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ATPase Associated with Ribosomal 30S-5SRNP Particles and 40S Subunits of Rat Liver

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The ATPase activity of rat liver 30S-5SRNP particles prepared by EDTA treatment of 80S ribosomes, and that of 40S subunits were investigated in correlation with polypeptide elongation. The ATPase activity of 30S-5SRNP particles was higher than that of 40S subunits. Poly(U) and TMV RNA stimulated the ATPase activity of 30S-5SRNP particles more markedly than that of 40S subunits. These two kinds of particles also showed intrinsic GTPase. Poly(U) enhanced the GTPase activity of 30S-5SRNP particles but not that of 40S subunits. An elongation factor (EF-1α, EF-2, or EF-1$\alpha\beta\gamma$) alone or in combination with poly(U) and/or other elongation factors stimulated the ATPase activities of both particles. The extent of stimulation of the ATPase activity by a combination of these components was usually somewhat higher than or similar to the sum of those with the individual components. The extents of stimulation by these components were higher in the case of 30S-5SRNP particles than that of 40S subunits, indicating the importance of the 5SRNP moiety in the former particles. The intactness of 18SrRNA was required for promotion of the ATPase activity of 30S-5SRNP particles by phe(+), (−)tRNA^{phe}. The ATPase activities of the two kinds of particles by themselves or those observed with the combinations of the components mentioned above were inhibited by several kinds of translation inhibitors. The degrees of inhibition were generally higher for 30S-5SRNP particles. The ATPase activity of 40S subunits was enhanced by spermidine, suggesting the importance of the conformational change induced by it. These results imply the participation of the intrinsic ATPase of 30S-5SRNP particles and 40S subunits in polypeptide elongation, and the important role of the 5SRNP moiety of 30S-5SRNP particles in the ATPase activity.

Key words: ATPase, elongation factors, 40S ribosomal subunits, 5S ribosomal RNA-L5 protein, 30S-5SRNP ribosomal particles.

Considerable information is now available concerning the participation of GTP and GTPases of polypeptide elongation factors in mammalian protein biosynthesis. On the contrary, only a few studies have been performed on the intrinsic ATPases or GTPases of mammalian ribosomes and ribosomal subunits (*1, 2*). Recently, it was indicated that not only mammalian 80S ribosomes, but also 40S and 60S subunits have intrinsic ATPase activities, although their role in the elongation reaction has not been elucidated (*3, 4*). Previously, it was reported that 5SrRNA-L5 protein particles (5SRNP) from rat liver exhibit low ATP- and GTP-hydrolyzing activities (*5-7*). On the other hand, we found that when rat liver 80S ribosomes or polysomes were dissociated into 30S and 50S subunits by EDTA-treatment, 5SRNP was present in the EDTA-derived 30S subunits, which are now designated as 30S-5SRNP particles (*8*), probably because 5SRNP is situated at the interface between the 60S and 40S subunits (*9*).

As shown later, the 30S-5SRNP particles exhibit higher ATP-hydrolyzing activity than that of 40S subunits, suggesting the importance of the 5SRNP moiety. The participation of 5SRNP in the ATPase of 30S-5SRNP particles in correlation with polypeptide elongation was expected because 5SRNP interacts with mRNA (*10, 11*) and EF-2 (*7*). Therefore, we comparatively investigated the properties of the ATPase of 30S-5SRNP and 40S particles, with specific reference to the stimulation of the activity through the interaction of these particles with messenger RNA, tRNA, and elongation factors. In this respect it is also important that 40S subunits contribute to the interaction of poly(U) and aminoacyl-tRNA because 40S subunits isolated from rat liver are able to bind poly(U) and two molecules of either phe(+)tRNA^{phe}, AcPhe-tRNA^{phe}, or phe(−)tRNA^{phe} in the presence of a saturated amount of poly(U). The addition of 60S subunits to 40S-poly(U)-

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Abbreviations: AMP-P(NH)P, 5′-adenylyl imidodiphosphate; ATA, aurintricarboxylic acid; DTT, dithiothreitol; GMP-P(NH)P, 5′-guanylyl imidodiphosphate; phe(+)tRNA^{phe}, phenylalanyl tRNA^{phe}; phe(−)tRNA^{phe}, deacylated tRNA^{phe}; TMV, tobacco mosaic virus; 5SRNP, 5SrRNA-L5 protein complex.

$(phe\text{-}tRNA^{phe})_2$ resulted in the quantitative formation of $(phe)_2$-tRNA, indicating that two binding sites for tRNA should be considered as the constituents of the P and A sites of 80S ribosomes (*12*).

The present experiments were undertaken to clarify the following: (1) the properties of the ATPases of 30S-5SRNP particles and 40S subunits, and their participation in polypeptide bond elongation, and (2) the role of the 5SRNP moiety of 30S-5SRNP particles in their ATPase activity.

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, the Hungarian Academy of Science. [γ-^{32}P]ATP and [γ-^{32}P]GTP were purified by column chromatography at 4°C on Dowex 1×2 (0.5×0.3 cm) to remove contaminating inorganic $^{32}PO_4$. Elution was performed with 0.01 N HCl–0.15 M NH_4Cl for ATP and with 0.01 N HCl–0.25 M NH_4Cl for GTP. Labeled nucleotides were used after neutralization of the ATP or GTP fraction thus obtained. The radioactivity of the contaminating $^{32}PO_4$ was about 0.1% of that of [^{32}P]ATP or [^{32}P]GTP. Poly(U), poly(C), and poly(A) were purchased from Yamasa Biochemicals (Tokyo). TMV RNA was prepared from TMV of Japanese type strain OM supplied by Dr. K. Hidaka, by the SDS-phenol method. Globin mRNA was prepared from a 0.5 M KCl wash of reticulocyte polysomes as described previously (*13*). tRNA from rabbit liver, and $tRNA^{phe}$ from brewer's yeast, AMP-P(NH)P, GMP(NH)P, ATA, tetracyclin, fusisic acid, puromycin, and spermidine were purchased from Sigma, and ammonium vanadate from Wako Pure Chemicals.

Medium—Medium A: 0.25 M sucrose, 5 mM $MgCl_2$, 50 mM KCl, and 50 mM Tris-HCl, pH 7.6. Medium II: 0.85 M KCl, 10 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.6.

Preparation of 30S-5SRNP Particles and 40S Subunits—At early periods of experiments, 80S(H) ribosomes were prepared from rat liver microsomes by a modification of the method (*14*) of Rendi and Hultin (*15*), and were found to be free from contamination by elongation factors (*16*). To obtain 30S-5SRNP particles, a modification of our previous method (*8*) was performed, as follows. EDTA was added to 80S(H) ribosomes with a 10 mM excess of $MgCl_2$. The ribosomes were layered on a 15–30% linear sucrose density-gradient containing 50 mM KCl, 1 mM EDTA, 2 mM DTT, and 20 mM Tris-HCl, pH 7.6. Centrifugation was carried out in a Hitachi SRP 28SA rotor at 27,000 rpm for 18 h at 2°C. The absorbance at 254 nm was continuously monitored with an ISCO automatic density-gradient fractionator. The fractions containing 30S-5SRNP particles were collected, dialyzed against Medium A minus sucrose containing 2 mM DTT for 1.5 h, and then concentrated with a Diaflow membrane. Particles thus obtained are designated as 30S-5SRNP(H) particles. Later, 80S(PM) ribosomes were used for the preparation of 30S-5SRNP(PM) particles, which were prepared from the rat liver postmitochondrial fraction by desoxycholate treatment, because they contained intact 18SrRNA owing to the presence of an RNase inhibitor in the rat liver cytosol. The 30S-5SRNP fractions were stored in small portions in liquid nitrogen.

