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Preparation of Biologically Active Ribosomal Subunits from Fungal Spores[1]

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Ribosomal subunits were prepared from fungal spore ribosomes and reassociated to yield biologically active ribosomes.

The dissociation of 80*S* ribosomes from eukaryotic organisms into subunits and the reassociation of these subunits into biologically active ribosomes has been reported (2, 5-11). In general, dissociation was achieved by incubation of the ribosomes in high KCl concentrations (5, 7-11) or by incubation with puromycin (6). The puromycin is believed to cause "polysome run-off," so that the detached monoribosomes are freed of messenger ribonucleic acid (mRNA) and peptidyl-transfer ribonucleic acid (tRNA), thus allowing the dissociation of the ribosomes into subunits.

The predominate ribosomal peak in spores from the mycelial fungus, *Botryodiplodia theobromae*, was in the monoribosomal region (4), and hence these ribosomes may be relatively free of mRNA and peptidyl-tRNA. This note describes the dissociation of these ribosomes into subunits and their subsequent reassociation into biologically active ribosomes.

The preparation of the ribosomes was identical to that previously reported (4), except that the extraction buffer contained 10^{-3} M magnesium acetate and 0.05 M NH_4Cl. The ribosomal pellet was suspended in 0.01 M tris(hydroxymethyl) aminomethane-chloride, *p*H 7.5; 0.05 M NH_4Cl; and 10^{-3} M magnesium acetate (standard buffer). Figure 1 demonstrates the effect of four magnesium acetate concentrations on the dissociation of the ribosomes. Complete dissociation into 36*S* and 60*S* subunits occurred at 3×10^{-4} M magnesium acetate.

For preparative purposes, the magnesium acetate concentration of the ribosomes was diluted to 3×10^{-4} M before placing on 10 to 35% linear sucrose gradient columns. The subunits were collected, diluted with an equal volume of standard buffer containing 3×10^{-3} M magnesium acetate, and pelleted by centrifugation at 133,000 × *g* for 5.5 hr. The 60*S* subunit fraction was recycled a second time through sucrose gradient columns equilibrated with standard buffer at 5×10^{-3} M magnesium acetate to obtain clean subunits.

The two subunits immediately reassociated at 4 C to form a normally sedimenting monoribosome when the magnesium acetate concentration was increased to 8×10^{-3} M. Analysis of the RNA obtained from the 36*S* and 60*S* subunits revealed very little cross contamination of the subunits (Fig. 2).

The transfer of phenylalanyl-tRNA into polyphenylalanine was also completely dependent on the presence of both subunits (Fig. 3). The characteristics and optimal conditions for transfer activity (Table 1) were very similar to those previously reported for native ribosomes (12).

Since the native ribosomes were always two to three times more active than the reassociated ribosomes, experiments were conducted to determine if one of the subunits was responsible for this decreased activity. Based on the *A* at 254 nm of the RNA extracted from the native ribosomes, there was a ratio of approximately 2.2:1 of large subunit to small subunit (Fig. 2A). The small subunit was held at a concentration of 0.3 A_{260nm} units, and increasing concentrations of the large subunit were added. Transfer activity increased until the ratio of large subunit to small subunit was about 4:1, thus indicating that the 60*S* subunit was at least partially responsible for this loss in activity.

The present experiments indicate that fungal spore ribosomes can be dissociated into subunits and reassociated into biologically active ribosomes by using very mild procedures. With the development of techniques for the preparation of biologically active subunits from 80*S* ribosomes, some of the elegant experiments which have been conducted with bacterial ribosomes may also be possible with ribosomes from eukaryotic organisms.

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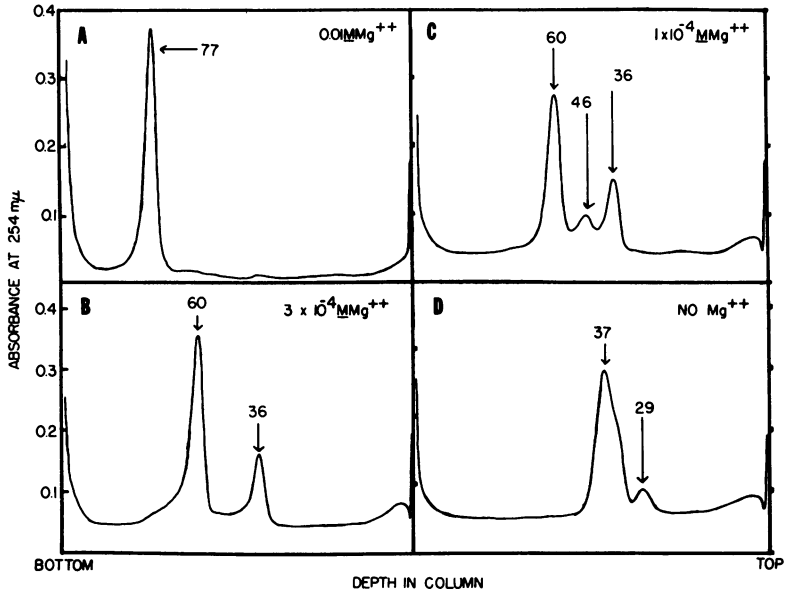

