Visualization of DNA-Containing Structures in Various Species of *Chlorophyta, Rhodophyta* and *Cyanophyta* Using SYBR Green I Dye

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ABSTRACT. We developed an alternative method of staining cell nuclei and chloroplast nucleoids of algal cells using SYBR Green I (the fluorescent dye used commonly for detecting dsDNA in agarose and polyacrylamide gels as an alternative to highly mutagenic ethidium bromide and for DNA staining of viruses and bacteria followed by flow cytometry, digital image analysis or real-time PCR), which enabled routine staining in vivo. Cells do not need to be fixed or treated chemically or physically before staining, thus the shape, size and position of DNA-containing structures are not affected. The fluorescence signal is sharp and reproducible. Examples of application of the method are shown in color microphotographs for representatives of eukaryotic algae from the taxa *Chlorophyta, Rhodophyta* and the prokaryotic *Cyanophyta*. The method is also useful for studying progress of the cell cycle in algal cells dividing by multiple fission, as shown by observation of changes in nuclear number during the cell cycle of the green alga *Chlamydomonas reinhardtii* and *Scenedesmus quadricauda*. Staining with SYBR Green I can be recommended as a fast, safe and efficient method for the detection of DNA-containing structures in vivo.

Immunofluorescence staining techniques and fluorescence microscopy constitute powerful tools for the cell biologist. Simultaneous staining of nuclei and protein structures with appropriate fluorescent dyes greatly facilitates the visualization of the location and shape of the cells. DNA in cells is usually stained with the fluorescent dye DAPI (4’,6-diamidino-2-phenylindole) for fluorescence microscopy (Williamson and Fenner 1975). When stained with DAPI, the DNA structures appear as blue-white fluorescence under UV illumination, and the positions of cell nuclei and organelle nucleoids can therefore be determined (Kuroiwa 1982; Suzuki et al. 1982).

For algal cells, DAPI staining of cell nuclei and nucleoids was introduced by Coleman (1978) and Kuroiwa and Suzuki (1980). The use of the method brought forth a series of papers on the morphology of chloroplast nucleoids and their localization and replication in the chloroplast of various green algae (Coleman 1978; 1979; Coleman et al. 1981; Lüttke and Bonotto 1981; Kuroiwa et al. 1981a,b; Coleman and Maguire 1982; Hashimoto and Murakami 1982; Hull et al. 1982; Cepák et al. 2002; Zachleder et al. 2004). DAPI staining has also made it possible to visualize structures containing DNA in some blue-green algae (Tschermak-Woess 1982; Cepák 1996).

A modified method of fluorescence staining of nuclei and nucleoids with DAPI was developed for chlorococcal algae (Zachleder and Cepák 1987). However, the method applied to algal cells required chemical or physical pretreatment. Moreover, the dye is highly mutagenic.

Recently, a variety of nucleic acid binding dyes has been developed, mostly for “in-gel” staining. The fluorescent dye SYBR Green I (*Molecular Probes*) was developed for detecting dsDNA in agarose and polyacrylamide gels as an alternative to highly mutagenic ethidium bromide. It has also been exploited for DNA staining of viruses and bacteria followed by flow cytometry (Chen et al. 1978; Broadaway et al. 2003), digital image analysis or real-time PCR (Aldea et al. 2002), and DNA staining for laser confocal
microscopy (Suzuki et al. 1997). It was also used for evaluation of the nuclear number in fungal mycelia (Meinhardt et al. 2001).

Here we show that SYBR Green I dye can be used to stain DNA-containing structures of various taxa of green, red and blue-green algae. It made staining in vivo possible without any chemical or physical pretreatment and the fluorescence signal was sharp and reproducible.