40S and 60S subunits were prepared by a slight modification of the method described previously (*14*), as follows. 80S(H) ribosomes in Medium A were incubated with 0.2 mM puromycin at 37°C for 15 min, and then 4 M KCl, 2 M $MgCl_2$, and 1 M DTT were added to the incubation mixture to give 1 M KCl, 10 mM $MgCl_2$, and 2 mM DTT. The mixture was layered on a 15–30% linear sucrose density-gradient containing Medium II, followed by centrifugation as described above. The subunits were stored in small portions at −80°C.

Preparation of Phenylalanyl tRNA—One milligram of phenylalanyl-tRNA (Sigma) was incubated with 1 ml of rat liver cytosol in Medium A (30 mg protein), which had been dialyzed against Medium A overnight, 60 nmol phenylalanine, 0.2 mM Tris-HCl, and 1 mM DTT, in a total volume of 5 ml. After incubation at 37°C for 10 min, the reaction mixture was subjected to purification by conventional SDS-phenol extraction followed by ethanol precipitation. After washing with 70% ethanol, the precipitate was dissolved in 400 μl of H_2O and then the mixture was subjected to Sephadex G-50 column chromatography (0.75×7 cm) to remove ATP. Elution was carried out with 5 mM Tris-HCl, pH 7.6. The RNA fraction was collected, subjected to ethanol precipitation, and then dissolved in 50 μl of water. Using [^{14}C]phenylalanine, it was calculated that about 30% of the $tRNA^{phe}$ contained phenylalanine.

ATPase and GTPase Assays—The standard incubation mixture comprised 2 pmol 30S-5SRNP particles or 40S subunits in 2 μl of Medium A, 50 mM Tris-HCl, pH 7.6, 2.5 mM DTT, 5 mM $MgCl_2$, 5 mM KCl, and 0.125 mM [γ-^{32}P]ATP or GTP (1 μCi) in a total volume of 20 μl. Incubation was carried out at 37°C for 30 min. The hydrolysis of [γ-^{32}P]ATP or GTP was followed by measurement of the amount of inorganic $^{32}PO_4$ released during the incubation by a slight modification of the isobutanol-benzene method (*17*) described previously (*7*), as follows. The reaction was stopped with 50 μl of a 0.02 M silicotungustenic acid solution in 0.02 N H_2SO_4, and 130 μl of 1 mM potassium phosphate buffer, pH 6.9, and then 50 μl of 5% ammonium molybdate in 4 N H_2SO_4 was added. Phosphomolybdate complexes were extracted with 250 μl of water-saturated isobutanol-benzene (1:1). After rigorous stirring for 3 min with an automatic mixer, the mixture was centrifuged at 15,000 rpm for 3 min. The radioactivity in a 50 μl aliquot of the isobutanol-benzene layer was measured with a Beckman LS3801 liquid scintillation system. The control values obtained without particles during incubation were low owing to the purification of [γ-^{32}P]ATP or [γ-^{32}P]GTP, and were subtracted from all values. The ATPase activity was expressed as pmol of inorganic $^{32}PO_4$ released per pmol particles per min. In this work we assumed that 1 A_{260} unit was equal to 48 pmol of 40S subunits and 30S-5SRNP particles, 27 pmol of 60S subunits, and 17 pmol of 80S ribosomes, according to Ref. *18*.

RESULTS

For the purpose of the present experiments it was desirable to prepare ribosomes, their subunits, and 30S-5SRNP(H) particles free from contamination by translation factors. Therefore, we chose 80S(H) ribosomes (*8, 14*) as the starting material for the preparation of these four kinds of particles, which were prepared from rat liver microsomes by desoxycholate treatment in the presence of 670 mM KCl followed by centrifugation through a sucrose

density-gradient containing 600 mM KCl (*14*), and shown to be free from contamination by elongation factors (*16*), although their 18SrRNA was partially degraded by RNase released from microsomes on the desoxycholate treatment.

The ATP-hydrolyzing activities of various particles under our standard conditions with 0.125 mM ATP are shown in Table I. It must be emphasized that the activity of 30S-5SRNP particles (H) was definitely higher than that of 40S subunits. Considering these findings together with the fact that 30S subunits prepared from 40S subunits by EDTA treatment exhibit no ATPase activity, it is reasonably considered that the 5SRNP moiety of 30S-5SRNP(H) particles contributes to their ATPase activity. Since 5SRNP is situated at the interface between the two subunits in 80S ribosomes (*9*), 5SRNP-containing small subunits may be important in the function of 80S ribosomes. In this respect, it is important that the ATP-hydrolyzing activity of 80S(H) ribosomes is definitely higher than that of 80S ribosomes assembled from 60S and 40S subunits *in vitro*, the ATPase activity of which is the sum of these of the individual subunits (data not shown), as also shown in Ref. *3*, indicating that the 5SRNP moiety in 80S ribosomes is involved in their high ATPase activity.

It must be added that the results of later experiments proved that the ATPase activity of 30S-5SRNP(PM) particles prepared from 80S(PM) ribosomes is significantly higher than that of 30S-5SRNP(H) particles, probably owing to the intactness of the 18SrRNA (Table I).

It was shown that 30S-5SRNP particles and 40S subunits also have GTPase activities. The GTPase activity of 30S-5SRNP particles was shown to be about 40% of the ATPase activity (the average of four independent experiments). The ATPase activities of these particles were strongly inhibited by GTP and *vice versa*, as described later.

Basic Properties of ATPase of 30S-5SRNP Particles—The time course of ATP hydrolysis by 30S-5SRNP particles is shown in Fig. 1 (1). The reaction proceeded almost linearly for at least 1 h at 37°C. The effect of the $MgCl_2$ concentration on the ATP hydrolysis is shown in Fig. 1 (2). In our standard system, in which 0.125 mM ATP was used, the activity was high in the range of 5 mM to 7.5 mM $MgCl_2$, and at 2.5 and 10 mM somewhat lower. The effects of the KCl concentration is shown in Fig. 1 (3). ATP hydrolysis was highest with 5 mM KCl, and decreased linearly from 10 to 100 mM KCl. The addition of NH_4Cl to

TABLE I. **Comparison of the ATPase activities of various ribosomal particles from rat liver.**

ATPase activity (pmol inorganic P/pmol particle/min)				
30S-5SRNP	30S-5SRNP(PM)	40S subunits	60S subunits	80S ribosomes
0.56 (14*)	1.1 (9*)	0.41 (7*)	0.4 (2*)	2.5 (4*)

The values obtained under the standard conditions with 0.125 mM ATP in the incubation mixture at 37°C for 30 min. *Number of independent experiments used to obtain average values.

