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# An ATPase Center of Rat Liver 30S-5SRNP Particles

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**Treatment of 30S-5SRNP with 1 M $Cs_2SO_4$ at 2°C overnight followed by sucrose density-gradient centrifugation yielded particles smaller than 30S-5SRNP, designated as CsS-particles. CsCl density-gradient centrifugation of CsS-particles showed the homogeneity of the particles containing about half the amount of proteins in 30S-5SRNP particles. The particles contained 18SrRNA, 5SRNP and about half the number of proteins in 30S-5SRNP. The ATPase activity of freshly prepared CsS-particles was about half the original 30S-5SRNP level although it was unstable even at 2°C. Poly(U) slightly enhanced the activity, and phe-tRNA$^{phe}$ stimulated it concentration-dependently. EF-1α alone enhanced it, and in combination with poly(U) and phe-tRNA$^{phe}$ stimulated it markedly. EF-2 alone markedly increased it. The activity with the full components for elongation described above became very high, being comparable to that of the original 30S-5SRNP and twice that of 40S subunits. A two-dimensional electrophoretogram of the protein in CsS-particles revealed 9 small subunit protein species, in addition to L5, which included proteins interacting with mRNA and two elongation factors. Taken together with the results of our preceding study indicating the participation of ATPase of 80S ribosomes in peptide elongation, the present results indicate CsS-particles may be a part of the ATPase centre of 80S ribosomes.**

**Key words: ATPase, CsS-particles, elongation factors, mRNA, 30S-5SRNP.**

It is known that mammalian ribosomes and both subunits contain intrinsic ATPase (*1–7*), although the role in peptide elongation only began to be clarified recently (*3–5*). In our preceding paper (*6*), it was shown that the ATPase activity of rat liver 30S-5SRNP particles, which were prepared by EDTA treatment of 80S ribosomes and consisted of 5SRNP-bound 40S subunits (*8*), was higher than that of 40S subunits. Poly(U), TMV RNA, tRNA, EF-1α, or EF-2 alone enhanced the ATPase activities of both 30S-5SRNP particles and 40S subunits. With combinations of these peptide-elongational components, the ATPase activities of these two particles were enhanced additively or synergistically. The extents of stimulation by elongation components were always higher for 30S-5SRNP particles than 40S subunits. Therefore, we considered that the individual components for polypeptide elongation induced positive conformational changes in different regions of the ATPase centre of these particles through the interaction with them. Furthermore, the ATPase activities of the two kinds of particles alone or those observed with combinations of the elongation components mentioned above were inhibited by several kinds of translation inhibitors. The extents of inhibition were generally higher for 30S-5SRNP particles than 40S subunits.

From these results we concluded the following. (i) The intrinsic ATPase of 30S-5SRNP particles or 40S subunits may be correlated in some way with peptide bond elongation, although the actual mode of correlation should be determined by use of 80S ribosomes. (ii) The 5SRNP moiety existing at the subunit interface (*10*) plays an important role in the ATPase activity of 30S-5SRNP particles. In this respect, in our preceding study (*7*), using rat liver 80S ribosomes, we showed that the intrinsic ATPase activity of 80S ribosomes was also enhanced by components for peptide bond elongation by themselves or in combination. Furthermore, after showing that GTPase of EF-1α or EF-2 is stringent as to GTP, it was found that two kinds of ATPase inhibitor, AMP-P(NH)P and vanadate, depressed the activity of poly(U)-dependent polyphe synthesis, suggesting that this ribosomal ATPase plays a role in peptide elongation through positive conformational changes of 80S ribosomes induced by binding the elongational components. Since the protein topographies of mammalian ribosomal subunits, the subunit interface, and ribosomal proteins interacted with mRNA, tRNA, EF-1α, and EF-2 were already known, we assumed an ATPase centre of 80S ribosomes, which is localized to a limited area including the interface between two subunits, and contains 5SRNP and a limited number of ribosomal proteins.

As the first step to find this ATPase centre, we tried to remove a part of the protein moiety from 30S-5SRNP particles, their ATPase activity remaining intact. At first, we treated 30S-5SRNP with high concentrations of KCl. How-

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Abbreviations: AMP-P(NH)P, 5′-adenyl imidodiphosphate; GMP-P(NH)P, 5′-guanyl imidodiphosphate; ATA, aurintricarboxylic acid; DTT, dithiothreitol; TMV, tobacco mosaic virus, 5SRNP, 5SrRNA-L5 protein particles; phe-tRNA$^{phe}$, phenylalanyl-tRNA$^{phe}$, tRNA$^{phe}$, deacylated tRNA$^{phe}$.

ever, we could not accomplished our purpose. For example, the pattern on sucrose density gradient centrifugation showed that treatment of 30S-5SRNP with 0.8 or 1.2 mM with 5 or 10 mM $MgCl_2$ did not result in the appearance of a sharp $A_{254}$ peak in the molecular weight region lower than that of the original 30S-5SRNP. We thought that this might be due to the instability of 5SRNP with high concentrations of KCl. It must be added that at higher KCl concentrations, RNA was removed from 30S-5SRNP, as shown by the low $A_{260}$ to $A_{280}$ ratio of the resulting $A_{254}$ peak. Since it was reported that $Cs_2SO_4$ density-equilibrium centrifugation of 60S subunits releases intact 5SRNP (*10*), we tried to use $Cs_2SO_4$ for this purpose. When 30S-5SRNP particles were treated with 1 M $Cs_2SO_4$ at 2°C overnight, followed by fractionation by sucrose density-gradient centrifugation, the resulting particles had lost about half the number of ribosomal proteins in 30S-5SRNP particles, sufficient ATPase activity being retained. We tentatively designated them as CsS-particles.

In this report, we describe (i) the purification procedures for CsS-particles, (ii) the homogenity of the particles shown on sucrose and CsCl density-gradient centrifugation, (iii) analysis of their RNA and protein moieties, (iv) some properties of their ATPase activity, especially stimulation of the activity by the components for polypeptide bond elongation, which were compared with those observed of the original 30S-5SRNP particles and 80S ribosomes, and (v) identification of the ribosomal proteins in CsS-particles by two-dimensional PAGE and their schematic representation in Cs-particles, showing the protein topography and the individual proteins interacting with peptide bond elongational components.

