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Sequence, modified nucleotides and secondary structure at the 3'-end of small ribosomal subunit RNA

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INTRODUCTION

The elucidation of the nucleotide sequence of the RNA of small ribosomal subunits (SSU-RNA) from a variety of organisms has shown that the structure of this RNA is highly conserved (1-3). This conservation is especially strong in the 3'-part of the molecule, the very end of which has attracted prime attention of investigators for several reasons: (a) it is involved in the initiation of protein synthesis, probably by basepairing with initiation sites on mRNA (4,5); (b) it is a determinant in the interaction of ribosomal subunits (6,7); and (c) the bacteriocins colicin E3 (8,9) and cloacin DF 13 (10) cleave prokaryotic 16S RNA *in situ* at about 50 nucleotides from the 3'-end, thereby enabling the isolation and characterization of this part of the molecule (11).

Sequences and secondary structures of the 3'-terminals of SSU-RNA's have been compared occasionally (*e.g.* 12-17). In this paper we will survey all the sequences, including modified nucleotides, of the 3'-ends of SSU-RNA's that have been published (October 1981). More or less arbitrarily we have taken the 3'-terminals to run (in *E. coli* 16S RNA) from nucleotide 1492 to the very 3'-end (nucleotide 1542). This part is strongly conserved and in models of the SSU-RNA's (1,2) it consists of a hairpin containing the characteristic structure $m_2^6Am_2^6A$ in the loop, flanked by two single stranded regions. In analogy with a previous comparison (17) the sequence has been subdivided into sections (Fig. 1): I. the "single stranded" region 5' to the hairpin; II. the stem of the hairpin, further subdivided in II_A and II_B corresponding to the 5' proximal and 3' proximal strands of the hairpin stem, respectively; III. the single stranded loop of the hairpin containing $m_2^6Am_2^6A$; IV. the conserved sequence GGA and V. the most variable part of the 3'-end both in sequence and in length.

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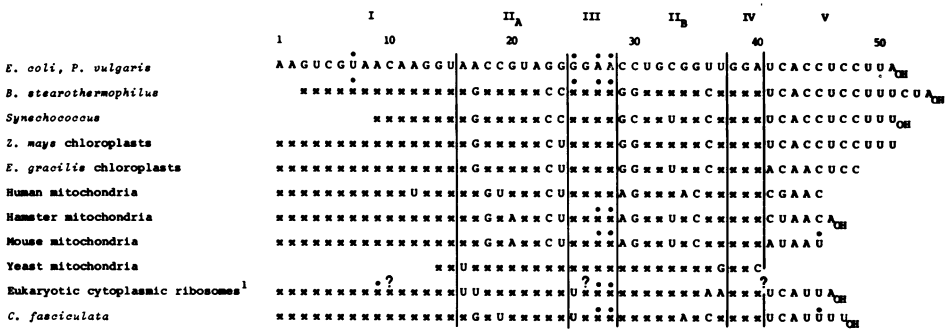

Figure 1. Sequences of 3'-terminal nucleotides of SSU-RNA's.
The sequences have been aligned such as to show maximal homology with the one of *E. coli* in sections I-IV. The way in which the sequences were determined (from RNA or DNA) and references to the original literature are given in the text. Modification of a residue is marked with a dot and question marks indicate that there is some doubt about the correct sequence (compare the text).

[1] Includes: hen reticulocyte, mouse sarcoma, rat liver, rabbit reticulocyte, barley embryo, *Bombyx mori*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, *Xenopus laevis*, *Triticum vulgare*.

