Ribosome specificity of archaebacterial elongation factor 2

Studies with hybrid polyphenylalanine synthesis systems

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Polyphenylalanine synthesis with ribosomes and two separated, partially purified elongation factors (EF) was measured in cell-free systems from the archaebacteria *Thermoplasma acidophilum* and *Methanococcus vannielii*, in an eukaryotic system from rat liver and an eubacterial one with *Escherichia coli* ribosomes and factors from *Thermus thermophilus*. By substitution of heterologous EF-2 or EF-G, respectively, for the homologous factors, ribosome specificity was shown to be restricted to factors from the same kingdom. In contrast, EF-1 from *T. thermophilus* significantly cooperated with ribosomes from *T. acidophilum*.

Archaebacteria Thermoplasma acidophilum Methanococcus vannielii *Elongation factor*
Ribosome specificity *Polyphenylalanine synthesis*

1. INTRODUCTION

With regard to ribosome specificity of elongation factors, all protein synthesis machineries have been divided into two classes: the prokaryotic–organellar and the eukaryotic–plasmatic [1,2]. For in vitro peptide elongation the supernatant factors are restricted to their own specificity class, and specificity apparently resides mainly in the translocating factor (EF-G in prokaryotes, EF-2 in eukaryotes) [3], though most investigators found tRNA-binding factors (EF-Tu or EF-1, respectively) also rather group-specific, displaying only marginal cross-activity, except for one special case [3–5]. However, factors and ribosomes of different species belonging to the same class are exchangeable with the exception of mammalian mitochondria [6]. The two classes of specificity coincided with the two classical kingdoms of organisms. Since 16 S-rRNA analyses revealed archaebacteria as a third primary kingdom [7,8], the question was open whether there exists a third type of protein synthesis machinery functionally incompatible with components of the other ones. The problem gained particular interest with respect to elongation factor 2 when a characteristic, unique feature of eukaryotic EF-2, ADP-ribosylation by diphtheria toxin, was found to be shared by the analogous factors from archaebacteria [9,10].

These results demonstrate that partially purified EF-2 preparations from the two archaebacterial species examined do not cooperate with other than archaebacterial ribosomes for polyphenylalanine synthesis; conversely, eubacterial EF-G or eukaryotic EF-2 had no significant effects on translation of polyuridylic acid on archaebacterial ribosomes.

2. EXPERIMENTAL

Factor-free ribosomes from *Thermoplasma acidophilum* DSM 1728 and *Methanococcus vannielii* DSM 1224 were prepared combining high salt washing and gradient centrifugation as described ([11,12]; in preparation). Purification of rat liver ribosomes was slightly modified after [13] (method C). Liver (25 g) yielded 185 A_{260} units of ribosomes (A_{260}/A_{280} = 1.79). *E. coli* ribosomes

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were prepared as in [14] except that the first NH_4Cl buffer was 10^{-3} M in magnesium acetate. From 6 g (wet wt) *E. coli* cells 177 A_{260} units resulted (A_{260}/A_{280} = 1.97).

Elongation factors from *T. acidophilum* were separated as detailed in [12]. Briefly, cells were homogenized (Potter-Elvehjem) at pH 6.5, ribosomes spun down, supernatant at pH 7.0 chromatographed on DE 23 cellulose (Whatman). Elongation factors were identified by GDP binding (EF-1, eluted with break-through protein) or by ADP-ribosylation (EF-2, eluted with 0.15 M KCl), respectively. Two factors from *M. vannielii* were separated with the same method. In an analogous manner but at pH 7.5, EF-1α and EF-2 from rat liver were separated. The high-M_r form of liver EF-1 (EF-1_H) was prepared as in [13]. EF-G from *Thermus thermophilus* and EF-Tu from *Thermus* and from *E. coli* were gifts from Dr M. Genz (Frankfurt). All factor preparations contained considerable amounts of other proteins but not of the complementary factors according to the identification reactions mentioned and to polyphenylalanine synthesis assays under standard conditions.