MATERIAL AND METHODS

Cells and culture conditions. Algal and cyanobacterial species Cosmarium holmiiense Vinatzer 1975/Innsbruck V 232, Cosmarium meneghiniii CZURDA-DENK 1927/Prague Ac.A 231, Merismopedia mituissima CEPAK 1994/1, Arthospira maxima LAPORTE 1963/M-132-2b, Pediasastrum boryanum KOVACIK 1979/4, Anabaena sp. TAKACOVA 1984/1, Fragilaria sp. (sample from nature), Pinnularia sp. (sample from nature), Botrydiopsis sp. KUBECKOVA 1999/22, Protosiphon botryoides PASHER/CAM 731-1a, Cyclotella meneghiniiana BUTTERWICK 1995/CCAP 1070-5, Pseudodendolonium sp. (sample from nature), Chlorhormidium pseudostichococcum PRINGSHEIM/Prague Ac.A 133, Euglena sp. (sample from nature), Porphyridium purpureum PRINGSHEIM 1951/Gott. 3290–1e, Haematococcus pluvialis TAKACOVA 1983/1, Chlamydomonas reinhardtii SMITH 1980/UTEX 2246, Scenedesmus obliquus TOMASELLI/Prague Ac.A 835, and Scenedesmus quadricauda Greifswald/15 were obtained from the Culture Collection of Autotrophic Organisms (Institute of Botany, Třeboň, Czechia).

For studying the progress of the cell cycle of algal cells dividing by multiple fission (C. reinhardtii, S. quadricauda), the cultures were synchronized by alternation of light and dark periods (14/10 h) in plate-parallel vessels (2.5- and 3.5-L). The vessels were illuminated from one side by fluorescent lamps (Osram L36W/41) at an incident irradiance of 100 W/m² of photosynthetically active radiation (PhAR) (400–720 nm) at the surface of the vessel and a temperature of 30 °C. Carbon dioxide concentration in the aerating gas mixture was 2 % (V/V). The inorganic nutrient solution was described by Zachleder and Šetlík (1982).

DNA structure staining. One part of 1 % (V/V) SYBR Green I ($E_x/E_m$: 497/520 nm) in TBE buffer (mmol/L: boric acid 90, Tris 40, EDTA 2; pH 7.6) was added to 2 parts of a cell suspension in nutrient solution; the mixture was incubated in darkness at room temperature. The staining time differed for different species. For chlorococcoid algae (Scenedesmus quadricauda), overnight treatment was used, for other algal species or cyanobacteria 2 h were sufficient.

For DAPI staining, 4’,6-diamidino-2-phenylindole hydrochloride (2 or 5 µg/mL) was used in S buffer (0.25 %, W/V, sucrose, 10 mmol/L Tris-HCl, 1 mmol/L EDTA, 0.6 mol/L spermidine, 0.05 %, W/V, 2-mercaptoethanol; pH 7.6).

Microscopy. Observation in transmitted light and fluorescence microscopy were carried out using a JenaLumar microscope (Carl Zeiss Jena) equipped with a filter set (excitation 350–500 nm, emission 510 nm) and a BX51 microscope (Olympus) equipped with a U-MWIBA2 filter block (excitation 460–490 nm, emission 510–550 nm). The photomicrographs were taken using a digital camera Camedia C-5050 Zoom (Olympus).

RESULTS AND DISCUSSION

Cell permeability to dyes. The cell walls of individual taxa of algae are distinctly different in their permeability to dyes, particularly, cell walls of chlorococcal algae, which are very strong, display low permeability to organic molecules. To test the permeability for the SYBR Green I dye, we chose the cenobial alga S. quadricauda which is known as a species with a cell wall that has a very low permeability to DAPI (Zachleder and Cepák 1987). Moreover, due to multiple fission of this species, cells have double cell walls before releasing daughter cenobia.

Samples from a synchronous culture taken successively during the cell cycle were stained with 0.3 % SYBR Green I at room temperature without any previous treatment. Potential bacterial contamination was eliminated by repeated washing (10×) with 0.01 % SDS. Because bacteria were easily stained with the dye, their presence was easily detectable. Samples were observed every hour by fluorescence microscopy to check the progress of staining. The permeability of the cell wall differed, depending on the age of the cells. The nuclei of daughter cells were stained after 6 h while mother cells after 10 or 12 h (Fig. 1). Thus, we found it convenient for this species to stain it overnight. The staining of the related species S. obliquus requires a shorter time of staining which is in agreement with the higher permeability for DAPI (Cepák et al. 2005).