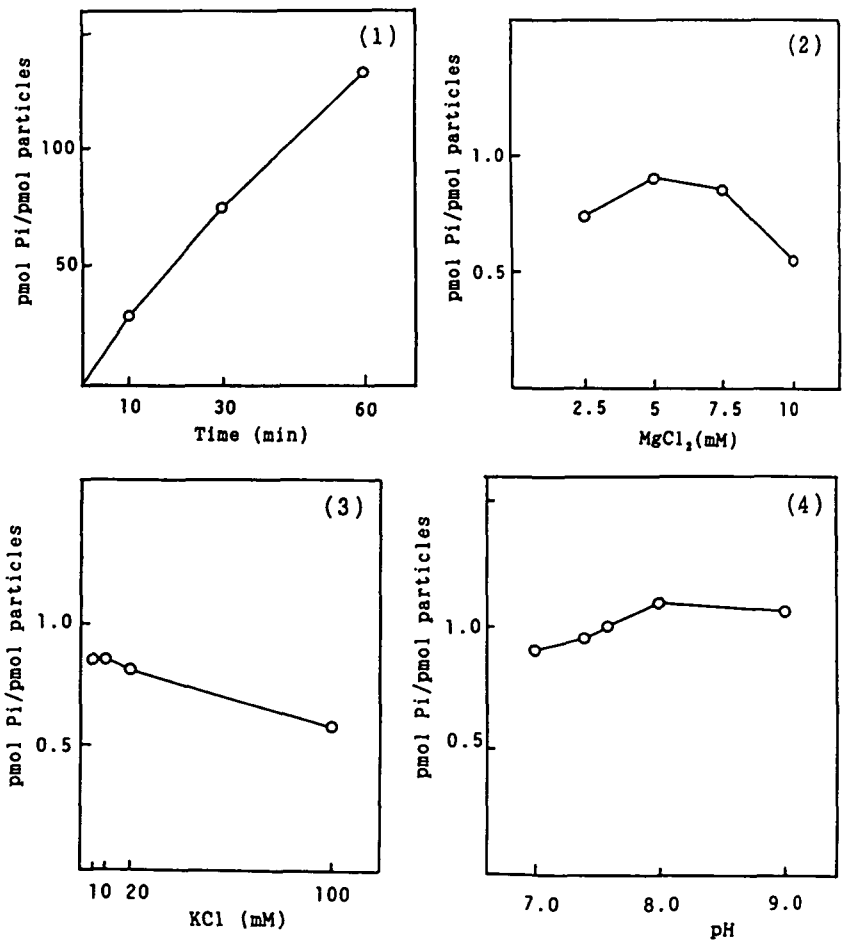


Fig. 1. **Basic properties of the ATPase of 30S-5SRNP(H) particles.** (1) Time course. (2) Effect of $MgCl_2$ concentration. (3) Effect of KCl concentration. (4) Effect of pH.

the standard reaction mixture to a final concentration of 50 mM markedly inhibited (about 40% of the control) the ATPase activity. The optimal pH was about 8.0 [Fig. 1 (4)]. The ATPase activity was somewhat unstable, decreasing to about 80% on standing at 0°C for 2 h.

Kinetic Parameters of Rat Liver 30S-5SRNP Particles—The kinetic parameters of the ATPase of 30S-5SRNP particles were estimated according to Michaelis-Menten equations. The dependence of the ATPase activity of 30S-5SRNP particles on the ATP concentration showed two kinetically different components for ATPase (K_{mI} and K_{mII}) as shown in Table II. The results obtained for 40S subunits also showed two kinetically different components, in agreement with the results of other investigators (*3, 4*) (data not shown).

Effects of mRNA on ATPase and GTPase Activities—We used poly(U), poly(A), and poly(C) as artificial mRNAs, and TMV RNA and globin mRNA as natural mRNAs. As shown in Fig. 2 (1), with an increase in the poly(U) concentration, the ATPase activity of 30S-5SRNP particles increased markedly up to 5 μg. Two micrograms poly(U) increased the hydrolysis of ATP by about 200% times (the average of twelve independent experiments). Poly(U) promoted ATP hydrolyzing activity with various concentrations of ATP, suggesting that poly(U) is a positive effector of the ATPase of 30S-5SRNP particles [Fig. 2 (2)]. Although poly(U) also enhanced the ATPase activity of 40S subunits, the extent of stimulation was markedly lower than that in the case of 30S-5SRNP particles. Two micrograms poly(U) promoted it about by only 127% times (the average of nine independent experiments). A similar stimulating effect of poly(U) was observed on the GTPase activity of 30S-5SRNP particles, whereas that of 40S subunits was not affected by poly(U) [Fig. 2 (1)]. These results indicate the importance of the 5SRNP moiety of 30S-5SRNP particles in poly(U) stimulation of ATPase and GTPase. In this respect, our previous work showed that 30S-5SRNP particles designated as EDTA-32S subunits at that time, participated in [^{3}H]poly(U) binding (*19*).

Poly(C) also promoted the ATPase activity of 30S-5SRNP particles, depending on its concentration, although the extent of promotion was smaller than that in the case of poly(U) [Fig. 2 (1)]. We did not observe a effect of poly(A). In this respect, it has been reported that poly(A) is not translated in eucaryotic cell-free systems including rat liver (*20*).

The effects of natural messenger RNAs were then examined. As shown in Fig. 2 (3), TMV RNA weakly stimulated the ATPase activities of the 30S-5SRNP(H) particles and 40S subunits, depending on its concentration. The extent of stimulation for the former particles was somewhat larger than that for the latter. Globin mRNA showed a negligible effect on the ATPase of 30S-5SRNP, probably due to the enrichment of its secondary structure, because the ATPase of the reticulocyte 43S initiation complex, which contains eIF-5A having helicase activity, was markedly stimulated by globin mRNA (to be published).

Effect of phe(+)tRNA^{phe} on the ATPase Activity of 30S-5SRNP Particles—It was very difficult to obtain consistent results showing the stimulative effect of phe-tRNA^{phe} on the ATPase activity of 30S-5SRNP(H) particles, probably owing to the partial degradation of 18SrRNA. By employing 30S-5SRNP(PM) particles containing intact 18SrRNA, a constant stimulative effect of phe(+)tRNA^{phe} was observed.

TABLE II. **Kinetic parameters of ATPase of rat liver 30S-5SRNP particles.**

	K_m (μM)	k_{cat} (m^{-1})
30S-5SRNP particles I	125	1.3
II	1,250	2.4

K_{MI} and K_{MII} correspond to two distinct slopes of Lineweaver-Burk plots.

