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Insensitivity of archaebacterial ribosomes to protein synthesis inhibitors. Evolutionary implications

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The effect on *Sulfolobus solfataricus* (an extremely thermoacidophilic archaebacterium) of selected inhibitors affecting reactions of the polypeptide elongation cycle has been tested by using poly(U) and poly(UG) directed cell-free systems. The results reveal a unique pattern of antibiotic sensitivity of *Sulfolobus* ribosomes with an inhibitory effect observed for only three of 60 compounds tested. Through comparison with suitable eubacterial and eukaryotic cell-free systems the insensitivity of *Sulfolobus* ribosomes to most inhibitors of protein synthesis appears to reflect a phylogenetic distinction of ribosome structure, rather than the high temperature conditions of the *Sulfolobus* assay system. In this respect ribosomes of thermoacidophilic archaebacteria differ not only from their eubacterial and eukaryotic counterparts, but also from ribosomes of archaebacteria belonging to the methanogenic-halophilic branch of the 'third' kingdom. The evolutionary implications of these findings are discussed.

Key words: thermoacidophilic archaebacteria/protein synthesis inhibitors/ribosomes/evolution

Introduction

Archaebacteria constitute a primary kingdom whose phylogenetic relationships with eubacteria and eukaryotes are still ill-defined, since in certain aspects they resemble the former whereas in others they resemble the latter (Woese, 1982). The two traditionally recognized lines of cellular descent have specific protein synthesizing machineries that are differentially affected by certain inhibitors (Vazquez, 1979). Drugs that specifically block protein synthesis in either eubacteria or eukaryotes provide sensitive tools both to probe the relatedness of archaebacteria to each of the other two aboriginal cell lineages and to investigate the depth of the phylogenetic split between the two main divisions of the archaebacterial domain (the methanogenic-halophilic and the sulphur-metabolizing archaebacteria).

Available data on the antibiotic sensitivity of poly(U)-directed cell-free systems of the methanogenic archaebacteria (Elhardt and Böck, 1982) indicate that their ribosomes lack binding sites for many drugs that inhibit eubacteria while being sensitive to certain drugs that inhibit eukaryotes. The development of a comparable cell-free system for the extreme thermoacidophilic archaebacterium *Sulfolobus solfataricus* (formerly named *Caldariella acidophila*) now allows an investigation of the antibiotic sensitivity of the sulphur-metabolizing branch of archaebacteria (Cammarano *et al.*, 1982).

The present survey, based on an extensive list of protein synthesis inhibitors, discloses a unique pattern of antibiotic sensitivity of *S. solfataricus* ribosomes, with an inhibitory effect observed for no more than three of the 60 compounds tested. Furthermore, characteristic differences that reflect the phylogenetic division within the archaebacterial kingdom become apparent through comparison with the methanogenic archaebacteria.

Results

The effect of selected inhibitors of poly(U)- or poly(UG)-directed cell-free systems from *S. solfataricus* are summarized in Tables I–III. The inhibitors chosen include representatives of the main classes of compounds affecting polypeptide elongation in eubacteria and/or eukaryotes. Due to the high optimal temperature of the *Sulfolobus* protein synthesis system (75°C), care was exercised to rule out any spurious effects that might have arisen from either (i) heat instability of the inhibitors; or (ii) low efficiency of interaction between the antibiotics and their target sites at high temperatures.

Thermal inactivation was ruled out by pre-incubating each compound at 75°C for 40 min and by subsequently testing its activity in the appropriate target systems (*Escherichia coli, Saccharomyces cerevisiae*, rabbit reticulocytes and rat liver) at their optimal temperatures. The results in Tables I–III show that most antibiotics tested in the present work are heat stable, except the plant polypeptide inhibitors ricin, gelonin, modeccin and pokeweed antiviral protein (PAP) which were partially or totally inactivated at 75°C. Consequently, it was possible to test all the antibiotics for their inhibitory action on protein synthesis at 75°C, except for the heat-sensitive ones, which could only be assayed at their 'stable' temperatures, as specified in Table II.

