

# SRP-RNA sequence alignment and secondary structure

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## ABSTRACT

The secondary structures of the RNAs from the signal recognition particle, termed SRP-RNA, were derived by comparative analyses of an alignment of 39 sequences. The models are minimal in that only base pairs are included for which there is comparative evidence. The structures represent refinements of earlier versions and include a new short helix.

## INTRODUCTION

Interplay between the signal recognition particle (SRP), the signal sequence, ribosomes, and the SRP-receptor is required to translocate secretory proteins across biological membranes (1, 2). The extensively studied canine SRP is composed of six proteins and an RNA-molecule, the SRP-RNA, which previously has been referred to as 7SL RNA (3). Typically, SRP-RNAs consist of about 300 nucleotides and are contacted directly by proteins SRP9, SRP14, SRP19, SRP68 and SRP72; a sixth protein SRP54 assembles only in the presence of bound SRP19 (4). The RNA exhibits extensive secondary structure (5) and it is also folded in three dimensions, as judged by the size and shape of the SRP particle (240×60 Å) (6, 7).

Understanding the function of SRP requires a detailed knowledge of the RNA structure. Comparative sequence analyses (8, 9) have proven particularly useful for determining secondary structures of small and large RNA molecules. Here, we apply this approach to the SRP-RNA, for which many sequences are now available. The compilation of the available SRP-RNA sequences, includes those from 6 bacteria (eubacteria), 9 archaea (archaeobacteria), and 24 eucaryotes. Since the last review of the structure and function of SRP-RNA (7), several archaeal sequences have appeared (10, 11, 12, 13, 14), and the similarity between bacterial 4.5S RNAs and the conserved portion of the other SRP-RNAs has been recognized (15, 16, 17).

## SRP-RNA sequences

The 39 known SRP-RNA sequences used in our analysis are listed in Table 1. Abbreviated and full names are shown, and whether the sequence derives from DNA, RNA, or SRP. Experimental evidence that SRP is involved in protein translocation has only

been provided for canine and several plant SRPs. Frequently, the precise ends of the RNA molecules were not determined, especially when the gene was sequenced. Four partial sequences from *Triticum aestivum* (c), *Crysanthemum morifolium*, *Benincasa hispida*, and *Gynura aurantiaca* are included.

We also searched for additional SRP-RNA sequences in a merged version of the GenBank and EMBL data bases (December 1989). Motif files describing common primary and secondary structural features of the SRP-RNAs were constructed as inputs for the search program ANALYSEQP (18). Only one human pseudogene was found which may not be expressed and, therefore, is not included here.

## Sequence alignment

In the alignment shown in Figure 1, the sequences are grouped as bacteria (top), archaea (middle) and eucaryotes (bottom) of each panel. In our alignment procedure, closest relatives were aligned first on the basis of primary structure similarity; each group of aligned sequences was then treated collectively and aligned against other groups. Sets of conserved nucleotides were then identified and used for aligning in the more variable regions. Finally, where little or no primary structure similarity existed, common secondary structural elements were used as additional markers.

## Comparative sequence analysis

In our derivation of secondary structure, we distinguish clearly between base pairs that are supported by covariances, and those that are not contradicted. A covariance is the observation that a base pair in one organism is different by both bases when compared to the equivalent base pair in another organism. If the two different pairs are of Watson-Crick type, we observe a compensating base change (CBC). Covariances and CBCs support the existence of a base pair, because during evolution, random single mutations that introduce an unstable pairing would not generally have been compensated for by a further mutation that restored the stability, unless it was required. Thus, such observations is positive evidence, and the more CBCs, the stronger the evidence. Negative evidence is a mismatch, which we define here as neither Watson-Crick pairs nor G-U pairs. In

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contrast, sequence conservation provides neither positive or negative evidence.

