Recognition of a Tetranucleotide Loop of Signal Recognition Particle RNA by Protein SRP19*

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The interaction of protein SRP19 with the RNA component of human signal recognition particle (SRP) RNA was studied by site-directed mutagenesis of the SRP RNA. The effects of nucleotide changes in the tetranucleotide loop (tetraloop) of helix 6 showed that SRP19 recognizes a tetraloop in a sequence-specific manner. Adenosine 149 at the third position of the tetraloop was essential for binding. In contrast, changes of the base at the second position had no effect. Mutations that disrupt or compensate individual SRP RNA helices were generated to investigate the importance of base pairing and to identify other binding sites. Considerable base pairing was essential in helix 6. Another SRP19-binding site was located in the distant part of helix 8. The primary sequences of the tetraloop-binding protein SRP19 and of bacterial ribosomal protein S15 are shown to be similar.

RNA tetranucleotide loops (tetraloops) are common to large RNA molecules (1, 2). Comparative sequence analysis of ribosomal RNAs shows that the majority of tetraloops exhibit either an UNCG or a GNRA motif, where N is any nucleotide and R is either G or A (3). The tetraloops contain an unusual base pair that increases their thermodynamic stability (4–6). Their primary role appears to be the formation of stable hairpins during RNA folding. This view is supported by the fact that the two motifs evolved independently and are phylogenetically interchangeable (7).

To investigate the possibility that tetraloops serve as recognition sites for RNA-binding proteins, I studied the interaction of protein SRP19 with signal recognition particle (SRP) RNA. SRP RNA is the central component of SRP, a ribonucleoprotein complex that plays an important role in the targeting of secretory proteins (8, 9). Homologous SRP RNAs were isolated from a wide variety of organisms. Comparative sequence analysis (10) allowed the establishment of the secondary structure of the SRP RNA (Fig. 1). Human SRP RNA has two tetraloops, one in helix 6 at position 147 (GGAG) and another in helix 8 at 198 (GAAA) (10). RNA footprinting showed that SRP19 protects the loops from digestion by α-sarcin (11), indicating protein–RNA interactions. From SRP RNAs that lack individual RNA-helices, it was determined that helix 6 contains the major SRP19-binding site, with minor contributions of helices 5, 7, or 8 (12). Amino acids adjacent to, but not including, the C terminus of SRP19 proved to be essential for the interaction with SRP RNA (13, 14).

Site-directed mutagenesis was used to define the binding site of SRP19 on the SRP RNA. I found that one of the tetraloops in SRP RNA is recognized by the protein in a sequence-specific manner. This result permitted the identification of other tetraloop-binding proteins of related structure and function.

EXPERIMENTAL PROCEDURES

Construction of Mutants—phR, a plasmid for transcription of authentic human SRP RNA by T7 polymerase (12) was altered using the polymerase chain reaction (PCR) essentially as described (15). Oligonucleotides were synthesized on an Applied Biosystems (PCR-mate) DNA synthesizer (trityl-on) using β-cyanoethylphosphoramidite chemistry. They were purified and detritylated on an oligonucleotide synthesizer (trityl-on). Site-directed mutagenesis was used to define the binding site of SRP19 on the SRP RNA. I found that one of the tetraloops in SRP RNA is recognized by the protein in a sequence-specific manner. This result permitted the identification of other tetraloop-binding proteins of related structure and function.

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‡ The abbreviations used are: SRP, signal recognition particle; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate.

