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A small basic ribosomal protein from the extreme thermophilic archaebacterium *Sulfolobus solfataricus* that has no equivalent in *Escherichia coli*

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The structure of the gene for a small, very basic ribosomal protein in *Sulfolobus solfataricus* has been determined and the structure of the protein coded by this gene has been confirmed by partial amino acid sequencing. The protein shows no sequence similarity to any of the ribosomal proteins from eubacteria (*Escherichia coli*) or to those that have been reported from eukaryotes.

Ribosomal protein; Gene; Archaebacterium; Evolution; *Sulfolobus solfataricus*

1. INTRODUCTION

Comparative analysis of the structure of the ribosome has revealed that there are significant differences both in the number and structure of the ribosomal proteins from the three kingdoms [1–5]. This probably means that during the evolution of the translational apparatus, some proteins may have been eliminated, others may have been added while still others may have been modified, perhaps to perform a different function. In order to understand the evolution of the translational apparatus, it will first be necessary to compare the primary structure of all of the ribosomal proteins from a member of each of the three kingdoms. In this paper, we present the structure of a gene that codes for a small very basic ribosomal protein in the archaebacterium, *Sulfolobus solfataricus*, that shows no sequence similarity to any of the ribosomal proteins reported present in eubacteria or eukaryotes. We have isolated this protein, which we will refer to as LX, and have determined that it is located in the large ribosomal subunit.

2. MATERIALS AND METHODS

2.1. *Sequencing of the LX gene*

A 6.90 kb *Eco*RI-*Bam*HI fragment from a *Sulfolobus solfataricus* DNA library, containing the LX gene as well as genes for ribosomal proteins L11, L1, L10 and L12 (Ramirez, Shimmin, Leggatt and Matheson, in preparation), was cloned in pUC 18 using standard methods [6]. A series of deletion plasmids were constructed [7] and used as templates to sequence this fragment using the dideoxychain termination method [8].

2.2. *Identification of the LX protein*

Sulfolobus solfataricus P1 was grown at 85°C in the medium described by Zillig et al. [9]. The ribosomal subunits were isolated as described in Matheson et al. [10]. The 50 S ribosomal subunits were extracted with acetic acid [11] and the ribosomal proteins fractionated in Sephadex G-75. The low molecular weight proteins were further fractionated by HPLC on a C-8 reverse phase column (data not shown). Proteins of the predicted size and relative charge were partially sequenced on an Applied Biosystems 470A Gas Phase Sequencer in a search for the LX protein. A protein with the predicted amino acid composition was identified and the sequence of the first 44 amino acid residues was determined.

2.3. *Computer analysis*

The amino acid sequence of the LX protein was compared to other proteins in the National Biomedical Research Foundation (NBRF) protein data bank using a FASTP computer program [12].

3. RESULTS AND DISCUSSION

Fig. 1 shows the nucleotide sequence of the LX gene and the predicted amino acid sequence of the protein. The codon utilization in this gene (see Table I), shows a bias towards A and T in the wobble position, reflecting the low G-C content (36%) of the DNA from this organism [9]. It should be noted that the CGN family of codons for arginine is not used. This set of codons has also been observed to be very seldom used in the L11, L1, L10, L12 and L46 ribosomal protein genes from this organism ([4,13,14], and Ramirez, Shimmon, Leggatt and Matheson, in preparation).

