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Fluorescent staining of Neurospora nuclei with DAPI

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Abstract

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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 pH7 (PB) and fixed by resuspending in sufficient fixative buffer (0.1 M phosphate buffer pH7 + 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 hyphoe were centrifuged at 3000rpm for 10 min, the hyphoe were resuspended in 10 ml of PB and recovered again by centrifugation. The washed, fixed hyphoe were then resuspended in 10 ml PB containing 0.2 µg/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 stared for weeks at -20° C. After staining, the hyphoe were recovered by centrifugation, washed twice with PB, resuspended in a small volume of buffer and mounted. Observations were made with on 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 fluroescence which are probably due to mitochondrial DNA. Septa are also visible as weak dark-blue liner.

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 con 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.