## MATERIALS AND METHODS

*Materials*—[γ-$^{32}$P]ATP (10 Ci/mmol) and [γ-$^{32}$P]GTP (10 Ci/mmol) were purchased from the Institute of Isotopes, Hungarian Academy of Science, which were purified as described in Ref. *6*, Cesium sulfate from Nacalai Tesque, Kyoto, and Pyronine Y from Wako Chemicals. Other chemicals were described in our preceding papers (*6*, *7*).

*Medium*—Medium A: 0.25 M sucrose, 5 mM $MgCl_2$, 50 mM KCl, and 50 mM Tris-HCl, pH 7.6.

*Preparation of CsS-Particles*—30S-5SRNP particles prepared from rat liver 80S(PM) ribosomes were used throughout this study (*6*); 3.7 M $Cs_2SO_4$ was added slowly with constant stirring to give a final concentration of 1 M and then the mixture was left standing at 2°C overnight. The mixture was then layered onto a 15–30% sucrose density-gradient containing sucrose-minus Medium A and 0.1 mM DTT. Centrifugation was carried out at 2°C in a Hitachi SRP28SA rotor at 28,000 rpm for 18 h or in a RPS 40T rotor at 40,000 rpm for 5 h. The latter centrifugation was used for assaying the ATPase activity. The absorbance at 254 nm was constantly monitored with an ISCO automatic density-gradient fractionator. The fractions containing CsS-particles (Fig. 1) were diluted twofold with sucrose-minus Medium A minus sucrose and then concentrated with a Diaflow membrane. The particles thus obtained were stored in small portions in liquid nitrogen.

*Cesium Chloride Density-Gradient Centrifugation*—After formaldehyde fixation (*11*), the CsS-fraction, 30S-5SRNP, and 40S subunits were subjected to CsCl density-gradient centrifugation (*11–14*) after dialysis of the samples against 0.02 M triethanolamine at 2°C for 24 h. After centrifugation in a RPS55T2 (Hitachi) at 50,000 rpm for 16 h, 10 drop fractions were collected, and then $A_{260}$ and $A_{280}$ of each fraction were measured. The CsCl density was calculated from the refractive index, using the table given in Ref. *15*. The percentage of protein was calculated with the empiric formula presented in Ref. *14*: protein % = 1.85 − ρ (buoyant density)/0.006.

*PAGE of RNA and Protein Moieties of CsS-Particles*—RNA of CsS-particles was prepared by the SDS-phenol method described previously (*16*). PAGE of 5SrRNA was carried out by a slight modification (*16*) of the method of Loening (*17*). PAGE of 18SrRNA was carried out by a slight modification of the agarose-acrylamide gel electrophoresis method of Peacock and Dingman (*18*), using a 1% agarose–3% acrylamide gel. The RNA bands were stained with ethidium bromide (0.5 μg/ml).

SDS-PAGE of protein was carried out by the method of Laemmli (*19*). Silver staining of protein on the gel was carried out with a reagent purchased from Daiichi Pure Chemical (Tokyo), using the procedures recommended by the manufacturer.

*ATPase Assay*—The standard incubation mixture contained 0.5 pmol of RNA of CsS-particles, 75 mM Tris-HCl (pH 7.6), 2.5 mM DTT, 7.5 mM $MgCl_2$, 12.5 mM KCl, and usually 0.062 mM [γ-$^{32}$P]ATP (1 μCi), in a total volume of 20 μl. In the experiments for comparison of the ATPase activity of CsS-particles with that of 30S-5SRNP particles, 0.125 mM [γ-$^{32}$P]ATP was used. Incubation was carried out at 37°C for 30 min. Hydrolysis of [γ-$^{32}$P]ATP was followed by determination of the amount of inorganic $^{32}PO_4$ released during the incubation time, by a slight modification of the isobutanol-benzene method described in the proceding paper (*6*). The ATPase activity was expressed as pmol of inorganic $^{32}PO_4$ per pmol particles per minute. In this work we assumed that 1 μg RNA was equal to 0.6 pmol of 30S-5SRNP particles and 1.7 pmol of CsS-particles from the compositions, molecular weights of the proteins (*20*, *21*), and RNA (*22*) of the particles.

*Micro-Scale Two-Dimensional PAGE of Ribosomal Proteins of CsS-Particles—1) Extraction of ribosomal proteins*: Ribosomal proteins were extracted from CsS-particles with acetic acid according to a modification (*23*) of the procedure of Hardey *et al.* (*24*), as follows. One molar $MgCl_2$ was added to the CsS-particles to give a final concentration of 20 mM, and then an equal volume of cold ethanol was added. The mixture was kept overnight at 0°C and then the particles were sedimented by centrifugation at 237,600 ×*g* for 70 min. The precipitated particles were suspended in 100 mM $MgCl_2$. After the addition of 2 volumes of glacial acetic acid, the mixture was stirred at 0°C for 48–72 h. Ribosomal proteins were obtained by centrifugation of this mixture in a microcentrifuge tube (1.5 ml) at 15,000 rpm for 30 min at 0°C. After the addition of 10 volumes of acetone, the mixture was stored at −30°C overnight. After centrifugation at 11,000 rpm for 30 min, the pellet was dissolved in a minimal volume (5 μl) of 6 M urea and then subjected to the following procedures.

*2) Microscale two-dimensional PAGE*: Two-dimensional PAGE of ribosomal proteins was carried out using an apparatus from TEF Corporation (1 mm, 15 wells). The same media as described in our previous report (*23*) were used:

an 8% acrylamide gel (pH 8.6) for the first dimensional gel with cytochrome c as a marker, and a 15% acrylamide gel (pH 4.6) for the second dimensional gel with pyronine Y as a marker. In the first-dimensional gel electrophoresis, 8 mA was applied for 21 h, and in the second-dimensional gel electrophoresis, 6 mA for 17 h. The gel was subjected to silver staining as described above.