Phenylalanine synthesis assay: for composition of the 125 μl standard assay mixture see fig.1. After incubation, radioactively labelled protein was precipitated and counted as in [15].

All materials were the same as used in [15] except of the Phe-tRNA which was prepared according to [16] from *E. coli* phenylalanine-specific tRNA with aminoacyl-tRNA ligase, both purchased from Sigma (St Louis MO).

3. RESULTS AND DISCUSSION

First hybrid polyphenylalanine synthesis systems were constructed with ribosomes from *E. coli*, *T. acidophilum* and rat liver; the homologous translocation factor (EF-G or EF-2, respectively) was exchanged for heterologous factors from other kingdoms, or in the case of archaebacteria, also from the same one. In all cases, the amounts of EF-2 or EF-G, respectively, applied in the heterologous systems were sufficient to catalyze maximal synthesis with the homologous partners under the same conditions. With *E. coli* ribosomes only eubacterial EF-G was found to be complementary to EF-Tu (fig.1A); with *Ther-*

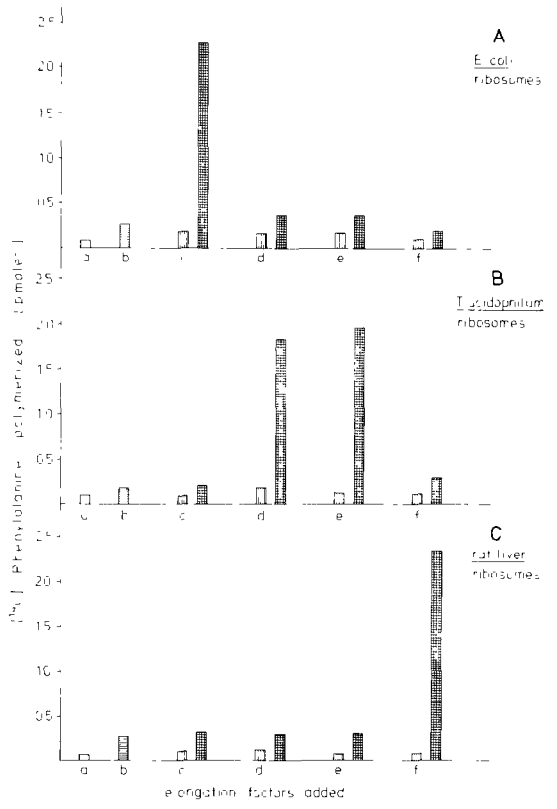

Fig.1. Polyphenylalanine synthesis with ribosomes from *E. coli* (A), *T. acidophilum* (B) and rat liver (C) with tRNA binding factor (EF-T or EF-1) homologous with ribosomes and translocating factor (EF-G or EF-2) from various sources as indicated below. Assay mixtures: 125 μl contained 50 mM Hepes [pH 7.1 (A) or pH 7.0 (B,C)]; 90 mM KCl, 10 mM magnesium acetate, 5 mM mercaptoethanol, 0.69 mM spermine (only in A,B), 1 mM GTP, 1 mM ATP, 40 μg polyuridylic acid, 28.8 nM [^{14}C]phenylalanyl-tRNA (spec. act. 500 Ci/mol), ribosomes (in A, 0.12 A_{260} units; in B, 0.14 units; in C, 0.18 units), and elongation factor preparations as follows: (a) without factors; (b) homologous binding factor alone; pairs of columns (c–f) translocating factor alone (left) and in combination with binding factor (right). Sources of EF-G or EF-2: (c) *T. thermophilus* (1.85 μg protein); (d) *T. acidophilum* (1.1 μg in A,B; 1.5 μg in C); (e) *M. vannielii* (21.8 μg); (f) rat liver (2.7 μg in A,C; 5.4 μg in B). Amounts of binding factor per assay: 2.5 μg EF-Tu from *T. thermophilus* (A), 5.2 μg EF-1 from *T. acidophilum* (B) and 6.0 μg EF-1_H from rat liver (C). Assays were incubated at 37°C for 15 min and further treated according to [15].

moplasma ribosomes, EF-2 factors from *Thermoplasma* and *M. vannielii* were effective in the presence of *Thermoplasma* EF-1 (fig.1B), and in the liver system only liver EF-2 stimulated synthesis. In the latter case, EF-1_H (as used in fig.1C) yielded similar results as the low-M_r from EF-1α (not shown). Likewise, liver polyribosomes programmed with endogenous mRNA were not stimulated by *Thermoplasma* EF-2 in the presence of liver EF-1 (not shown).