For each base pair in Figure 2 we estimated positive and negative evidence by counting the number of CBCs and mismatches. The set of sequences that aligned unambiguously at a given alignment position was first identified; the most conserved base pair in this set was then found and the remaining pairs added as CBCs where they covaried. Our guideline was to consider base pairs supported, if there was at least twice as much positive evidence as negative. As a general rule, when there was less, we preferred not to include a base pair. However, when a base pair was supported in one primary kingdom (now termed domain, 19) and disproven in others, we included it as specific for that group.

### Secondary structure

The derived secondary structure models are presented as diagrams in Figure 2a, b and c for a bacterium (*Bacillus subtilis*), an archaeum (*Halobacterium halobium*) and a eucaryote (*Canis species*), respectively. Supported base pairs are juxtapositioned and connected with a line; bases of unsupported pairings at helical ends are placed adjacently with no line between them. Finally, when there is more negative than positive evidence, the base symbols are spaced apart with no line between them. In addition to the secondary structure diagrams, Watson-Crick and G-U pairs of supported helices of all SRP-RNAs are shown in reverse print in Figure 1.

Below, the eight identified helices of the SRP-RNAs, numbered one to eight starting at the 5'-end, are discussed, and

characteristics of each helix and the connecting bulges and loops, as well as their domain-specific peculiarities, are considered.

**Helix 1:** Is present in archaea and in *Bacillus subtilis*, but may also exist in the remaining bacteria, since the terminal nucleotides of 4.5S RNA (position 1 to 8 in *Escherichia coli*) may correspond to nucleotides 2 to 9 in *Bacillus subtilis*. The helix contains between three and nine base pairs which link the two ends of the SRP-RNA. Its variability in length suggests, as for many RNA molecules, that the main function may be to close the ends of the RNA.

**Helix 2:** Is universal and generally consists of three base pairs. It defines the 5'-domain of the SRP-RNA, which also includes helices 3 and 4. In archaea, helix 2 may be extended at its proximal end (the end with the base closest to the 5'-end) by up to three base pairs. More sequences will be required to provide conclusive evidence for this putative extension.

**Helix 3:** In eucaryotes, helix 3 consists of four proximal base pairs, two unpaired bases and a single distal base pair. Only the four proximal base pairs are present in the archaea although there is some support for a fifth. The helix constitutes only two base pairs in *Bacillus subtilis*, and is absent from the lower eucaryotes *Schizosaccharomyces pombe* and *Yarrowia lipolytica*.

**Helix 4:** Extends through all three domains except in *Schizosaccharomyces pombe*. A constant number of four base pairs occur in the helix, with a variable number of nucleotides in the loop. The large loops of *Bacillus subtilis* and the archaea participate in tertiary interactions (discussed below). The animal sequences possess a loop of six or eight nucleotides which apparently is compensated for by the loss of three or four nucleotides following helix 2. Plants and fungi exhibit only two nucleotides in the terminal loop and there is strong support for the last distal base pair of helix 4. Whether this is sterically feasible will have to be verified experimentally, but a similar two base loop was detected in the 23S-like mitochondrial rRNAs of higher eucaryotes (20).

Helix 4 and helix 2 are continuous on the 3' strand in all SRP-RNAs and may stack coaxially. Evidence for this concept is provided by the even distribution of CBCs along the seven continuous base pairs of the two helices. In contrast to most other RNA helices (e.g., those of 5S RNA), where such changes generally occur with highest frequency at helix centers, in a coaxially stacked helix the ends are stabilized, and could thus better accommodate base changes.

**Helix 5:** Consists of several base paired regions with a variable number of bulges. A highly variable region encompassing nucleotides at positions 48 to 97 and 253 to 298 (referring to the secondary structure of *Canis species*, Figure 2c), is separated from a conserved section (positions 102 to 127 and 223 to 250) by a micrococcal nuclease hypersensitive bulge. The former region varies not only in base composition, but also in size. Thus, for eucaryotic and archaeal SRP-RNAs (Figure 1), the alignment reveals that nucleotides corresponding to about one helical turn are lacking from the latter SRP-RNAs. The conserved part of helix 5 starts with a region of 6 or 7 base pairs, generally followed by a single bulged pyrimidine on the 5'-strand and four nucleotides at the 3'-strand. The next pyrimidine on the 5'-strand is base paired and followed by another small bulge, with one or two nucleotides on the 5'-strand and a single nucleotide on