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Amplified mutant DNAs were restricted with the enzymes EcoRI and primer CGCCAGGGTTTTCCCAGTCACGAC. This thiotic adaptor replaced this portion and introduced the de-
Biolabs), purified on 2% agarose gels (16), extracted, and ligated to RamHI (all restriction enzymes were obtained from New England
for 25 cycles with EcoRI- and BamHI-digested phR-DNA. Competent
using Sequenase (U. S. Biochemical Corp.),
10 min at 72 °C. This was followed by a second PCR with the unique
plasmid DNAs were prepared from individual colonies and sequenced (15) using Sequense (U. S. Biochemical Corp.).
The 149U mutant was obtained by restricting phR-DNA with the enzymes SmaI and Bsu36I to remove a small DNA frag-
plasmid sequences
polymerase chain reaction was used to introduce
synthesis with the polymerase chain reaction was used to introduce
molecules and Binding of Protein SRP19—RNAs were synthesized in vitro by run-off transcription with T7 polymerase (obtained from Stratagene). DNAs were digested with the restriction enzyme DraI, excised with phenol and chloroform, concentrated by ethanol precipitation, and dissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. RNA synthesis and preparation were carried out as described previously (12). SRP19 was translated and labeled with [35S]methionine in a rabbit reticulocyte free system. Binding of SRP19 to mutant RNAs was monitored by centrifugation and retardation of SRP19-RNA complexes on DEAE-Sepharose and followed by SDS gel electrophoresis also as described (15, 20). Gels were fixed, dried, and exposed to x-ray film. Individual bands were quantitated using an exposure times in the linear response range of an Abaton 300/GS scanner.
RESULTS
Synthesis of Mutant SRP RNAs—Site-directed mutagene-
sis with the polymerase chain reaction was used to introduce mutations into the gene for human SRP RNA. This approach was chosen in order to analyze portions of the RNA that are inaccessible to enzymatic or chemical modification. Four oligonucleotides had to be prepared for the first construct; only one new oligonucleotide was required for each additional mutant (15). An upstream oligonucleotide that is character-
ized by a unique 5’-portion and a mutagenic oligonucleotide, located downstream, were applied in the first amplification. The purified amplified DNA was used to prime a single-step extension reaction for synthesis of the complete gene. This was followed by a second PCR with the unique oligonucleotide and an oligonucleotide complementary to plasmid sequences located further downstream. The DNA from the second amplifica-
tion was restricted with EcoRI and BamHI, purified by electrophoresis on agarose gels, and ligated to phR-DNA that had been linearized with EcoRI and BamHI. Plasmid DNAs from the transformants were prepared on a small scale and sequenced directly (18). Usually, at least half of the trans-
forms contained the expected change.
Four “mixed” oligonucleotides were synthesized to intro-
duce every possible single base alteration in the tetraloop of helix 6 (Fig. 1). They were heterogenous at positions 147, 148, 149, and 150, respectively. Unexpectedly, the PCR approach failed to generate the U149 mutant, but produced a deletion mutant (Δ149). U149 was constructed by restricting phR with the enzymes SmaI and Bsu36I to remove a small DNA frag-
ment corresponding to SRP RNA positions 146-170. A syn-
thetic adaptor replaced this portion and introduced the de-
sired change. All other mutations, indicated in Fig. 1 and listed in Table I, were constructed using the PCR approach.
