Ribosomes and Ribosomal Proteins from

Neurospora crassa

II. Ribosomal Proteins in Different Wild-Type Strains and During Various Stages of Development

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Cultures of N. crassa were grown at 30 C in 80 to 100 liters of minimal medium in a Ferva-cell fermentor (New Brunswick Scientific Co., New Brunswick, N.J.). The various wild-type strains were harvested at 18 hr, or at several times during growth in the case of wild-type 74A. The washed mycelia were frozen in liquid nitrogen and stored at -60 C. Conidia were lyophilized and stored under the same conditions.

The cells were disrupted by grinding 200 to 300 g of frozen mycelia or 20 g of conidia with sea sand and buffer in a mortar and pestle, and the ribosomes were isolated by differential centrifugation in a Spinco (model L) ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). Ribosomal proteins were extracted from twice-washed ribosomes with 5 and 7% sucrose in standard ribosomal buffer solution by the lithium chloride-urea method (P. S. Leboy, E. C. Cox, and J. G. Flax, Proc. Natl. Acad. Sci. U.S. 52:1367, 1964), and the proteins were concentrated and dialyzed against 8 m urea in Sartorius membrane filter colloidion bags (F. A. M. Alberghina and S. R. Suskind, J. Bacteriol. 94:630, 1967).

Samples of 20 to 50 μl, containing 30 to 150 μg of ribosomal proteins, were incorporated into the sample gel and analyzed by polyacrylamide gel disc electrophoresis at pH 4.5 and pH 8.7 in a Canalco unit, according to the method of Leboy et al. Electrophoresis was carried out at 2 to 4 C at 3 ma for about 3 hr at pH 4.5 and for about 2 hr at pH 8.7, i.e., until the tracking dye, 0.5% pyronine red at pH 4.5 and 0.5% bromophenol blue at pH 8.7, had reached the end of the gel. Gels were stained with Amido Black 10B dye in 7% acetic acid and destained with the solvent.

The electrophoretic profile of ribosomal proteins of N. crassa at acid and alkaline pH exhibits the extensive heterogeneity reported for other species, but there is remarkable qualitative similarity of profile within the species (Fig. 1) and throughout the various stages of development from conidia to mature hyphae (Fig. 2). All samples were compared at a minimum of three protein concentrations before a meaningful evaluation of the electrophoretic profile could be made. At pH 4.5, 22 to 26 bands were detected; at pH 8.7, 6 to 9 bands were observed, depending on the protein concentration employed.

When tested by the Ouchterlony immunodiffusion method, with rabbit antisera against wild-type Neurospora ribosomes and ribosomal proteins, no antigenic differences were noted among the ribosomes or ribosomal proteins of the 10 Neurospora strains or the conidial and hyphal samples of strain 74A.

Rabbit antisera against N. crassa ribosomes and N. crassa and Escherichia coli ribosomal proteins were also tested for cross-reaction with
Fig. 1. pH 4.5 acrylamide gel disc electrophoresis of ribosomal proteins prepared from 18-hr mycelia of 10 wild-type strains of Neurospora crassa. (A) 74A; (B) Fiji; (C) Puerto Rico; (D) Costa Rica; (E) New Zealand; (F) Java; (G) Liberia; (H) Philippines; (I) North Africa; (J) Singapore.

Fig. 2. pH 4.5 acrylamide gel disc electrophoresis of Neurospora crassa 74A ribosomal proteins prepared from conidia and from mycelia of different ages. (A) 9 hr; (B) 12 hr; (C) 18 hr; (D) 36 hr; (E) 42 hr; (F) 96 hr; (G) conidia.

Xenopus laevis and sheep reticulocyte ribosomes and ribosomal proteins by agglutination and agar diffusion tests. Cross-reactions were observed between N. crassa and E. coli ribosomal protein components and ribosomes, but neither X. laevis nor sheep reticulocyte ribosomes or ribosomal proteins gave positive reactions with these antisera against microbial ribosomes or ribosomal proteins.

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