Bacteria			
THE. THE.	<i>Thermus thermophilus</i>	(33)	DNA
LEG. PNE.	<i>Legionella pneumophila</i>	(34)	DNA
PSE. AER.	<i>Pseudomonas aeruginosa</i>	(35)	DNA
ESC. COL.	<i>Escherichia coli</i>	(36)	DNA
MIC. LYS.	<i>Micrococcus lysodeikticus</i>	(34)	DNA
BAC. SUB.	<i>Bacillus subtilis</i>	(15)	RNA
Archaea			
MET. VOL.	<i>Methanococcus voltae</i>	(10)	DNA
MET. FER.	<i>Methanothermobacter ferredoxinus</i>	(11)	DNA
MET. THE.	<i>Methanobacterium thermoautotrophicum</i>	(12)	DNA
MET. ACE.	<i>Methanosarcina acetivorans</i>	(13)	DNA
HAL. HAL.	<i>Halobacterium halobium</i>	(37)	DNA
ARC. FUL.	<i>Archaeoglobus fulgidus</i>	(13)	DNA
SUL. SOL.	<i>Sulfolobus solfataricus</i>	(13)	DNA
PYR. OCC.	<i>Pyrodicticum occultum</i>	(14)	DNA
THE. CEL.	<i>Thermococcus celer</i>	(13)	DNA
Eucaryotes			
ZEA. M. A-H	<i>Zea mays</i>	(38)	SRP
TRI. A. A-C	<i>Triticum aestivum</i>	(39)	SRP
CRY. MOR.	<i>Crysanthemum morifolium</i>	(40)	RNA
LYC. ESC.	<i>Lycopersicon esculentum</i>	(41)	RNA
CIN. HYB.	<i>Cineraria hybrida</i>	(40)	RNA
HOM. S. A	<i>Homo sapiens</i>	(42)	RNA
HOM. S. B	<i>Homo sapiens</i>	(43)	DNA
CAN. SPE.	<i>Canis species</i>	(44)	SRP
RAT. RAT.	<i>Rattus rattus</i>	(45)	RNA
XEN. LAE.	<i>Xenopus laevis</i>	(46)	RNA
DRO. MEL.	<i>Drosophila melanogaster</i>	(47)	RNA
SCH. POM.	<i>Schizosaccharomyces pombe</i>	(48, 49, 50)	SRP
YAR. LIP.	<i>Yarrowia lipolytica</i>	(48)	SRP
BEN. HIS.	<i>Benincasa hispida</i>	(40)	RNA
GYN. AUR.	<i>Gynura aurantiaca</i>	(40)	RNA

**Table 1.** Catalog of the SRP-RNA sequences used in this survey. Abbreviated and full species names were grouped as bacteria (top), archaea (middle) and eucaryotes (bottom) as in Figure 1. References containing sequence information appear in brackets. Sources of the isolates appear in the right column: DNA, implies that the sequence of the gene was determined; RNA, indicates that RNA was isolated from a crude mixture and either sequenced directly or from its cDNA; SRP, shows that the RNA was isolated from a purified particle.

the other. The next bulge, composed of four or three and four to six nucleotides, is preceded by a four base pair helix. Four to six base pairs, sometimes containing a single nucleotide bulge, terminate helix 5.

*Helix 6:* Is exclusive to the eucaryotes and archaea. Its sequence is variable except for two bases in the terminal loop (Figure 3, left), which may be involved in protein binding, possibly SRP19 (21). The CBCs generally occur throughout most of the helix, and in the archaea *Archaeoglobus fulgidus*, *Sulfolobus*

*solfatarius*, and *Pyrodictium occultum* also within the internal loop (Figure 1). This may reflect that helix 6 is continuously stacked.

