Presence of DNA in the pyrenoid matrix of the siphonous green alga, Caulerpa okamurae Web. v. Bos.

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Summary The specific localization and unique shape of the chloroplast nucleoid (ct-nucleoid) in the pyrenoid of C. okamurae Web. v. Bos. were revealed by fluorescence microscopy after staining with the DNA specific fluorochrome 4'6-diamidino-2-phenylindole (DAPI).

Key words: Caulerpa okamurae, chloroplast DNA, chloroplast nucleoid, DAPI, fluorescence microscope, pyrenoid, siphonous green alga.

The pyrenoid is an integral part of the chloroplast, which is found in many eukaryotic algae and a few cryptogamic plants. Its duplication is linked to chloroplast division, in that chloroplast cleavage often takes place through the pyrenoid (Goodenough 1970, Hori and Green 1985). Although the main component of pyrenoids has been shown to be Ribulose-1,5-Bisphosphate carboxylase (RuBisco) (Holdsworth 1971, Satoh et al. 1984, Lacoste-Royal and Gibbs 1987, Kuchitsu et al. 1988), a key enzyme in carbon dioxide fixation, other constituents and functions of the pyrenoid are unknown. Most investigations have revealed that pyrenoids do not contain DNA except in Streptotheca thamesis in which electron microscopic observation combined with DNase treatment indicated the possible presence of DNA in the pyrenoids (Esser 1967). However, it is in general very difficult to demonstrate conclusively the presence of DNA in pyrenoids by electron microscopy. Recently, DNA within chloroplasts has been observed by fluorescence microscopy after staining with DAPI (Coleman 1978, James and Jope 1978). Kuroiwa et al. (1981) and Coleman (1985) showed the diversity of the distribution, number, size, and shape of ct-nucleoids in variety of plants using this technique. In the present study, as the result of a survey of ct-nucleoid arrangements in many algae, we were able to demonstrate the

presence of DNA (ct-nucleoid) in the pyrenoid matrix of C. okamurae by fluorescence microscopy.

C. okamurae was collected at Shichirigahama, Kanagawa Prefecture, in July, 1988, and was maintained in an aquarium under natural daylight conditions. Thalli of C. okamurae were cut into small pieces, fixed with 1% glutaraldehyde and stained with 1 µg/ml DAPI dissolved in S buffer as described by Kuroiwa and Suzuki (1980). Vital staining was carried out with 1 µg/ml DAPI dissolved in S buffer. For DNase treatment, 13 units/ml of DNase I (type IV, Sigma) dissolved in 0.08 M CH₃COONa (pH 5.0), 4 mM MgSO₄ and 0.025 M NaCl were added to the specimens after staining with DAPI. Observations were made with an Olympus BHS-RFK fluorescence microscope equipped with phase contrast optics.

In chloroplasts of C. okamurae, the pyrenoid exists in the centre of a large chloroplast and its matrix is usually surrounded by a horseshoe-shaped starch plate with a single orifice (Figs. 1 and 2). The pyrenoid matrix is composed of fine granular material and is not surrounded by any type of membranous component. A large spherical fluorescent spot was observed in the pyrenoid matrix after staining with DAPI (Figs. 3 and 4). This fluorescent spot had a projection which penetrated the orifice of the starch sheath. In a further experiment, chloroplasts were treated with DNase after vital staining with DAPI in order to know whether this fluorescence is derived from DNA, from starch sheath or from an unknown substance in the pyrenoid matrix. Brilliant fluorescent spots in the chloroplasts (Figs. 5 and 6) disappeared after DNase treatment (Fig. 7). It may be reasonably concluded, therefore, that each fluorescent spot represents a ct-nucleoid (ct-DNA).

The arrangement of ct-nucleoids has been investigated in many plant species. Kuroiwa et al. (1981) showed that most plants could be classified into five types according to difference in the shape, size and distribution of the ct-nucleoid in their mature chloroplast. Coleman (1985) also observed the diversity of ct-nucleoid configuration in 100 species of eukaryotic algae, representing all classes. However, ct-nucleoid have never been observed in the pyrenoid matrix.

Figs. 1-7. Chloroplasts of *C. okamurae*. 1. A chloroplast observed by phase contrast microscopy. x1750. 2. A chloroplast observed by electron microscopy. x7200. 3. The same chloroplast as shown in Fig. 1 observed by DAPI-fluorescence bright field microscopy. x5350. 4. The same chloroplast as in Fig. 1 observed by DAPI-fluorescence and phase contrast microscopy. x5350. 5. Chloroplasts observed by DAPI-fluorescence microscopy before DNase treatment. x2100. 6. The same chloroplasts as in Fig. 5 observed by DAPI-fluorescence and phase contrast microscopy. x2100. 7. The same chloroplasts as in Fig. 5 observed by DAPI-fluorescence and phase contrast microscopy after DNase treatment. x2100. P:Pyrenoid matrix. S:Starch sheath around the pyrenoid matrix.

More recently, the behavior of the ct-nucleoids in Chlamydomonas reinhardtii has been shown to be of great significance (Nakamura et al. 1986). In this species, several ct-nucleoids are distributed throughout the mature chloroplast. In one-month old culture, they become assembled into one large ct-nucleoid near the pyrenoid. Thus, it seems likely that there is a functional relationship between the arrangement of the ct-nucleoid, the role of pyrenoid and the physiological condition of the cell.

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宮村 新一・堀 輝三(筑波大・生物科学系): 囊状緑藻フサイワヅタのピレノイドに局在するDNA

DNA特異的蛍光色素 4',6-diamidino-2-phenylindole (DAPI) 染色による蛍光顕微鏡観察法によって、藻類の葉緑体核様体の形状、分布について広く調査した結果、囊状緑藻の一種であるフサイワヅタ Caulerpa okamurae Web. v. Bos. のピレノイドにDNAの局在することをみいだした。

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