A sequence complementary to the 3′ end of the *S. solfataricus* 16 S rRNA (3′ ACUCCACUAGC) [15]

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GGACAGAGATTTTAAGAATCCAAAGAGCATTTAGTGATCAGT      42
ATGGCTGAAGTAAAAATTTTCATGGTCAGAGGAACTGCCATA      84
M  A  E  V  K  I  F  M  V  R  G  T  A  I
TTTAGTGCGTCAAGATTTCCTACAAGTCAAAAATATGTTAGA     126
F  S  A  S  R  F  P  T  S  Q  K  Y  V  R
GCTTTAAATGAAAAACAAGCAATCGAATACATTTATAGTCAA     168
A  L  N  E  K  Q  A  I  E  Y  I  Y  S  Q
CTTGGTGGAAAAAATAAAATTAACGATACAACATACACATAC     210
L  G  G  K  N  K  I  N  D  T  T  Y  T  Y
AAGAGATCAAAGAAGTTAAGGAAGATGAAATCACAGACAAGA     252
K  R  S  K  K  L  R  K  M  K  S  Q  T  R
CAATAA                                         258
Q  *
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Fig. 1. Sequence of the Sso LX Gene. The predicted amino acid sequence of the protein is shown below the nucleotide sequence. The residues confirmed by amino acid sequencing of the isolated protein are underlined. A putative Shine-Dalgarno sequence is overlined.

was identified upstream of the LX gene (see Fig. 1). However, it should be stated that, although an interaction between these sequences and the 3′ end of the 16 S rRNA is possible, such an interaction has yet to be demonstrated in the archaebacteria.

The gene codes for a small (71 residues), very basic (pI = 12.02) protein with a molecular weight of 8329 Da. Partial sequencing of the isolated protein confirmed the first 44 amino acid residues and showed that the N-terminal methionine is removed post-translationally (see Fig. 1).

Table I

Codon utilization in the LX gene

UUU	Phe	2	UCU	Ser	0	UAU	Tyr	2	UGU	Cys	0
UUC	Phe	1	UCC	Ser	0	UAC	Tyr	3	UGC	Cys	0
UUA	Leu	2	UCA	Ser	3	UAA	*	1	UGA	*	0
UUG	Leu	0	UCG	Ser	0	UAG	*	0	UGG	Trp	0
CUU	Leu	1	CCU	Pro	1	CAU	His	0	CGU	Arg	0
CUC	Leu	0	CCC	Pro	0	CAC	His	0	CGC	Arg	0
CUA	Leu	0	CCA	Pro	0	CAA	Gln	4	CGA	Arg	0
CUG	Leu	0	CCG	Pro	0	CAG	Gln	1	CGG	Arg	0
AUU	Ile	3	ACU	Thr	1	AAU	Asn	2	AGU	Ser	3
AUC	Ile	1	ACC	Thr	0	AAC	Asn	1	AGC	Ser	0
AUA	Ile	1	ACA	Thr	5	AAA	Lys	6	AGA	Arg	5
AUG	Met	3	ACG	Thr	0	AAG	Lys	4	AGG	Arg	1
GUU	Val	1	GCU	Ala	2	GAU	Asp	1	GGU	Gly	1
GUC	Val	1	GCC	Ala	1	GAC	Asp	0	GGC	Gly	0
GUA	Val	1	GCA	Ala	1	GAA	Glu	3	GGA	Gly	2
GUG	Val	0	GCG	Ala	1	GAG	Glu	0	GGG	Gly	0

* Chain termination

When the amino acid sequence of the *Sulfolobus* LX protein was compared to the structures of the eubacterial (*E. coli*) ribosomal proteins, no sequence similarity was evident, suggesting that this protein has no equivalent in the eubacterial ribosome. Several other examples of archaebacterial ribosomal proteins which appear to be absent in the eubacterial ribosome have been reported in *Methanococcus vannielii* [16] and in *Halobacterium marismortui* [1,17]. In some cases these proteins show sequence similarity to eukaryotic ribosomal proteins [2,16,17], but in others, like in the case of the LX protein, no similarity to any of the sequenced proteins in eukaryotes is evident [2]. Since all of the ribosomal proteins from a eukaryotic ribosome have not as yet been sequenced, the possibility remains that an equivalent protein may still be found in eukaryotes. Nevertheless, there is also the possibility that some of these proteins may be unique to the archaebacteria. It will be of interest, therefore, to determine the function of this protein in the ribosome.

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