PRIMARY STRUCTURE AND BASE MODIFICATIONS

1. Escherichia coli

The entire sequence shown in Fig. 1 was established in its final version by sequencing of the gene for 16S RNA (18,19) and by sequencing of the RNA itself (10,21). Parts of this sequence were already known from earlier work (4, 22,23). The 3' ends of all SSU-RNA's contain modified bases, the most conspicuous of which are the two adjacent dimethyladenines ($m_2^6Am_2^6A$) at position 27 and 28 in Fig. 1 (24). These A residues are unmodified in a kasugamycin resistant strain of *E. coli* (25,56). A methylated U residue (position of the methyl-group unknown), found in an oligonucleotide in earlier work (27) was later on placed at position 7 of Fig. 1 (28). In a recent study (29) these modifications have been confirmed and it was also shown that the G residue at position 25 is methylated (m^2G). The $m_2^6Am_2^6A$ feature is strongly conserved among prokaryotes (30-33) and eukaryotes (34). The situation with the other modifications is less clear: in the oligonucleotide UAACAAG (positions 7-13 in Fig. 1) the first three positions can be posttranscriptionally modified depending on the species (32).

E. coli contains 7 genes for ribosomal RNA's (35,36), resulting in several heterogeneities in the 16S RNA sequence (21). None of these, however, are present in the region shown in Fig. 1 (21).

2. Proteus vulgaris

Sequence obtained directly from the 16S RNA (37). The part shown in Fig. 1 is completely identical with the one of *E. coli*. Modified nucleotides were found at the same positions, including a modified G at position 25.

3. Bacillus stearothermophilus

The sequence in Fig. 1 is the one reported for the colicin fragment of this bacterium (38). It is three nucleotides (UCU) longer than the corresponding part in *E. coli*. The nucleotides marked with a dot in Fig. 1 are modified (38), probably methylated. The sequence of the 3'-terminal nonanucleotide was derived earlier (39,40) and showed heterogeneity, partly lacking the 3'-terminal A (39).

4. Synechococcus AN PCC 6301

The sequence of the 3'-terminal 43 nucleotides of 16S ribosomal RNA from the cyanobacterium *Synechococcus* AN PCC 6301 has been determined (41). The adenosines at position 27 and 28 were identified as m_2^6A.

5. Zea mays chloroplasts

This sequence corresponds to the one determined from a cloned chloroplast ribosomal RNA gene (12,42). On the basis of a comparison with the *E. coli* sequence the RNA is supposed to end with the terminal U, but this is not certain. The strong homology between *E. coli* and *Zea mays* chloroplasts in their SSU-RNA has been discussed (43). No reports were made about possible modifications.

6. Euglena gracilis chloroplasts

Similar to *Z. mays* the sequence was derived from the ribosomal RNA gene (43,44). The 3'-terminal RNase T_1 oligonucleotide of the 16S RNA was established as $AACAACUCN_{OH}$ (N = unknown nucleotide) (45). The presence of modified bases has not been reported.

7. Human mitochondria

The entire sequence of ribosomal RNA genes was determined from a plasmid containing human placental mitochondrial DNA (16). The exact ending of the 12S RNA is not known; it would probably not extend beyond the sequence shown, since it then would overlap with the sequence of a tRNA gene (16).

8. Hamster mitochondria

The 3'-terminal 220 nucleotides of hamster mitochondrial 13S ribosomal RNA have been sequenced (46). An incorrection in an earlier published sequence (47) has been corrected (46). The presence of the dimethylated A's at the positions indicated has been established (46,48).

9. Mouse mitochondria

Here the sequence has been derived from cloned ribosomal RNA genes as well as from direct sequencing of the mature RNA (14). The 3'-terminal U, as well as the adjacent A's at positions 27 and 28 seem to be posttranscriptionally modified (14), although the nature of these modifications has not been established.

10. Yeast mitochondria

Only a part of the 3'-terminal sequence, derived from the gene (49) is shown in Fig. 1. There is so little homology in sections I and V with the other SSU-RNA's that a comparison is meaningless. The exact ending of the mature SSU-RNA is not known and neither are the positions and the nature of base modifications. However, it was reported that there were probably no dimethylated adenosines (50).

