEC) and the image was rotated in space until a proper angle of view was obtained.

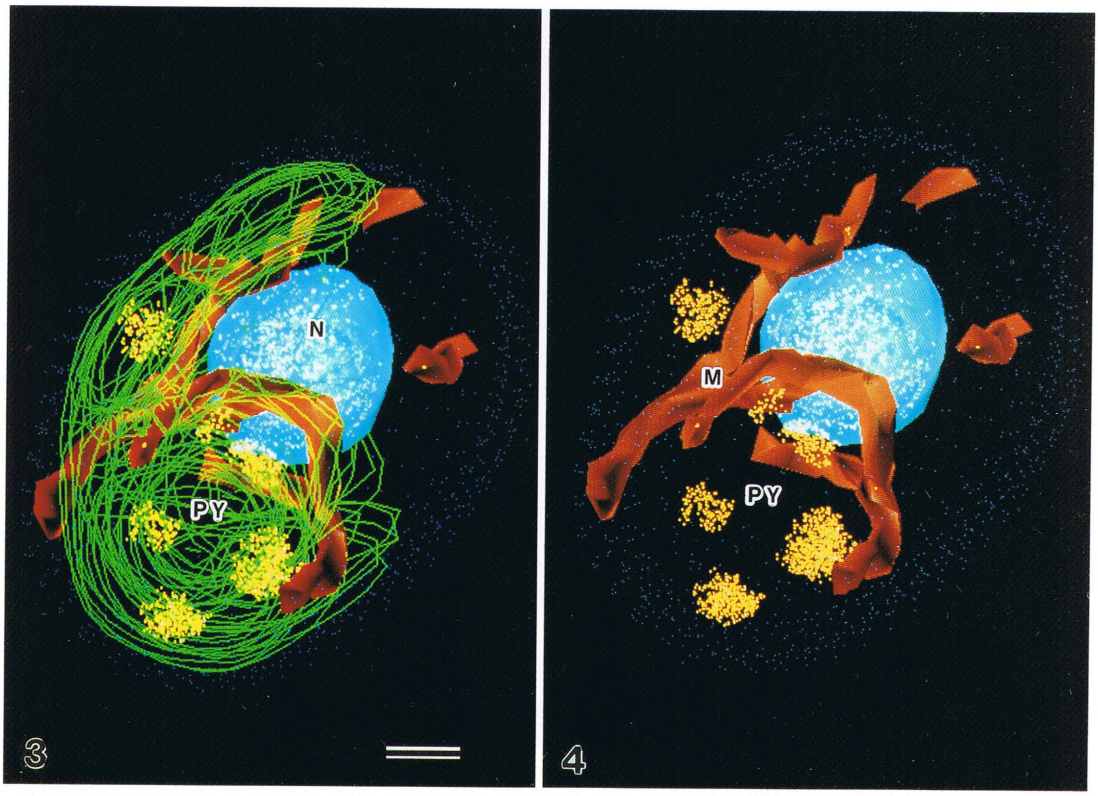

Fig. 3. An inside view of a three-dimensional model of a cell 4 hrs into the light period reconstructed with the aid of a computer. A sky blue color shows nucleus, mitochondria are red and the chloroplasts are green. Yellow dots show gold particles (cp-nucleoids) and blue dots indicate the membrane. Note that large clusters of gold particles present near the pyrenoid region. The white-arrow indicates region of basal body that no DNA could be seen around them. ×11,500.
Fig. 4. Chloroplast (green) was eliminated in the same cell shown in Fig. 3. It is clearly seen that the pyrenoid regin is surrounded by 5 clusters of gold particles. ×11,500.

Results and Discussion

A monoclonal antibody has been described which specifically reacts with DNA both in double and single-stranded forms but not with other molecules and structures[16, 17]. In Fig. 1, gold particles can be seen over the nucleus, mitochondria (as the arrowheads indicate) and chloroplast (the arrows indicate) of a cell of *Chramydomonas reinhardtii* 4 hrs after the onset of light. No gold is visible in the same locations in sections exposed to DNase prior to the application of the primary anti-DNA monoclonal antibody MAB 030 (data not shown). A small amount of gold label is seen over mitocondria, but the chloroplast is labeled more heavily than the nucleus. The arrows show that the large clusters of gold are present over in the vicinity of the pyrenoid in the chloroplast (Fig. 1). The results in this work are in accordance with our previous observations that in synchronized *C. reinhardtii* cells, mots of the cpnucleoids gathered around the pyrenoid forming a compact mass (cf. Ehara *et al.*, Fig. 6-B)[7] twice during the light period. Ehara[7] *et al.* also suggested possible functional

relations between the cp-nucleoids and pyrenoid. Miyamura and Hori[6] reported the presence of DNA in the pyrenoid matrix of *Caulerpa okamurae,* but it is difficult to study the cpnucleoid structrues and distributions in chloroplast using DAPI-fluorescence microscopy. As seen from Fig. 2, gold particles (cp-nucleoids) are in large clusters over stroma region (as the arrow indicates) near the pyrenoid (the pyrenoid is surrounded by the several clusters of gold) and no gold (anti-DNA labeling) is clearly detected over the pyrenoid or thylakoid membranes.