Since ribosomes or factors from mesophilic organisms ruled out incubation of the respective assays at >40°C, all experiments in fig.1 were performed at 37°C. One crucial experiment showing the lack of activity of EF-G from *T. thermophilus* with *Thermoplasma* ribosomes was repeated under thermophilic conditions (fig.2a–f).

The ionic milieu used represented a compromise between the considerably differing demands of the individual homologous systems which had been found for *Thermoplasma* to be pH 6.4, 30–80 mM K^+ and 15 mM Mg^{2+} [12], for liver and *E. coli* (pH 7.8–8.0), 100–120 mM K^+ and 5–10 mM Mg^{2+} [17], and for *M. vannielii* (pH 7.6–7.8) at least 120 mM K^+ or NH_4^+ and 10 mM Mg^{2+} (unpublished).

In the 3 homologous systems synthesis proceeded at about half-maximal rate even under the compromising conditions of pH and temperature. For quantitative comparisons of elongation factor effects in heterologous systems *M. vannielii* seemed more suitable than *T. acidophilum* as source of archaebacterial ribosomes. Therefore, increasing amounts of translocating factors from *M. vannielii*, *T. acidophilum*, rat liver and *T. thermophilus* were applied to systems with ribosomes from *M. vannielii* and liver (fig.3). This time, reaction conditions lay near the optimal values for

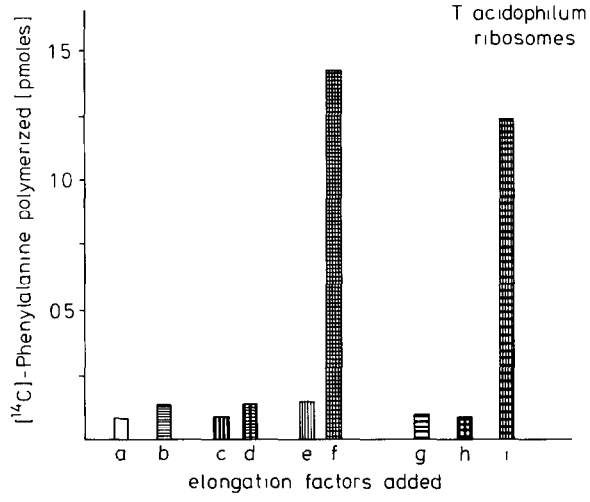

Fig.2. Ribosome specificity of elongation factors from thermophilic eu- and archaebacteria. All assays were incubated for 10 min at 56°C and pH 7.2. They contained 0.14 A_{260} units of *T. acidophilum* ribosomes and factors as follows: (a) none; (b) 5.2 μg *T. acidophilum* EF-1; (c) 3.6 μg *T. thermophilus* EF-G; (d) factor combination of (b,c); (e) 1.1 μg *T. acidophilum* EF-2; (f) combination of (b,e); (g) 1.25 μg *T. thermophilus* EF-Tu; (h) combination of (c,g); (i) combination of (e,g). For other components see fig.1.

