Reconstitution of Functional Eukaryotic Ribosomes from *Dictyostelium discoideum* Ribosomal Proteins and RNA*

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40 and 60 S ribosomal subunits have been reconstituted in vitro from purified ribosomal RNA and ribosomal proteins of *Dictyostelium discoideum*. The functionality of the reconstituted ribosomes was demonstrated in in vitro mRNA-directed protein synthesis. The reassembly proceeded well with immature precursors of ribosomal RNA but poorly if at all with mature cytoplasmic RNA species. Reassembly also required a preparation of small nuclear RNA(s), acting as morphopoietic factor(s).

In vitro reconstitution of functional ribosomal subunits from free Escherichia coli rRNA and proteins was reported almost 30 years ago (1–3). More recently it has been accomplished also with archaeabacteria (4, 5). However, all attempts to reconstitute eukaryotic ribosomes have failed, although their biogenesiss in vitro has been well characterized (6–13). Here we describe the in vitro reassembly of functional ribosomal subunits from free ribosomal RNA and proteins of *Dictyostelium discoideum*

The pioneer work of Nomura and co-workers (1, 2) has established for the first time that the information sufficient to assemble a cell organelle are contained in its components. It has been extremely useful in studying the function of single ribosomal proteins both in ribosome assembly and in their interactions with the other components of the translation machinery. Nevertheless, the experimental conditions used to reconstitute bacterial ribosomal subunits in vitro were probably much different from those in which the assembly process occurs in vivo. We have shown that a step requiring high energy and that could be accomplished in vitro only by the exposure of the reconstitution mixture to high, nonphysiological temperatures (1, 2) was probably not the limiting step in ribosome assembly in vivo (14). Although ribosome assembly also occurs in *E. coli* cells in discrete steps (15), the high energy requirement is probably overcome in vivo by some factor(s) not present among the components derived from mature 70 S ribosomes.

And we others have found that in *E. coli* newly formed 30 S ribosomal subunits still contain immature pre-16 S rRNA (16, 17). The extra sequences present in immature rRNA must facilitate the assembly process, since in vitro pre-16 S rRNA, but not mature 16 S rRNA, was incorporated instantaneously into 30 S particles in the presence of ribosomal proteins even at 0 °C (18).

Our interest in the in vitro reconstitution of eukaryotic ribosomes has been prompted by the finding that during development of *D. discoideum* the stability of a class of mRNAs is controlled by a mechanism that involves the modification of one or several components of 40 S ribosomal subunits (19–21). In trying to accomplish the reconstitution of eukaryotic ribosomes, we took advantage of our previous studies on ribosome assembly in *E. coli*. Here we report that *D. discoideum* immature precursor rRNAs are also better substrates than mature cytoplasmic 17 and 26 S rRNAs for the in vitro reconstitution process. Furthermore, a nuclear component, probably one or several small nucleolar RNAs, which are not present in 40 or 60 S ribosomal particles, plays an essential role.

**EXPERIMENTAL PROCEDURES**

**Cell Culture Conditions—**Dictyostelium discoideum AX2 cells were grown as described previously (22).

**Differential Radioactive Labeling and Isolation of Nuclear and Cytoplasmic rRNA—**Growing cells at a density of 2 × 10⁶/ml were washed twice by centrifugation in Sorensen buffer and resuspended in the same buffer at a concentration of 2 × 10⁶ cells/ml. In a typical experiment, 200 µCi of [³H]uracil were added to 10 ml of cell suspension for 30 min. Cells were then pelleted and lysed in ice-cold 25 mM HEPES/KOH, pH 7.5, 25 mM potassium acetate, 10 mM magnesium acetate, 5% sucrose, and 2% Tergitol (buffer A). The lysate was spun in an Eppendorf microcentrifuge at top speed for 20 s to remove cell debris and then for 5 min to pellet the nuclei, which contained most of the incorporated label. Nuclei were lysed by resuspension in buffer A not containing sucrose and Tergitol, and the lysate was centrifuged in a Beckman SW40 rotor at 170,000 × g for 18 h at 4 °C through a 15–35% sucrose gradient in 20 mM Tris-HCl, pH 7.8, 0.5 mM MgCl₂, 50 mM magnesium acetate, 6 mM β-mercaptoethanol (buffer B). Fractions sedimenting at 40 and 60 S were collected separately, ethanol-precipitated, resuspended in buffer B, and centrifuged again through a sucrose gradient to isolate pure 40 and 60 S particles. From each of these preparations, RNA was extracted with Ultraspec-II RNA (Biotec Laboratories), following the procedure suggested by the manufacturer. RNA (about 3–4 µg from 40 S particles and 6–8 µg from 60 S particles) was ethanol-precipitated and redissolved in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA (TE buffer).