The possibility of secondary effects arising from the high temperature of the *S. solfataricus* system, such as low efficiency of the inhibitor interaction with its target site(s) was ruled out by using appropriate controls. Thus, eubacterial targeted antibiotics (group I inhibitors) (Table I) as well as eubacterial and eukaryotic targeted antibiotics (group III inhibitors) (Table III) are able to block polypeptide synthesis in *Bacillus stearothermophilus* and *Thermus thermophilus* at 75°C with an efficiency comparable with that seen in the mesophilic control systems. Although we lack a high temperature control assay for the eukaryotic targeted antibiotics (group II inhibitors) (Table II) we assume that the results obtained for the group I and group III inhibitors in the thermophilic systems can be safely extrapolated to the eukaryotic targeted drugs. Representative inhibition curves showing the effect of antibiotics from the three different groups on the *S. solfataricus* protein synthesis system, together with the different types of controls related to the specificity of the antibiotics and to the high temperature conditions of the assay, are shown in Figure 1.

The most significant result in Tables I–III is the evidence that under optimum conditions for *in vitro* activity the translational apparatus of *S. solfataricus* is essentially insensitive to most

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Table I. Inhibition of protein synthesis in *S. solfataricus* by eubacterial targeted antibiotics (Group I inhibitors)

Antibiotics	*S. solfataricus*					Controls					Other archaebacteria[a]			
Group I	Type of inhibitor	Inhibitory effect	Incubation temperature	Maximum drug concentration used	Drug concentration producing 50% inhibition	*E. coli*	*B. stearothermophilus*	*T. thermophilus*	*S. cerevisiae*	Activity of pre-heated antibiotic[b]	*M. vannielii*	*M. barkeri*	*M. formicicum*	*H. cutirubrum*
Bluensomycin	A	–	75°C	1 mM	–	++	++		–	++				
Gentamicin	A	±	75°C	–	10^{-3} M	++	++	++	–	++	+	+	n.t.	
Kanamycin	A	–	75°C	1 mM	–	++	++	++	–	++	–			
Kirromycin	A	–	75°C	1 mM	–	++	++	++	–	++				–
Neamine	A	–	75°C	1 mM	–	++	++		–	++	±			
Neomycin	A	±	75°C	–	2×10^{-4} M	++	++	++	–	++	+	+	n.t.	
Paromomycin	A	±	75°C	–	5×10^{-4} M	++	++		–	++	+			
Pulvomycin	A	–	45°C	1 mM	–	++	++		–	–				
Ribostamycin	A	–	75°C	1 mM	–	++	++		–	++				
Streptomycin	A	–	75°C	1 mM	–	++	++	++	–	++	–	–	n.t.	
Sisomycin	A	±	75°C	–	3×10^{-3} M	++	++		–	++	±			
Thiostrepton	A	–	75°C	1 mM	–	++	++	++	–	++	+	n.t.	+	
Tobramycin	A	±	75°C	–	10^{-4} M	++	++		–	++	±			
Althiomycin	B	–	75°C	1 mM	–	++	++		–	++				
Carbomycin-A	B	–	75°C	1 mM	–	++	++		–	++				
Chloramphenicol	B	–	75°C	1 mM	–	++	++	++	–	++	–	–	n.t.	+
Griseoviridin	B	–	75°C	1 mM	–	++	++		–	++				
Tylosin	B	–	75°C	1 mM	–	++	++		–	++				
Virginiamycin-M	B	–	75°C	1 mM	–	++	++	++	–	++	+	n.t.	n.t.	
Mikamycin-A+B	B+C	–	75°C	1 mM	–	++	++		–	++				
Spectinomycin	C	–	75°C	1 mM	–	++	++		–	++				
Viomycin	C	–	75°C	1 mM	–	++	++		–	++	–			
Viridogrisein	C	–	75°C	1 mM	–	++	++		–	++				

Protein synthesis of cell-free systems from different organisms were prepared as described in Materials and methods. Antibiotics are classified according to the specific reaction of the polypeptide elongation cycle that is affected: (A) binding of aminoacyl-tRNA; (B) peptide bond formation; (C) translocation (Vazquez, 1979). The stability of each compound was tested by pre-incubation at 75°C for 40 min and by subsequent incubation of the pre-heated antibiotic in the appropriate control system. The thermal stability is defined under the 'controls' column as (++) no inactivation; (±) partial inactivation; (–) complete inactivation after pre-incubation at 75°C for 40 min. The inhibitory effects in *S. solfataricus* are defined as (–) no inhibition at the maximum concentration indicated in the table; (++) same inhibition as in the control assays; (+) inhibition occurring at concentrations at least one order of magnitude greater than that needed to produce the same effect as in the control assay; (±) some inhibition produced at the concentrations given in the table.

