Archaebacterial and eukaryotic ribosomal subunits can form active hybrid ribosomes

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Purified ribosomal subunits from the extremely thermoacidophilic archaebacterium *Sulfolobus solfataricus* are able to recognize ribosomal subunits from the yeast *Saccharomyces cerevisiae* forming hybrid monosomes that can be revealed by sucrose gradient analysis and are active in peptide bond formation. Both reciprocal combinations (archaebacterial 30 S + eukaryotic 60 S and archaebacterial 50 S + eukaryotic 40 S) are functional. In contrast, no hybrid couples are formed between subunits of yeast and *Escherichia coli* ribosomes. These results indicate that ribosomes of at least one archaebacterial species share specific structural features with those of the lower eukaryote *S. cerevisiae*.

Sulphur-dependent archaebacteria *Ribosomal subunit exchange* *Peptidyltransferase* *Evolution*

1. INTRODUCTION

Although the design of the translational apparatus is the same in all living systems, individual translation components have evolved somewhat differently in the two classically recognized kingdoms, the eubacteria and the eukaryotes. Notably, eubacteria harbour ribosomes that are smaller and contain less protein than those of eukaryotes. Within each kingdom, however, ribosome architecture is conserved to the extent that large and small ribosomal subunits from distantly related organisms can be combined into functionally active hybrid monomers [1–3].

Less is known about the ribosomes of the recently discovered kingdom of archaebacteria, except that they fall into two size classes. One, which can be found in halophiles and most methanogens, comprises particles that are physicochemically indistinguishable from 70 S ribosomes of eubacteria. The other, found in Methanococcales and sulphur-dependent thermophiles, comprises ribosomes that are heavier and richer in protein than their eubacterial counterparts, but still considerably smaller than the 80 S particles of eukaryotic cytosol [4]. Also, it has been reported that specific details of the subunits' shapes correlate the ribosomes of methanogens and halophiles to those of eubacteria, and the ribosomes of sulphur-dependent thermophiles to those of eukaryotes [5]. These correlations and their phylogenetic implications, however, have been challenged on the grounds that the distinguishing morphological features are too elusive and ill-defined [6].

In the present paper we report that the ribosomal subunits of the thermophilic sulphur-dependent archaebacterium *Sulfolobus solfataricus* interact with those of the eukaryote *Saccharomyces cerevisiae* to form hybrid monomers which are active in peptide bond formation. This finding represents the first direct evidence of a specific affinity between ribosomal subunits of organisms belonging to different kingdoms.

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Abbreviations: Phe, phenylalanine; acPhe, *N*-acetylphenylalanine; PTF, peptidyltransferase

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2. MATERIALS AND METHODS

2.1. *Isolation of ribosomal subunits*

Ribosomes and subunits of *Sul. solfataricus* (DSM 1616) and *Escherichia coli* (MRE 600) were prepared as detailed in [7] and [8], respectively. Ribosomes of *S. cerevisiae* were prepared as described [9]. To isolate subunits, yeast ribosomes were suspended in 500 mM KCl, 20 mM Tris-HCl (pH 7.2), 3 mM Mg acetate, 10 mM β-mercaptoethanol and centrifuged on 38.5 ml, linear, 10–30% (w/v) sucrose density gradients made in the above solvent containing 300 mM KCl instead of 500 mM. After centrifugation in an SW 27 rotor at 4°C and 18000 rev/min for 16 h, particles corresponding to the 40 S and 60 S peaks of A_{260} were collected by high-speed centrifugation (150000 × g and 4°C for 20 h), dissolved in 100 mM KCl, 20 mM Tris-HCl (pH 7.2), 3 mM Mg acetate, 10 mM β-mercaptoethanol, 50% (v/v) glycerol and stored at −25°C.

