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Abstract

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The study of precursor ribosomal RNA (pre-rRNA) maturation in ribosome biosynthesis mutants of N.crassa is facilitated by the isolation of RNA from purified nuclei. Problems have been encountered in attempts to purify nuclei with Ludox gradients. Specifically, Ludox precipitates at low temperatures when exposed to Triton X-100, which is an essential component of the buffer used in the nuclei isolation steps. Therefore, a new gradient medium, Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) was tested for its applicability. The use of Percoll rather than Ludox eliminated probelms with precipitation. In addition it was possible to determine the buoyant density of the nuclei accurately, since the colloidal silica particles are coated with polyvinylpyrrolidone to which the nuclear membrane is impermeable.

Flasks of liquid Vogel's minimal medium inoculated with wild type conidia ($2x10^7$ ml^{-1}) were incubated for 8 h. at 25°C. Crude nuclear pellets were prepared from these mid-logarithmic phase cultures using a modified version of the procedure described by Hautala et al. (1977 J. Bacteriol. 130:704). As in the original method, a French pressure cell was used for efficient cell breakage. Modifications inculded centrifuge of the supernatant liquid from the post-Omnimixer homogenized cell suspension at 2,300 g (r_{av} 8.26cm) rather than 500 g for each centrifugation. For subsequent steps, changes in buffer B were necessary to maintain the correct osmolality for the Percoll gradient step. To generate a medium having an osmolality of 320 mOs/kg H_2O, it is necessary to mix Percoll with 2.5 M sucrose in a 9:1 ratio. Lower starting densities of Percoll can be obtained by adding the appropriate amount of 0.25 sucrose. Since, in the Hautala method, the crude nuclear pellet is suspended in buffer B which contains 1 M sucrose (i.e., 50 mM Tris-HCl, pH 7.5; 5 mM $MgCl_2$; 10 mM $CaCl_2$; 1 M sucrose; and 1% (v/v) Triton X-100), it was necessary to reduce the sucrose concentration in the experiments reported here from 1.0 to 0.25 M, while keeping the other ingredients the same.

Thus, the crude nuclear pellets that were obtained were suspended in 8-10 vol of the modified buffer B and homogenized in 40-ml Potter-Elvehjem tissue grinders. The suspensions were then mixed with the appropriate amount of Percoll (isotonic in 2.5 M sucrose) in Beckman 1.6 X 7.62 cm, 10.4.ml polycarbonate bottle assemblies

which were centrifuged at 4 C for 45 min at 58,300 g (r_{av} 6.66 cm) using a DuPont-Sorvall T865.1 rotor in a DuPont-Sorvall OTD-2 ultracentrifuge. Owing to the size heterogeneity among Percoll particles, they sediment (and diffuse) at different rates in a gravitational field, thereby creating a density gradient. The biological material in the gradient, in this case nuclei, bands isopycnically, so that the sample particles reach a position where their densities and that of the surrounding Percoll medium are equal. As is the case with isopycnic separation using cesium chloride gradients, a fixed angle rotor has advantage over a swinging bucket rotor since with fixed angle rotors reorientation of the tube contents does not occur to alter the final separation of the zones and there is better resolution of the experimental materials since they are banded over a larger cross sectional area.

A range of starting densities of Percoll from 1.05 to 1.12 gm ml^{-1} were tested in separate experiments to determine the most useful for banding Neurospora nuclei. After each experiment the tube contents were fractionated into 12 fractions, and their refractive index determined with an A/O Refractometer. The results showed that the centrifugation generated adequate Percoll gradients. The nuclei banded to one region of the gradient but the band was not homogeneous: the upper part was relatively disperse, the center was dense and homogeneous, and the lower part exhibited some clumps. Based on refractive index measurements, the density of the nuclei was determined to be 1.078 gm ml^{-1} The nuclei may be recovered by centrifuging gradient fractions containing nuclei for 2 h at 100,000 g (r_{av}) in a swinging bucket rotor. Under these conditions, the silica particles pellet and the nuclei remain above the gel formed. The nuclei nay then be pelleted from the supernatant liquid by centrifugation for 20 min at 5,000 g (r_{av}).

In conclusion, the results indicate that Percoll is an effective alternative to Ludox for the purification of Neurospora nuclei from crude nuclear preparations. The absence of large osmotic effects such as is observed with other gradient materials has allowed the density of wild type nuclei to be determined. Finally, although RNA extracted from crude nuclei includes high molecular weight species that are presumptive precursors to mature rRNA (K. Talbot 1980 Baccalaureate Thesis, Reed College), studies of pre-rRNA processing in the nucleus will be greatly facilitated now that pure nuclei can be obtained. (Supported by NIGMS, NIH grant GM22488). - - - Biology Department, Reed College, Portland, Oregon 97202.