[a]Data from the literature; results for methanogenic archaebacteria are from Elhardt and Böck (1982) and Böck *et al.* (1983); data for halophilic archaebacteria are from Bayley and Griffith (1968) and by Kessel and Klink (1981). Original values are maintained: inhibition at antibiotic concentrations below 10 μg/ml (++); inhibition between 10 and 50 μg/ml (+); inhibition above 50 μg/ml (±); no effect at 125 μg/ml (–); n.t., not tested.

[b]Assayed by pre-incubating each compound at 75°C for 40 min and by subsequently testing its activity in the *E. coli* cell-free system.

known inhibitors of protein synthesis. Nevertheless, certain antibiotics were active on the *S. solfataricus* system, although at concentrations significantly higher than those normally required to block polypeptide synthesis in the control assays. These exceptions are discussed below for each of the three groups of inhibitor compounds.

Group I (eubacterial targeted antibiotics) (Table I)

The disubstituted 2-deoxystreptamine (DOS) aminoglycosides neomycin, paromomycin, gentamycin, tobramycin and sisomycin are able to inhibit polyphenylalanine synthesis in the *S. solfataricus* system at concentrations at least two orders of magnitude greater ($>10^{-4}$ M) than those required to inhibit the eubacterial control systems. The concentration *versus* activity curves in Figure 1a show that one representative compound of this group, neomycin, affects polypeptide synthesis in the *Sulfolobus* system, within the same range of concentration that is effective in inhibiting a eukaryotic (yeast) control system. Therefore, the specificity of the inhibitory effects exerted by the DOS aminoglycosides remains in doubt.

The remaining antibiotics of this group were ineffective even at concentrations as high as 10^{-3} M which, in our experimental conditions, is equivalent to an antibiotic/ribosome ratio of ~3000 (Figure 1b and 1c).

Group II (eukaryotic targeted antibiotics) (Table II)

Only α-sarcin, a cytotoxic protein with specific RNase activity (Endo and Wool, 1982) is able to suppress protein synthesis in *S. solfataricus* at rather low concentrations, although still higher than those required to block translation in the yeast system. In these conditions a specific cut is produced in the 23S rRNA of the 50S subunit of *Sulfolobus* ribosomes (Sanz and Amils, 1984), suggesting that the very same sequence recognized by the toxin in different eukaryotic ribosomes must be present in *S. solfataricus*. Interestingly, the two polypeptide inhibitors, mitogillin and restrictocin, which are closely related to α-sarcin (Fando, personal communication), were completely ineffective on *S. solfataricus* while being as effective as α-sarcin when assayed in the yeast system. The remaining antibiotics of group II were ineffective even at concentrations as high as 10^{-3} M (Figure 1d, e, f). Some inhibition is observed at very high concentrations for pederine.

Group III (eubacterial and eukaryotic targeted antibiotics) (Table III)

Few inhibitors of this group, puromycin (Figure 1g), sparsomycin (Figure 1h) and tetracycline, were active in the *S. solfataricus* system, although their effective concentration range was at least one order of magnitude greater in *Sulfolobus* than in the control assays. Surprisingly, even the 'universal' inhibitor puromycin was less effective in *Sulfolobus* than in either the eubacterial or the eukaryotic control systems. The inhibition curve for puromycin (Figure 1g) reveals a gradient of sensitivity to this antibiotic, with eubacterial and *Sulfolobus* ribosomes being respectively the most and the least sensitive. This observation may hint at some phylogenetic specificity of the ribosomal do-

Table II. Inhibition of protein synthesis in *S. solfataricus* by eukaryotic targeted antibiotics (Group II inhibitors)