Binding of Protein SRP19 to Mutant SRP RNAs—The binding of in vitro translated SRP19 to the various mutant RNAs was determined by centrifugation and DEAE chromatography and followed by SDS-gel electrophoresis and autoradiography. SRP19 was translated in a wheat germ cell-free system, the reaction mixture was adjusted to 300 mM potassium acetate and incubated either in the absence or in the presence of in vitro transcribed SRP RNA or mutant RNAs. Constant amounts of SRP19 and SRP RNA were used; the RNA was in excess. Ribosomes were removed by centrifugation, and the supernatant was loaded onto a DEAE-Sepharose column. The column was washed with a buffer containing 300 mM or 2 M potassium acetate. The amount of 35S-labeled SRP19 was measured in the pellet (P) of the high speed spin, the low salt flow-through (F), and the high salt eluate (E) from the DEAE column (20, 13). In the absence or presence of tRNA (not shown), the vast majority of SRP19 was in the pellet and the flow-through. In the presence of SRP RNA, most of SRP19 stayed in the supernatant, and the amount found in the eluate of the high salt wash was increased (Fig. 2).
Fig. 3 summarizes the results obtained by binding of SRP19 to mutant SRP RNAs that contain single nucleotide changes in tetraloop 147. Changes of the guanosine at position 148, as well as altering guanosine 147 to an adenosine, did not affect the binding of SRP19 significantly. Slightly impaired binding was seen by altering guanosine 147 to a pyrimidine or by changing guanosine 150 to any other base. Any modification at position 149, including Δ149, abolished the interaction with SRP19. Table I shows that changing the tetraloop sequence from GGAG to UUCG also prevented the interaction with SRP19. Introducing two changes simultaneously to generate a GAAA-tetraloop reduced binding to a degree that was comparable with the A150 RNA. These findings demonstrate that SRP19 recognizes the tetraloop of helix 6 in a sequence-
specific manner. In contrast, altering the 4 bases in tetraloop 198 (mutant 8T) had no effect on the interaction with SRP19, indicating that the tetraloop of helix 8 is not involved in an interaction with the protein (Table I).
To determine the need for base pairing in the stem of helix 6 and to identify additional SRP19-binding sites, mutations were tested that disrupt a limited number of base pairs (Fig. 1 and Table I). Analysis of six mutants in helix 5 (5A to 5F) demonstrated that helix 5 is to a large part dispensable. Binding of SRP19 to mutant 5C RNA was slightly impaired; mutant RNAs 7A or 7B (which disrupt or compensate helix 7) retained full binding capacity. Four of the six helix 6-disrupting mutations (6A to D) completely abolished binding to SRP19, and binding to 6E and 6F RNA was reduced. Two compensating mutations (C2 and 3) fully restored binding, demonstrating the need for an intact stem. The mutant C1 RNA had a significantly reduced affinity to SRP19.
The efficient binding to the compensatory mutants of helix 6 shows that a particular sequence is not required for the interaction with SRP19. By comparing the secondary structures of the helix 6 mutants (Fig. 4) with the differential results obtained with 6A and 6F RNA (Table I), it appeared that SRP19 bound preferentially to the 5’ half of helix 6; although 6A and 6F disrupt the proximal part of helix 6, only one (6A) completely abolished binding.
Binding to 8C and 8D RNA was reduced to almost background level. The mutants are changed at sites that face each other in the secondary structure of SRP RNA (Fig. 1), demonstrat-
ing the involvement of the distal part of helix 8 in the binding to SRP19. With the exception of 8F, the other mutant RNAs (8A, 8B, and 8E) did not substantially interfere with binding.
**FIG. 1. Secondary structure of human SRP RNA.** The seven helices are numbered from 2 to 8 (10). Arrows indicate hypersensitive cutting sites by micrococcal nuclease (32), which divide SRP into two domains. Only the larger domain (right) was studied here. The locations of the helix-disrupting mutations (5A to 5F, 6A to 6F, 7A, and 8A to 8F) and the two tetraloops are indicated by the brackets.

