Visualizing Mitochondrial and Plastid Nuclei in Thin Sections of DAPI-stained Cells Using a Confocal 405-nm Laser Scanning Microscope

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Summary Ten-day-old Pelargonium zonale embryos were fixed in formaldehyde and embedded in Technovit 7100 resin. Sections were cut to a thickness of 0.6 μm and stained with 1 μg/ml DAPI. The samples were observed by epifluorescence microscopy using 365-nm excitation and by confocal laser scanning (CLS) microscopy using 405-nm excitation. The mitochondrial and plastid nuclei (nucleoids, DNA and protein complex) were visualized more clearly by CLS microscopy than by traditional fluorescence microscopy.

The DNA-specific dye DAPI has been used to view mitochondrial DNA in squashed yeast cells (Williamson and Fennel 1975) and chloroplast DNA in squashed plant cells, using epifluorescence microscopy (James and Jope 1978). However, it is very difficult to visualize organelle nuclei in tissue cells in vivo using this method. Kuroiwa (1990) was the first to view organelle and cell nuclei in higher plant tissues embedded in Technovit 7100 resin. This resin does not emit autofluorescence under epifluorescence microscopy with excitation at 365 nm, and it is permeable to water. The Technovit-DAPI method has since been used in various studies of organelle division (Fujie et al. 1993) and inheritance, in combination with light microscopy, autoradiography, microphotometry, immunoelectron microscopy (Kuroiwa et al. 1993), and in situ hybridization (Kuroiwa et al. 1992). However, the Technovit-DAPI method has not been applied to confocal laser scanning microscopy (CLSM) because of its high cost. Furthermore, the sections of Technovit resin were thought to be too thin for CLSM. DAPI dye is excited by light at wavelengths between 300 and 410 nm, with the peak of excitation at 365 nm. Recently, a 405-nm blue laser (LAVIOS-tentative) was developed by Sigma Koki, which can be used to cause DAPI to emit blue-white fluorescence.

This study examined whether cell and organelle nuclei could be visualized in DAPI-stained 0.6-μm-thick Technovit sections by using confocal scanning microscopy with a 405-nm excitation laser. We obtained clear images of mitochondrial and plastid nuclei in tissue cells in vivo by this method.

Materials and methods

Fixing and embedding samples

Ten-day-old Pelargonium zonale embryos were fixed in 4% paraformaldehyde, which was buffered with 10 mM cacodylic acid at pH 7.2, for 12 h at 4°C, and dehydrated through an ethanol series. The samples were then embedded in Technovit 7100 resin in accordance with the manufacturer's instructions (Kulzer and Co., GmbH, Wehrheim, Germany; Kuroiwa 1990). Sections were cut to a thickness of 0.6 μm with a glass knife on an MT-6000 XL ultramicrotome (RMC-Eiko Corp, Japan), placed on a drop of distilled water on a cover glass, and dried by heating.
Confocal laser-scanning microscopy

The cells were stained with 1 μg/ml DAPI and observed under a Nikon Eclipse E600 fluorescence microscope, using 365-nm excitation, and by a Nipkow Disk Confocal scanner CSU10 (Yokogawa Electronic Corp., Tokyo, Japan) with a 405-nm excitation laser (LAVIOS-tentative, Sigma Koki, Tokyo). Images were acquired by a high-resolution CCD camera (C4742-95; Hamamatsu Photonics, Hamamatsu, Japan) and processed using IPLAB software (Scanalytics, VA, USA).

Results

The same thin sections of DAPI-stained P. zonale were examined by both epifluorescence and CLSM.

Figs. 1 and 2 show low-magnification images of cell, mitochondrial, and plastid nuclei (nucleoids) in interphase cells observed by epifluorescence (Fig. 1) and CLSM (Fig. 2). In Fig. 1, the small dense areas of chromatin in the nuclei cannot be distinguished, although some dense chromatin is visible in the cell nuclei. By contrast, the entire image in Fig. 2 is clear and we can easily identify the small dense areas of chromatin. A remarkable difference in the sharpness of the fluorescence and CLSM images can be seen in the organelles, the edges of which are very distinct in the latter.

Figs. 3 to 6 show higher magnification images of interphase (Figs. 3, 4) and metaphase (Figs. 5, 6) cells in 10-day-old embryos by epifluorescence (Figs. 3, 5) and CLSM (Figs. 4, 6). The dense and dispersed chromatin in the cell, mitochondrial, and plastid nuclei in interphase cells are not clearly visualized by epifluorescence microscopy (Fig. 3), whereas these organelle nuclei and their localizations are clearly visualized by CLSM using a 405-nm excitation laser (Fig. 4). The plastid nuclei are located at the edge of the plastids (Fig. 4). Similar findings are observed in metaphase cells (Figs. 5, 6); each chromosome and the mitochondrial and plastid nuclei in each cell are clearly visualized by CLSM (Fig. 6). These results demonstrate that the chromosomes, cell, mitochondrial,
Figs. 3-6. Higher magnification images of interphase (3, 4) and metaphase (5, 6) cells after staining with DAPI. The cells were embedded in Technovit 7100, sectioned with an ultramicrotome, and observed by epifluorescence (3, 5) or CLSM (4, 6). Figs. 3-6, are the same field and magnification. A small plastid nucleus (arrowhead in 4), mitochondrial nuclei (double arrowheads in 4, 6), plastid nuclei (arrowhead in 4), and metaphase chromosomes (triple arrowheads in 6) clearly appear as tiny bright spots by CLSM. Bar=10 μm.

and plastid nuclei in thin sections stained with DAPI can be clearly visualized by CLSM using a 405-nm excitation laser. CLSM proved very useful for examining 0.6-μm-thick sections.

Discussion

It has been very difficult to visualize mitochondrial and plastid nuclei in tissue cells in vivo using DAPI staining. Kuroiwa (1990) was the first to view mitochondrial, chloroplast, and cell nuclei in DAPI-stained thin sections of Technovit 7100 resin, and the Technovit-DAPI method has been used in various fields. However, the updated Technovit-DAPI method has not been applied to CLSM because CLSM using a UV laser system is expensive, and it was not considered useful for thin sections of Technovit resin.
DAPI produces emissions when excited at wavelengths between 300 and 410 nm. At wavelengths over 400 nm, the power of the emissions weakens and the blue-white fluorescence from mitochondrial and plastid nuclei is likely to be faint. Nevertheless, the shape and localization of mitochondrial and plastid nuclei were clearly revealed by CLSM using a 405-nm excitation laser (Figs. 4–6). In addition, we had expected that although CLSM is a powerful tool for thick sections, whole mount tissues, and cells, it would not be useful for observing 0.6-μm-thick sections. However, compared with traditional fluorescence microscopy, CLSM using a 405-nm excitation laser proved very useful for detecting small amounts of DAPI-stained DNA (Figs. 4–6).

Since 405-nm lasers are not very expensive and are easy to handle, this method can be used to observe cells in many branches of cell biology.

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References