11. Eukaryotic cytoplasmic SSU-RNA's

The 3'-terminal sequences of 18S ribosomal RNA from hen reticulocyte, mouse sarcoma, rat liver, rabbit reticulocyte and barley embryo (only partly sequenced, but compare ref. 51) are completely homologous (15). At positions 10 and 40-41 the published sequences would be at variance with other 18S RNA's. The authors (15), however, have some problems with the identification of the nucleotides at these positions. Using the same chemical method as these workers to check the sequence in *E. coli*, we also found "artificial" G's around positions 10 and 41 in addition to the correct nucleotides C and U, respectively (unpublished). We believe therefore that the sequences are as given in Fig. 1.

Part of the sequence of the 3'-end of rat liver 18S RNA (positions 11-42) was determined earlier (52). The sequence from the 3'-end up till the $m_2^6Am_2^6A$ sequence was established by sequencing cDNA of the ribosomal RNA's from the mouse, silk worm (*Bombyx mori*), wheat embryo and slime mold (*Dictyostelium discoideum*) (53). Two deviations from the "common" sequence of eukaryotic cytoplasmic SSU-RNA's (Fig. 1) were found: *D. discoideum* contains two additional U residues between positions 45 and 46 and the wheat RNA ends with a G in contrast to the usual A. The sequence $GAUCAUUA_{OH}$ was also reported for 18S ribosomal RNA from *Drosophila melanogaster* and *Saccharomyces cerevisiae* (54). Of the former organism the region of the gene corresponding with the 3'-end of 18S RNA was sequenced (55). This sequence, as well as the one determined from the 18S ribosomal RNA gene of *Xenopus laevis* (56) concurs with the sequence shown in Fig. 1. The 3'-terminus of *Bombyx mori* 18S RNA, deduced from the sequence of the gene (13) would be identical to the other eukaryotic 18S RNA

were it not that the G at position 26 was not found. In view of the conservation in this region and because of the strain that a 3-membered loop would pose on the hairpin (57) it would be very peculiar when this residue is missing.

The sequence of the 3'-terminal 21 nucleotides of 17S ribosomal RNA of *Saccharomyces carlsbergensis* was determined (58) as was the complete nucleotide sequence of the corresponding gene for *Saccharomyces cerevisiae* (59). *S. carlsbergensis* was reported to have the sequence CUC at positions 29-30-31 instead of CCU for all other eukaryotic cytoplasmic SSU-RNA's (58). A distinct difference between eukaryotic cytoplasmic SSU-RNA's and those of bacteria, chloroplasts and mitochondria occurs at position 25: SSU-RNA's of the cytoplasm have a U here instead of a (modified) G.

Dimethylation of the two A's at positions 27 and 28 has been firmly established in rat liver (52) and in yeast (58,59). However, the presence of the oligonucleotide $m^6_2Am^6_2ACCUG$ in RNase T_1 digests of SSU-RNA's has been established for numerous organisms, both eukaryotes (34) and prokaryotes (30-33). The A at position 9 in eukaryotic cytoplasmic SSU-RNA's is probably methylated: the unique T_1 oligonucleotide $UAm^6ACAAGp$ was found in rat 18S RNA (60), yeast 18S RNA (59) and *Xenopus* 18S RNA (56).

12. Crithidia fasciculata

This protist eukaryote has such a deviant 3'-terminal sequence in the 18S RNA in comparison to other eukaryotes that it is listed separately in Fig. 1. The data were derived directly from the 18S RNA (17). As compared to the other eukaryotic 18S RNA's, the one of *C. fasciculata* contains three basepair changes in the hairpin stem (*vide infra*) and the replacement of the terminal A_{OH} by UU_{OH}. In addition to the dimethylated A's, a modified U (possible Ψ) is found at position 45, but no modification of A at position 9 was reported (17).