*Helix 7:* This new helix is exclusive to eucaryotic SRP-RNAs and supported by several covariances. In the plant SRP-RNAs, A-C pairs might occur in helix 7, as they do sporadically elsewhere in the SRP-RNA. Since such atypical A-C and A-G pairings appear occasionally in well supported secondary structural regions (Figure 1) it is likely that they base pair.

Sequence alignment showing nucleotide conservation across various species. The alignment is divided into two blocks. The first block shows conserved regions around positions 22, 33, 333, 444, 444, and 22. The second block shows conserved regions around positions 555, 555, 555, 555, and 555. Conserved regions are highlighted with bold text. Species abbreviations are listed on the left, and line numbers are on the right.

THE	THE	22	33	33	444	444	22	83		
LEG.PNE	LEG.PNE									
PSE.AER	PSE.AER									
ESC.COL	ESC.COL									
MIC.LYS	MIC.LYS									
BAC.SUB	BAC.SUB									
MET.VOL	MET.VOL							80		
MET.FER	MET.FER							82		
MET.THE	MET.THE							83		
MET.ACE	MET.ACE							85		
HAL.HAL	HAL.HAL							81		
ARC.FUL	ARC.FUL							84		
SUL.SOL	SUL.SOL							84		
PYR.OCC	PYR.OCC							82		
THE.CEL	THE.CEL							87		
ZEA.M.A	ZEA.M.A	222	3333	3	3333	4444	4444222	55555555	55555	62
ZEA.M.B	ZEA.M.B									62
ZEA.M.C	ZEA.M.C									62
ZEA.M.D	ZEA.M.D									62
ZEA.M.E	ZEA.M.E									62
ZEA.M.F	ZEA.M.F									62
ZEA.M.G	ZEA.M.G									62
ZEA.M.H	ZEA.M.H									62
TRI.A.A	TRI.A.A									62
TRI.A.B	TRI.A.B									61
TRI.A.C	TRI.A.C									
CRY.MOR	CRY.MOR									17
LYC.ESC	LYC.ESC									60
CIN.HYB	CIN.HYB									62
HOM.S.A	HOM.S.A									65
HOM.S.B	HOM.S.B									65
CAN.SPE	CAN.SPE									65
RAT.RAT	RAT.RAT									65
XEN.LAE	XEN.LAE									63
DRO.MEL	DRO.MEL									62
SCH.POM	SCH.POM									29
YAR.LIP	YAR.LIP									47
BEN.HIS	BEN.HIS									57
GYN.AUR	GYN.AUR									58

Continuation of sequence alignment showing nucleotide conservation across various species. Conserved regions are highlighted with bold text. Species abbreviations are listed on the left, and line numbers are on the right.

THE	THE	555555555555555	5	5555	555	55	24
LEG.PNE	LEG.PNE						30
PSE.AER	PSE.AER						29
ESC.COL	ESC.COL						29
MIC.LYS	MIC.LYS						78
BAC.SUB	BAC.SUB						140
MET.VOL	MET.VOL						150
MET.FER	MET.FER						151
MET.THE	MET.THE						152
MET.ACE	MET.ACE						155
HAL.HAL	HAL.HAL						148
ARC.FUL	ARC.FUL						154
SUL.SOL	SUL.SOL						153
PYR.OCC	PYR.OCC						148
THE.CEL	THE.CEL						158
ZEA.M.A	ZEA.M.A						145
ZEA.M.B	ZEA.M.B						142
ZEA.M.C	ZEA.M.C						143
ZEA.M.D	ZEA.M.D						146
ZEA.M.E	ZEA.M.E						146
ZEA.M.F	ZEA.M.F						143
ZEA.M.G	ZEA.M.G						148
ZEA.M.H	ZEA.M.H						143
TRI.A.A	TRI.A.A						143
TRI.A.B	TRI.A.B						141
TRI.A.C	TRI.A.C						47
CRY.MOR	CRY.MOR						89
LYC.ESC	LYC.ESC						140
CIN.HYB	CIN.HYB						142
HOM.S.A	HOM.S.A						144
HOM.S.B	HOM.S.B						144
CAN.SPE	CAN.SPE						144
RAT.RAT	RAT.RAT						144
XEN.LAE	XEN.LAE						142
DRO.MEL	DRO.MEL						144
SCH.POM	SCH.POM						107
YAR.LIP	YAR.LIP						126
BEN.HIS	BEN.HIS						93
GYN.AUR	GYN.AUR						66