## RESULTS

*The Pattern on Sucrose Density-Gradient Centrifugation of $Cs_2SO_4$-Treated 30S-5SRNP Particles*—The pattern on sucrose density-gradient centrifugation of $Cs_2SO_4$-treated 30S-5SRNP particles (PM) is shown in Fig. 1(1). A sharp $A_{254}$ peak was present in a molecular weight region (fractions 15–17) lower than that of the original 30S-5SRNP particles (fractions 22–24). The fractions in this region were collected and concentrated with a Diaflow membrane. The resulting fraction was used as CsS-particles throughout the present experiments. It must be added that the intactness of 18S rRNA in 30S-5SRNP particles was necessary to obtain homogeneous CsS-particles, since when 30S-5SRNP-(H) (*6*) was used, a diffuse distribution of $A_{254}$ was observed in the molecular weight region lower than that of 30S-5SRNP particles.

*CsCl Density-Gradient Centrifugation of CsS-Particles*—The homogenity of CsS-particles was further examined by CsCl density-gradient centrifugation. The main peak at $A_{260}$ of CsS-particles was situated at $\rho = 1.71$ [Fig. 1(2)]. It was shown that $\rho$ of 30S-5SRNP and 40S subunits were 1.58 and 1.6, respectively (data not shown).

Judging from these results together with the pattern on sucrose density-gradient described above, CsS-particles were thought to be homogeneous. From these $\rho$ values, protein % of CsS-particles, 30S-5SRNP and 40S subunits were calculated to be 23, 45, and 47%, respectively. Thus, the protein content of CsS-particles is about 50% of that of 30S-5SRNP.

*Basic Properties of CsS-Particles*—The $A_{260}/A_{280}$ ratio of CsS-particles was about 1.9, indicating that RNA is abundant in these particles. As shown in Fig. 2(1), the pattern of RNA prepared from CsS-particles on agarose-acrylamide gel electrophoresis (*18*) showed the presence of 18SrRNA [lane 3 in Fig. 2(1)].

Electrophoretograms obtained on SDS-PAGE of RNA from CsS-particles are presented in Fig. 2(2)(3). As shown in lanes 1 and 3 in Fig. 2(2), similar band patterns were observed for RNA of CsS-particles and that of 30S-5SRNP, although the 5SrRNA bands were faint. When a large amount of RNA of CsS-particles was applied, as shown in lane 1 in Fig. 2(3), a distinct 5SrRNA band was observed, which was different from the tRNA bands observed for marker 5SrRNA [see lane 2 in Fig. 2(2)], and from the 5.8SrRNA band observed for marker rRNA [see lane 4 in Fig. 2(2)]. It must be added that 5SrRNA prepared from CsS-particles mainly gave one band corresponding to the slower moving 5SrRNA band of marker 5SrRNA [lanes 1, 3, and 4 in Fig. 2(3)]. Since two bands of 5SrRNA were reported to be due to different conformations of 5SrRNA (*25*, *26*), conformational changes of 5SrRNA occurred during the preparation of CsS-particles. Such a phenomenon was observed previously on PAGE of RNA prepared from the methionyl-tRNA synthetase complex which was purified from the macromolecular aminoacyl-tRNA synthetase complex (*27*).

Figure 3(1) shows a silver staining pattern of protein on

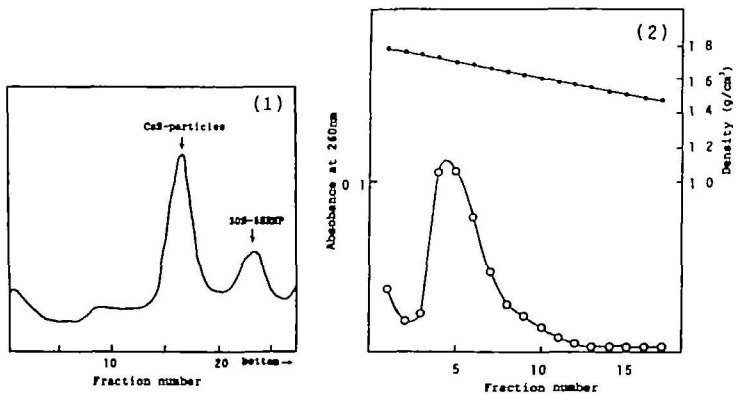


Fig 1 **(1) Sucrose density-gradient centrifugation of 30S-5SRNP particles treated with $Cs_2SO_4$.** 30S-5SRNP-(PM) particles (250 μg of RNA) were treated with 1 M $Cs_2SO_4$ at 2°C overnight and then subjected to sucrose density-gradient centrifugation, with monitoring with an ISCO automatic density gradient fractionater. **(2) CsCl density-gradient centrifugation of CsS-particles.** CsS-particles (19 μg RNA) were used.

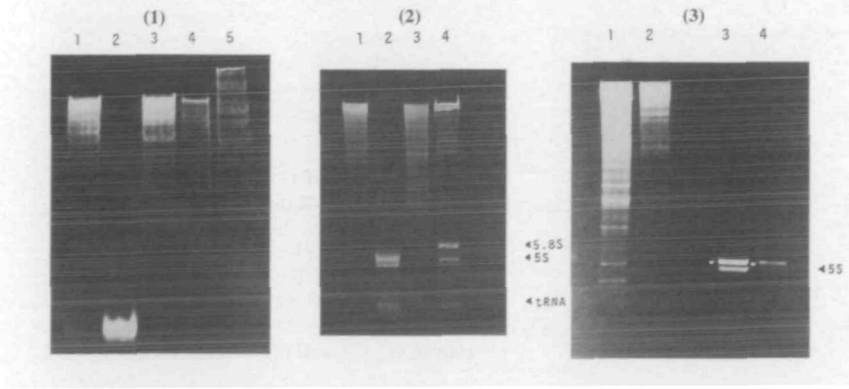


Fig. 2. **(1) Staining pattern with ethidium bromide of RNA on 1% agarose–3% acrylamide gel electrophoresis, and (2) and (3) staining pattern with ethidium bromide of RNA on 7.5% PAGE.** (1) Lane 1, 18SrRNA (5 μg); lane 2, 5SrRNA (5 μg); lane 3, RNA of CsS-particles (5 μg); lane 4, RNA of CsS-particles (2 μg), and lane 5, 28SrRNA (5 μg). (2) Lane 1, 30S-5SrRNA (10 μg), lane 2, 5SrRNA (5 μg); lane 3, RNA of CsS-particles (10 μg); and lane 4, marker rRNA (19 μg). The dot shows 5SrRNA. (3) Lane 1, RNA of CsS-particles (38 μg); lane 2, RNA of CsS-particles (8 μg); lane 3, 5SrRNA (2 μg); and lane 4, 5SrRNA (0 4 μg). The dot shows 5SrRNA.