**TABLE I**
Description of SRP RNA mutations

Changes in the helix-disrupting and -compensating RNAs (5A to 5F, 6A to 6F, 7A and 7B, 8A to 8F, and C1 to C3) and the helix 8 tetraloop mutation (8T) and their effect on base pairing are listed. Impact on binding to SRP19 is shown on the right. ++++, more than 75%; +++, 50-75%; ++, 25-50%; +, less than 25%. No background (+) that is due to the interaction of SRP19 to a component in the wheat germ translation system (37) was subtracted. The degree of binding was determined from at least two independent binding experiments.

| Name | Nucleotide changes (from \( \rightarrow \) to) | Effect | Binding |
|------|---------------------------------|--------|--------|
| 5A   | UCGGGUGU\(130\) \( \rightarrow \) GUCCGG | Disrupts a segment of helix 5 | ++++   |
| 5B   | CCGCAUAA\(138\) \( \rightarrow \) GGGUGAU | Disrupts a segment of helix 5 | ++++   |
| 5C   | UGGCGCAU\(128\) \( \rightarrow \) CCAGUGUG | Disrupts a segment of helix 5 | +++    |
| 5D   | GUAGUGGUA\(151\) \( \rightarrow \) UACGCUCCGC | Disrupts a segment of helix 5 | ++++   |
| 5E   | GUAGUGGUA\(143\) \( \rightarrow \) AAUACCCUC | Disrupts a segment of helix 5 | ++++   |
| 5F   | GCGGCUG\(152\) \( \rightarrow \) UUGUGGU | Disrupts a segment of helix 5 | +++    |
| 6A   | CAUAU\(154\) \( \rightarrow \) GUUGGA | Disrupts proximal segment of helix 6 | +      |
| 6B   | GGUGAC\(160\) \( \rightarrow \) CCACCA | Disrupts central segment of helix 6 | +      |
| 6C   | CUCGCC\(148\) \( \rightarrow \) GGGGG | Disrupts distal segment of helix 6 | +      |
| 6T   | GAAG\(166\) \( \rightarrow \) UUGG | Mutates tetraloop of helix 6 | +      |
| 6G\(A\) | GAG\(173\) \( \rightarrow \) GAAA | Mutates tetraloop of helix 6 | +++    |
| 6D   | CUG\(192\) \( \rightarrow \) CUG | Disrupts distal segment of helix 6 | +      |
| 6F   | AGGUUG\(211\) \( \rightarrow \) UAAAC | Disrupts proximal segment of helix 6 | +      |
| C1   | CAUAU\(154\) \( \rightarrow \) GUUGGA | Compensates proximal segment of helix 6 | ++     |
| C2   | GGUGAC\(160\) \( \rightarrow \) CCACCA | Compensates central segment of helix 6 | ++++   |
| C3   | CUCGCC\(148\) \( \rightarrow \) GGGGG | Compensates distal segment of helix 6 | ++++   |
| 7A   | CCUAC\(175\) \( \rightarrow \) GG | Disrupts helix 7 | ++++   |
| 7B   | CCUAAG\(175\) \( \rightarrow \) GUUAACC | Compensates helix 7 | ++++   |
| 8A   | AGGCCG\(193\) \( \rightarrow \) GCCUCAAG | Disrupts proximal segment of helix 8 | +++    |
| 8B   | GGUGGCA\(193\) \( \rightarrow \) GACUCUAG | Disrupts central segment of helix 8 | ++++   |
| 8C   | AGGUGG\(175\) \( \rightarrow \) CCAGGC | Disrupts distal segment of helix 8 | +++    |
| 8T   | GAAA\(219\) \( \rightarrow \) UCUG | Mutates tetraloop of helix 8 | ++++   |
| 8D   | GGAG\(207\) \( \rightarrow \) GCCUGCA | Disrupts central segment of helix 8 | ++++   |
| 8E   | AGGCUCAA\(214\) \( \rightarrow \) CCCGGGC | Disrupts proximal segment of helix 8 | +++    |

In summary, the results demonstrate that there are two distinct binding sites of protein SRP19 on the SRP RNA. The major site (12) involves tetraloop 147, with adenosine 149 being the most important base, and numerous base pairs in the stem. A minor site is located in helix 8 adjacent to, but not including, the tetraloop at position 198. A hypothetical three-dimensional model of the SRP RNA in the vicinity of protein SRP19 is shown in Fig. 5 (see "Discussion").
**FIG. 2. Affinity of protein SRP19 to mutant SRP RNAs.** Complexes were analyzed by their retardation on DEAE-Sepharose. Polypeptides from equal aliquots of the ribosomal pellet (P), the flow-through (F), and the high salt eluate (E) were separated on SDS-polyacrylamide gels and exposed to x-ray film. Only the portions of the autoradiograms that contained the SRP19 polypeptide are shown. The absence of SRP RNA (no RNA) and the presence of SRP RNA (SRP RNA), helix 6 tetraloop mutants (A147, C147, U147, A148, C148, U148, G149, C149, U149, A150, C150, U150, A149, C150, A149, 6T, and 6A), helix-disrupting mutants (5A to 5F, 6A to 6F, 7A and 7B, and 8A to 8F), helix-compensating mutants (C1 to C3), and helix 8 tetraloop mutant (8T) are shown. The mutations are described in more detail in Table I.

