The 3′-terminal nucleotide sequence of the *Halobacterium halobium* 16 S rRNA

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1. INTRODUCTION

RNA of the small subunit of ribosome may play an important role in ribosomal structure and function. Development of rapid nucleic acid sequencing techniques has allowed us to gain access to the primary structures of a number of eubacterial and eukaryotic rRNAs [1–4]. This calls for comparative analysis which will furnish information on the structure–function relationship of these molecules [5–9]. There are abundant data indicating that a certain region of eubacterial 16 S rRNA near the 3′-end (the so-called Shine–Dalgarno sequence) mediates the recognition of the initiation site of mRNA by ribosome. 18 S rRNA of eukaryotic ribosomes, however, lacks such sequence and some other mechanisms of the initiation of protein synthesis are proposed for eukaryotes (review [20]). Almost nothing is known about the structure and function of ribosome of archaebacteria which represents a third separate line of descent [10].

Here we present the primary and secondary structure of the 3′-end proximal region of 16 S rRNA of *Halobacterium halobium*, which belongs to archaebacteria. It contains the Shine–Dalgarno sequence and resembles structurally and functionally, in this respect, eubacterial 16 S rRNA.

2. MATERIALS AND METHODS

Cells of *Halobacterium halobium*, strain R1, were kindly provided by Dr L. Minaeva. T4 RNA-ligase was a gift from Dr L. Tjulkina.

2.1. *Isolation of 16 S rRNA*

Halobacterium halobium 70 S ribosomes were prepared as in [11]. 16 S rRNA was isolated from the 70 S ribosomes by phenol extraction and purified by sucrose density gradient centrifugation.

2.2. *Determination of the 16 S rRNA 3′-terminal nucleotide sequence*

Halobacterium halobium 16 S rRNA was partially digested for 30 min at 0°C with RNase T_1 (Worthington) in 20 mmol Tris–HCl buffer (pH 7.6), containing 10 mmol $MgCl_2$, at an enzyme:substrate ratio of 1/1000 (w/w). The mixture was subjected to phenol extraction and RNA was precipitated with ethanol.

The labelling of the resultant RNA fragments was performed by ligation with [5′-^{32}P]-cytidine-5′, 3′-diphosphate (Amersham, 2000–3000 Ci/mmol) as in [12].

RNA fragments (20–80 nucleotides) were electrophoretically separated in 8% polyacrylamide gel and chemically sequenced as in [13].

3. RESULTS AND DISCUSSION

The 16 S rRNA of the small ribosomal subunit of *H. halobium* was isolated by a conventional technique and after mild RNase T_1 hydrolysis was labelled at its 3′-end by ligation with [5′-^{32}P]-cytidine-5′,3′-disphosphate. T_1 ribonuclease treatment decreased the length of the labelled fragments facilitating its elution from the gel after electrophoretic purification. All RNase T_1-generated products

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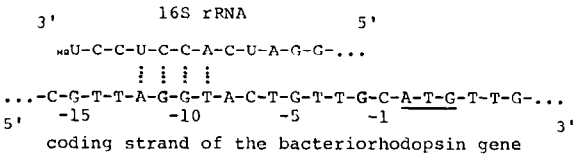

Fig.1. The primary and secondary structures of the 3′-end regions of *H. halobium* 16 S rRNA (A), *E. coli* 16 S rRNA (B) and *S. cerevisiae* 18 S rRNA (C).

contained the phosphorylated 3′-ends except those which possessed the native 3′-termini of 16 S rRNA which had been demonstrated not to be phosphorylated [14]. It is the latter that should be labelled by ligation. The majority of sequenced fragments had an identical 3′-end primary structure which was that of the 16 S rRNA native termini. Moreover, the sequence of 10 3′-end nucleotides determined here is exactly that of the 3′-terminal decanucleotide listed in the RNase T_1 oligonucleotide catalogue of *H. halobium* 16 S rRNA [14].

We have determined the primary structure of the 3′-terminal 42 nucleotide long segment of *H. halobium* 16 S rRNA (fig.1). Fig.1 also represents the primary and secondary structure of the same regions of the respective rRNAs from the organisms belonging to other 2 lines of descent, eubacterial (*Escherichia coli*) [1] and eukaryotic (*S. cerevisiae*) [3]. The sequence of this part of *H. halobium* 16 S rRNA is similar to that of *E. coli* except for 3 base substitutions that do not disturb the complementarity in the hairpin stem, and for the absence of dinucleotide UA in its 3′-termini.

We did not identify the chemical nature of 2 modified nucleotides in the hairpin loop as they had not split during the standard chemical sequencing. This led to characteristic 'gaps' at the certain positions in the sequencing gel. Such gaps are present in sequencing gels when 3′-terminal regions of all eubacterial and eukaryotic small subunit rRNAs are analyzed; for these RNAs these modified nucleotides proved to be $_{m2}{}^{6}$A [15,16]. These 'gap' sites in *H. halobium* 16 S rRNA may also be occupied by *N*-6-dimethyladenines.