SDS-PAGE of CsS-particles. While about 20 protein bands were detected for 40S subunits [lane 3 in Fig. 3(1)], about 10 were observed in addition to a sharp band located at the position of L5 for CsS-particles [lane 2 in Fig. 3(1)]. It must be mentioned that the protein species in the lowest molecular weight region on sucrose density-gradient centrifugation of $Cs_2SO_4$-treated 30S-5SRNP showed a different band pattern from that of CsS-particles on SDS-PAGE, as shown in lanes 3 and 4 in Fig. 3(2), indicating that $Cs_2SO_4$ treatment selectively removed protein species from 30S-5SRNP particles.

From these results, it is reasonably assumed that CsS-particles contain 18SrRNA, 5SRNP, and about half the number of proteins in 30S-5SRNP particles.

*The ATPase Activity of CsS-Particles*—Since we intended to prove that CsS-particles are an ATPase centre of the 30S-5SRNP particles, the following experiments were carried out. When the ATP-hydrolyzing activity was measured under our standard conditions with 0.125 mM ATP, the activity of freshly prepared CsS-particles by themselves was 0.51 pmol inorganic P per pmol particles per min (average of eight independent experiments). It was about half that of the original 30S-5SRNP particles (PM) and somewhat higher than that of 40S subunits, which were 1.1 pmol and 0.4 pmol inorganic P per pmol particles per min, respectively. It must be added that the activity varied (from 0.82 to 0.27 pmol inorganic P per pmol particles per min) among individual preparations owing to the instability of the ATPase activity of CsS-particles, as described later.

*Basic Properties of ATPase of CsS-Particles*—The time course of ATP hydrolysis by CsS-particles is shown in Fig. 4(1). The reaction proceeded almost linearly at least for 30 min at 37°C. The effect of the $MgCl_2$ concentration on ATP hydrolysis is shown in Fig. 4(2). The optimal $MgCl_2$ concentration was 7.5 mM, in agreement with that in the case of 30S-5SRNP particles. The ATPase activity was highest with 12.5 mM KCl [Fig. 4(3)]. The addition of $NH_4Cl$ to the standard reaction mixture at the final concentration of 50 and 100 mM markedly decreased the ATPase activity to 63 and 53% of the control level, respectively. The ATPase activity was unstable, decreasing to about 25% of the original level after standing at 2°C for 2 h.

*Effects of Components for Peptide Bond Elongation*—While poly(U) markedly stimulated the ATPase activity of 30S-5SRNP particles (*6*), the extent of its stimulation of the activity of CsS-particles was lower and somewhat variable. One microgram of poly(U) increased the hydrolysis of ATP by 114% (average of twelve independent experiments). Figure 5(1) shows a dose-response curve for the effect of phe-tRNA$^{phe}$ on the ATPase activity of CsS-particles. It stimulated the ATPase activity markedly up to 15 μg, depending on the concentration. The extent of stimulation by phe-tRNA$^{phe}$ was greater than that by tRNA$^{phe}$ (Fig. 6, Exp. 3).

*Effects of Elongation Factors on the ATPase Activity of CsS-Particles*—To examine the correlation of ATPase of CsS-particles with polypeptide elongation, we investigated the effects of elongation factors on the ATPase activity of

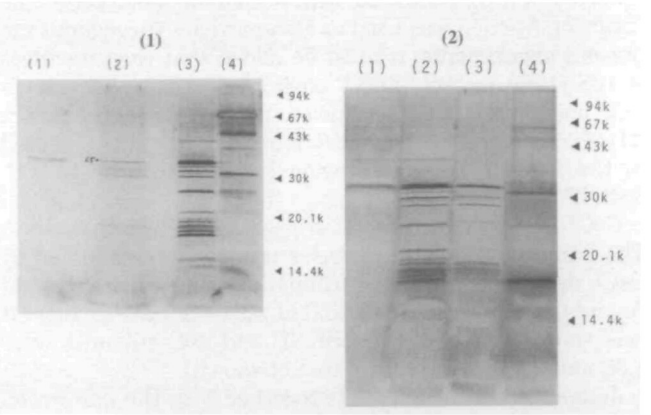


Fig 3. (1) **SDS-PAGE of CsS-particles and (2) of the lowest molecular weight fraction released from 30S-5SRNP on $Cs_2SO_4$ treatment and of CsS-particles.** (1) Lane 1, 5SRNP (0 6 μg of RNA), lane 2, CsS-particles (2 3 μg, RNA), lane 3, 40S subunits (2 μg, RNA); and lane 4, markers (0 5 μg of each), phosphorylase *b* ($M_r$ 94,000), bovine serum albumin ($M_r$ 69,000), ovalbumin ($M_r$ 43,000), carbonic anhydrase ($M_r$ 30,000), and soy bean trypsin inhibitor ($M_r$ 20,100). (2) Lane 1, 5SRNP (0.6 μg of RNA); lane 2, 40S proteins (2 μg of RNA), lane 3, CsS-particles (5 μg of RNA), and lane 4, low molecular weight fraction (2 μg of protein).