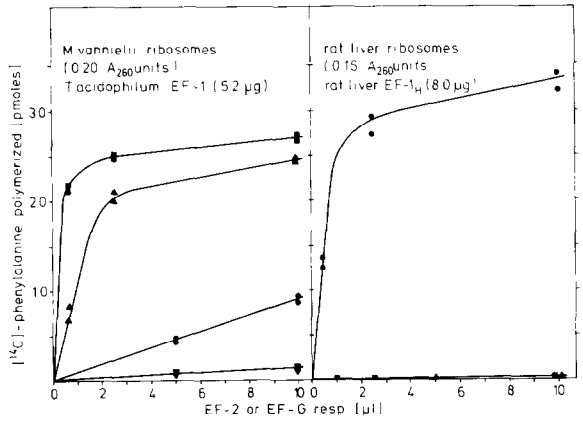

Fig.3. Amounts of various translocating factors plotted against polyphenylalanine synthesis in combination with ribosomes and EF-1 from rat liver (right) and with *M. vannielii* ribosomes and *T. acidophilum* EF-1 (left). Translocating factor preparations used: (■) *M. vannielii* EF-2 (4.1 μg protein/μl); (▲) *T. acidophilum* EF-2 (0.5 μg/μl); (●) rat liver EF-2 (0.36 μg/μl); (▼) *T. thermophilus* EF-G (1.1 μg/μl). Assay mixtures: 125 μl contained 30 mM Tris–HCl (pH 7.6), 10 mM magnesium acetate, 5 mM mercaptoethanol, 1 mM GTP, 1 mM ATP, 40 μg polyuridylic acid, 28.8 nM [^{14}C]phenylalanyl-tRNA (spec. act. 500 Ci/mol), ribosomes and factors as indicated below or in the drawing, and for the *Methanococcus* system 100 mM NH_4Cl, 30 mM KCl and 0.23 mM spermine; for the liver system 100 mM KCl but no NH_4Cl or spermine. Incubation was for 20 min at 37°C. Further processing according to [15]. Background effects without translocating factors, 0.06 pmol (left) and 0.46 pmol (right), had been subtracted.

M. vannielii and rat liver. The results corroborated the findings of fig.1 inasmuch as amounts of EF-2 producing nearly maximal synthesis with homologous reaction partners had marginal or insignificant effects with ribosomes from the other kingdom. Very large quantities of liver EF-2 gave substantial synthesis in the archaebacterial system; no analogous result could be found reversing the procedure. This asymmetric behavior has to be confirmed using purified eukaryotic EF-1α instead of the EF-1_H preparation applied here; likewise, the effect of a large amount of EF-G from a mesophilic eubacterium on *M. vannielii* ribosomes remains to be examined.

Nevertheless, the present results clearly suggest that the translocating factors from two archaebacteria differ from eubacterial and eukaryotic factors in ribosome specificity. Our findings fit into the proposal of 3 general structural patterns of ribosomal small subunits drawn from electron micrographs [18]. Possibly due to phylogenetic changes of ribosomes, no functional equivalent seems to exist to the structural similarity between archaebacterial and eukaryotic EF-2 suggested by the common feature to be substrates for diphtheria toxin [10].

It remains uncertain for the present whether the third class of ribosome specificity comprises the entire archaebacterial kingdom or has to be subdivided. The EF-2 preparation from *M. vannielii* had an ~4-fold lower 'specific activity' (ADP-ribosylation/mg protein) than the factors from *T. acidophilum* and rat liver. Hence, in the *Thermoplasma* system at pH 7.0, ~5-fold molar amounts of *Methanococcus* EF-2 were necessary to reach the synthesis value given by the homologous factor (fig.1B); on the other hand, using the same basis of calculation, roughly equal molar amounts of both factors yielded similar effects with *Methanococcus* ribosomes at pH 7.6 (fig.3, left). For comparison, according to fig.3, a 25-fold molar amount of liver EF-2 would be needed in the *Methanococcus* system to reach the synthesis effect of the homologous factor.

Thus, *T. acidophilum* and *M. vannielii* though phylogenetically rather distant according to 16 S rRNA analysis [19] indeed seem to belong to one class of ribosome specificity. We have no information as yet about the functional behavior of factors from the aerobic and anaerobic sulphur-metabolizing archaebacteria.

The question whether the tRNA-binding factors from archaebacteria show ribosome specificity to a comparable extent as the analogous factors from the other kingdoms [5] is under study. From the results of an exchange experiment between the two thermophilic organisms (fig.2g–i) it may be concluded that *Thermoplasma* ribosomes possess no marked specificity with respect to prokaryotic tRNA binding factors, but as yet we have no complete picture.

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