Base-paired interaction of the 5′-strand of the 'dimethyladenine hairpin' of eubacterial and eukaryotic 16 S (18 S) rRNAs has been proposed in [16] with a certain region in the corresponding 5 S

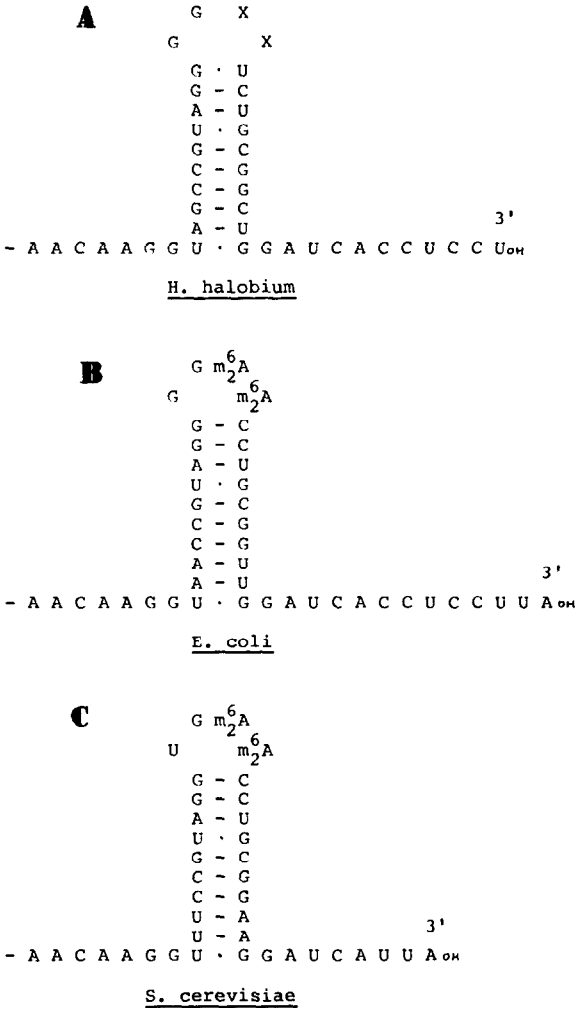

Fig.2. The possible complementarity of the Shine–Dalgarno sequence of *H.halobium* 16 S rRNA and the preinitiator region of *H. halobium* bacteriorhodopsin gene. (The initiating codon is underlined.)

rRNA. According to [16] such interaction can mediate the association of the ribosomal subunits. Although there is no direct evidence that such mechanism really acts in ribosome assembly, we have found that similar complementarity also exist between *H. halobium* 16 S and 5 S rRNAs. Namely, the sequence UAGGGG 5′-proximal to the modified adenines in the hairpin loop of the 16 S rRNA is complementary to the 5 S rRNA sequence [17] CCUCUG (nucleotides 95–100).

Comparing the 3 structures shown in fig.1 one can see that, in general, the primary and secondary structure motifs of the 3′-end proximal region of small ribosomal subunit rRNA are rather conservative in eukaryotes, eubacteria and archaebacteria.

Eubacterial and eukaryotic 3′-terminal rRNA regions differ significantly in that there is no Shine–Dalgarno polypyrimidine (CCUCC) sequence in eukaryotic 18 S rRNA. This polypyrimidine track is involved in ribosome–mRNA recognition by Watson–Crick basepair interaction with an initiator region of messenger RNA in eubacteria, while some other mechanism may function in the protein synthesis initiation events in eukaryotic ribosomes (review [20]).

As suggested [18] and directly shown here the Shine–Dalgarno sequence is also present in archaebacterial 16 S rRNA. This polypyrimidine track in *H. halobium* 16 S rRNA is not only identical to that of eubacteria but is also localized at the same distance from the extremely conservative '*N*-6-dimethyladenine hairpin' as the typical Shine–Dalgarno sequence of other bacteria. Thus, such a structure of the 5′-end of the halobacterial 16 S rRNA provides evidence that the mechanism of mRNA recognition may be similar in eubacterial and archaebacterial ribosomes.

The bacteriorhodopsin gene from *H. halobium* was sequenced in [19]. While analyzing the primary structure of the region preceding the initiating ATG codon, we have found a purine-rich sequence (AGGT, nucleotides – 9– – 12) complementary to the 3′-end segment of *H. halobium* 16 S rRNA (fig.2). It is located at 9 nucleotides from the initiator ATG, which is close to average (9.8) for Shine–Dalgarno sequence in eubacterial mRNAs [20]. The complementarity to 16 S rRNA in this case (4 basepairs) is shorter than the average for other prokaryotes (4.8) [20]. However, in halophilic organisms the Shine–Dalgarno duplex should be stabilized by very high internal salt concentration.

The situation is somewhat complicated by the fact that bacteriorhodopsin mRNA isolated from *H. halobium* had a very unusual feature: It contained at its 5′-terminus not more than 3 nucleotides upstream to the initiating AUG codon and thus had no preinitiator region complementary to the 16 S rRNA 3′-end pyrimidine stretch [19,21]. The bacteriorhodopsin mRNA described [19,21] could be shorter than the native one (i.e., it lacked its native 5′-end). If it were the case, our study would strongly support the view that the 'standard' Shine-Dalgarno mechanism of translation initiation is inherent in all prokaryotes.

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