SECONDARY STRUCTURE

As has been pointed out (1), strong support for secondary structure models in ribosomal RNA comes from phylogenetic comparison of sequences. In the 3'-end of SSU-RNA's the variations in the regions II_A and II_B (Fig. 1) support the idea that these sections are the two antiparallel strands of a hairpin stem. A nucleotide change in II_A leads to a concommittant substitution in II_B such that basepairing is maintained or to the introduction of a U-G pair at the expense of a Watson-Crick pair. Fig. 2 summarizes the secondary structure models. With respect to *E. coli* the greatest variation is found in the mito-

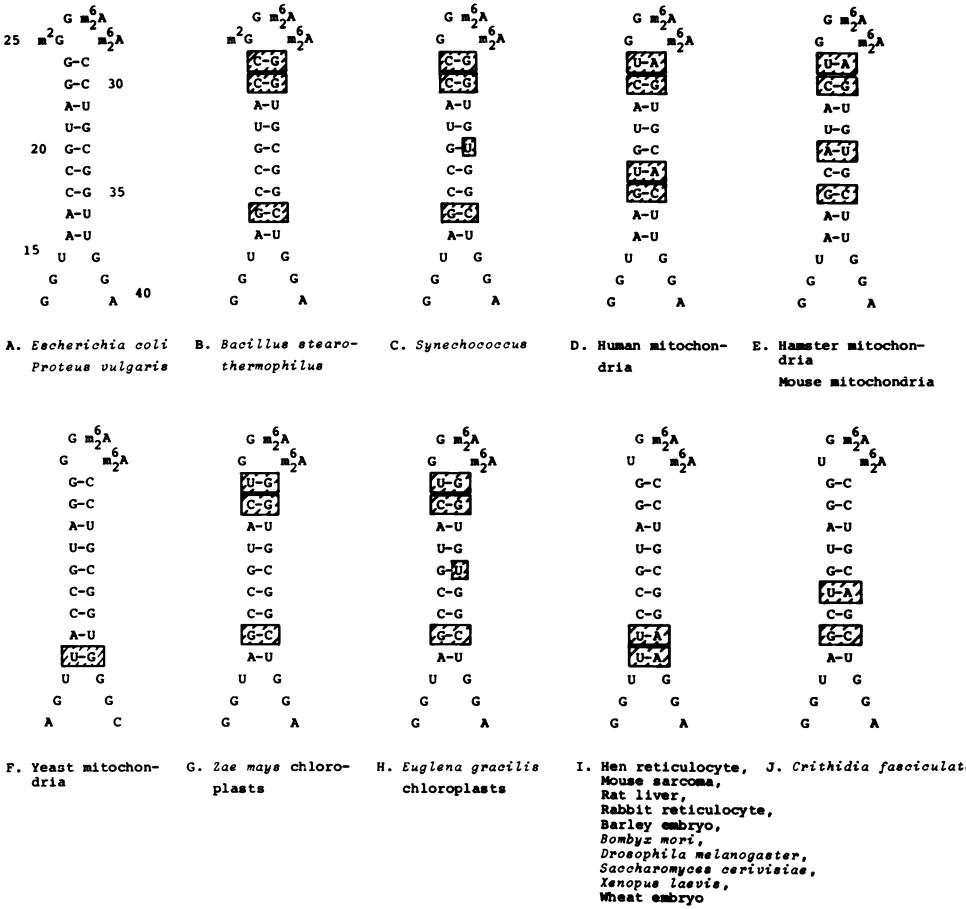

Figure 2. Hairpin structures of the sequences shown in Fig. 1 by basepairing section II_A with section II_B.
The alterations with respect to *E. coli* have been boxed. It has been assumed that the modified G residue at position 25 in the bacteria *P. vulgaris* (37) and *B. stearothermophilus* is methylated in the amino group, although this has proven strictly only for *E. coli* (63). No G residue was reported at position 26 for *Bombyx mori* (13). It has also been assumed that the adjacent adenosines in the loop are dimethylated, although this may not be justified in the case of yeast mitochondria (56).