Antibiotics	*S. solfataricus*					Controls				Other archaebacteria[a]			
Group II	Type of inhibitor	Inhibitory effect	Incubation temperature	Maximum drug concentration used	Drug concentration producing 50% inhibition	*E. coli*	Lysate of rabbit reticulocytes	*S. cerevisiae*	Activity of pre-heated antibiotic[b]	*M. vannielii*	*M. barkeri*	*M. formicicum*	*H. cutirubrum*
Alpha-sarcin	A	+	75°C	–	5 µg/ml	–		++	++				
Dianthin-32	A?	–	45°C	15 µg/ml	–	–	++		–				
Gelonin	A?	–	45°C	15 µg/ml	–	–	++		–				
Harringtonine	A+B	–	75°C	1 mM	–	–		++	++				
Homoharringtonine	A+B	–	75°C	1 mM	–	–		++	++				
Mitogillin	A	–	75°C	100 µg/ml	–	–		++	++				
Momordica	A?	–	45°C	15 µg/ml	–		++		–				
PAP	A	–	60°C	15 µg/ml	–	–	++		±				
Restrictocine	A	–	75°C	100 µg/ml	–	–		++	++				
Ricin	A	–	60°C	15 µg/ml	–	–	++		±	–	n.t.	–	
Streptovitacin-A	C	–	75°C	1 mM	–	–		++	++				
Anisomycin	B	–	75°C	1 mM	–	–		++	++	±	±	+	+
Bruceantin	B	–	75°C	1 mM	–	–		++	++				
Haemanthamine	B	–	75°C	1 mM	–	–		++	++				
Narciclasine	B	–	75°C	1 mM	–	–		++	++				
Pretazetine	B	–	75°C	1 mM	–	–		++	++				
Tenuazonic acid	B	–	75°C	1 mM	–	–	++	–	++				
Toxin-T2	B	–	75°C	1 mM	–	–		++	++				
Trichodermin	B	–	75°C	1 mM	–	–		++	++	–	–	n.t.	
Verrucarin-A	B	–	75°C	1 mM	–	–		++	++	–	–	±	
Cycloheximide	C	–	75°C	1 mM	–	–		++	++	–	–	–	
Cryptopleurine	C	–	75°C	1 mM	–	–		++	++				
Emetine	C	–	75°C	1 mM	–	–		++	++				
Pederine	C	±	75°C	–	10^{-4} M	–		++	++				
Streptimidone	C	–	75°C	1 mM	–	–		++	++				
Tubulosine	C	–	75°C	1 mM	–	–		++	++				
Tylophorine	C	–	75°C	1 mM	–	–		++	++				
Other data from the literature													
Diphteria toxin	C	Elongation factor EF-G ADP-ribosylated				–	++	++		++			++

The inhibitory effects in the *S. solfataricus* and the reference assay systems are quantified as described in the legend to Table I.
[a]From the literature: literature data for methanogenic archaebacteria are from Elhardt and Böck (1982) and Böck *et al.* (1983); literature data for halophilic archaebacteria are from Bayley and Griffiths (1968), Kessel and Klink (1980,1981).
[b]Assayed by pre-incubating the inhibitory compound at 75°C for 40 min and by subsequently testing its activity in the *S. cerevisiae* cell-free system.

Table. III. Inhibition of protein synthesis in *S. solfataricus* by antibiotics affecting protein synthesis in both eubacteria and eukaryotes (Group III inhibitors)

Antibiotics	*S. Solfataricus*					Controls					Other archaebacteria[a]			
Group III	Type of inhibitor	Inhibitory effect	Incubation temperature	Maximum drug concentration used	Drug concentration producing 50% inhibition	*E. coli*	*B. stearothermophilus*	*T. thermophilus*	*S. cerevisiae*	Activity of pre-heated antibiotic[b]	*M. vannielii*	*M. barkeri*	*M. formicicum*	*H. cutirubrum*
Fusidic acid	A	–	75°C	1 mM	–	++	++	++	++	++				
Tetracycline	A	±	75°C	–	1.1×10^{-3} M	++	++		++	++	–	±	n.t.	
Actinobolin	B	–	75°C	1 mM	–	++	++		++	++				
Amicetin	B	–	75°C	1 mM	–	++	++		++	++				
Anthelmycin	B	–	75°C	1 mM	–	++	++		++	++				
Blasticidin-S	B	–	75°C	1 mM	–	++	–	++	++	++				
Gougerotin	B	–	75°C	1 mM	–	++	++		++	++				
Puromycin	B	+	75°C	–	1.1×10^{-3} M	++	++		++	++				+
Sparsomycin	B	±	75°C	–	1.2×10^{-4} M	++	++		++	++				
Hygromycin-B	A+C	–	75°C	1 mM	–	++	++		++	++	–	±	n.t.	

