

Fluorescent staining of *Neurospora* nuclei with DAPI

E. Martegani
Universita di Milano

F. Trezzi
Universita di Milano

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Abstract

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Martegani, E. and F. Trezzi.

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nuclei with DAPI.

A simple procedure is described for fluorescent staining of *Neurospora crassa* nuclei with the biscationic dye DAPI (4', 6' diamidino-2-phenylindole • 2 HCl). DAPI binds selectively to DNA, and the intensity of the blue fluorescence obtained is proportional to the DNA content of each nucleus (Schneidel et al., 1977 *Cytobiologie* 15: 357). A technique was developed to determine microfluorimetrically the relative DNA content of nuclei in exponentially growing hyphae.

Mycelia were washed with cold 0.1 M phosphate buffer pH 7 (PB) and fixed by resuspending in sufficient fixative buffer (0.1 M phosphate buffer pH 7 + 0.3% Formolin) to obtain a suspension with an absorbance at 450 nm (A_{450}) of 0.250.

After two hours at room temperature, 10 ml of the fixed hyphae were centrifuged at 3000 rpm for 10 min, the hyphae were resuspended in 10 ml of PB and recovered again by centrifugation. The washed, fixed hyphae were then resuspended in 10 ml PB containing 0.2 $\mu\text{g/ml}$ of DAPI and left for 15-16 hours at 4°C. Although the dilute DAPI solution is unstable, a stock solution of 1 mg/ml in distilled water can be stored for weeks at -20°C. After staining, the hyphae were recovered by centrifugation, washed twice with PB, resuspended in a small volume of buffer and mounted. Observations were made with an optical fluorescence microscope (Leitz Ortholux equipped with 1 mm UG-1 excitor filter, 5 mm BG-38 red absorbing filter and a barrier filter K.430 or K.460). Nuclei appear as bright, light-blue spherical bodies, while the cytoplasm is almost completely dark, except for small foci of fluorescence which are probably due to mitochondrial DNA. Septa are also visible as weak dark-blue lines.

With a suitable microfluorimeter (we employed a Leitz-MPV microphotofluorimeter with a KNOTT-MFLK photoelectric unit) the intensity of the fluorescence of individual nuclei can be measured, and the relative DNA content of each nucleus can therefore be determined. The staining with DAPI appears very stable under UV light, with no appreciable fading. - - - Centro del C.N.R. per la Biologia Cellulare e Molecolare delle piante; Istituto di Scienze Botaniche, Università di Milano, 20133 Milano, Italy.