chondrial RNA's (except yeast) with four basepair changes in the stem. The chloroplast RNA's have three basepair substitutions in comparison with *E. coli* and in addition a C → U alteration at position 33 in the chloroplast RNA of *Euglena gracilis*. The latter RNA would thereby have 3 U-G pairs in the stem. Although the sequence of the 3'-end of SSU-RNA has been completely conserved between the bacteria *E.coli* and *P. vulgaris* the thermophile *B. stearothermophi-*

lus has basepair changes at 3 positions. These alterations strongly influence the stability of the hairpin (38). The cyanobacterium *Synechococcus* has the same basepair changes as *B. stearothermophilus* and in addition a C → U alteration at position 33. Remarkably conserved is the composition of the hairpin stem among the eukaryotic cytoplasm SSU-RNA's except *Crithidia fasciculata* (a trypanosomatid). The difference between the eukaryotic RNA's and *E.coli* is the replacement of two A-U pairs at the base of the stem in *E. coli* by two U-A pairs in the eukaryote.

The secondary structure model of the 3'-end of 16S RNA has been substantiated in *E. coli*. RNase T_1 cleavage patterns of 16S RNA in 30S ribosomes are consistent with the presence of such a hairpin configuration *in situ* (61). The G specific chemical reagent kethoxal has been used to probe the accessibility of the G residues in active ribosomes (6,62). In agreement with the secondary structure model (Fig. 2) the G residues at positions 13, 14, 25, 26, 38 and 39 were accessible, while those at positions 20, 23, 24, 32, 34 and 35 were not.

A fragment of 49 nucleotides, that is cleaved off from the 3'-end of 16S ribosomal RNA by treatment of ribosomes with the bacteriocins colicin E_3 or cloacin DF_{13} (8,10) has been studied extensively by physical techniques (63-65). All the results agree on the presence of the central hairpin in the isolated colicin fragment. The methyl groups on the A's in the loop appear to destabilize the hairpin (65) and the basepair alterations from *E. coli* to *B. stearothermophilus* strongly stabilize the structure (38).

Although measurements on the colicin fragment show that it contains additional secondary structure (64,65) it is neither clear whether this is due to basepairing between nucleotides in section I and section IV as has been proposed (66), nor is there evidence that 16S RNA in the ribosome adopts such structures.

CONCLUSIONS

The 3'-ends of SSU-RNA's are strongly conserved in sequence (with the possible exception of the RNA of the small subunit of yeast mitochondria) and in secondary structure. Preceeding the conserved hairpin at the 5'-side, is a stretch of 15 nucleotides which is practically identical in all organisms. This suggests a vital function of this sequence in protein biosynthesis. The hairpin has, though with basepair substitutions from one species to the other, always a stem of 9 basepairs and a loop of 4 nucleotides. In bacteria, chloroplasts and mitochondria, the sequence in the loop is $(m^2)GGm^6_2Am^6_2A$ (with the

possible exception of yeast) and in cytoplasmic ribosomal RNA UGm^6_2Am^6_2A. At the 3'-side, the hairpin is followed by the sequence GGAUCA in the RNA's of bacterial, eukaryotic, cytoplasmic and maize chloroplast ribosomes. Mitochondria (except those of yeast) have only conserved GGA here. SSU-RNA's from bacteria and *Zea mays* chloroplasts contain the "Shine and Dalgarno" sequence CCUCC; all other SSU-RNA's lack this sequence.

The secondary structure models for this part of the RNA are supported by chemical and physical studies with *E. coli* ribosomes and ribosomal RNA.

Abbreviations: SSU-RNA : small ribosomal subunit RNA (16S RNA in prokaryotes, 16S RNA in chloroplasts, 12-13S RNA in mitochondria, 17-18S RNA in cytoplasmic ribosomes from eukaryotes).

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