The inhibitory effects in the *S. solfataricus* and the control assay systems are quantified as described in the legend to Table I.
[a]From the literature: literature data for methanogenic archaebacteria are from Elhardt and Böck (1982) and Böck *et al.* (1983); literature data for the halophilic archaebacteria are from Bayley anbd Griffith (1983) and Kessel and Klink (1981).
[b]Assayed by pre-incubating each compound at 75°C for 40 min and by subsequently testing its activity in the *E. coli*, the *S. cerevisiae*, or in both cell-free systems.

main involved in the binding of the antibiotic. The effective concentration range for tetracycline on *Sulfolobus* ribosomes was about three orders of magnitude greater than those required to inhibit eubacterial and eukaryotic ribosomes; therefore, it should be regarded as a non-specific inhibitor of archaebacterial protein synthesis.

Discussion

The generalized pattern of insensitivity of *S. solfataricus* to a large number of antibiotics that inhibit eubacterial and eukaryotic protein synthesis has a phylogenetic, rather than adaptive, significance since the reference thermophilic eubacteria (*B. stearothermophilus* and *T. thermophilus*) are as sensitive to pro-

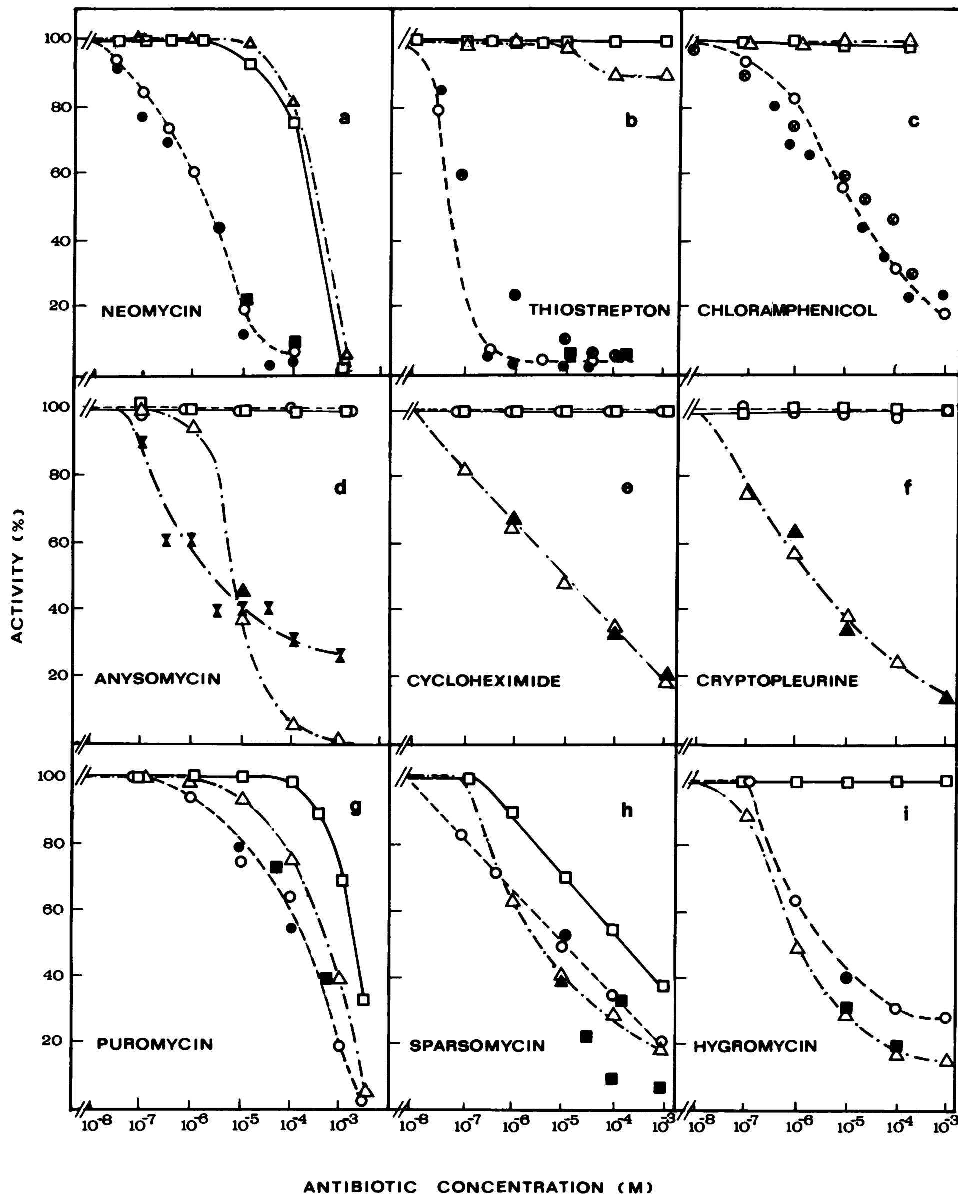

Fig. 1. Effect of selected antibiotics on phenylalanine incorporation directed by poly(U), or poly(UG), in *S. solfataricus* and control cell-free systems. Keys: (□ —) *S. solfataricus*, assayed at 75°C; (○ - - -) *E. coli*, assayed at 37°C wtih unheated antibiotic; (● - - -) *E. coli*, assayed at 37°C with antibiotic pre-heated at 75°C for 40 min; (△ · — · —) *S. cerevisiae*, assayed at 30°C with unheated antibiotic; (▲ · — · —) *S. cerevisiae*, assayed at 30°C with antibiotic pre-heated at 75°C for 40 min; (■) *B. stearothermophilus*, assayed at 75°C; (⊗) *T. thermophilus*, assayed at 75°C; (⧓) rat liver, assayed at 37°C. (**a**–**c**) eubacterial-targeted antibiotics; (**d**–**f**) eukaryotic-targeted antibiotics; (**g**–**i**) antibiotics affecting both eubacterial and eukaryotic ribosomes. All antibiotics were tested with poly(U) as the template, except chloramphenicol which was assayed by using poly(UG) (4:1).

tein synthesis inhibitors as their mesophilic counterparts. This provides further evidence for the existence of at least three primary kingdoms (Woese, 1982).