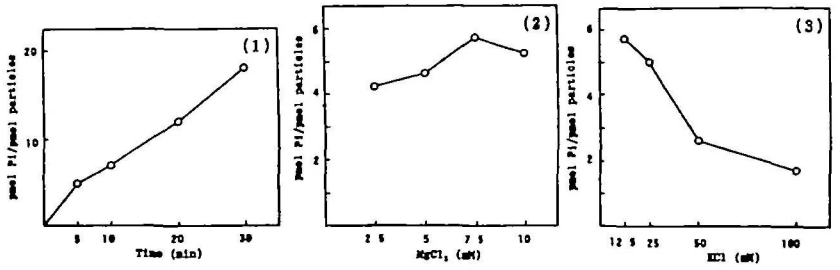


Fig 4. **Basic properties of ATPase of CsS-particles.** (1) Time course, (2) effect of the $MgCl_2$ concentration, and (3) effect of the KCl concentration. The results are expressed as pmol $P_i$ per pmol particles per 30 min.

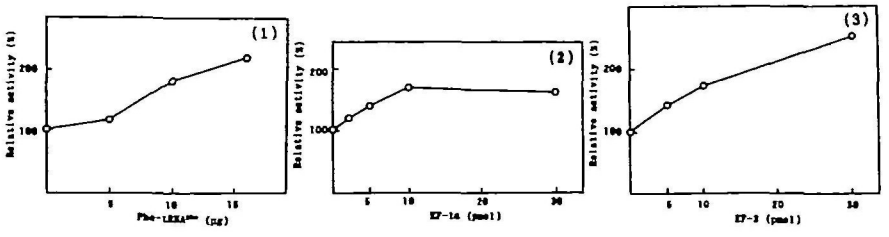


Fig. 5. **Effects of (1) phe-tRNA$^{phe}$, (2) EF-1α, and (3) EF-2 on the ATPase activity of CsS-particles.** Dose response curves for the effects of (1) phe-tRNA$^{phe}$, (2) EF-1α, and (3) EF-2 The ATPase activity of CsS-particles themselves is set as 100%, which is 0 32, 0 54, and 0 75 pmol $P_i$ per pmol particles per min in (1), (2), and (3), respectively

CsS-particles, using highly purified preparations of EF-1α (*28*) and EF-2 (*29*) from pig liver, which were found to be free from contamination by ATPase (*6*). The dose-response curve for the effect of EF-1α is shown in Fig. 5(2), EF-1α stimulated the ATPase activity up to 10 pmol, depending on its concentration. It must be added that the extent of stimulation somewhat varied among the preparations of CsS-particles, as described later. As shown in Fig. 5(3), EF-2 markedly stimulated the ATPase activity of CsS-particles, depending on its concentration, at least up to 30 pmol. It must be mentioned that EF-2 enhanced the ATPase activity of partially inactivated CsS-particles, as shown later in Fig. 8.

The effects of combinations of elongation components were then investigated. Since it is well-known that EF-1α , phe-tRNA$^{phe}$, and GTP form the ternary complex which binds to poly(U)-containing ribosomes, the effects of the combination of poly(U), EF-1α, and phe-tRNA$^{phe}$ on the

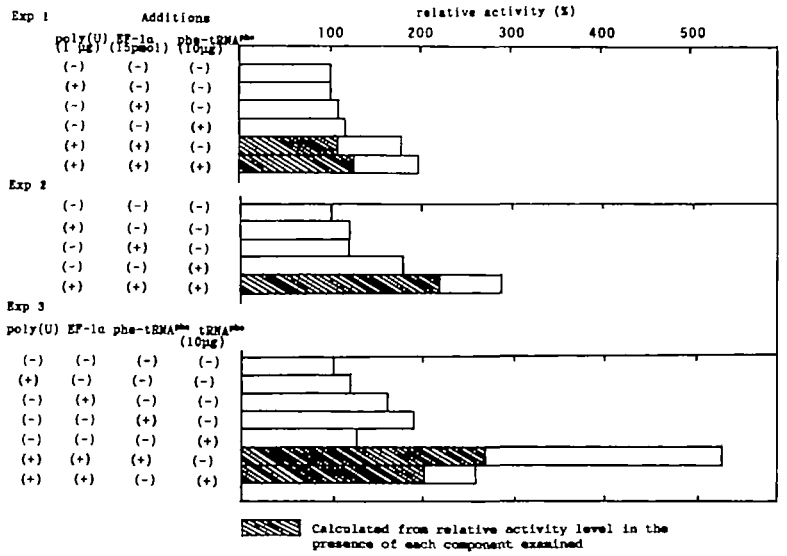


Fig. 6. **Effect of the combination of EF-1α, poly-(U), phe-tRNA$^{phe}$, and tRNA$^{phe}$ on the ATPase activity of CsS-particles.** The ATPase activity of CsS-particles alone is set as 100%, which is 0.32, 0 06, and 0 32 pmol $P_i$ per pmol particles per min in Exp 1, Exp 2, and Exp 3, respectively