To estimate the probability that proteins equivalent to SRP19 are present in other species, the identified SRP19-binding sites were superimposed onto the secondary structure of SRP RNA, showing overall sequence conservation (Fig. 5, left). Interestingly, the invariant adenosine at position 149 is also the most crucial base for the interaction with SRP19 (Fig. 3). Guanosine 148 at the second tetraloop position is very variable; this correlates with the finding that changing guanosine 148 to any other base has no effect. Guanosine 150 is moderately conserved and has an intermediate affinity to SRP19. In evolution, many compensatory base changes occurred throughout helix 6, emphasizing the need to form a continuously stacked helix independent of a particular sequence. Analysis of the compensatory mutations that exhibit full binding capability supports this idea. The second SRP19-binding site contains 4 invariant bases that could potentially interact, but their role was not studied in detail. In general, there is an excellent agreement between the conservation pattern and the experimentally determined binding requirements.

The majority of tetraloops in the ribosomal RNAs conforms to one of two general motifs, GNRA or UNCG. They are frequently interchangeable; therefore, their predominant function appears to be the formation of a stable hairpin (1-3) and not the sequence-specific interaction with proteins. However, in SRP RNA, SRP19 interacted with a tetraloop, demanding an adenosine at the third tetraloop position (Fig.

**FIG. 3. Affinity of protein SRP19 to single-site mutations in tetraloop 147.** Complexes were analyzed by retardation on DEAE-Sepharose. Polypeptides from equal aliquots of the ribosomal pellet, the flow-through, and the high salt eluate were separated on SDS-polyacrylamide gels, exposed to x-ray film and quantitated by scanning of the autoradiogram. The degree of binding is plotted in reference to the affinity of SRP RNA, arbitrarily set to 100%. The background (20-30%) that is due to the binding of SRP19 to a SRP-like component in the wheat germ translation system (37) was subtracted. The wild-type sequence GGAG is shown at the top of the figure, with single nucleotide changes indicated at the bottom. A deletion of A149 (Δ) is included.
Fig. 4. Secondary structures of helix 6 mutants. Structure of helix 6 (top left). Base pairs supported by comparative sequence analysis (10) are connected by lines or circles. Secondary structures of helix-compensating (C1 to C3, top) and helix-disrupting (6A to 6F, bottom) mutant RNAs.

Fig. 5. Binding sites of protein SRP19 on SRP RNA. Left, secondary structure of the SRP RNA in the large domain of SRP showing overall sequence conservation between the corresponding bases in archaea and eucarya (10). Invariant bases are shown by letters; dot diameters are proportional to the extent of conservation. Only one Zea mays sequence, one Triticum aestivum sequence, and one human sequence were used to avoid a biased high degree of conservation due to overrepresentation of these closely related sequences. Helices are numbered from 5 to 8. The two areas required for the interaction of protein SRP19 with the RNA are shaded in gray. Right, hypothetical three-dimensional model of the SRP RNA showing SRP19 (shaded area) sandwiched between helix 6 and helix 8. For the purpose of clarity, helix 5 was omitted.

Comparative analysis of more than 30 SRP RNA sequences confirmed the experimental results by identifying the helix 6 tetraloop motif as GNAR (10). Sequence comparison also showed that, unlike in some of the rRNA tetraloops, the GNAR motif of SRP RNA helix 6 is never interchangeable with the dissimilar UNCG motif.