One mCi of [¹⁴C]uracil was added to another 10-ml cell suspension for 8 h, followed by a large excess of unlabeled uracil for 2 h. Cells were lysed, nuclei and cytoplasm were separated, and rRNA was extracted from isolated cytoplasmic 40 and 60 S ribosomal subunits as described above.

The effectiveness of nuclei and cytoplasm separation was verified by the fact that nuclei did not contain any [¹⁴C]-labeled RNA, while cytoplasm did not contain any [³H]-labeled 36 S RNA (the large precursor of ribosomal RNAs).

**Gel Electrophoretic Analysis of rRNA—**[³H]-Labeled (nuclear) and [¹⁴C]-labeled (cytoplasmic) rRNAs, isolated as described above, were mixed together in an appropriate ratio, denatured by heating at 65 °C in 50% formamide for 5 min, and analyzed by electrophoresis on 5% polyacryl...
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amid gels in 6 μL urea. At the end of the run, the gel was cut into 1.5-mm slices, which were counted in Triton solution (Packard). 3H-Labeled RNA was also extracted from in vitro reconstituted ribosomal particles isolated by sedimentation through sucrose gradients, and it was analyzed by gel electrophoresis as described above.

In Vitro Reconstitution of Precursor and Mature rRNAs—109 cells were starved, plated on a 2.8-cm Millipore filter embedded with 10 μCi of 32P in MES-PDP buffer (19). After 2 h, cells were collected and lysed, and RNA was extracted from nuclear and cytoplasmic 40 and 60 S particles as described above. Each RNA preparation was sedimented through sucrose gradients to display nuclear precursor 19 and 28 S RNAs and cytoplasmic mature 17 and 26 S RNAs (23–25). Each fraction was concentrated by ethanol precipitation, digested with RNase T1, and analyzed as described by Batts-Young et al. (25).

In Vitro Labeling and Analysis of a 7 S RNA Fraction—Total RNA was extracted with Ultraspec-II RNA from nuclei derived from 109 unlabeled growing cells, ethanol-precipitated, dissolved in TE buffer, and chromatographed on an oligo(dT) cellulose column to eliminate poly(A)+ RNA, as described previously (19). Poly(A)+ RNA was fractionated on a 15–35% sucrose gradient containing 20 mM Tris-HCl, pH 7.3, and 0.5 M NH4Cl in a Beckman SW40 rotor at 170,000 × g for 36 h to display 19 S RNA and smaller RNAs. Each fraction from the top half of the gradient was tested for its ability to support in vitro ribosome reconstitution. A fraction moving at about 7 S, which turned out to be active, was labeled with [35S]pCp (26) and analyzed by electrophoresis on a 5% polyacrylamide gel in 6 μL urea, followed by autoradiography.

Recovery of 7 S RNA from the Polyacrylamide Gel—The nuclear RNA fraction sedimenting at about 7 S and labeled in vitro with [35S]pCp was separated by gel electrophoresis into two major bands. A faint band was visible in front of each of the two major bands, while another 13 bands appeared after an exposure longer than the one shown in Fig. 5. However, the two major bands in the latter case obscured part of the gel. Therefore, the number of different small nuclear or nucleolar RNAs present in the fraction sedimenting at about 7 S could not be determined precisely. RNA was extracted as described in Ref. 24 from the regions of the gel corresponding to each of the two major bands and the regions containing the faint bands. The RNA corresponding to each of the regions of the gel was tested both for its ability to substitute for nuclear lysate in supporting in vitro reconstitution of ribosomal subunits (see below) and to hybridize to cloned DNAs containing rRNA genes.