Perhaps the most significant result in the present report is the evidence that *Sulfolobus* ribosomes differ not only from those of eubacteria and eukaryotes in their sensitivity to protein synthesis inhibitors, but also from those of archaebacteria belonging to the methanogenic-halophilic branch of the 'third kingdom'. To facilitate comparison, the results of Elhardt and Böck (1982) and Böck *et al.* (1983) are included in Table I. Although a generalized pattern of insensitivity for the antibiotics tested seems to exist in the methane-producers, the translational apparatus of these latter organisms is affected by thiostrepton, virginiamycin and, to different extents, by the 4,5 and 4,6 disubstituted derivatives of 2-deoxystreptamine (neomycin, paromomycin, gentamycin, tobramycin, all of which belong to the class of eubacterial targeted inhibitors) and by anysomycin (a member of the eukaryotic-targeted group of antibiotics). These differences in antibiotic sensitivity may reflect structural distinctions between the ribosomes of the extremely thermoacidophilic archaebacteria and those of the methane-producing archaebacteria.

The antibiotic sensitivity spectra of the *S. solfataricus* protein synthetic machinery suggests that *Sulfolobus* ribosomes are no more closely related to eukaryotic ribosomes than to the eubacterial ones, although a greater degree of relatedness between the ribosomes of the sulphur-dependent archaebacteria and those of eukaryotic cytosol has been proposed by Lake *et al.* (1984).

The comparison of the antibiotic sensitivity patterns in Tables I–III may provide some clue to the evolutionary branching order of the urkingdoms. A possible model would hold that a com-

mon ancestral phenotype (protocell) that emerged with the transition from progenotes to true organisms (Fox *et al.*, 1982) lacked ribosomal sites having the binding potential for both eubacterial and eukaryotic targeted drugs. These sites would have accumulated gradually, at later stages during the evolutionary course of the common ancestral cell line, with the majority of them arising after the branching that led to eubacteria and eukaryotes. According to this model *S. solfataricus*, with its apparent insensitivity to most antibiotics, would be one of the earliest derivatives of the protocell whereas methanogenic and halophilic archaebactera would have diverged at some later stage, after the appearance of ribosomal sites having the binding potential for viriginiamycin, thiostrepton, neomycin, gentamycin and anysomycin. An alternative model would be that most of the antibiotic binding sites found in extant organisms were already present in the progenote but were lost differentially during the divergences of the main lines of cellular descent, i.e., eubacterial ribosomes lost all of the eukaryotic sites and *vice versa* whereas the methanogenic archaebacteria retained a mixture of both.