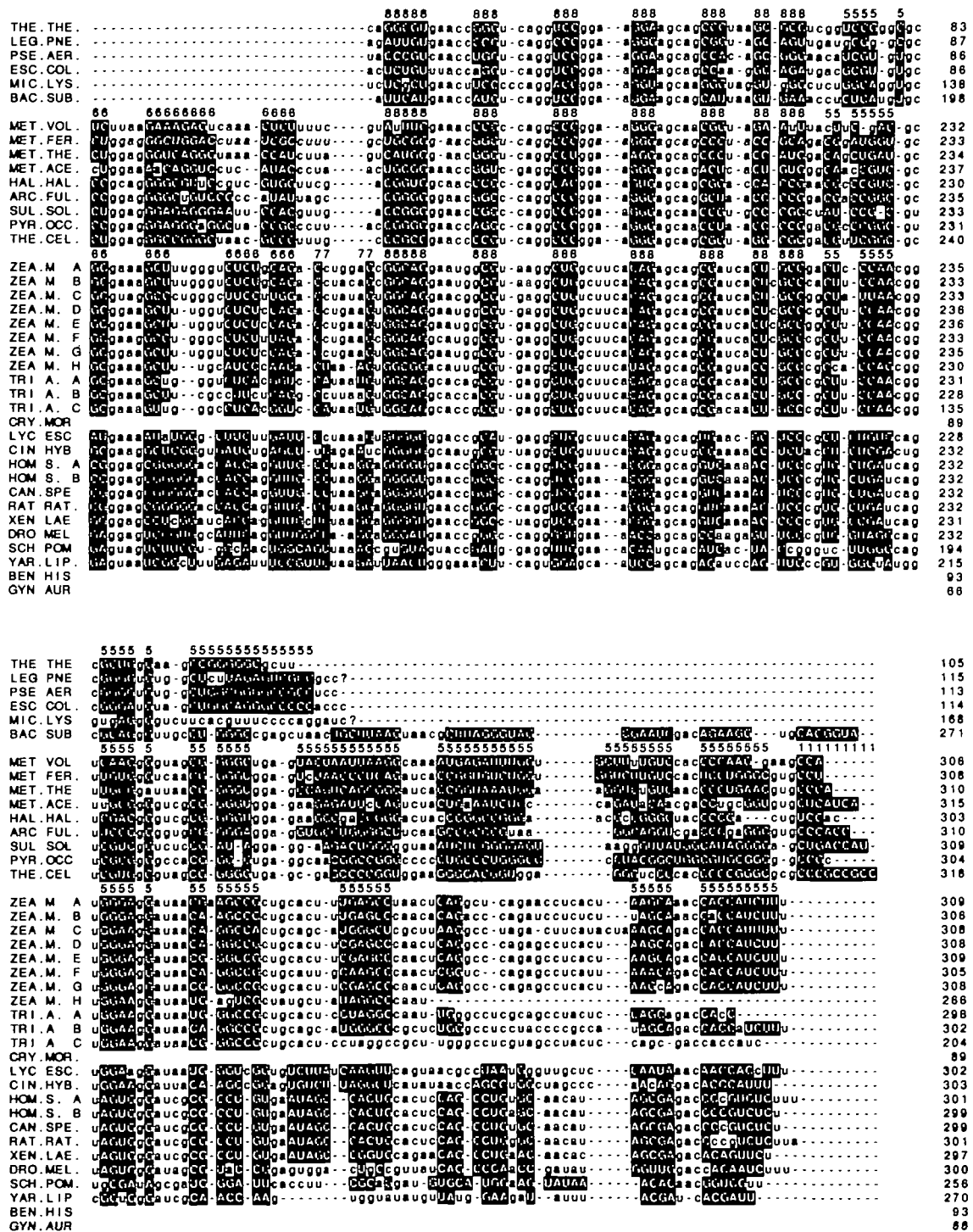


Figure 1. Alignment of SRP-RNA sequences. The abbreviated species names correspond to those listed in Table 1. Sequences are grouped as bacteria (top), archaea (middle) and eucaryotes (bottom). Helices are shown in reverse print for each group and are numbered from one to eight (starting from the 5'-end) in lines with blank species name fields. The alignment was created using the sequence editor ALMA (30), and a PostScript file was printed on a photosetting machine.

**Helix 8:** In contrast to helix 6, helix 8 is separated into three base paired regions and two internal loops (Figure 3, right). The nucleotides in the