Hybridization of 7 S Nuclear RNA Fraction to Ribosomal DNA—Phage DNAs each containing a different gene for rRNAs (17, 5.8, 26, and 5 S rRNA) were cloned with the hybridization competition technique described by Mangiarotti et al. (27). The 7 S nuclear RNA corresponded to the regions of the gel shown in Fig. 5 which separated closest to each of the cloned DNA containing an rRNA gene, in the absence or in the presence of an excess of the corresponding unlabeled rRNA species, following the procedure described in Ref. 19.

Isolation of Nucleoli—Nucleoli were isolated following the procedure described in Ref. 27, as modified by Frankel et al. (24).

Preparation of Ribosomal Proteins—Growing cells were collected and lysed, in buffered E, containing 2% Tergitol. Ribosomal 40 and 60 S subunits were isolated by centrifugation through sucrose gradients as described above. To extract ribosomal proteins, ribosomal subunits (50 A260/ml) were adjusted to 0.1 μM magnesium acetate, and 2.2 volumes of glacial acetic acid were added dropwise with constant stirring in the cold, as described in Ref. 28. Staining was continued for 1 h, and rRNA was removed by centrifugation at 27,000 × g for 10 min. The extracted proteins were dialyzed against 300 volumes of 6 μL urea, 6 μM d-mercaptoethanol for 18 h in the cold.

Labeling of Ribosomal Proteins—To facilitate the analysis of ribosomal proteins by two-dimensional gel electrophoresis, growing cells were resuspended in Sorensen buffer and labeled with 250 μCi of [35S]methionine and cysteine for 3 h. The labeled proteins were extracted as described above from native or in vitro reconstituted 40 and 60 S ribosomal subunits and analyzed as described by Ramagopal and Ennis (28).

In Vitro Reconstitution of Ribosomal Subunits—In a typical experiment, 10 μg of 40 S ribosomal proteins in 0.2 ml of 6 μL urea were mixed with 4 μg of [3H]-labeled 19 S RNA or [14C]-labeled 17 S RNA in 0.1 ml of TE buffer and 760 S ribosomal proteins in 0.4 ml of TE buffer. The rRNA and protein mixtures were dialyzed at 23 °C for 12 h against a buffer containing 20 mM Tris-HCl, pH 7.3, 0.5 M NH4Cl, 20 mM MgCl2, 10 mM d-mercaptoethanol.

Preparation of Nuclear Lysate—Growing cells were washed twice in Sorensen buffer and lysed in buffer B containing 2% Tergitol. Ribosomal 40 and 60 S subunits were isolated by centrifugation through sucrose gradients as described above. To extract ribosomal proteins, ribosomal subunits (50 A260/ml) were adjusted to 0.1 μM magnesium acetate, and 2.2 volumes of glacial acetic acid were added dropwise with constant stirring in the cold, as described in Ref. 28. Staining was continued for 1 h, and rRNA was removed by centrifugation at 27,000 × g for 10 min. The extracted proteins were dialyzed against 300 volumes of 6 μL urea, 6 μM d-mercaptoethanol for 18 h in the cold.

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Preparation of Nuclear Lysate—Growing cells were washed twice in Sorensen buffer and lysed in buffer B. The lysate was spun in an Eppendorf microcentrifuge at top speed for 20 s to remove cell debris and then for 5 min to pellet nuclei. The nuclei were lysed by resuspension in a buffer containing 2% Tergitol and centrifuged in a 55 Ti rotor at a TL Beckman ultracentrifuge at 250,000 × g for 2 h to discard any particle the size of ribosomal subunits or larger. When nuclear lysate was added to ribosomal subunit reconstitution mixtures, the amount of nuclear lysate and the amount of rRNA utilized were derived from a comparable number of cells.

Preparation of a Cell-free System for Protein Synthesis—Cells were lysed in buffer A. The lysate was spun in an Eppendorf microcentrifuge at top speed for 10 min to pellet microsomes and lysosomes. The supernatant was centrifuged in a 55 Ti rotor in a TL Beckman ultracentrifuge at 250,000 × g for 5 h to pellet all ribosomal particles. The supernatant (S55) was dialyzed for 3 h against buffer A not containing sucrose and Tergitol. S55 represented the source of all factors required for protein synthesis except ribosomes and mRNAs.