FIG. 1. *Effect of four magnesium acetate concentrations on the dissociation of fungal spore ribosomes. Ribosome samples on each gradient column were equivalent to about 45 μg of ribosomal RNA. Centrifuged on sucrose "linear log" gradient columns (3) equilibrated with standard buffer at the magnesium acetate concentrations shown. The sedimentation constants of the subunits were obtained by using Escherichia coli ribosomal subunits as markers. The direction of sedimentation is from right to left.*

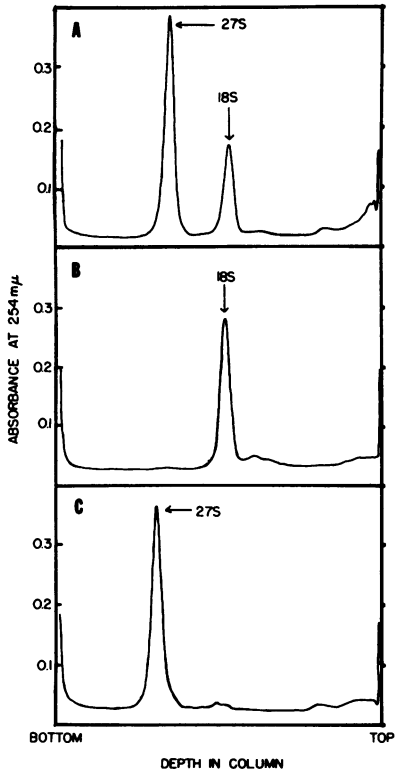

FIG. 2. *Sedimentation pattern of ribosomal RNA from spore ribosomes and ribosomal subunits. Centrifuged on sucrose "linear log" gradient columns (3) equilibrated with 0.15 M NaCl and 0.015 M sodium citrate (pH 7.0). Brome mosaic virus and tobacco mosaic virus RNA species were used as markers for determining the sedimentation constants of the ribosomal RNA species. The ribosomal RNA was analyzed after stripping the ribosomes of protein by incubation in 0.01 M tris(hydroxymethyl)aminomethane-chloride (pH 9.0); 1% sodium dodecyl sulfate; 10^{-4} M ethylenediaminetetracetic acid; 0.2 M NaCl; and 100 μg of bentonite/ml for 1 hr and then layering this mixture directly on top of the gradient columns. (A) Sample was equivalent to 45 μg of RNA from the native ribosome. (B) Sample was equivalent to 19.4 μg of RNA from the small subunit. (C) Sample was equivalent to 35 μg of RNA from the large subunit.*

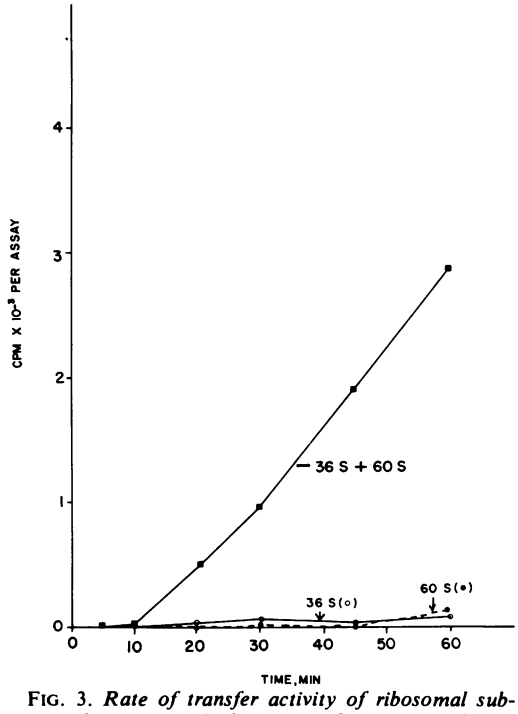

FIG. 3. *Rate of transfer activity of ribosomal subunits and reassociated ribosomes. The assay conditions were identical to those described in Table 1.*

TABLE 1. *Effect of various ingredients in the assay mixture on the transfer activity of the reassociated ribosomes*

Assay mixture	Counts/min[a]	Per cent of control
Complete[b]	2,491	—
Minus enzyme	0	0
Minus magnesium acetate	102	4.1
Minus spermine	442	17.8
Minus guanosine triphosphate	36	1.4
Minus NH_4Cl[c]	17	0.7
Minus polyuridylic acid	0	0
Complete plus 100 μg of chloramphenicol	2,540	102
Complete plus 50 μg of cycloheximide	1,420	57.6
Complete plus 50 μg of puromycin	1,525	61.3

[a] Figures represent counts per minute per assay after 30-min incubation.

[b] The standard assay in 0.5-ml quantities contained 25 μmoles of ammonium maleate (*p*H 6.5); 4 μmoles of magnesium acetate; 1 μmole of guanosine triphosphate; 20 μg of spermine; 1.5 μmoles of 2-mercaptoethanol; 60 μg of polyuridylic acid; 250 μg of 105,000 × *g* supernatant protein (12); 10,000 counts/min of ^{14}C-phenylalanyl-tRNA (12); 0.3 and 0.6 $A_{260\ nm}$ units of the small and large subunits fractions, respectively. The reaction mixtures were incubated at 37 C; 0.05-ml fractions were taken at various time intervals, placed on filter paper discs, and then processed as described by Bollum (1).

[c] Assay conducted in Tris-maleate buffer, *p*H-6.6.

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