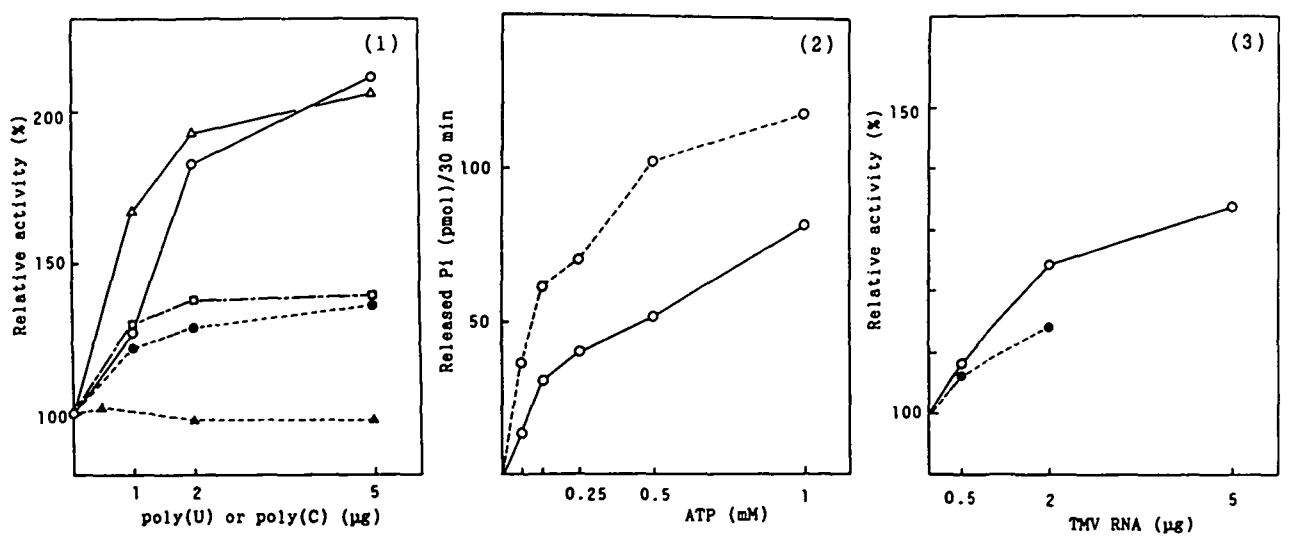


Fig. 2. **Effects of mRNA on the ATPases of 30S-5SRNP(H) particles and 40S subunits.** (1) Dose-response curves for the effects of poly(U) and poly(C). The ATPase activity of 30S-5SRNP particles or 40S subunits by themselves is set as 100%. Values are the means of duplicate runs. —○—, ATPase of 30S-5SRNP, and ···●···, ATPase of 40S in the presence of poly(U); —△—, GTPase of 30S-5SRNP, and ···▲···, GTPase of 40S in the presence of poly(U); -—□—-, ATPase of 30S-5SRNP in the presence of poly(C). (2) Effects of poly(U) on the ATPase activity of 30S-5SRNP particles in the presence of various concentrations of ATP. —○—, ATPase of 30S-5SRNP; ···○···, ATPase of 30S-5SRNP in the presence of poly(U) (2 μg). (3) Effect of TMV RNA. —○—, ATPase of 30S-5SRNP; ···●···, ATPase of 40S.

Figure 3 shows a dose-response curve for the effect of phe(+)tRNA^{phe} on the ATPase activity of 30S-5SRNP-(PM) particles. It stimulated the ATPase activity, depending on its concentration, although the extent of stimulation was rather small. Four micrograms of phe-tRNA^{phe} increased the ATPase activity by 131% times (the average of six independent experiments). It must be added that phe(−)tRNA^{phe} also stimulate the activity: 4 μg of phe(−)-tRNA^{phe} increased the activity by 120% times (the average of three independent experiments). These results may indicate that the intactness of 18SrRNA is required for the stimulation, and that the extent of stimulation by phe(+)-tRNA^{phe} is larger than that by phe(−)tRNA^{phe}. The effects of phe-tRNA^{phe} in the presence of poly(U) plus EF-1α will be described later.

Effects of Elongation Factors on the ATPase Activity of 30S-5SRNP Particles or 40S Subunits—To examine the participation of the ATPase of 30S-5SRNP particles and 40S subunits in polypeptide elongation, we investigated the effects of elongation factors on the ATPase activities of these particles. We used highly purified preparations of EF-1α (*21*), EF-2 (*22*), and EF-1$\alpha\beta\gamma$ (*23*) from pig liver, which were found to be free from contamination by ATPase except for EF-1$\alpha\beta\gamma$, which contained a small amount of ATPase.

Effect of EF-1α—The effect of EF-1α, which is known to catalyze the GTP-dependent binding of aminoacyl-tRNA to the A site of ribosomes, on the ATPase activity of 30S-5SRNP particles was investigated in the presence and absence of poly(U) or TMV RNA. As shown in Fig. 4 (1), the ATPase activity of 30S-5SRNP particles or 40S subunits was increased by EF-1α, depending on its concentration, although the extent of the increase was higher for the former particles.

As shown in Table III (1, 3), in the presence of both poly(U) and EF-1α, the ATPase activity of 30S-5SRNP particles was markedly stimulated. The extent of stimulation by poly(U) plus EF-1α was somewhat larger than the sum of that with poly(U) alone and that with EF-1α alone. Such cooperative stimulation was also observed in the

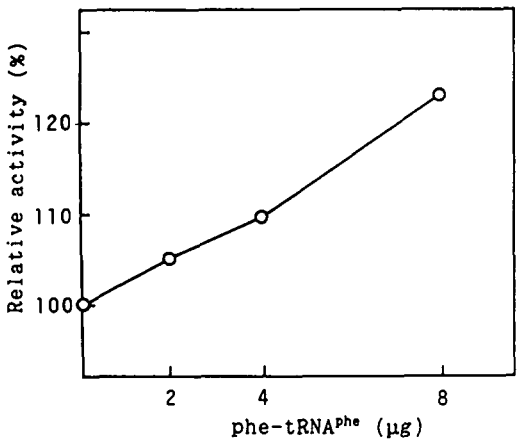
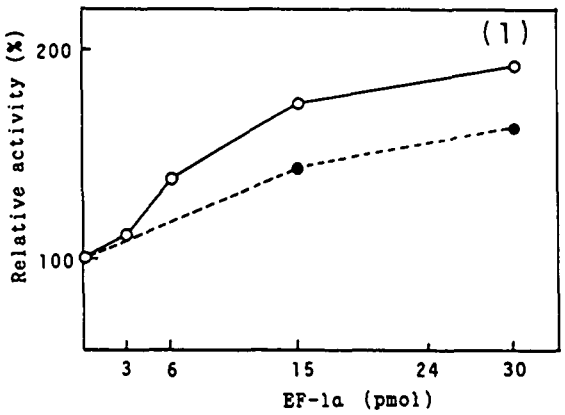
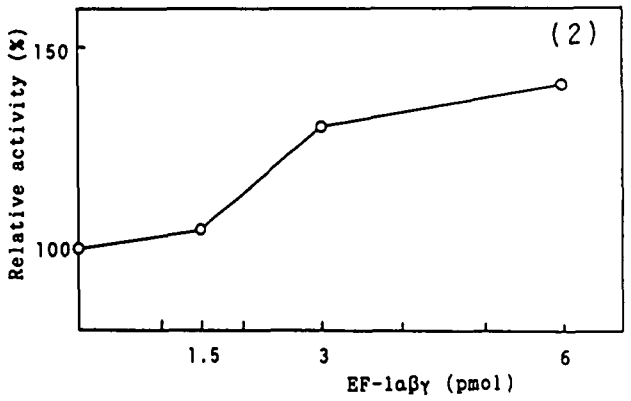


Fig. 3. **Effect of phe-tRNA^{phe} on the ATPase activity of 30S-5SRNP(PM).**





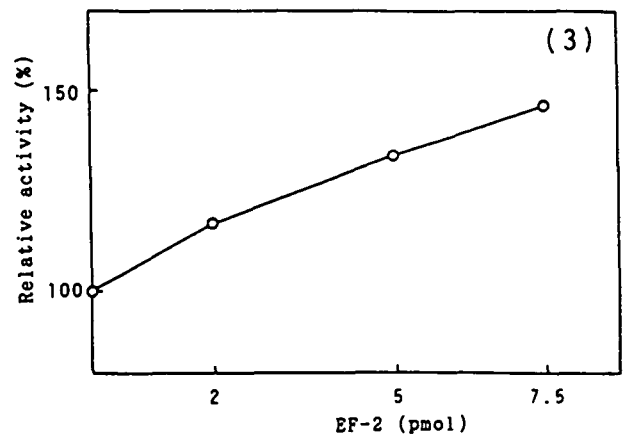


Fig. 4. **(1) Effects of EF-1α on the ATPase activities of 30S-5SRNP(H) particles and 40S subunits.** —○—, 30S-5SRNP; ···●···, 40S subunits. **(2) Effect of EF-1$\alpha\beta\gamma$ on the ATPase activity of 30S-5SRNP particles. (3) Effect of EF-2 on the ATPase of 30S-5SRNP particles.**

presence of EF-1α and TMV RNA [Table III (1-b)]. It must be added that similar stimulation by poly(U) and/or EF-1α was observed in the case of a high concentration of [^{32}P]ATP (1 mM) (data not shown).