Amino Acid Incorporation System—Protein synthesis was measured at 23 °C for 15 min in a total reaction volume of 100 μl containing 10 μg of S55 proteins in the presence of the following components: 20 mM HEPES/KOH, pH 7.5, 100 mM potassium acetate, 15 mM magnesium acetate, 2 mM dithiothreitol, 0.5 mM spermidine, 2 mM ATP, 0.5 mM GTP, 20 mM creatine phosphate, 3 μg of creatine phosphokinase, 250 mM each of 19 amino acids (excluding methionine), 200 μg of Dictostelium.
RESULTS

Nuclear 40 and 60 S Ribosomal Subunits Contain Immature rRNA—Nuclear and cytoplasmic ribosomal particles were labeled differentially with \(^{3}H\)uracil and \(^{14}C\)uracil. Nuclear and cytoplasmic ribosomal particles were labeled with \(^{3}H\)uracil and \(^{14}C\)uracil. Nuclear and cytoplasmic ribosomal particles were labeled with \(^{3}H\)uracil and \(^{14}C\)uracil. Nuclear and cytoplasmic ribosomal particles were labeled with \(^{3}H\)uracil and \(^{14}C\)uracil.

Ribosome Assembly Requires Pre-rRNAs and Morphopoietic Factors—When the labeled rRNAs were incubated with 40 and 60 S ribosomal subunit proteins under different salt and temperature conditions, no particles sedimenting at 40 or 60 S were formed (Fig. 1A). However, when a small amount of nuclear lysate depleted of ribosomal subunits (see “Experimental Procedures”) was added to the incubation mixture, \(^{3}H\)-labeled 19 S RNA was incorporated into particles sedimenting at 40 S and \(^{3}H\)-labeled 5, 5.8, and 28 S RNAs were incorporated into particles sedimenting as 60 S with an efficiency of 80–90%, while \(^{14}C\)-labeled mature RNAs were incorporated into similar particles with an efficiency of at most 10% (Fig. 3B). The amount of nuclear lysate required was that derived from a number of cells comparable with the one from which the rRNA had been derived. The amount of ribosomal proteins needed was twice that of the rRNA, probably because some proteins were partially denatured during the extraction procedure.

Ribosome reconstitution therefore proceeds more efficiently with nuclear immature rRNA than with cytoplasmic mature rRNA, and a nuclear component plays an important role in the assembly process. The required nuclear lysate could be replaced by an RNA fraction that was extracted from nuclei with guanidine and phenol and sedimented at about 7 S in zonal sedimentation in a sucrose gradient (Fig. 4). The 7 S fraction contained two major RNA species plus several minor species, which could be separated by gel electrophoresis (Fig. 5, right lane). The minor species are only partially visible in Fig. 5, but they became visible with a longer exposure of the gel. The number of small nuclear or nucleolar species present in the 7 S fraction was at least 17, but we cannot exclude the possibility that several species comigrated in our gel, masking an even greater complexity of this RNA fraction.

The 7 S Fraction Contains a Precursor of 5.8 S rRNA and Many Small Nuclear RNAs—The RNAs corresponding to each of the two bands shown in Fig. 5 (right lane) and to the faint bands were recovered from the gel and hybridized to cloned DNAs containing the gene of 17, 5.8, 26, or 5 S RNA. The RNA corresponding to the upper major band hybridized only to DNA containing the 5.8 S RNA gene, but its hybridization was completely prevented by the addition of an excess of unlabeled 5.8 S RNA (Fig. 6). The RNA corresponding to the lower major...
band and to the faint bands did not hybridize to any ribosomal DNA. Thus, the first RNA is probably a precursor of 5.8 S rRNA, not previously detected in D. discoideum but already described in other eukaryotic species (29, 30), while the other RNAs must be small nuclear RNAs.

The Morphopoietic Factor Is Probably a Small Nucleolar RNA—The RNAs corresponding to the two major bands shown in Fig. 5 (right lane) and to the faint bands were separately tested for their ability to promote ribosomal particle reconstitution in the absence of nuclear lysate. In the presence of the RNA corresponding to the upper major band, no ribosomal particles formed. Thus, the requirement for the 7 S nuclear fraction is not due to the requirement for an immature 5.8 S rRNA. In the presence of the RNA corresponding to the lower major band, nuclear \(^{3}H\)-labeled rRNA was incorporated into 40 and 60 S particles to an extent comparable to that with nuclear lysate (Fig. 7). Thus, the small nuclear RNA corresponding to the lower major band in Fig. 5 can fully replace the nuclear lysate in the reconstitution system. In the presence of the RNAs corresponding to the faint bands, no ribosomal particles formed.

