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RESEARCH NOTE

Use of immunofluorescence microscopy to compare small nuclei in two populations of *Caulerpa taxifolia* (Caulerpales, Chlorophyta)

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Nuclei from two populations of the siphonous green alga *Caulerpa taxifolia* (Vahl) C. Agardh were labeled with fluorescent antihistone antibodies and examined by confocal microscopy. Because the nuclei are small (2 μm diameter), it is difficult to study them by conventional methods, and DAPI staining does not distinguish the nuclei from intracellular bacteria. Using immunofluorescent microscopy, it was possible to recognize nuclei and chromosomes in dividing nuclei. This technique is useful for studying nuclei and counting chromosomes to assess ploidies of life history stages of algae, especially those with small nuclei.

INTRODUCTION

Studies of the nuclei of siphonous green algae have progressed by innovative application of standard methods to large coenocytic cells (De & Berger 1990; Kapraun 1993, 1994). The general problem of localizing nuclei in aseptate cells is compounded by special cellular characteristics, such as lack of karyokinesis periodicity, viscosity of the cytoplasm, thick cell walls, cell wall extensions into the cytoplasm, small nuclei, and the presence of intra- and extracellular bacteria.

Caulerpa is a large (up to 2 m long), coenocytic, unicellular, green alga distributed throughout the tropical and subtropical zones. The siphonous thallus is organized into a stolon with apical growth and photosynthetic blades with species-specific morphologies. Delicately branched wall thickenings called trabeculae are intimately associated with the thin layer of cytoplasm. *Caulerpa taxifolia* (Vahl) C. Agardh, a common species in the tropics, has been artificially introduced into the Mediterranean Sea (Meinesz & Hesse 1991) and has thrived as an invasive weed with growth and developmental characteristics different from those of natural populations of the same species (Caye *et al.* 1996; Komatsu *et al.* 1997). The true geographical origin of the Mediterranean population is not known. The present study compares the nuclear characteristics of populations from the tropics and the Mediterranean Sea using anti histone immunofluorescence microscopy.

In his ultrastructural study of *Caulerpa prolifera* (Forsskal) Lamouroux, Sabnis (1969) pointed out the small size of the nuclei, 1.5–2.0 μm diameter, and their sparse distribution in the cytoplasm relative to other organelles. Hori (1981) showed that the nuclear envelope of the 2-μm-diameter nucleus in *Caulerpa brachypus* Harvey remains intact throughout mitosis. Kapraun (1994) has done the only measurements of DNA content of *Caulerpa* nuclei by cytophotometry of DAPI (4′,6-diamidino-2-phenylindole 2HCl) staining. The four species he studied showed considerably smaller nuclear DNA contents than other members of the Caulerpales. Also, in *Caulerpa prolifera* the 'supposedly haploid' macrothallus contained nuclei with 2C and 4C DNA amounts and nuclei in the 'supposedly diploid' microthallus had 4C and 8C amounts. Kapraun interpreted his results as indicating a degree of 'polyploidy or endopolyploidy' in both stages. Preliminary studies in our laboratory showed that nuclei in *C. taxifolia* stolons are about 2 μm in diameter (Caye *et al.* 1996). Extra- and intracellular bacteria have been isolated from *C. taxifolia* (Chisholm *et al.* 1996). Because DAPI staining does not readily distinguish bacteria from nuclei, immunofluorescent antihistone labeling of eukaryotic nuclear chromatin (Montag *et al.* 1990) is an efficacious way to study nuclear development in *Caulerpa.* These techniques have been applied successfully in a study of the large (> 100 μm diameter) primary nucleus of *Acetabularia acetabulum* (L.) Silva (Liddle *et al.* 1997).

MATERIAL AND METHODS

Cultures of *Caulerpa taxifolia* from the Port of Villefranche-sur-Mer (France) and Martinique (Caribbean Sea) were maintained in filtered seawater at 25°C under c. 150 μmol m^{-2} s^{-1} (Blue Moon Fluorescent, General Electric, UK). Cytoplasm from apical regions of the stolons of healthy cells was pipetted first into seawater, then transferred to 7 mM phosphate buffer containing 100 mM Pipes, 1 mM $MgCl_2$, 10 mM EGTA, 100 mM KCl, and 5% DMSO, pH 6.8, in Eppendorf tubes. Solution changes were made without centrifugation to avoid disruption of the viscous cytoplasm. Best results for DAPI staining were obtained with solutions of 10–20 μg ml^{-1} on unfixed cytoplasm on glass slides.