loops are highly conserved, perhaps reflecting their interactions with SRP-proteins, possibly SRP64 and SRP72 (21), or because tertiary structure forms in this region (22, 23). One A-G pairing is supported in the central helical region (Figure

1). The terminal loop of helix 8 usually contains four purines, but for plants six pyrimidines are present.

In *Escherichia coli*, helix 8 contains the sequence GAAGC-AGCCA (matching GAAGCAGCAU in *Bacillus subtilis* at positions 168 to 177, Figure 2a), which also occurs in the 23S rRNA at positions 1068–1077. It has been proposed that 4.5S

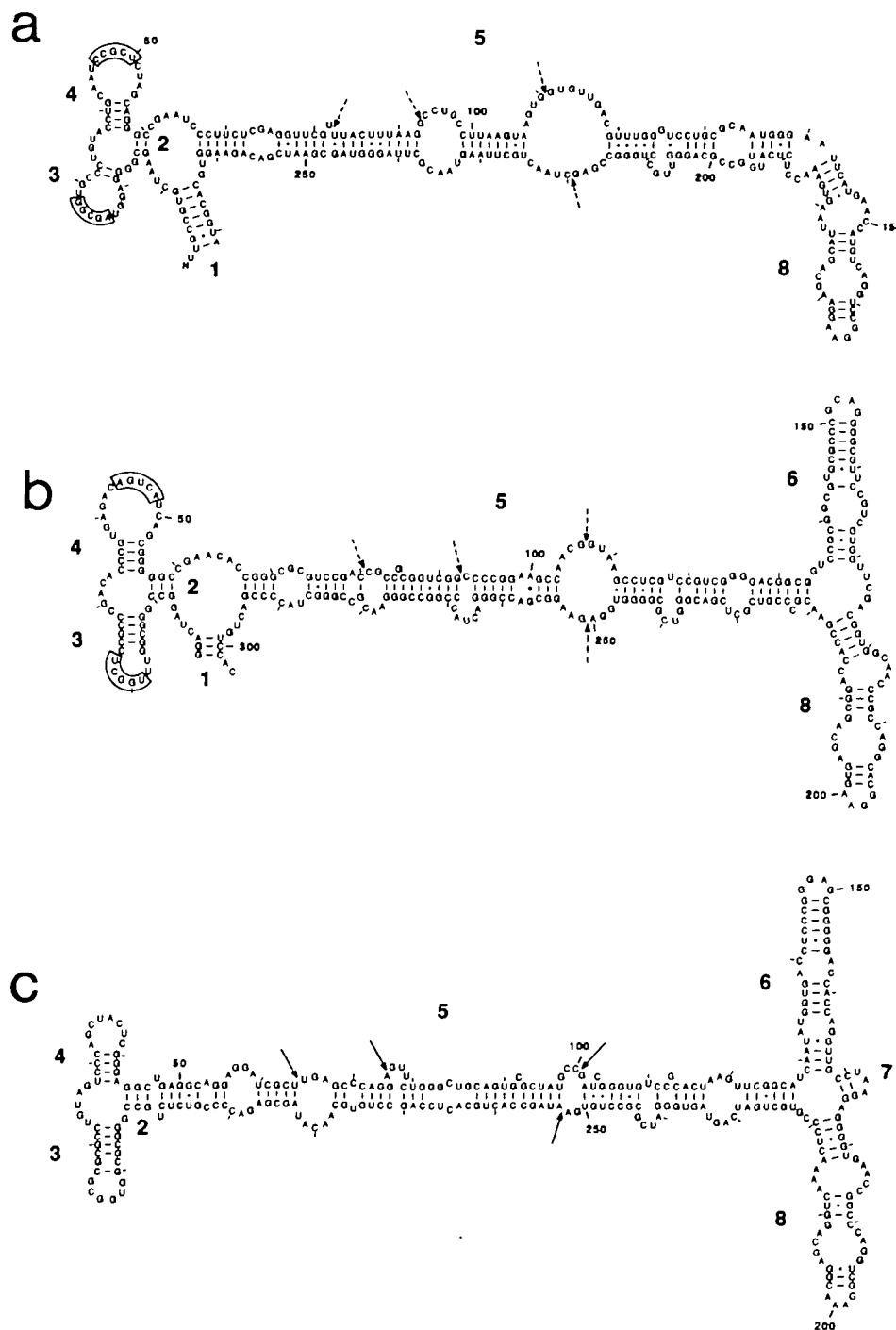
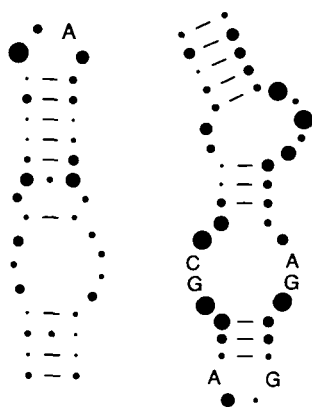