In the case of *Sulfolobus* the adaptation to extreme environmental conditions could account for the lack of binding sites for most antibiotics. However, we do not favour this view because all of the antibiotic binding sites found in *E. coli* ribosomes appear to have been conserved in the extreme thermophilic eubacteria.

Given the present state of the art it is difficult to choose between either of these alternative models. In any case the pattern of insensitivity to different protein synthesis inhibitors displayed by *Sulfolobus* reveals a unique ribosomal structure. It remains to be established whether the same pattern is shared by other thermoacidophilic archaebacteria such as the moderate thermoacidophile *Thermoplasma acidophilum* and the extreme thermoacidophilic archaebacteria belonging to the order Thermoproteales (Zillig *et al.*, 1981). Comparative analysis of antibiotic sensitivity spectra may provide a valuable tool to unravel the phylogenetic relationships among the main lines of cellular descent.

Materials and methods

Preparation of ribosomes and supernatant fractions

Crude ribosomes and a 105 000 *g* supernatant (termed S-100) from *S. solfataricus*, *E. coli* (RNase I_{10}^- and MRE 600 strains) and *T. thermophilus* HB8 were obtained according to Nirenberg and Matthaei (1961). The ribosomes were resuspended in a high salt buffer (Tris-HCl, pH 7.4, 20 mM; $MgO(Ac)_2$, 10 mM; NH_4Cl, 500 mM; dithiothreitol, 0.5 mM) and further purified by centrifugation through a 7.0 ml pad of 0.5 M sucrose in high salt buffer according to Cammarano *et al.* (1982). A soluble protein fraction was obtained by precipitation of the S-100 supernatant with 70% saturated ammonium sulphate as described elsewhere (Cammarano *et al.*, 1982). The precipitated protein was resuspended in 20 mM Tris-HCl, pH 7.4; 1 mM $MgO(Ac)_2$; 10% glycerol and extensively dialyzed against the same buffer.

Ribosomes and S-100 from *B. stearothermophilus* were obtained by a modification of the method of Algranati and Lengyel (1966). The cells were suspended in 3 ml/g wet weight of a medium containing Tris-HCl, pH 7.8, 20 mM; KCl, 50 mM; $MgO(Ac)_2$, 10 mM; 2-mercaptoethanol, 7 mM; bentonite, 8 mg/ml. The cells were incubated for 30 min with 100 μg/ml of lysozyme while sonicating the suspension every other minute for 10 min. The cell lysate was centrifuged at 30 000 *g* and the supernatant (termed S-30) was centrifuged at 48 000 r.p.m. for 2.5 h. The ribosome pellet was resuspended in a high salt buffer (Tris-HCl, pH 7.4, 20 mM; $MgO(Ac)_2$ 10 mM; NH_4Cl, 1.0 M) and the crude ribosomes were purified by centrifugation through a 7 ml pad of 20% sucrose in high salt buffer. The S-100 supernatant was dialyzed overnight against Tris-HCl, pH 7.8, 20 mM; KCl, 25 mM; $MgO(Ac)_2$, 5 mM; 2-mercaptoethanol, 5 mM, and stored in small aliquots at −70°C.

Rat liver microsomes and S-100 fractions were prepared as follows: the tissue was homogenized in a Potter Elvehjem homogenizer with 2.5 volumes of 0.25 M sucrose in Tris-HCl, pH 7.5, 20 mM; NH_4Cl, 100 mM; $MgO(Ac)_2$, 4 mM; dithiothreitol, 1 mM; and centrifuged at 20 000 *g* for 20 min. The upper two thirds of the supernatant were centrifuged for 12 h at 100 000 *g* in a 40K Spinco rotor to yield a microsome pellet and a S-100 supernatant. Microsomes were resuspended in 5 ml per 25 g of tissue wet weight of extraction buffer lacking sucrose.

Ribosomes and S-100 proteins from *S. cerevisiae* were prepared as described by Sanchez-Madrid *et al.* (1979). A rabbit reticulocyte lysate was prepared according to Stirpe *et al.* (1980).