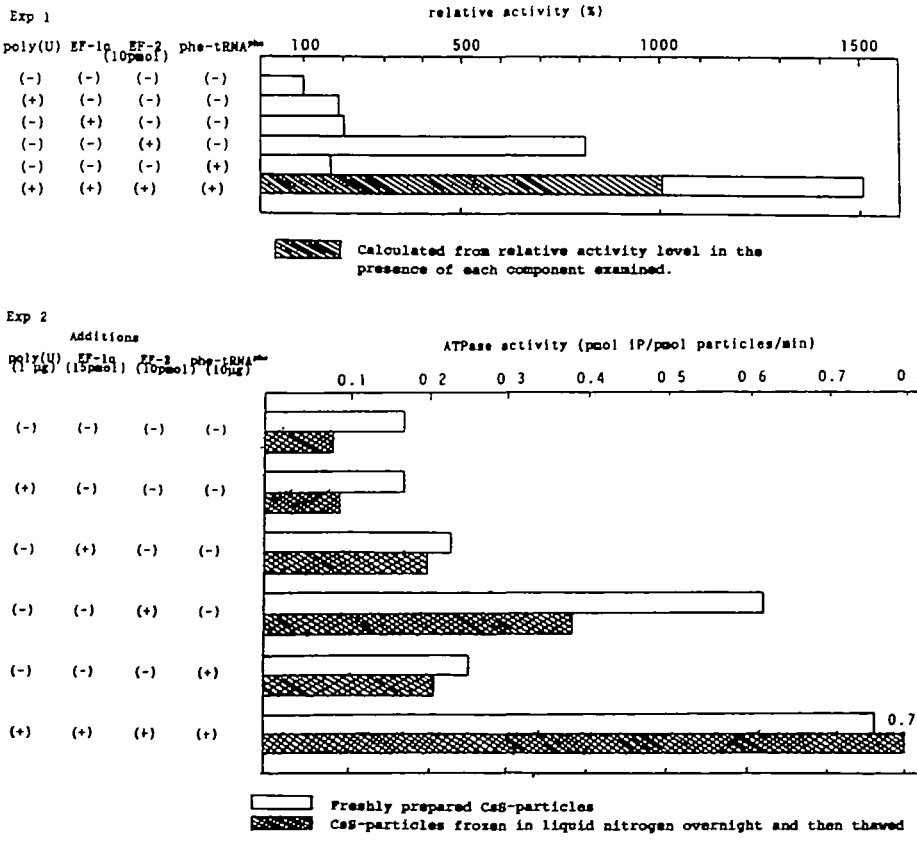


Fig 7 **(Exp. 1) Effects of the full components for translation on the ATPase activity of CsS-particles and (Exp. 2) ATPase activities of CsS-particles freshly prepared and after storage in liquid nitrogen, and the effect of the full components for translation.** Exp 1 The ATPase activity of CsS-particles themselves is set as 100%, which is 0 07 pmol $P_i$ per pmol particles per min.

ATPase of CsS-particles were examined. The results are shown in Fig. 6. The extent of stimulation by each of poly(U), EF-1α and phe-tRNA$^{phe}$ alone was small, especially in Exp. 1 in Fig. 6. However, when poly(U) plus EF-1α, or poly(U) plus EF-1α plus phe-tRNA$^{phe}$ were added, they markedly increased the ATPase activity and the extent of stimulation was greater than the sum of that observed with each component alone (Exp. 1 and 2 in Fig. 6), as in the case of 30S-5SRNP (*6*). Furthermore, phe-tRNA$^{phe}$ promoted the ATPase activity more markedly than tRNA$^{phe}$ in the absence and presence of poly(U) plus EF-1α , as shown in Exp. 3 in Fig. 6. Such cooperative stimulation by the combination of poly(U), EF-1α, and phe-tRNA$^{phe}$ was also observed for the original 30S-5SRNP particles (*6*).

The effects of the full components for peptide bond elongation on the ATPase activity of CsS-particles were examined. As shown in Fig. 7, Exp. 1, when the particles were incubated with the combination of poly(U), EF-1α, EF-2, and phe-tRNA$^{phe}$, the latter markedly stimulated the ATPase activity of the particles and the extent of stimulation was greater than the sum of that observed with each of the components alone (synergic stimulation).

Since the ATPase activity of CsS-particles was unstable, we compared the activity of freshly prepared CsS-particles with that of freshly prepared particles kept in liquid nitrogen overnight and then thawed at 2°C. The results are presented in Exp. 2 in Fig. 7. The ATPase activity of the thawed particles by themselves was about half that of the freshly prepared particles. However, when these two kinds of particles were incubated with poly(U), phe-tRNA$^{phe}$, EF-1α, or EF-2 alone, the extent of stimulation by each of them, especially that by EF-1α or EF-2, was greater in the case of the thawed particles. Furthermore, the ATPase activity of the two kinds of particles in the presence of the full components for elongation became almost the same for the two types of particles and their activity, 0.8 pmol inorganic $^{32}PO_4$ released per pmol particles per min with 0.062 mM [γ-$^{32}$P]ATP, was comparable to that of the original 30S-5SRNP particles, 1.6 pmol with 0.125 mM [γ-$^{32}$P]ATP, under similar assay conditions, considering the concentration of [γ-$^{32}$P]ATP used [Tables I and IV in Ref. *6*] (*6*). The results indicate that although the ATPase activity of CsS-particles is unstable, the activity of the partially inactivated particles is able to react well with the components for peptide bond elongation, and the restored activity is the same as that of freshly prepared particles and comparable to that of the original 30S-5SRNP particles.

*Effects of Translation Inhibitors on the ATPase Activity of CsS-Particles*—As shown in Table I, ATA, ammonium vanadate and tetracyclin inhibited the ATPase activity of CsS-particles, as in the case of 30S-5SRNP particles (*6*).

*Effects of GTP on the ATPase Activity and ATP on GTPase Activity of CsS-Particles*—CsS-particles showed both ATPase and GTPase activities, like 30S-5SRNP particles and 40S subunits. As shown in Fig. 8(1), the ATPase activity of CsS-particles was strongly inhibited by GTP and somewhat less efficiently by GMP-P(NH)P. Figure 8(2) shows that the GTPase activity was strongly inhibited by ATP and somewhat less efficiently by AMP-P(NH)P The results indicate the wide substrate specificity of ATPase of CsS-particles, as also shown for 30S-5SRNP particles (*6*) and 80S ribosomes (*3*, *7*). It is of interest that the inhibition of ATP hydrolysis by GTP was stronger than that of GTP hydrolysis by ATP with the same competitor/substrate ratio.