Figure 2. SRP-RNA secondary structure diagrams of a) *Bacillus subtilis* (bacterium), b) *Halobacterium halobium* (archaeum) and c) *Canis species* (eucaryote). Perfect base pairs are connected with a line, G-U and A-G pairs with a filled and an open circle, respectively. Paired bases involved in tertiary interaction are boxed, and helices are numbered one to eight from the 5'-end. Solid arrows in Figure 2c indicate experimentally determined cut sites by micrococcal nuclease (31), while dashed arrows in Figures 2a and 2b point at the aligning nucleotides. Figures were edited by the editor EDSTRUC (32) and a PostScript file was printed on a photosetting machine.

RNA competes for an EF-G binding site using this decamer sequence (24) since it can interact with EF-G (25). The sequence AGCAG is highly conserved in the SRP-RNAs and in the 23S RNAs. However, since we could not detect any covariation among the SRP-RNA and rRNA sequences in the remainder of the decamer, the hypothesis cannot be universally valid.

The overall implications of the deduced SRP-RNA secondary structure will be addressed in the following:

Evidence for division of living matter into three major domains (bacteria, archaea and eucaryotes) is based primarily on the analyses of ribosomal RNA sequences. The archaeal SRP-RNA structures all resemble the single bacterial representative in their 5'-ends (Figure 1), and are distinct from the eucaryotes. On the other hand, they resemble the eucaryotes in that they possess helix 6; similar features can be discerned at the more detailed level. Any alternative grouping would include a mixture of these features, and is therefore not equally supported.



**Figure 3.** Secondary structure of helix 6 (left) and 8 (right) of *Halobacterium halobium* showing overall sequence conservation between the corresponding bases in archaea and eucaryotes: invariant bases are shown by letters, dot diameters are proportional to the extent of conservation. Only one *Zea mays* sequence (ZEA. M. A) and one *Triticum aestivum* sequence was used to avoid a biased high degree of conservation due to overrepresentation of these closely related sequences.