Cell-free polypeptide synthesis

The *S. solfataricus* translation system (63 μl) contained per ml: Tris-HCl (pH 7.3) 15 μmol, NH_4Cl 6 μmol, $MgO(Ac)_2$ 18 μmol, dithiothreitol 1 μmol, spermine 3 μmol, ATP 2.4 μmol, GTP 1.6 μmol, poly(U) 160 μg or, when indicated, poly(UG) (4:1 base ratio) 160 μg, [^{3}H]phenylalanine (100 μCi/μmol) 20 nmol, ribosomes 10 A_{260} units (240 pmol) and S-100 supernatant protein 2.0 mg. Incubations were at 75°C for 40 min, unless otherwise specified.

The *E. coli* translation system (63 μl) contained per ml: Tris-HCl (pH 7.5) 50 μmol, NH_4Cl 70 μmol, $MgO(Ac)_2$ 15 μmol, dithiothreitol 1 μmol, ATP 1 μmol, GTP 0.5 μmol, tRNA (*E. coli*) 80 μg, creatine phosphate 16 μmol, creatine phosphate kinase 30 μg (or, alternatively, 6 μmol phosphoenolpyruvate and 50 μg of pyruvate kinase), poly(U) 200 μg or, when indicated, poly(UG) (4:1 base ratio) 200 μg, ribosomes 10 A_{260} units (250 pmol), S-100 supernatant protein 100−200 μg and [^{3}H]phenylalanine (100 μCi/μmol) 20 nmol. Incubations were at 37°C for 20 min.

The *B. stearothermophilus* cell-free system (100 μl) contained per ml: Tris-HCl (pH 7.4) 30 μmol, NH_4Cl 50 μmol, $MgO(Ac)_2$ 21 μmol, dithiothreitol 1.5 μmol, spermine 0.1 μmol, ATP 2 μmol, GTP 0.3 μmol, poly(U) 300 μg or, when indicated, poly(UG) (4:1 base ratio) 300 μg, tRNA (*E. coli*) 300 μg, ribosomes 180 pmol and an optimum amount of S-100 supernatant solution. The mixture was incubated for 15 min at 75°C.

The *S. cerevisiae* cell-free system (50 μl) contained per ml: Tris-HCl (pH 7.4) 48 μmol, KCl 80 μmol, $MgO(Ac)_2$ 12 μmol, 2-mercaptoethanol 5 μmol, ATP 1 μmol, GTP 0.05 μmol, creatine phosphate 4 μmol, creatine phosphate kinase 80 μg, poly(U) 300 μg, tRNA (yeast) 500 μg, [^{3}H]phenylalanine (100 μCi/μmol) 26 nmol, ribosomes 160 pmol and a volume of S-100 fraction optimized for maximum activity. The system was incubated at 30°C for 20 min.

The *T. thermophilus* translation system (63 μl) contained per ml: Tris-HCl (pH 7.0) 53 μmol, NH_4Cl 100 μmol, $MgO(Ac)_2$ 15 μmol, dithiothreitol 2 μmol, ATP 4 μmol, GTP 0.6 μmol, tRNA (*T. thermophilus*) 300 μg, poly(U) 160 μg or, when indicated, poly(UG) (4:1 base ratio) 160 μg, spermine 3 μmol, [^{14}C]-phenylalanine (513 μCi/μmol) 3 nmol, ribosomes 10 A_{260} units (250 pmol) and a volume of S-100 supernatant yielding optimum activity. Incubations were at 75°C for 40 min.

The rat liver poly(U)-directed system (125 μl) contained per ml: Tris-HCl (pH 7.4) 30 μmol, KCl 135 μmol, $MgO(Ac)_2$ 10 μmol, dithiothreitol 5 μmol, ATP 2.4 μmol, GTP 2.4 μmol, creatine phosphate 16 μmol, creatine phosphate kinase 30 μg, poly(U) 50 μg, [^{14}C]phenylalanine (513 μCi/μmol) 3 nmol, microsomes 240 μl and S-100 supernatant 240 μl. Incubations were at 37°C for 40 min.

Total tRNA from different sources was prepared according to Zubay (1962).

Hot trichloroacetic acid insoluble radioactivity was assayed according to Mans and Novelli (1961). The efficiency for protein synthesis of the different systems ranged from 20 to 40 pmol of phenylalanine polymerized per pmol of ribosome. The level of protein synthesis in *S. solfataricus* was 16% at 45°C and 60% at 60°C with respect to the control at 75°C. When antibiotics were dissolved in either dimethylsulphoxide or ethanol, the incorporation values of control assays containing the same amount of solvent were taken as 100% values.

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