*Identification of Ribosomal Proteins in CsS-Particles*—Recently, many reports have been available concerning the topography and functions of mammalian ribosomal proteins, as described later. Therefore, it was thought to be important to identify the protein species in CsS-particles. For this purpose, two-dimensional PAGE of ribosomal proteins of CsS-particles was carried out, using the basic-acidic system. The electrophoretogram is shown in Fig. 9(1). In comparison with the electrophoretogram of proteins of 30S-5SRNP particles [Fig. 9(2)], which is the same as that of 40S subunits, except for L5, the following nine proteins were identified; S2, S3/3a, S4, S6, S8, S11, S16, S17a, and S24, while 23 kinds of 40S proteins were detected in the electrophoretogram of 30S-5SRNP proteins [Fig. 9(2)]. The spot of protein L5 detected was faint, probably owing to the extremely poor solubility of this protein. It must be added that the L5 spot was also faint in the electrophoretogram of 30S-5SRNP [Fig. 9(2)]. There have been several reports indicating the extremely poor solubility of L5. Previously, using a 5SrRNA immobilized Sepharose 4B column (*47*) or the nitrocellulose membrane assay (*48*), the binding of L5 to 5SrRNA was not detected. Later, the failure to detect the binding was concluded to be due to the notorious insolubility of L5 (*49*). The insolubility of L5 was also reported by Isoda *et al.* (*10*). We also encountered difficulties in detecting the L5 protein on SDS-PAGE of the methionyl-tRNA synthetase-5SRNP complex, which was prepared from the macromolecular aminoacyl-tRNA synthetase complex, owing to the adhesion of L5 to the dialysis membrane, to a

TABLE I **Effects of ATA, ammonium vanadate, and tetracycline on the ATPase activity of CsS-particles.**

| | Activity | | | | |
|---|---|---|---|---|---|
| | None | ATA 100 μM | Ammonium vanadate 0.1 mM | 0.5 mM | Tetracyclin 0.7 mM |
| CsS-particles | 100 | 85 | 40 | 24 | 87 |

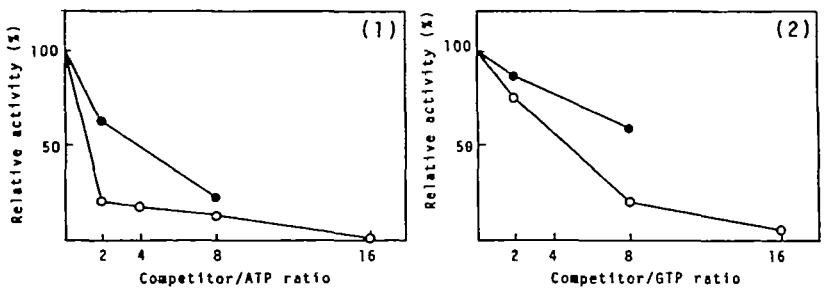


Fig. 8. **(1) Inhibitory effects of GTP and GMP-P(NH)P on the ATPase activity of CsS-particles. (2) Inhibitory effects of ATP and AMP-P(NH)P on the GTPase activity.** (1) ○, inhibitory effect of GTP; ●, inhibitory effect of GMP-P(NH)P (2) ○, inhibitory effect of ATP; ●, inhibitory effect of AMP-P(NH)P.

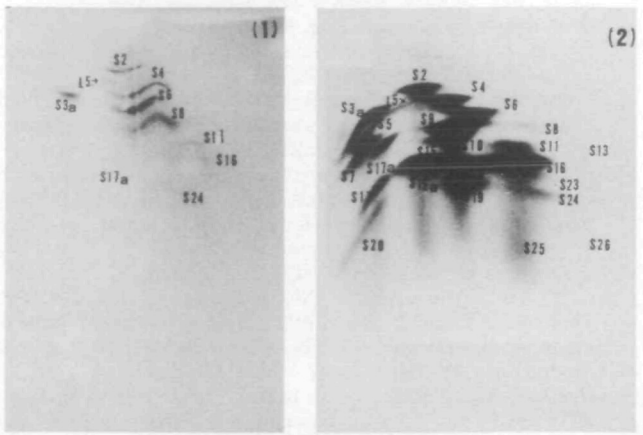


Fig. 9. **Two-dimensional electrophoretograms of liver ribosomal proteins.** (1) Proteins from CsS-particles (4 μg of protein). (2) Proteins from 30S-5SRNP particles (21 μg of protein). The arrow indicates L5

Diaflow membrane and to the lyophilizing vessel (*27*). When serum albumin was added to the sample, followed by concentration with a microconcentrator, L5 was detected as a distinct band [Fig. 3B(1) in Ref. *27*].

## DISCUSSION

The purpose of the present experiment was to find an ATPase centre of 30S-5SRNP particles by removing partially ribosomal proteins, leaving 18SrRNA and 5SRNP intact. At first we did not succeed with high concentrations of KCl, probably owing to the instability of 5SRNP in 30S-5SRNP particles. Next, we accomplished the purpose by using $Cs_2SO_4$. In this respect, it has been reported that $Cs_2SO_4$ density-gradient equilibrium centrifugation of 60S subunits released intact 5SRNP from the particles (*10*). Treatment of 30S-5SRNP with 1 M $Cs_2SO_4$ at 2°C overnight, followed by sucrose density-gradient centrifugation resulted in the separation of CsS-particles in the molecular weight region lower than that of 30S-5SRNP as a sharp $A_{254}$ peak. It was important that intact 18SrRNA was necessary to obtain this sharp peak. The homogeneity of the CsS-particles and their protein content were further analyzed by CsCl density-gradient centrifugation. The CsS-particles sedimented at $\rho = 1.71$ while 30S-5SRNP sedimented at $\rho = 1.58$. From these values, the protein content of CsS-particles was assumed to be about 50% of that of 30S-5SRNP. Analysis of the RNA moiety of CsS-particles by PAGE showed that they contained 18SrRNA and 5SrRNA, like the original 30S-5SRNP particles. SDS-PAGE of the particles showed that, in addition to a sharp protein band at the same position as L5, about half the number of the protein bands in the case of 40S subunits was detected, in agreement with the results obtained on CsCl density-gradient centrifugation of both types of particles described above. It was important that SDS-PAGE of the materials in the lowest molecular weight region on sucrose density-gradient centrifugation of $Cs_2SO_4$-treated 30S-5SRNP showed a different pattern of protein bands from the band pattern of CsS-particles, indicating the release of specific kinds of 40S proteins on $Cs_2SO_4$ treatment of 30S-5SRNP.