Similar cooperative stimulation of the ATPase activity by EF-1α plus poly(U) or TMV RNA was observed in the case of 40S subunits, although the extent of stimulation was smaller than that observed with 30S-5SRNP particles [Fig. 4 (1) and Table III (2)(a)(b)].

Since it is well-known that EF-1α, phe(+)tRNA^{phe}, and GTP form a ternary complex which binds to poly(U) on ribosomes, the effects of poly(U), EF-1α, and phe-tRNA^{phe} combined were examined using 30S-5SRNP(PM). As shown in Table III (3), an additive effect was observed when these three components were present in the reaction mixture.

Effect of EF-1$\alpha\beta\gamma$—EF-1$\alpha\beta\gamma$ was purified from pig liver to apparent homogeneity as a high molecular weight form of EF-1, and shown to be a 1:1:1 complex of EF-1α, EF-1β, and EF-1γ, which was designated as EF-1$\alpha\beta\gamma$ (*23*). Since the preparation was contaminated by a small amount of ATPase, we subtracted this value from that of the ATPase activity determined in the experiment.

As shown in Fig. 4 (2), EF-1$\alpha\beta\gamma$ promoted the ATP-hydrolyzing activity of 30S-5SRNP particles, depending on its concentration. It also stimulated the activity of 40S subunits [Table IV (2)]. In the presence of both poly(U) and EF-1$\alpha\beta\gamma$, the ATPase activity of 30S-5SRNP particles or 40S subunits was higher than the sum of that with poly(U) alone and that with EF-1$\alpha\beta\gamma$ alone [Table IV (1) (2)]. The extent of stimulation was larger for 30S-5SRNP particles than 40S subunits.

Effect of EF-2—EF-2 is known as a factor participating in the translocation reaction. Previously, we observed that EF-2 plus tRNA stimulated the ATPase activity of 5SRNP (*7*). EF-2 enhanced the ATPase activity of 30S-5SRNP particles, depending on its concentration [Fig. 4 (3)] and that of 40S subunits [Table V (2)]. As shown in Table V (1-a,b), EF-2 plus poly(U) or EF-2 plus rabbit liver tRNA markedly stimulated the ATPase activity of 30S-5SRNP, although the extent of stimulation was similar to the sum of that observed with EF-2, tRNA, or poly(U) alone. Similar additive stimulation of the ATPase activity by EF-2 plus poly(U) or by EF-2 plus rabbit liver tRNA was observed in the case of 40S subunits [Table V (2)].

Effects of the Full Components of Translation—As shown in Table VI (A-1), the incubation of 30S-5SRNP particles with the combination of poly(U), EF-1$\alpha\beta\gamma$, EF-2, and phe(+)tRNA^{phe} resulted in marked stimulation of the ATPase activity, the extent of stimulation being similar to the sum of that observed with each of the components alone. Similar additive stimulation by the full components of translation was observed in the case of 40S subunits, although the extent of stimulation was lower than in the case of 30S-5SRNP particles [Table VI (A-2)]. These results indicate the importance of the 5SRNP moiety in these particles for ATPase activity in the presence of the full components of translation [Table V (A-2)].

It is of interest that when 30S-5SRNP(PM) was used, the extent of stimulation by phe-tRNA^{phe} in the presence of the other full components was consistently somewhat larger than that observed with phe(−)tRNA^{phe} [Table VI (B)].

Effects of Translation Inhibitors on the ATPase Activities of 30S-5SRNP Particles and 40S Subunits—The effects of several inhibitors of the translation reaction [see review article (*24*)] on the ATPase of 30S-5SRNP particles and 40S subunits were examined.

ATA: It has been reported that at low concentrations, ATA specifically inhibits the initiation reaction in eucaryotic protein biosynthesis, but at high concentrations it also inhibits the elongation reaction (*25*). The inhibition is

TABLE III. **Effects of EF-1α on ATPase activity in the presence of mRNA, and/or phe-tRNA^{phe}.**
(1) 30S-5SRNP(H) and (2) 40S subunits

	Additions		Relative activity	
(a)	EF-1α (30 pmol)	poly(U) (2 μg)	(1) 30S-5SRNP(H)	(2) 40S
	(−)	(−)	100	100
	(−)	(+)	195	122
	(+)	(−)	159	164
	(+)	(+)	339 (254*)	239(186*)
(b)	EF-1α (15 pmol)	TMV RNA (5 μg)	(1) 30S-5SRNP(H)	(2) 40S
	(−)	(−)	100	100
	(−)	(+)	136	115
	(+)	(−)	152	166
	(+)	(+)	218 (202*)	187 (167*)

(3) 30S-5SRNP(PM)

EF-1α (30 pmol)	poly(U) (0.5 μg)	phe-tRNA^{phe} (4 μg)	Relative activity (3) 30S-5SRNP (PM)
(−)	(−)	(−)	100
(−)	(+)	(−)	169
(+)	(−)	(−)	110
(−)	(−)	(+)	153
(+)	(+)	(−)	225 (179*)
(+)	(+)	(+)	238 (232*)

The values in parentheses denoted by * were calculated from the relative activity values in the presence of each component examined.

TABLE IV. **Effects of EF-1$\alpha\beta\gamma$ on ATPase activity in the presence and absence of poly(U).** (1) 30S-5SRNP and (2) 40S subunits.

Additions		Relative activity	
EF-1$\alpha\beta\gamma$ (3 pmol)	poly(U) (2 μg)	(1) 30S-5SRNP	(2) 40S
(−)	(−)	100	100
(−)	(+)	173	114
(+)	(−)	194	154
(+)	(+)	308 (267*)	203 (171*)

*See the legend to Table III.

TABLE V. **Effects of EF-2 on ATPase activity in the presence and absence of poly(U) or rabbit liver tRNA.** (1) 30S-5SRNP and (2) 40S subunits.

	Additions		Relative activity	
(a)	EF-2 (5 pmol)	poly (U) (2 μg)	(1) 30S-5SRNP	(2) 40S
	(−)	(−)	100	100
	(−)	(+)	203	127
	(+)	(−)	175	165
	(+)	(+)	271(278*)	178(192*)
(b)	EF-2 (7.5 pmol)	Liver tRNA (25 μg)	(1) 30S-5SRNP	(2) 40S
	(−)	(−)	100	100
	(−)	(+)	149	132
	(+)	(−)	146	160
	(+)	(+)	195(195*)	207(192*)

*See the legend to Table III.

induced through the interaction of ATA with ribosomal proteins, which alters the conformation of ribosomes (*26*). As shown in Fig. 5 (1), ATA inhibited the ATPase activities of both 30S-5SRNP particles and 40S subunits, depending on its concentration. The extent of inhibition was larger for 30S-5SRNP particles than for 40S subunits.

Tetracyclin: Tetracyclin blocks protein synthesis in bacterial and mammalian systems (*27*). Since it prevents non-enzymatic binding of tRNA to bacterial 30S subunits, it is assumed that its main effect is on 30S subunits. Furthermore, the EF-Tu-dependent binding of aminoacyl-tRNA to 70S ribosomes is inhibited by much lower concentrations of tetracyclin than non-enzymatic binding to 30S subunits, suggesting that the specific enzymatic step described above is inhibited by tetracyclin (*28*).

As shown in Table VII-1(a), tetracyclin (0.7 mM) inhibited the ATPase activity of 30S-5SRNP particles by themselves, and that stimulated by poly(U), phe-tRNA^{phe}, EF-1α, or EF-1α plus phe-tRNA^{phe}. Tetracyclin also inhibited the ATPase activity of 40S subunits alone and with the components described above, although the extent of the inhibition was smaller than that observed in the case of 30S-5SRNP particles [Table VII-1 (b)].