2.2. *Peptidyltransferase (PTF) activity of subunit combinations*

The reaction mixtures for the PTF assay (150 μl) contained: 70 mM KCl, 20 mM triethanolamine-HCl (pH 7.2), 18 mM Mg acetate, 2 mM dithiothreitol, 2 mM spermine tetrahydrochloride, 2 mM puromycin, 5000–7000 cpm of *E. coli* [^{3}H]acPhe-tRNA (spec. act. 20 counts·min^{-1}·pmol^{-1} total tRNA), 0.2 A_{260} units (~12 pmol) small subunits, 0.5 A_{260} units (~20 pmol) large subunits. Homologous and heterologous mixtures containing *E. coli* subunits lacked spermine. The samples were incubated at 43°C for 20 min and formation of [^{3}H]acPhe-tRNA was determined as described [10]. [^{3}H]acPhe-tRNA was prepared from *E. coli* bulk tRNA (Boehringer) as detailed in [11].

2.3. *Sucrose gradient analysis of subunit combinations*

Large and small subunits were incubated at 43°C for 30 min as described above, except that puromycin was substituted with unlabelled Phe-tRNA (*E. coli*, 0.1 A_{260} units). The reaction mixtures (150 μl) were then supplemented with 25 μl of 36% (v/v) formaldehyde in 200 mM triethanolamine-HCl (pH 7.2) and allowed to stand 30 min at 20°C. The distribution of the monomers and the free subunits was monitored by centrifuging the samples for 180 min at 10°C (SW 41 rotor) on 11.5 ml, linear, 10–30% (w/v) sucrose density gradients made in 30 mM KCl, 20 mM triethanolamine-HCl (pH 7.2), 5 mM Mg acetate, 5 mM β-mercaptoethanol.

3. RESULTS AND DISCUSSION

The compatibility between ribosomal subunits of distantly related species belonging to the same kingdom can be conveniently assayed by measuring their poly(Phe) synthesizing capacity in a poly(U)-programmed cell-free system. This simple approach, however, could not be used to test whether subunits from yeast and *Sulfolobus* complement one another. First, cell-free systems from these two organisms differ widely in their ionic and temperature optima (30°C and 80°C, respectively) [12]. Secondly, eukaryotic and archaebacterial elongation factors only function with ribosomes of their own lineage [13]. To overcome these difficulties, the interaction between the ribosomal subparticles from the two sources was assayed by means of the PTF reaction under conditions in which the formation of AcPhe-puromycin on the large subunit can occur only in the presence of small subunits and template [14]. In addition to eliminating the requirement of added supernatant factors, this assay has the advantage of being efficient at both high (65°C) and low (37°C) temperatures with *Sulfolobus* ribosomes [10].

The ionic milieu for the PTF reaction represented the best compromise between the optimal conditions for peptide bond formation in the two homologous systems. The *Sulfolobus* assay system is absolutely dependent upon spermine (3 mM), but is drastically inhibited by relatively low (greater than 20 mM) concentrations of monovalent cations [10]. The yeast system, on the other hand, is unaffected by spermine (up to 2 mM) but requires a relatively high (150 mM) K^+ concentration. With 70 mM K^+ and 2 mM spermine, the activity of the yeast and *Sulfolobus* systems was 50% that observed under optimum conditions. *E. coli* acPhe-tRNA was compatible with both archaebacterial and yeast ribosomes and therefore was used in all the experiments.

The PTF activity of homologous and heterologous combinations of *S. cerevisiae* and

Table 1

Activity of various combinations of ribosomal subunits in the peptidyltransferase assay

Assay mix	cpm ^{3}H/ assay mix
All components except ribosomes	250
Yeast 60 S	350
Yeast 40 S	400
Yeast 60 S + yeast 40 S (no puromycin)	300
Sulfolobus 30 S	450
Sulfolobus 50 S	350
Sulfolobus 50 S + *Sulfolobus* 30 S (no puromycin)	200
Yeast 60 S + yeast 40 S	1600
Sulfolobus 50 S + *Sulfolobus* 30 S	2300
Yeast 60 S + *Sulfolobus* 30 S	1400
Yeast 40 S + *Sulfolobus* 50 S	2500
Yeast 60 S + *E. coli* 30 S	400
Yeast 40 S + *E. coli* 50 S	500
E. coli 50 S + *E. coli* 30 S	1700