The active small nuclear RNA fraction has not yet been further characterized. Though it gives origin to only one band by gel electrophoresis analysis, it might contain several small nuclear RNAs that comigrate under our electrophoretic conditions. Thus, although the small nuclear RNA fraction is required for the reconstitution of both 40 and 60 S ribosomal subunits, we cannot know whether this function is carried out by the same RNA species.

We cannot determine the stoichiometry of the small nuclear RNA and of the rRNA in the assembly process, because we cannot detect the first RNA by the \(A_{260}\) absorbance in the preparative sucrose gradient. However, since we have labeled with \([^{32}P]pCp\) the 7 S RNA fraction in parallel with known amounts of 5 and 5.8 S RNAs, we can roughly estimate that the labeled major 7 S lower band contained \(10^{-2}\) μg of RNA, judging by the amount of the other two RNAs run on the gel to obtain bands of intensity comparable with the one of the 7 S RNA. Thus, the stoichiometry of the assembly reaction should be 1 small nuclear RNA/100–200 rRNA molecule species. It is evident that the 7 S RNA functions catalytically in stimulating ribosome assembly.

We have repeated the experiments shown in Figs. 4 and 7 with RNA extracted from nuclei. This RNA contained a fraction that sedimented at 7 S on a sucrose gradient, could be labeled with \([^{32}P]pCp\), and gave an electrophoretic pattern similar to the one shown in Fig. 5 (right lane). The lower major band could support the in vitro reconstitution of 40 and 60 S ribosomal subunits with the same efficiency as the 7 S nuclear band in the experiment of Fig. 7. This suggests that the active nuclear 7 S fraction is probably a small nucleolar RNA.

In Vitro Reconstituted Ribosomal Subunits Contain a Full Complement of Ribosomal Proteins—Ribosomal proteins labeled with \([^{35}S]\)methionine and cysteine were extracted from cytoplasmic 40 and 60 S subunits and analyzed by two-dimensional gel electrophoresis (Fig. 8, A and C). An aliquot of these proteins was used to reconstitute in vitro 40 and 60 S particles starting from nuclear immature rRNA and in the presence of the 7 S small nuclear RNA fraction. As shown in Fig. 8, B and D, the reconstituted particles contained all of the ribosomal proteins required for the reconstitution of both 40 and 60 S ribosomal subunits.

**Fig. 4. Identification of a 7 S nuclear RNA fraction active in ribosome reconstitution.** RNA was extracted from whole nuclei as described under "Experimental Procedures" and chromatographed on oligo(dT) columns. Poly(A)\(^+\) RNA was sedimented in a 10–35% sucrose gradient in 20 mM Tris-HCl, 0.5 M NH$_4$Cl in a Beckman SW40 rotor at 170,000 \(\times g\) for 36 h. The first 22 fractions were tested for their ability to substitute for nuclear lysate in promoting the reconstitution of ribosomal particles. Only fraction 18 (indicated by an arrow) was active.

**Fig. 5. Analysis of the 7 S nuclear RNA fraction.** RNA contained in fraction 18 of the gradient shown in Fig. 3 was labeled with \([^{32}P]pCp\) and electrophoresed on an 8% polyacrylamide gel (right lane). On a parallel lane (left lane) a sample containing 5 S RNA (lower band) and 5.8 S RNA (upper band) also labeled with \([^{32}P]pCp\) was electrophoresed. The gel was autoradiographed. In an exposure longer than the one shown here, several other bands were visible in the right lane.

**Fig. 6. Hybridization of 7 S RNA to DNA containing a 5.8 S RNA gene.** RNA was isolated from the regions of the gel shown in Fig. 4 corresponding to the two major bands of the right lane. The RNAs were hybridized to 2 μg of spotted DNA containing a 5.8 S RNA gene, in the absence (upper row of spots) and in the presence (lower row) of 10 μg of unlabeled 5.8 S RNA. The second spot in each row corresponds to the 7 S RNA from the upper band, while the third spot corresponds to the 7 S RNA from the lower band. The first spot of each row is given by the hybridization of \([^{32}P]pCp\)-labeled 5.8 S RNA.