Emetine: This alkaloid is known as an effective inhibitor

TABLE VI. **(A) Effects of the full components of translation and (B) comparison of the effects of phe-tRNA^{phe} with tRNA^{phe} on 30S-5SRNP(PM) ATPase with the full components of translation.** (1) 30S-5SRNP(PM) and (2) 40S subunits.

(A)

Additions				Relative activity	
poly(U) (2 μg)	EF-1$\alpha\beta\gamma$ (3 pmol)	EF-2 (5 pmol)	phe-tRNA^{phe} (5 μg)	(1) 30S-5SRNP	(2) 40S
(−)	(−)	(−)	(−)	100	100
(+)	(−)	(−)	(−)	222	137
(−)	(+)	(−)	(−)	140	102
(−)	(−)	(+)	(−)	152	150
(−)	(−)	(−)	(+)	122	125
(+)	(+)	(+)	(+)	346(336*)	200(214*)

(B)

	Additions					Relative activity
	poly(U) (μg)	EF-1$\alpha\beta\gamma$ (pmol)	EF-2 (pmol)	phe-tRNA^{phe} (μg)	tRNA^{phe} (μg)	30S-5SRNP
Exp. 1*	1	1.5	5	5	(−)	232
	1	1.5	5	(−)	5	202
Exp. 2	1	1.5	5	5	(−)	214
	1	1.5	5	(−)	5	169
Exp. 3*	0.5	3	5	5	(−)	199
	0.5	3	5	(−)	5	172

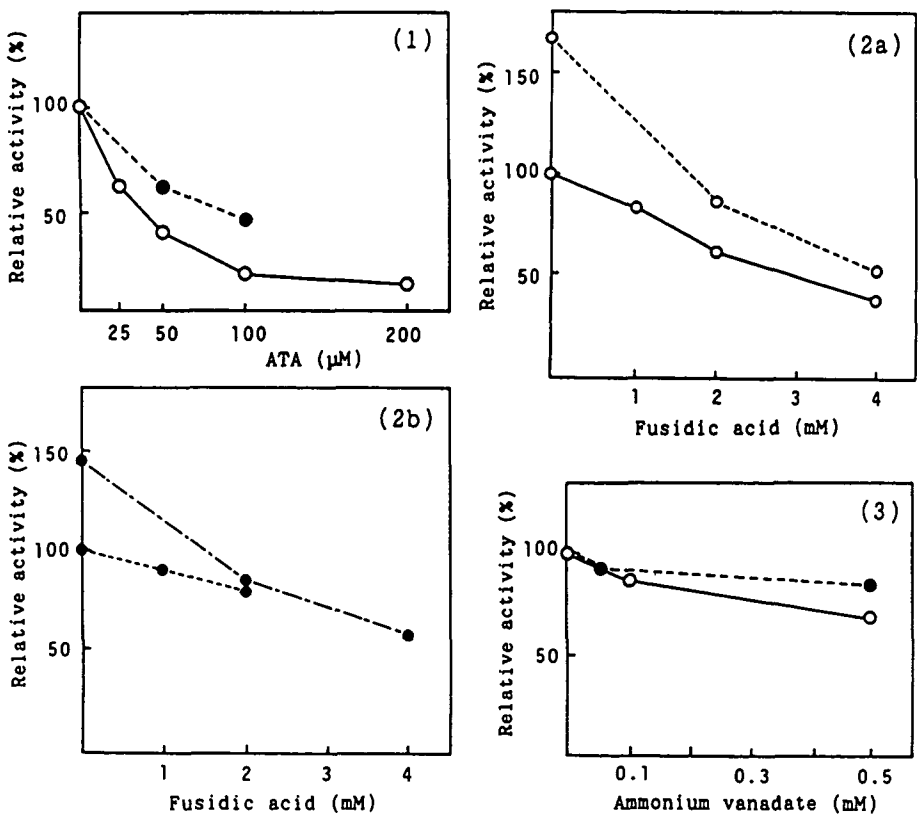


Fig. 5. **Effects of translation inhibitors on the ATPase activities of 30S-5SRNP(H) particles and 40S subunits.** (1) ATA: —○—, 30S-5SRNP; ···●···, 40S subunits. (2a) Fusidic acid: —○—, 30S-5SRNP; ···○···, 30S-5SRNP+EF-2+rabbit tRNA. (2b) Fusidic acid: ···●···, 40S subunits; – –●– –, 40S+EF-2+rabbit liver tRNA. (3) Ammonium vanadate: —○—, 30S-5SRNP; ···●···, 40S subunits.

of EF-2-dependent translocation in a eucaryotic cell-free system (*24*). As shown in Table VII-2(a), emetine inhibited the ATP hydrolysis by 30S-5SRNP particles alone and in combination with EF-2, rabbit liver tRNA or poly(U). Similar inhibition was also observed with 40S subunits, although the extent of the inhibition was smaller than that in the case of 30S-5SRNP particles except in the presence of EF-2 [Table VII-2 (b)]. The inhibition of the ATPase activities of 80S ribosomes, 40S subunits and 60S subunits by emetine was reported previously (*3*).

Fusidic acid: Fusidic acid was reported to inhibit the EF-2-dependent translocation and uncoupled GTP hydrolysis in a mammalian cell-free elongation system (*24*). Grummt *et al.* reported that the hydrolysis of GTP or ATP by rat liver 5SRNP was inhibited by fusidic acid (*5*), and Grummt and Speckbacher reported that GTPase of 80S ribosomes reassembled from the two subunits was also inhibited (*2*). We showed previously that the ATPase activity of 5SRNP enhanced by eIF-2 plus aminoacyl-tRNA was inhibited by fusidic acid (*5*).

Firstly, we investigated the effects of fusidic acid on the ATPase activities of 30S-5SRNP particles or 40S subunits alone and with EF-2 plus rabbit liver tRNA. As shown in Fig. 5 (2-a), ATP hydrolysis by 30S-5SRNP particles alone and in combination with EF-2 plus tRNA was inhibited by fusidic acid, depending on its concentration. A larger extent of inhibition was observed in the presence of poly(U), phe-tRNA^{phe}, EF-2, or EF-1$\alpha\beta\gamma$ than in its absence [Table VII-3(a)].

Similar inhibition was observed in the case of 40S subunits, although the extents of inhibition were somewhat smaller than those observed for 30S-5SRNP particles [Fig. 5 (2b) and Table VII-3(b)].

Ammonium vanadate: Employing a rabbit reticulocyte cell-free system, it was found that ammonium vanadate at concentrations higher than 0.05 mM inhibited polypeptide elongation (*29*). As shown in Fig. 5 (3), the ATPase activity of 30S-5SRNP particles was decreased concentration-dependently by ammonium vanadate at concentrations higher than 0.1 mM. Similar inhibition was observed for 40S

TABLE VII. **Effects of tetracycline (TC), emetine (EM), fusidic acid (FA), and KF on the ATPase activities of 30S-5SRNP particles and 40S subunits.** The figures in parentheses represent per cent inhibitions by inhibitors.