For immunofluorescence labeling, cytoplasm was fixed for 15 min in 4% formaldehyde and 2% glutaraldehyde in phosphate buffer. It was then washed three times in buffer, 10 min each, followed by 30 min in 1% Triton in phosphate buffer, which was rinsed out by three 10-min washes in buffer/0.063 M glycine. Aldehydes were reduced with 0.1% sodium borohydride in buffer/glycine for 15 min followed by three 10-min washes in buffer/glycine. The cytoplasm was then incubated with sheep anti-histone core (Interchim, Montluçon, France) diluted 1 : 100 in buffer/glycine for 60 min at 37°C or overnight at room temperature. Antibodies were rinsed by three 20-min washes of buffer/glycine. Incubation in the second antibody, an FITC-conjugated affini pure rabbit anti-sheep IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA), was at 37°C for 60 min. Excess second antibody was rinsed out by three 20-min buffer/glycine washes. To stabilize the fluorescent label, aliquots of 0.7 µl fixed and labeled cytoplasm were transferred to multiwell slides (Poly Labo, Strasbourg, France), each 7-mm-diameter well containing an antibleaching solution of 2% DABCO diazabicyclo (2.2.2) octane (Sigma), 49% Vectashield (Biosys, France) and 49% glycerol (fluorescence microscopy grade). Coverslips were applied and then sealed with nail polish.

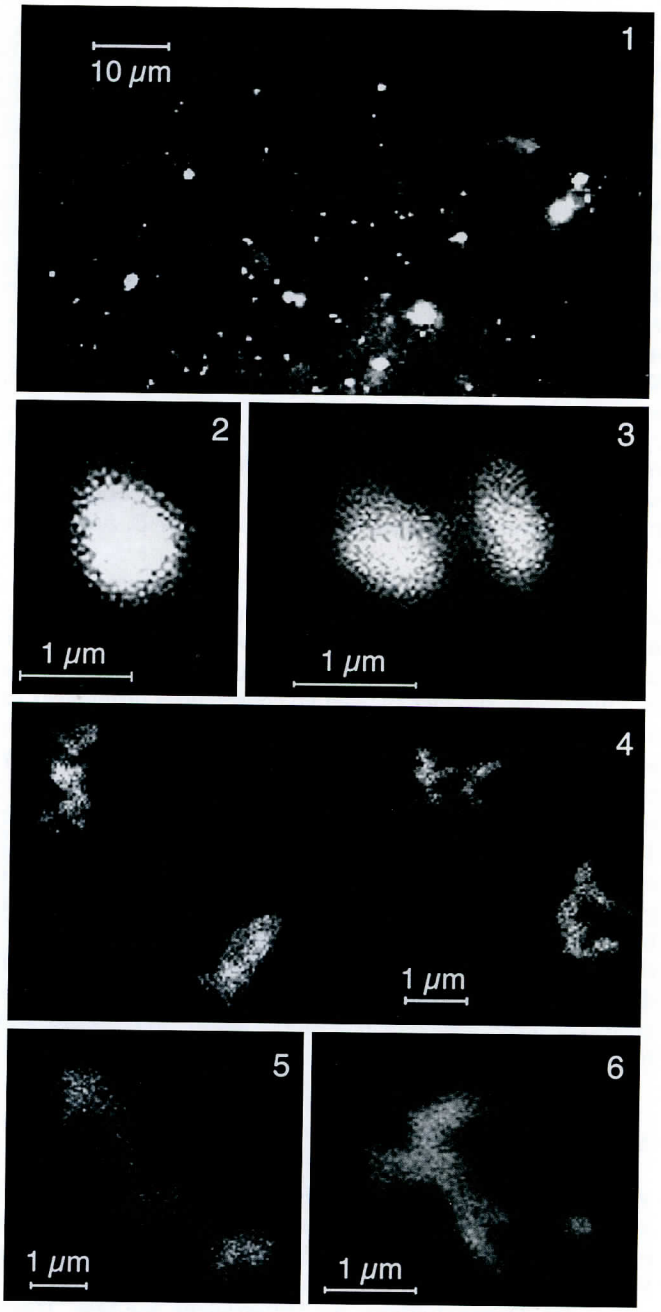
DAPI-stained material was observed with the epifluorescent system of an inverted Nikon Diaphot microscope with a xenon 100 W lamp. A CCD black and white 10^{-6} lux camera captured the images, which were displayed on a computer monitor. The excitation filter was 360 nm and the emission filter was 450 nm, with a 400 nm dichroic mirror. Images were saved using Fluo software (IMSTAR, Paris, France).