The RNA region corresponding to helix 8 and the conserved part of helix 5 is common to all SRP-RNAs and present in most bacteria as the 4.5S RNA. The large size of the SRP-RNA of *Bacillus subtilis* could be explained by the fact that the 4.5S RNA termini occur within a bulge of the *Bacillus subtilis* RNA and align precisely with the hypersensitive cutting sites of micrococcal nuclease in canine SRP-RNA (indicated in Figure 2); perhaps, a bacterial 4.5S RNA has originated by degradation of a larger bacterial SRP-RNA equivalent and insertion into the genome after reverse transcription (26). We do not know if the terminal sequences of the *B. subtilis* RNA were rescued and constitute a separate molecule in the other bacteria.

### Tertiary interactions in SRP-RNA

Of known higher order interactions, the five Watson-Crick base pairs that form between the loops of helices 3 and 4 in *Bacillus subtilis* and the archaea (marked by >>>>> <<<<< in Figure 1 and boxes in Figures 2a,b) are supported by conclusive phylogenetic evidence. Similar pairings occur between the loops of 23S rRNA helices 5 and 7 (bases 50–120 in *Escherichia coli* numbering), and it may also be regarded as reminiscent of the pairing between the D- and T-loop of tRNA.

We subjected the unpaired regions of the structure to a search for tertiary pairings using a recently developed computer program CBCFOLD (27). This program is well suited for searching regions which cannot be aligned with certainty. Watson-Crick base pairs and G-U pairs were permitted and one out of five sequences were allowed to mismatch. No additional tertiary interactions were found, for which there is conclusive phylogenetic evidence. A few weakly supported interactions were located, however, including the pairing of C9 with G14 and of C190 with G243 (*Canis species* numbering). The first is supported by three CBCs and is exclusive to eucaryotes; like the five base pair tertiary interaction, this pairing could contribute to a more tightly structured 5'-domain. The second interaction would involve backfolding of helix 8 to lie adjacent to helix 5.

It is supported by several covariances within both archaea and eucaryotic RNAs, but mismatches and non-covariant changes also occur. Such folding would bring the size of the structure in closer agreement with what is observed in the electron microscope (6). However, both pairings receive insufficient support at present to be included in the model.

### Interactions with ribosomal RNA

SRPs associate with polysomes (28) during translocation of secretory proteins, and therefore we searched—using CBCFOLD—for phylogenetically supported SRP-RNA—rRNA interactions. For the large subunit, the pairing of U94-A95 (U206-U207 in *Bacillus subtilis*, Figure 2a) with U1273-A1274 (*Escherichia coli* numbering), receives some support. In *Escherichia coli* 23S rRNA, such pairing would involve a tract of bases that link structure domains 2 and 3; the nucleotides are reactive towards both nucleases and chemical reagents in the naked RNA (29), which suggests they are at the subunit surface and could potentially interact. We emphasize however, that we have not found firm evidence for any RNA-RNA contacts with the ribosome.

### Outlook

Analysis of the available SRP-RNAs using the phylogenetic approach has provided a solid secondary structural model of the SRP-RNA. The data base is sufficiently large to identify major group-specific structural features clearly and even reveal details between minor phylogenetic divisions. Currently, the data base is too small to prove possible intra- or intermolecular interactions, more sequences are needed.

In the absence of sufficient amounts of material for structure determination by X-ray crystallography, biochemical methods (enzymatic and chemical modification, site-directed mutagenesis, cross-linking) and molecular modeling will be useful for resolving the three-dimensional structure of the RNA. The present sequence alignment and structures should form a solid basis for design and interpretation of such experiments. Ultimately, such experiments should also provide insight into how the SRP-proteins are assembled in the SRP, and how SRP interacts with the other participants of the translation-translocation machinery.

### Data distribution

The sequence alignment and secondary structure diagrams are available from the second author upon request. We invite submission of new SRP-RNA sequences (as hard copies or by electronic mail to 'zwieb%jason.decnet@utadnx.cc.utexas.edu'; in return, submitters will receive the updated alignment.

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