VII-I. Effect of TC (0.7 mM)

(a) Relative activity of 30S-5SRNP(PM)

	Additions				
	None	poly(U) (0.5 μg)	phe-tRNA^{phe} (4 μg)	EF-1α (15 pmol)	EF-1α+phe-tRNA^{phe}
30S-5SRNP	100	182 (100)	143 (100)	117 (100)	154 (100)
30S-5SRNP plus TC	77	139 (76)	96 (67)	84 (71)	103 (67)

(b) Relative activity of 40S subunits

	Additions			
	None	poly(U) (2 μg)	phe-tRNA^{phe} (4 μg)	EF-1α (15 pmol)
40S	100	168 (100)	107 (100)	146 (100)
40S plus TC	87	129 (76)	100 (93)	112 (77)

VII-2. Effect of EM (0.4 mM)

(a) Relative activity of 30S-5SRNP

	Additions					
	None	poly(U) (2 μg)	Rabbit liver tRNA (25 μg)	EF-2 (5 pmol)	poly(U)+tRNA	poly(U)+tRNA+EF-2
30S-5SRNP	100	235 (100)	135 (100)	130 (100)	267 (100)	283 (100)
30S-5SRNP plus EM	70	147 (62)	99 (73)	124 (95)	197 (74)	215 (76)

(b) Relative activity of 40S

	Additions				
	None	poly(U) (2 μg)	Rabbit liver tRNA (25 μg)	EF-2 (5 pmol)	poly (U)+tRNA
40S	100	129 (100)	114 (100)	110 (100)	138 (100)
40S plus EM	83	122 (95)	109 (97)	86 (78)	128 (93)

VII-3. Effect of FA (2 mM)

(a) Relative activity of 30S-5SRNP(PM)

	Additions				
	None	poly(U) (0.5 μg)	phe-tRNA^{phe} (3 μg)	EF-2 (5 pmol)	EF-1$\alpha\beta\gamma$ (5 pmol)
30S-5SRNP	100	211 (100)	175 (100)	246 (100)	283 (100)
30S-5SRNP plus FA	66	58 (27)	71 (40)	105 (43)	105 (37)

(b) Relative activity of 40S subunits

	Additions				
	None	poly(U)	phe-tRNA^{phe}	EF-2	EF-1$\alpha\beta\gamma$
40S	100	145 (100)	100 (100)	123 (100)	147 (100)
40S plus FA	65	75 (51)	68 (68)	97 (79)	75 (51)

VII-4. Effect of KF (24 mM)

(a) Relative activity of 30S-5SRNP

30S-5SRNP	100
30S-5SRNP plus KF	13

(b) Relative activity of 40S subunits

40S	100
40S plus KF	24

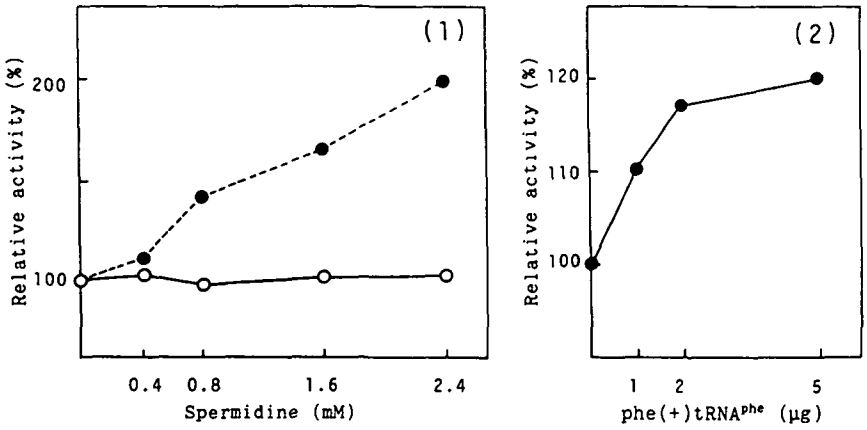


Fig. 6. **(1) Effects of spermidine on the ATPase activities of 40S subunits and 30S-5SRNP(H) particles.** Spermidine was added to the incubation mixture containing 2.5 mM $MgCl_2$. —○—, 30S-5SRNP; ···●···, 40S subunits. **(2) Effect of phe(+)tRNA^{phe} on the ATP activity of 40S subunits in the presence of 2.5 mM $MgCl_2$ and 1.6 mM spermidine.**

subunits, although the extent of inhibition was somewhat smaller than that observed for 30S-5SRNP particles. The inhibitory effects of ammonium vanadate on the ATPase activities of rabbit liver 80S ribosomes and ribosomal subunits were reported previously (*3*).

It must be added that pactamycin (10 μM), an inhibitor of the initiation of protein biosynthesis, and sparsomycin (8 mM), which inhibits peptidyl transferase through an interaction with large subunits [see review article (*24*)], did not inhibit the ATPase activity of 30S-5SRNP particles (data not shown). On the other hand, KF (24 mM), which is known as an inhibitor of the initiation reaction (*24*), inhibited the ATP hydrolysis by 30S-5SRNP particles and 40S subunits (Table VII-4).

Effects of Spermidine on the ATPase Activities of 40S Subunits and 30S-5SRNP Particles—Much evidence has been accumulated indicating that polyamines stimulate protein biosynthesis through positive conformational changes of ribosomes [see review article (*30*)]. In an eucaryotic system, spermidine enhances globin synthesis in a rabbit reticulocyte cell-free system (*31*) and protein biosynthesis in a wheat-germ cell-free system (*32*). Therefore, we examined the effects of spermidine on the ATPase activities of 30S-5SRNP particles and 40S subunits. A dose-response curve for the effect of spermidine on the ATP-hydrolyzing activity of 40S subunits is shown in Fig. 6 (1). Spermidine enhanced it, depending on its concentration. On the other hand, spermidine had little effect on the ATPase activity of 30S-5SRNP particles, as also shown in this figure. Spermidine (1.6 mM) at 2.5 mM $MgCl_2$ increased the ATPase activity of 40S subunits by 150% times (the average of four independent experiments), but only by 108% times in the case of 30S-5SRNP particles (the average of nine independent experiments). From these results, it may be deduced that spermidine stimulates the ATPase activity of 40S subunits by inducing positive conformational changes. The weak stimulation in the case of 30S-5SRNP particles may be explained by assuming that they have a conformation favorable for an ATPase activity without spermidine, probably owing to the presence of a 5SRNP moiety.

It must be added that, in the presence of 2.5 mM $MgCl_2$ and 1.6 mM spermidine, phe(+)tRNA^{phe} stimulated the ATPase activity of 40S subunits, depending on its concentration, as shown in Fig. 6 (2), although the extent of stimulation was rather small.

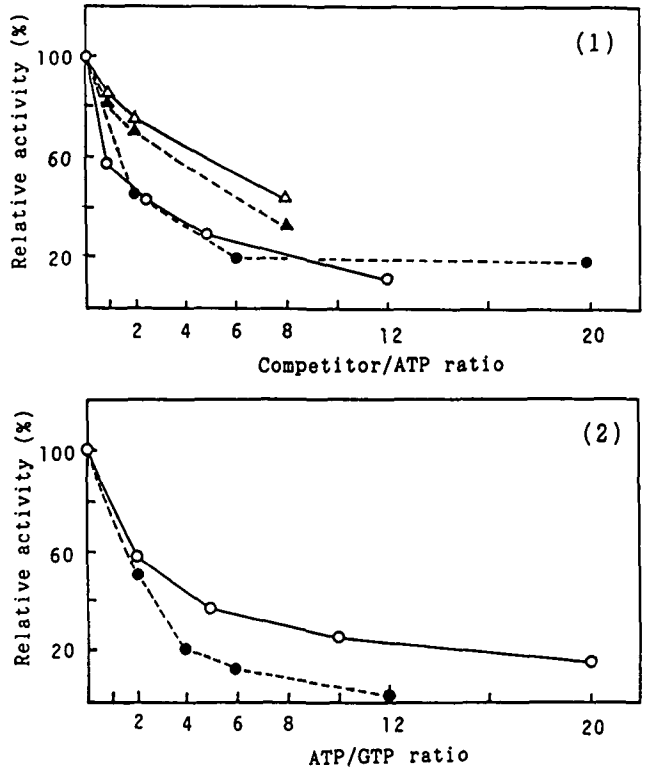


Fig. 7. **(1) Inhibition of the ATPase activity of 30S-5SRNP(H) particles by GTP, AMP·PNP, and GMP·PNP, and that of the ATPase activity of 40S subunits by GTP.** 30S-5SRNP: —○—, 30S-5SRNP+GTP; —△—, 30S-5SRNP+AMP-P(NH)P; ···▲···, 30S-5SRNP+GMP-P(NH)P. 40S: ···●···, 40S+GTP. **(2) Inhibition of the GTPase activities of 30S-5SRNP(H) particles and 40S subunits by ATP.** —○—, 30S-5SRNP; ···●···, 40S subunits.