Sul. solfataricus subunits is summarized in table 1. As the results show, mixtures containing all the components required for the reaction but lacking either of the two subunits are inactive in peptide bond formation. The two heterologous combinations of yeast and *Sulfolobus* particles, however, are just as active as the homologous ones (i.e. 40 S + 60 S and 30 S + 50 S).

The specificity of the assay was checked by determining the PTF activity of combinations of eubacterial (*E. coli*) and eukaryotic (yeast) ribosomal subunits. These reactions were performed under the same conditions as described above, but in the absence of spermine which strongly inhibits *E. coli* PTF (P. Londei, unpublished results). As the results in table 1 show, both heterologous mixtures are essentially inactive in acPhe-puromycin formation, in contrast to mixtures containing homologous subunits.

Sucrose gradient analysis of the reaction mixtures was used to demonstrate that the synthesis of acPhe-puromycin by heterologous and homologous combinations of ribosomal subunits reflects the formation of monosomes (fig.1). The subunits were incubated in the standard reaction mixture for the PTF assay, with Phe-tRNA instead of puromycin; samples were treated with formaldehyde prior to zone-velocity centrifugation. Fixation with formaldehyde is required to stabilize subunit couples against pressure-induced dissociation which occurs during density-gradient centrifugation of *Sulfolobus* ribosomes even when these carry long poly(Phe) chains [10].

As fig.1 (mid panels) shows, prominent peaks of hybrid monomers are apparent in the sedimentation diagrams of samples containing reciprocal combinations of yeast and *Sulfolobus* ribosomal subunits. Interestingly, the hybrid monomers are more resistant to pressure-induced dissociation than the *Sulfolobus* 70 S monomers, 50% of which dissociate into free subunits upon centrifugation, even though fixed with formaldehyde (fig.1 top left panel). The possibility that the free subunits may represent particles that never associated rather than products of monomer dissociation can be discounted by the results in table 1 which show that homologous combinations of *Sulfolobus* 50 S and 30 S subunits are as active as heterologous combinations of *Sulfolobus* and yeast ribosomal subunits.

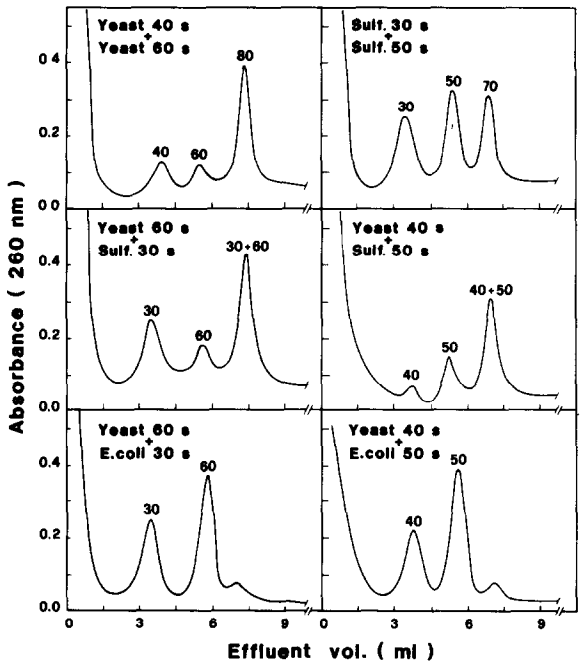

Fig.1. Sedimentation patterns of ribosomal subunit mixtures incubated under conditions for peptide bond formation and fixed with formaldehyde prior to zone-velocity centrifugation. Direction of sedimentation is from left to right.

