

## Isolation and Characterization of Ribosomes from Nongerminated Conidia of *Erysiphe graminis* f. sp. *tritici*

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### ABSTRACT

Ribosomes were extracted from nongerminated conidia of *Erysiphe graminis* f. sp. *tritici*. Sucrose density-gradient profiles indicated the presence of 80S monosomes and polysomes which were shown to be ribonuclease-sensitive. Isopycnic density-gradient centrifugation of the ribosomes in cesium chloride was performed to determine the RNA to protein ratio. *Phytopathology* 61:1030-1031.

The infection process of wheat (*Triticum aestivum* L.) by the powdery mildew fungus (*Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal) has been described as a distinct series of morphological changes in the fungus (3, 5). However, little is known about the molecular basis of these developmental changes or of the infection process itself. The results reported here on the isolation and characterization of ribosomes by centrifugation represent attempts to provide a base for a study of the molecular changes associated with the process of primary infection by *Erysiphe* conidia.

Large quantities of nongerminated conidia were obtained from 2,000 infected Little Club wheat plants. The conidia were harvested in sterile distilled water containing Tween 80 (polyoxyethylene sorbitan monooleate) with a vacuum apparatus. The collecting apparatus was kept chilled in ice throughout the operation. The conidia were then centrifuged at 3,000 g, and the pellets washed with sterile distilled water and recentrifuged twice, all at 0 C. The washed pellets were lyophilized for 48 hr. The grinding of the lyophilized conidial powder, the isolation of the ribosomes, and the density-gradient centrifugation in linear 5-20% sucrose gradients were performed according to the methods described by Leary et al. (2). Ribosomes from rabbit reticulocytes and *Schizosphyllum commune* were centrifuged as separate samples for comparison.

A typical sucrose density-gradient profile of ribosomes from ungerminated conidia is shown in Fig. 1. By comparison with the ribosomes from reticulocytes and *Schizosphyllum*, the predominant peak appears to consist of 80S monosomes. However, a significant amount of material can be seen to have sedimented faster than the monosomes. From their position in the gradient and the resolution of this material into minor peaks, these peaks are presumed to represent polysomes.

To determine if these were indeed polysomes, a sample of the ribosomes was treated with 4  $\mu$ g/ml of pancreatic ribonuclease for 20 min at 4 C, then layered over a 5-20% linear gradient. A nontreated sample and a reticulocyte-ribosome control were centrifuged at the same time. The results are shown in Fig. 2. Though the profile for the nontreated sample shows far less polysomal material than is evident in Fig. 1, the ribonuclease treatment can be seen to have resulted in the shift of considerable A<sub>260</sub>-absorbing material from the

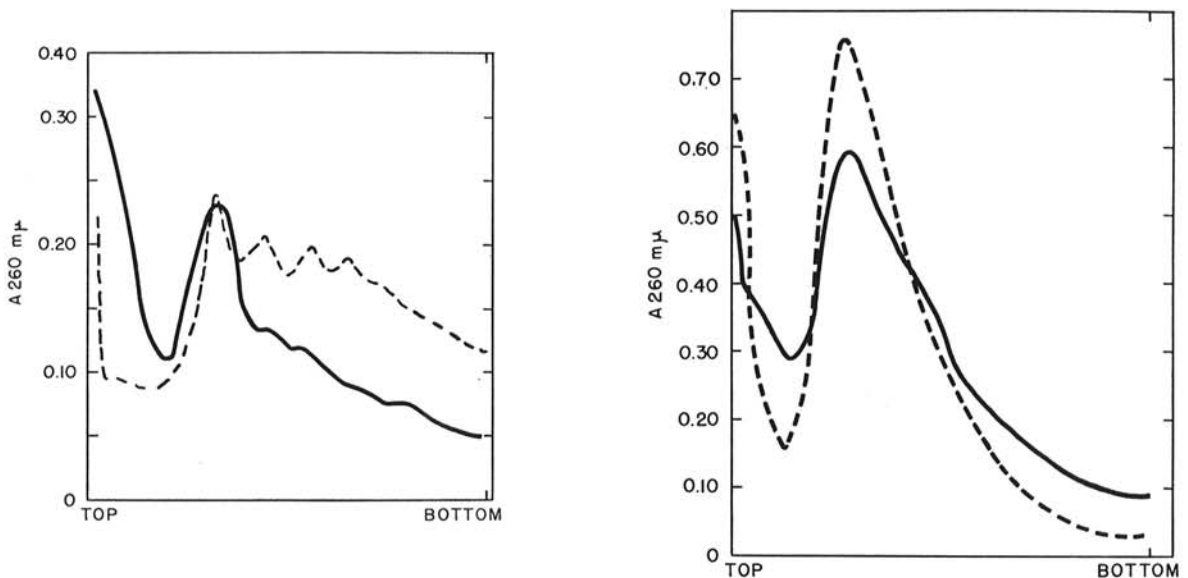


Fig. 1-2. 1) Sucrose density-gradient profile (5 to 20% sucrose) of ribosomes isolated from nongerminated conidia of *Erysiphe graminis* (—) and rabbit reticulocytes (----). 2) Sucrose density-gradient profile (5 to 20% sucrose) of untreated and ribonuclease-treated ribosomes from nongerminated conidia of *Erysiphe graminis* (— = untreated sample; ---- = treated sample).