**Fig. 7. In vitro reconstitution of ribosomal particles in the presence of 7 S small nuclear RNA.** Ribosomal particles were reconstituted as described under "Experimental Procedures" in the absence of nuclear lysate but in the presence of the 7 S small nuclear RNA corresponding to the lower band shown in the gel of Fig. 5 (right lane). The incubation products were analyzed as in Fig. 3. \(\bullet\), \(^{3}H\)-labeled 19 S RNA; \(\triangle\), \(^{3}H\)-labeled 5, 5.8, and 28 S RNAs; \(\oplus\), \(^{14}C\)-labeled 17 S RNA; \(\bigtriangleup\), \(^{14}C\)-labeled 5, 5.8, and 26 S RNAs; \(\blacksquare\), \(A_{260}\).
proteins present in native subunits at comparable amounts.

In Vitro Reconstituted Ribosomal Subunits Function in Protein Synthesis—To test their functionality, the \(^{3}H\)-labeled particles reconstituted in the presence of nuclear lysate or of the 7 S small nuclear RNA were incubated in a cell-free system for protein synthesis (31) lacking endogenous ribosomes and programmed with poly(A)"RNA extracted from Dictyostelium cells. We tested native subunits alone and several amounts of each species of reconstituted particles in the presence of an excess of the other species of native subunit (Table I). As we mentioned under “Experimental Procedures,” the added ribosomes are the only limiting factor of the rate of \(^{35}S\)methionine incorporation. The relative activity of the tested ribosomes can therefore be directly compared. With all types of reconstituted particles, the efficiency of incorporation was 85–90% of that obtained with native ribosomal subunits.

In additional experiments, the \(^{3}H\)-labeled ribosomal subunits reconstituted in the presence of nuclear lysate or of 7 S small nuclear RNA entered polyribosomes in a cell-free system to the same extent as native cytoplasmic ribosomal subunits (Fig. 9). The three kinds of subunits entered polyribosomes at an efficiency of 50–60%, a level close to the one found in growing cells. Thus, the original activity of the subunits used for the reconstitution experiment, the efficiency of the reassembly, and the functionality of reconstituted particles were comparable with those obtained with E. coli ribosomes (1).

Data to be published elsewhere\(^2\) show that the in vitro reconstituted 40 S ribosomal subunits are also functional in an in vitro test of their ability to destabilize specific mRNAs, when their protein components are obtained from cells at a developmental stage in which the same mRNAs are highly unstable in vivo.

Pre-rRNA Does Not Mature during the Reconstitution Process—Since in vitro reconstitution of ribosomal subunits requires small nuclear RNA and small nuclear (or nucleolar) RNA is known to be involved in rRNA processing (32–38), one possibility to test was that ribosome reconstitution is dependent upon or concomitant with rRNA maturation. On the contrary, Fig. 1B shows that the rRNA molecules contained in reconstituted particles retained the same size they had in vivo.

Nuclear Ribosomal Subunits Containing Immature rRNA Can Function in Protein Synthesis—The results reported above suggested the possibility that in vivo assembled ribosomal subunits still present in nuclei and containing immature RNA can already function in protein synthesis. To examine this possibility, we tested \(^{3}H\)-labeled ribosomal particles isolated from nuclei in the in vitro protein synthesis system described under “Experimental Procedures.” They were as active as unlabeled ribosomal subunits isolated from polyribosomes (Table I).

**DISCUSSION**

The data reported here show that, in the presence of a nuclear component, eukaryotic 40 and 60 S ribosomal subunits can be reconstituted in vitro from free *D. discoideum* rRNA and ribosomal proteins. This should provide a new opportunity to study the structure and function of eukaryotic ribosomes.

The *in vitro* reconstituted ribosomal subunits have a full complement of ribosomal proteins and are fully functional in protein synthesis. Data to be published elsewhere\(^2\) show that they are active also in a newly discovered function, the control of the stability of a class of developmentally regulated mRNAs.