As might be expected on the basis of the results of the functional assays, in samples containing homologous combinations of eubacterial or eukaryotic subunits (*E. coli* 30 S + 50 S and yeast 40 S + 60 S) most particles reassociate into 70 S (not shown) and 80 S ribosomes (fig.1 top left panel). In contrast, essentially no monomers are formed in samples containing reciprocal combinations of *E. coli* and yeast ribosomal subunits (fig.1 bottom panels). The small monomer peaks seen in the sedimentation diagrams of both heterologous combinations of eubacterial and eukaryotic subunits almost certainly represent *E. coli* 70 S and yeast 80 S ribosomes arising from contamination of the subunit preparations by trace amounts of complementary subunits. This conclusion is well supported by the sedimentation diagrams in fig.2 which show that yeast 60 S subunits (fig.2 bottom right panel) and both subunits of *E. coli* ribosomes

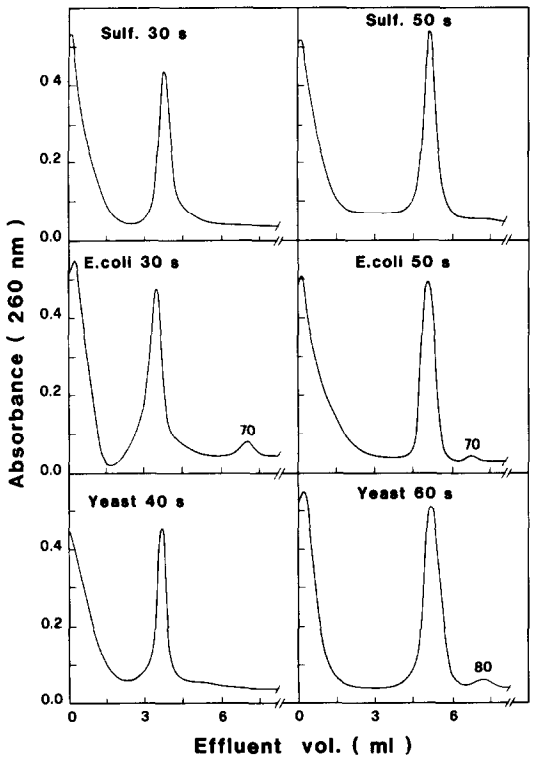

Fig.2. Sedimentation patterns of subunit preparations incubated in the presence of acPhe-tRNA and Phe-tRNA, and centrifuged in sucrose density gradients after fixation with formaldehyde. Direction of sedimentation is from left to right.

(fig.2 mid panels) yield small amounts of monomeric particles following incubation with acPhe-tRNA and Phe-tRNA. These account for the small monomer peaks seen in the sedimentation diagrams of reciprocal combinations of *E. coli* and yeast ribosomal subunits (fig.1 bottom panels).

On the whole, our results indicate that ribosomal subunits of *Sul. solfataricus* (an extremely thermophilic, acidophilic archaebacterium) and *S. cerevisiae* (a lower eukaryote) have specific affinities for one another, at least insofar as they form hybrid particles active in peptide bond formation.

It has been proposed, on the basis of similarities in certain structural features (5 S rRNA secondary folding [15], subunit composition of the DNA-dependent RNA polymerases [16], details of ribosome shape [5]), that eukaryotes and eubacteria are phylogenetically related, though in a way that remains to be defined, to the sulfur-dependent and the methanogen branches of the archaebacterial kingdom, respectively [5]. Although the interchangeability of *Sulfolobus* and yeast ribosomal subunits lends support to this hypothesis, we feel that it is unwise to posit ancestral relationships until the compatibility of the ribosomal subunits of other archaebacteria (e.g. methanogens) with those of eukaryotes is assayed. More importantly, subunit compatibility must be tested between eubacteria and archaebacteria belonging to the two branches of the third kingdom. These issues are presently being explored in our laboratory.

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