TABLE II. **The individual ribosomal components of CsS-particles, (1) their localization and (2) interaction with components for peptide bond elongation.**

| | | | | | | |
|---|---|---|---|---|---|---|
| (1a) Interaction with 60S proteins (*9*) | S2, | S3a, | S4, | S6, | | 5SRNP |
| (1b) Interaction with 28SrRNA (*34*) | S2, S3, | | S4, | S6 | | |
| (1c) A site (*35*) | S3 | | | | | |
| (1d) P site (*36*) | | S3a, | | S6, | | 5SRNP |
| (2) mRNA (*37–41*) | | S3a, | | S6, | S11, | 5SRNP |
| phetRNA (*42*) | S2, | S3a, | | S6 | S24 | |
| EF-2 (*44–46*) | | S3a, | | S6, | S24 | 5SRNP |
| EF-1α (*43*) | | | | | S24 | |

(1a) Subunit interface shown by cross-linking with 2-iminothiolane, and (1b) crosslinked to 28SrRNA by diepoxybutane.

It was found that the ATPase activity of CsS-particles was stimulated by the elongation components by themselves and in combination, as in the case of the original 30S-5SRNP particles. It is important that the combination of the elongation components necessary for ternary complex formation, poly(U), EF-1α, and phe-$tRNA^{phe}$, synergically stimulated the ATPase activity and that a higher degree of promotion of the ATPase activity was observed with phe-$tRNA^{phe}$ than $tRNA^{phe}$ alone, and especially in the presence of poly(U) and EF-1α. Furthermore, the ATPase activity became very high in the presence of the full components for poly(U)-dependent polyphe synthesis and even partially inactivated CsS-particles showed a similar value to the original particles or 30S-5SRNP. These results may indicate that a positive conformation of CsS-particles for ATPase is induced by the translational components, resulting in synergic stimulation of the ATPase activity. Such stimulation of ATPase by the full elongation components has been observed for the ATPase of 30S-5SRNP (*6*) and 80S ribosomes (*7*).

In the case of 80S ribosomes, because two kinds of ATPase inhibitor, AMP-P(NH)P and vanadate, which did not inhibit the GTPase activity of EF-1α and EF-2, inhibited poly(U)-dependent polyphe synthesis, we implied that ribosomal ATPase plays a role in peptide bond elongation (*7*). Therefore, it is possible that the ATPase of CsS-particles may participate in peptide elongation.

Many reports are available concerning the ribosomal proteins of mammalian cells, as follows: (i) a topographical map of ribosomal proteins of 40S subunits (*30–33*), (ii) protein localized at the subunit interface (*9*, *34*), (iii) proteins localized at the A site (*35*) and P site (*36*), and (iv) proteins interacting with elongational components; mRNA (*37–41*), tRNA (*42*), EF-1α (*43*), and EF-2 (*44–46*, *50*). To clarify the function of CsS-particles, we identified ribosomal proteins in the particles by two-dimensional PAGE. Nine species of 40S proteins were identified in addition to L5. The localization and interaction with elongation factors are presented in Table II. It is important that CsS-particles contain proteins localized at or near the subunit interface, and at the A site and P site, and interacting with mRNA, tRNA, EF-1α, and EF-2. We further constructed a schematic representation of CsS-particles, which is shown in Fig. 10. The following points may be important: (i) mRNA interacts with 5SRNP, and S3a, S6, and S11 localized at or near the subunit interface, (ii) tRNA interacts with S2, S3a, S6, and S24 (*42*), (iii) EF-1α interacts with S24, which also interacts

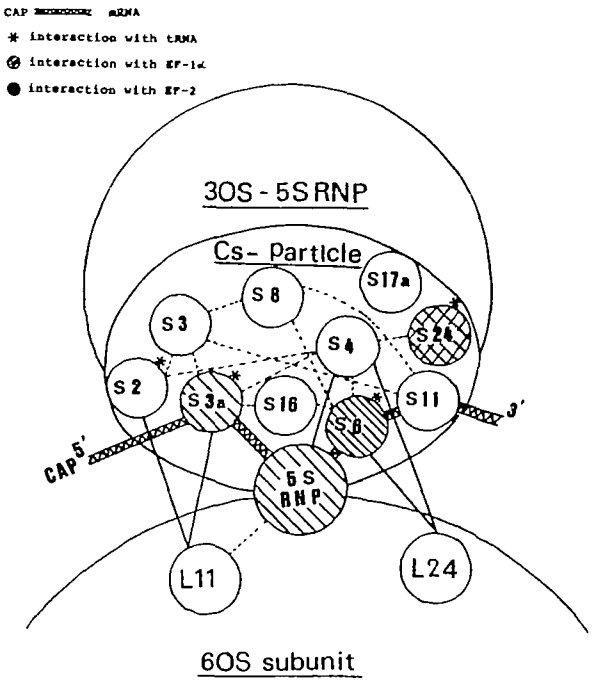


Fig 10 **Schematic representation of proteins in CsS-particles.** (see also Table II )

with tRNA, since both components are essential for ternary complex formation, (iv) EF-2 interacts with S3a, S6, S24, and 5SRNP, (v) S24 is an overlapping protein that interacts with both EF-1α and EF-2, and (vi) proteins of CsS-particles form a network by connecting with each other, except for S17a, the function of which has not been reported. Considering these facts, the stimulation of the ATPase activity of CsS-particles by peptide elongation components may be explained by the positive conformational changes of the CsS-particles induced by the interaction of different ribosomal proteins with elongation components, as in the cases of 30S-5SRNP particles (*6*) and 80S ribosomes (*7*).

Taking the situations described above together, it may be reasonably considered that CsS-particles are the ATPase centre of 30S-5SRNP. We considered that 30S-5SRNP represents the small subunit part of 80S particles containing 5SRNP although it is unfolded on EDTA treatment of 80S ribosomes. On the other hand, high KCl (and puromycin) treatment of 80S ribosomes or polysomes breaks the interaction of 5SRNP with 40S subunits, 5SRNP remaining bound to 60S subunits. Therefore, it may be assumed that 80S ribosomes consist of 60S subunits-5SRNP-40S subunits and that CsS-particles are the ATPase centre of 5SRNP containing small subunits, although further study involving 80S ribosomes must be performed to confirm the assumptions described above.

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