Computer-aided construction of three-dimensional models of chloroplasts, nuclei and mitochondria as well as distribution of DNAs from serial section profiles supported (Figs. 3 and 4) supported the inference already mentioned[7]. Chloroplast-nucleoids are clustered in stroma around the pyrenoid; five cp-nucleoids in the form of compact aggregates surrounded the pyrenoid region (Fig. 3 and 4). The arrangement of gold particles (cp-nucleoids) are seen to be spherical or oval in shape by rotating the images on a color display in the computer. In addition, no DNA could be detected in or around basal bodies on computer-aided reconstructions of *C. reinhardtii* (Fig. 4).

The present study clearly showed that cp-nucleoids are clustered around the pyrenoid using DNA-immunoelectron microscopy. As shown previously,[7] cp-nucleoids in the form of compact aggregates surrounding the pyrenoid region occurred with maximal frequency (about 20%) around the 4th hrs and around 9th hrs into the light period in *C. reinhardtii* (when cells were on a 12 : 12 hrs light : dark regimen). Zhang and Wu[8] suggested that the amount of frx B protein around the pyrenoid is related to the cellular content and the distribution of cp-nucleoid in *C. reinhardtii.* Further experiments are necessary to explore the functional relations between cp-nucleoids and the pyrenoid.

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References

1) Ris, H. and Plaut, W.: Ultrastructure of DNA-containing areas in the chloroplast of *Chlamudomonas.* J. Cell Biol., **13**, 383–391 (1962).
2) Goodenough, UW.: Chloroplast division and pyrenoid formation in *Chlamydomonas reinhardtii.* J. Phycol., **6**, 1–6 (1970).
3) Coleman, AW.: Visualization of chloroplast DNA with two fluorochromes. Exp. Cell Res., **114**, 95–100 (1978).
4) Coleman, AW.: Dicersity of plastid DNA configuration among classes of eukaryote algae. J. Phycol., **21**, 1–16 (1985).
5) Kuroiwa, T., Suzuki, T., Ogawa, K. and Kawano, S.: The chlorplast nucleus: distribution, number, size, and shape, and a model for the multiplication of the chloroplast genome during chloroplast development. Plant Cell Physiol., **22**, 381–396 (1981).
6) Miyamura, S. and Hori, M.: Presence of DNA in the pyrenoid matrix of the siphonous geen algae, *Caulerpa okmurae* Web. v. Bos. Plant Morphol., **1**, 19–22 (1989).
7) Ehara, T., Ogasawara, Y., Osafune, T. and Hase, E.: Behavior of chloroplast nucleoids during the cell cycle of *Chlamydomons reinhardtii.* (Chlorophyta). J. Phycol., **26**, 317–323 (1990).
8) Zhang, Y. and Wu, M.: Fluorescence microscopy on dynamic changes of frx B distribution in *Chlamydomonas reinhardtii.* Protoplasma, **172**, 57–63 (1993).
9) Osafune, T., Mihara, S., Hase, E. and Ohkuro, I.: Electron microscope studies on the vegetative cellular life cycle of *Chlamydomonas reinhardtii* Dangeard in synchronous culture. I. Some characteristics of changes

in subcellular structures during the cell cycle, especially in formation of giant mitochondria. Plant Cell Physiol., **13**, 211–227 (1972).

10 Uchida, H., Nozue, F., Kuroiwa, H., Osafune, T., Sumida, S., Ehara, T. and Kuroiwa, T.: Evidence for preferential digestion of male-derived chloroplast DNA in young zygotes of *Chlamydomonas reinhardtii* by histochemical immunogold electron microscopy. Cytologia, **57**, 463–470 (1992).

11) Osafune, T., Ehara, T., Hase, E. and Schiff, JA.: Stage-dependent association of nuclear and chloroplast DNA molecules through bridges transiently formed between the two organelles in synchronized cells of *Euglena gracilis*. J. Electron Microsc., **42**, 197–201 (1993).

12) Osafune, T., Hase, E. and Schiff, JA.: Stage-dependent localization of LHCP II apoprotein in the Golgi of synchronized cells of *Euglena gracilis* by immunogold electron microscopy. Exp. Cell Res., **193**, 320–330 (1991).

13) Osafune, T., Hase, E. and Schiff, JA.: Accumulation of LHCP II apoprotein in wax-rich cells of *Euglena* in low light or in the presence of streptomycin. J. Struct. Biol., **109**, 97–108 (1992).

14) Osafune, T., Sumida, S., Ehara, T. and Hase, E.: Three-dimensional distribution of ribulose-1,5-bisphosphate carboxylase/oxygenase in chloroplasts of actively photosynthesizing cell of *Euglena gracilis*. J. Electron Microsc., **38**, 399–402 (1989).

15) Osafune, T.: Three-dimensional distribution of LHCP II apoprotein immunoreaction in synchronized cells of *Euglena*. Plant Morphol., **3**, 49–55 (1991).

16) Hansmann, P., Falk, H., Scheer, U. and Sitte, P.: Ultrastructural lcalization of DNA in two *Cryptomonas* species by use of a monoclonal DNA antibody. Eur. J. Cell Biol., **42**, 152–160 (1986).

17) Johnson, KA. and Rosenbaum, JL.: The basal bodies of *Chlamydomonas reinhardtii* do not contain immunologically detectable DNA. Cell, **62**, 615–619 (1992).

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