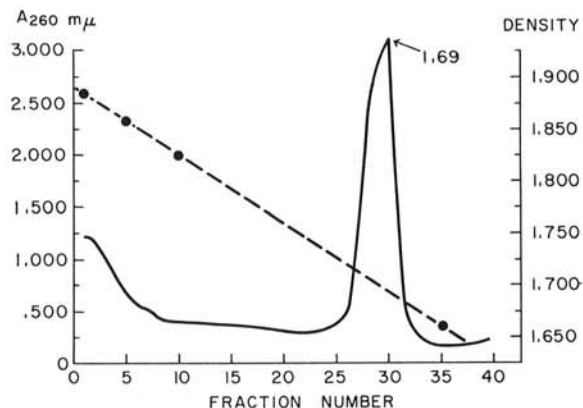


Fig. 3. Absorbancy profile of ribosomes subjected to equilibrium density-gradient centrifugation in CsCl (— =  $A_{260}$  nm; ---- = density). The points on the density line were calculated by the method of least squares where  $y = 1.89 - 0.0064 X$ .

heavier region of the gradient into the monosome peak. The difference in the profiles of nontreated samples from Fig. 1 and 2 is thought to be due to (i) freezing and thawing the resuspended ribosome samples between experiments; and (ii) the amount of material loaded on the gradient. The effect of ribonuclease on the ribosomes does offer further indication that polysome species are present in the nongerminated conidia.

Further characterization of the ribosomes isolated from the nongerminated conidia was achieved by equilibrium density-gradient centrifugation in cesium chloride. Ribosomes were resuspended in 3 ml 5 mM Tris [tris(hydroxymethyl)amino methane]-HCl buffer (pH 7.4) containing 5 mM  $MgCl_2$ , and 4.0 g CsCl were added with rapid stirring to give a starting density of 1.75 g/cc. Four ml of this mixture were centrifuged at 130,500 g (40,000 rpm) for 18 hr at 10 C in the SW50L rotor of the Spinco ultracentrifuge. The bottoms of the tubes were punctured, and eight-drop fractions collected automatically with a LKB fraction collector. The refractive index of the nondiluted samples was determined and the density calculated from the equation of Ifft et al. (1). The fractions were diluted to a volume of 0.3 ml, and the absorbancy of each fraction at 260 nm was taken.

The results of a CsCl density-gradient centrifugation are shown in Fig. 3. The single band of ribosomes visible in the tube after centrifugation shows a sharp peak at an interpolated density of 1.69 g/cc. This density indicates that the particles equilibrating in this region of the gradient are composed of ca. 75% RNA and 25% protein (6). The high RNA/protein ratio may be the result of the instability of the ribosomal protein in CsCl (4).

We consider the primary significance of these results to be the demonstration of the feasibility of studies on the protein synthetic apparatus in *Erysiphe*, an obligate parasite. Of considerable interest also is the evidence for the presence of polysomes in nongerminated conidia. Similar results have been reported by Staples et al. (7) in bean rust uredospores, and by Leary et al. (2) in *Schizophyllum commune* basidiospores. If the presence of a complete, functional, protein synthetic apparatus can be demonstrated in the conidia of *Erysiphe graminis*, the possibilities for further studies on the molecular basis of the primary infection process are numerous.

#### LITERATURE CITED

1. IFFT, J. B., D. H. VOET, & J. VINOGRAD. 1961. The determination of density distribution and density gradients in binary solutions at equilibrium in the ultracentrifuge. *J. Phys. Chem.* 65:1138-1145.
2. LEARY, J. V., A. J. MORRIS, & A. H. ELLINGBOE. 1969. Isolation of functional ribosomes and polysomes from lyophilized fungi. *Biochim. Biophys. Acta* 182: 113-120.
3. MASRI, S. S., & A. H. ELLINGBOE. 1966. Germination of conidia and formation of appressoria and secondary hyphae in *Erysiphe graminis* f. sp. tritici. *Phytopathology* 56:304-308.
4. MESELSON, M., M. NOMURA, S. BRENNER, C. DAVERN, & D. SCHLESSINGER. 1964. Conservation of ribosomes during bacterial growth. *J. Mol. Biol.* 9:696-711.
5. NAIR, K. R. S., & A. H. ELLINGBOE. 1965. Germination of conidia of *Erysiphe graminis* f. sp. tritici. *Phytopathology* 55:454-457.
6. PERRY, R. P., & D. E. KELLEY. 1966. Buoyant densities of cytoplasmic ribonucleoprotein particles of mammalian cells: distinctive character of ribosomes subunits and rapidly labelled components. *J. Mol. Biol.* 16:255-268.
7. STAPLES, R. C., D. BEDIGIAN, & P. H. WILLIAMS. 1968. Evidence for polysomes in extracts of bean rust uredospores. *Phytopathology* 58:151-154.