Antibody-labeled nuclei were observed using confocal laser scanning immunofluorescence microscopy (CLSM) on a Leica Diaplan microscope with a Leica spot system. The fluorescence was excited with an argon/krypton ion laser with three wavelengths: 488, 568, and 647 nm. The FITC excitation filter, KP510 (Leica), and emission filters OG 515 or OG 530 (Leica) were used. Images were processed with NIH software (National Institutes of Health, Bethesda, MD, USA). Observations were recorded as a series of optical slices.

RESULTS

Abundant nuclei were observed in cytoplasm from the stolons and fronds of *C. taxifolia* prepared by DAPI staining and by immunofluorescent labeling. DAPI-stained nuclei were also observed during gametogenesis and in whole rhizoids. Nuclei were spherical or ovoid (Fig. 1) and of comparable sizes from all parts of the organism. In stolon cytoplasm, DAPI-stained nuclei, because of their small sizes, were distinguishable from bacteria primarily by their location. Bacteria were observed in the vacuole and on the surface of intact cell walls, especially on rhizoids, whereas nuclei were in the droplets of cytoplasm. The mixing of the two in making preparations was likely (Fig. 1).

Anti-histone immunofluorescent-labeled nuclei were observed (Figs 2, 3) and chromosomes could be distinguished (Figs 4–6). The average area of median optical sections of nuclei from Mediterranean specimens was 2.0 ± 0.2 µm² (mean ± SE, n = 61) and from Martinique specimens was 2.0 ± 0.3 µm² (n = 28). The range in diameter was 0.6–4.7 µm and 0.6–4.2 µm, respectively. A wide range of sizes due to the expansion and separation of chromatin were observed and measured, presumably because the nuclear envelope remains intact (Hori 1981). Large nuclei (5 µm diameter) were in the late anaphase or early telophase stages. Three condensed chromosomes (Figs 5, 6) were clearly observed in four



Figs 1–6. Fluorescent images of nuclei and chromosomes extracted from stolons of Mediterranean specimens of *Caulerpa taxifolia.*
Fig. 1. DAPI-stained nuclei and bacteria.
Figs 2–6. Histone immunofluorescent-labeled nuclei and chromosomes viewed by confocal microscopy.
Fig. 2. Single interphase nucleus.
Fig. 3. Two interphase nuclei in close proximity.
Fig. 4. Two pairs of late mitotic nuclei.
Figs 5, 6. Chromosomes reconstructed from numerous optical sections.

of the 27 scans from the Mediterranean and in two of the five scans from Martinique.

DISCUSSION

Anti-histone immunofluorescent labeling of the chromatin of nuclei of *C. taxifolia* is an effective technique for studying nuclear development and chromosomes in small nuclei. Although the relationship between nuclear size and DNA content varies among taxonomic groups, a linear relationship has been suggested for many coenocytic green algae (Kapraun 1994; Kapraun & Nguyen 1994). Our observations of small nuclei with a wide range of sizes in *C. taxifolia* conform to previous cytophotometric measurements of *Caulerpa prolifera* and *Halimeda macrophysa* Askenasy (Kapraun 1994). In addition, we observed three chromosomes in *C. taxifolia* compared to four chromosomes reported by Hori (1981) for *C. brachypus* Harvey.

Caulerpa taxifolia in the Mediterranean is more morphologically and physiologically robust than specimens from the tropics, the natural habitat. The observations of nuclei from natural populations in Martinique and introduced populations in Villefranche-sur-Mer showed no conspicuous differences in nuclear size or chromosome numbers. Further studies following Kapraun's suggestion that there is polyploidy in *Caulerpa* could help characterize the differences in DNA content between the *C. taxifolia* populations from all parts of the world. The amount of variation of nuclear DNA content among Pacific Ocean populations of *C. taxifolia* remains unknown. The present study shows that immunofluorescence is a useful tool for studying small nuclei. Further observations and quantification of fluorescence characteristics of nuclei at all stages of development could help establish a karyological profile of the genus *Caulerpa,* which includes more than 75 species and 100 forms.

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