Competitive Effects of GTP, AMP-P(NH)P, and GMP-P(NH)P on the ATPase, and the Effect of ATP on GTPase of 30S-5SRNP Particles—30S-5SRNP particles show both ATPase and GTPase activities. Therefore, it appears important to clarify the relationship between the activities. For this purpose, we used a competitive method. As shown in Fig. 7 (1), the ATPase activity of 30S-5SRNP particles was strongly inhibited by GTP, and somewhat less efficiently by AMP-P(NH)P and GMP-P(NH)P. On the other hand, the GTPase activity of the particles was strongly

inhibited by ATP. It must be added that ATP hydrolysis by 40S subunits was strongly inhibited by GTP, and that GTP hydrolysis was strongly inhibited by ATP, as shown in Fig. 7 (2). These findings indicate the intimate relationship between the two enzymes in these two kinds of particles. Strong inhibition of the ATPase activity of 80S ribosomes reassembled from the two subunits by GTP and nucleotide analogues was reported by Rodnina *et al.* (*3*).

DISCUSSION

Much evidence has been accumulated concerning the properties and role of GTPase in eucaryotic polypeptide elongation. It was reported that the GTPases participating in it are localized to elongation factors, although their activities are low in the absence of ribosomes (*33, 34*). On the other hand, eucaryotic ribosomes and both subunits were shown to have intrinsic ATPase and GTPase (*1-4*). However, their properties, especially their role in the elongation reaction, have not been clarified.

While EDTA treatment of 60S ribosomal subunits releases 5SRNP (*35*), 5SRNP is attached to 30S subunits when 80S(H) ribosomes or polysomes are treated with EDTA (*8*). Recently, important findings have been made for 5SRNP, as follows. It is situated at the interface of the two subunits (*9*), and at the P site of 60S subunits (*36*). It interacts with EF-2 (*37, 38*) and mRNA (*10, 11*).

We attempted to clarify the following two points. (I) The possible correlation of the ATPases of 30S-5SRNP particles and 40S subunits with peptide bond elongation. (II) The role of the 5SRNP moiety in 30S-5SRNP particles in their ATPase activity.

(I) Firstly we investigated the effects of artificial and natural mRNAs on the ATPase activities of the two kinds of particles. It was found that mRNAs stimulate their ATPase activities. Later, employing 30S-5SRNP(PM) particles containing intact 18SrRNA, it was shown that phe(+) and (−)tRNA^{phe} stimulate their ATPase activity, and that the extent of stimulation by phe(+)tRNA^{phe} is larger than that by phe(−)tRNA^{phe}. Furthermore, phe(+)tRNA^{phe} promoted the ATPase activity of 40S subunits in the presence of spermidine. These findings indicate that mRNAs and tRNA^{phe} bind to these particles, and modulate their conformation, resulting in the enhancement of their ATPase activities.

Using the nitrocellulose membrane filtration techniques, the interaction of poly(U) and phe-tRNA^{phe} with rat liver 40S subunits was studied by Semenkov *et al.* (*12*). It was found that 40S subunits bind poly(U) with a stoichiometry of 1:1, and with saturating concentrations of poly(U), each 40S subunit binds two molecules of either phe-tRNA^{phe}, acetyl-phe-tRNA^{phe} or deacylated phe-tRNA^{phe} at the P and A sites (*12*).

Next, we investigated the effects of elongation factors on ATPase activity. It must be mentioned that systematic studies on this subject have not yet been reported. The stimulative effect of EF-1α, EF-1$\alpha\beta\gamma$, or EF-2 may be explained by the conformational changes of these particles on the binding of these factors, as in the case of mRNA and tRNA.

We further investigated the effects of the combination of mRNA, phe-tRNA and elongation factors. There are two modes of stimulation of the ATPase activities of these particles by the combination of mRNA and elongation factors. The one is that the extent of stimulation by the combined components is similar to the sum of that observed with the individual components alone. Since crosslinking studies on rat liver ribosomes indicated that mRNA, EF-1α, and EF-2 interact with different ribosomal proteins, although some of them overlap [*10, 39-41*, see also review article (*42*)], the individual components induce conformational changes in different regions of the ATPase center of these particles, resulting in an additive stimulatory effect on the ATPase activity.

The other more interesting one is that the extent of stimulation by the combination of mRNA and elongation factors is larger than the sum of that observed with each component alone as in the case of stimulation by poly(U) plus EF-1α or EF-1$\alpha\beta\gamma$. These components induce cooperative changes in the conformation of the ATPase center of these particles, resulting in the synergic stimulation of the ATPase activity.

It is of interest that the ATPase activity of 30S-5SRNP particles or 40S subunits alone and in combination with elongation components is inhibited by several kinds of inhibitors in the elongation reaction.

It must be emphasized that the behavior of these two types of particles in the stimulation by elongation components and in the inhibition by elongation inhibitors is qualitatively similar. Therefore, the two particles have the same ATPase center. Taken together, these findings may indicate that the ATPase of 30S-5SRNP particles and 40S subunits participate in some way in the peptide bond elongation, although the actual mode of participation should be determined by employing 80S ribosomes.

(II) Concerning the role of the 5SRNP moiety in 30S-5SRNP in the ATPase activity, the results of the present experiments can be summarized as follows: (1) The ATPase activity of 30S-5SRNP particles is significantly higher than that of 40S subunits. (2) The extents of stimulation of ATPase by mRNA, elongation factors or combinations of them are definitely larger for the former particles. (3) The extents of inhibition by inhibitors in elongation are larger in the case of 30S-5SRNP particles. Therefore, it is reasonably considered that the conformation of the ATPase center in 30S-5SRNP particles is more favorable for the ATPase activity, and more sensitive to the stimulation by components in the elongation reaction and to the inhibition by inhibitors in elongation than that in 40S subunits. Considering these findings together with the fact that 30S subunits prepared by EDTA treatment of 40S subunits exhibit no ATPase activity, it is reasonably considered that the 5SRNP moiety in 30S-5SRNP particles maintains the active conformation of the ATPase center in these particles.

It is important that the intactness of 18SrRNA in 30S-5SRNP particles is required for the stimulation of the ATPase activity by phe(+) and (−)tRNA^{phe}.

Lastly, it must be mentioned that we have explained the alteration of the ATPase activities of these particles by elongation components through the conformational changes induced by them. This explanation may be supported by the fact that spermidine enhances the ATPase activity of 40S subunits since much evidence has been accumulated that polyamine stimulates protein synthesis through positive conformational changes of ribosomes (*30*).

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