The *in vitro* system described here for ribosome reconstitution indicates some principles that should be applicable to

**TABLE I**

| Incorporation of \(^{35}S\)methionine in trichloroacetic acid-precipitable material by native and in vitro reconstituted ribosomal subunits |
|---------------------------------------------------------------|
| Experiment^a |
| 1       | 2       | 3       |
| 15 \(\mu\)g of native cytoplasmic subunits | 52.205  | 63.250  | 64.720  |
| 2.5 \(\mu\)g of 40 S reconstituted with nuclear lysate plus 10 \(\mu\)g of native 60 S | 24.488  | 25.602  | 25.290  |
| 5 \(\mu\)g of 40 S reconstituted with nuclear lysate plus 10 \(\mu\)g of native 60 S | 58.810  | 49.780  | 62.110  |
| 2.5 \(\mu\)g of 40 S reconstituted with 7 S small nuclear RNA plus 10 \(\mu\)g native 60 S | 31.640  | 26.760  | 32.430  |
| 5 \(\mu\)g of 40 S reconstituted with 7 S small nuclear RNA plus 10 \(\mu\)g native 60 S | 53.570  | 61.750  | 63.330  |
| 5 \(\mu\)g of 60 S reconstituted with nuclear lysate plus 5 \(\mu\)g of native 40 S | 29.880  | 30.150  | 32.220  |
| 10 \(\mu\)g of 60 S reconstituted with nuclear lysate plus 10 \(\mu\)g of native 40 S | 51.150  | 58.970  | 64.060  |
| 5 \(\mu\)g of 60 S reconstituted with 7 S small nuclear RNA plus 5 \(\mu\)g of native 40 S | 24.560  | 29.800  | 31.250  |
| 10 \(\mu\)g of 60 S reconstituted with 7 S small nuclear RNA plus 10 \(\mu\)g of native 40 S | 52.450  | 61.340  | 63.660  |
| 10 \(\mu\)g of immature nuclear subunits | 48.565  | 55.760  | 61.350  |
| Ribosomal subunits omitted | Poly(A)"RNA omitted in the presence of native subunits | 255     | 310     | 285     | 120     | 150     | 210     |

^a^ Values above represent counts/min incorporated in a 15-min incubation.
show that in Dictostelium, as in E. coli (14, 18, 39), ribosomal subunits still containing immature rRNA enter polysomes directly as they join the cytoplasm. Maturation of rRNA would therefore occur in polysomes.

In vitro reconstitution of ribosomal particles does not occur autonomously but instead requires morphopoietic factors that are contained in the nucleus. Among these factors there is/or one or several small nuclear RNAs, since the nuclear lystate that drives the reconstitution reaction in our experimental conditions can be substituted by a small nuclear RNA fraction. Since rRNA incorporated in reconstituted particles is still immature, the small nuclear RNA that functions in our system is probably not involved in rRNA trimming but rather in the assembly process. In any case, the principle that the assembly of many cell organelles is facilitated and/or controlled by morphopoietic factors appears to be valid also for eukaryotic ribosomes, as already suggested by in vivo studies (33, 34).

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FIG. 9. Formation of polyribosomes by native and in vitro reconstituted ribosomal subunits. Ribosomal subunits reconstituted from nuclear RNA and ribosomal proteins in the presence of nuclear lysate (A) or of 7 S small nuclear RNA (B) and native ribosomal subunits (C) were incubated for 15 min in the absence (A and B) or in the presence (● and ○) of poly(A)– RNA in the in vitro system for protein synthesis described under “Experimental Procedures.” The incubation mixtures were sedimented through 15–35% sucrose gradients in 20 mM Tris-HCl, pH 7.5, 50 mM KCl, 20 mM MgCl2 at 170,000 g for 2 h to display polyribosomes. If pancreatic RNase was added to the mixtures at the end of the incubation, no material sedimented faster than 80 S.

other eukaryotic organisms and help to solve the problem of the in vitro reconstitution of their ribosomal particles. One is that mature rRNA, extracted from cytoplasmic ribosomes, is an inadequate substrate for the reconstitution reaction. A much better substrate is immature precursor rRNA extracted from nuclei. It is likely that the extra sequences present in precursor RNA play some role in the assembly process. This is in agreement with the finding that nuclear particles sedimenting at 40 and 60 S and therefore presumably fully assembled still contain immature RNA. The same is true for ribosomal particles reconstituted in vitro and containing a full complement of ribosomal proteins. Both nuclear and in vitro reconstituted particles containing immature rRNA are active in protein synthesis. This suggests that in Dictostelium the last step in ribosome assembly is RNA maturation, as in E. coli (17, 18, 39) and probably in yeast (40). Data to be published elsewhere.