The nature and distribution of GNAR tetraloops in the 16S ribosomal RNA as described by others (3) show that two tetraloops at positions 159 (GAAA) and 898 (GCAA) (E. coli numbering) are absolutely conserved, whereas a third alternates between GAAG and GAAA (position 863), but is not present in the bacteria. Only the tetraloop at position 727 alternates and invariably conforms to the GNAR motif in the archaea and the bacteria. Interestingly, tetraloop 727 is part of the binding site of ribosomal protein S15 (21–24). Sequence comparison between SRP19 and ribosomal protein S15 revealed a significant similarity. Fig. 6 shows an alignment of the SRP19 sequence with that of ribosomal proteins S15 from two bacteria and an archae. Similarities were 45% between SRP19 and E. coli ribosomal protein S15, as compared with
thirds of the SRP19 polypeptide is shared with S15, excluding comparative sequence analysis (10). However, the conserved be essential, whereas guanosine 148 could be altered without affecting binding. The results on the tetraloop reduced binding slightly. The results obtained by analysis of helix 6—proximal part.

**Fig. 6. Alignment of protein sequences.** Human SRP19 (top), S15 ribosomal proteins from E. coli (38) and B. steaoroermophilus (B.ste.), and H-S11 ribosomal protein of H. marismortui (H.mar.) (25). Amino acid positions in SRP19 are marked at multiples of 10. The alignment was generated with the program GAP (39). Dashes represent alignment gaps. Identical amino acids are shown in boxes. Close and more distant evolutionary conservations between neighboring sequences are indicated by double and single dots, respectively (40). 42 of the H-S11 N-terminal amino acids that do not align with SRP19 are not shown (+).

**DISCUSSION**

**Binding Sites of SRP19 on the SRP RNA**—To investigate the possibility that RNA tetraloops serve as recognition sites for proteins, I studied the interaction of protein SRP19 with signal recognition particle RNA. Human SRP RNA contains two tetraloops, one at position 147 (GGAG) and another at 198 (GAAA). Site-directed mutagenesis was used to alter the tetraloops and revealed that SRP19 recognizes tetraloop 147 in a sequence-specific manner. Adenosine 149 was found to be essential, whereas guanosine 148 could be altered without affecting binding. Introducing changes into the first and last position of the tetraloop reduced binding slightly. The results agree well with the consensus motif GNAR, derived by comparative sequence analysis (10). However, the conserved guanosine 147 could be altered without affecting binding seriously. The GNAR-motif of SRP RNA (10) is slightly different from GNRA, which dominates the small subunit ribosomal RNA (3). Although the three-dimensional structure of a GNAG tetraloop is unknown, the presence of a guanosine at the fourth tetraloop position is unlikely to change the tetraloop structure significantly. This is indicated by the fact that SRP19 retained about 50% of its capacity when binding to GGAA. A similar result was obtained when 2 nucleotides were altered simultaneously in order to obtain a GAAA tetraloop, the structure of which is known (5). The inability of SRP19 to bind to a UUCG tetraloop proves that the protein does not simply recognize a helix that is stabilized by a loop, but rather that recognition occurs in a sequence-dependent manner. Guanosine 147 at the second tetraloop position protrudes (5) and, although freely accessible, was not required for binding. Thus, the mode of SRP19 binding probably excludes the very tip of helix 6 but includes several base pairs of the stem. The results obtained by analysis of helix 6—disrupting and -compensating mutations are compatible with this interpretation. Although mutant RNAs 6A and 6F disrupt the same base pairs, there is a significant difference of their affinity to SRP19. This could be explained by SRP19 lying parallel to helix 6, where it prefers one RNA strand in the proximal part.

It was shown previously that an SRP RNA that lacked helix 8 retained much of its ability to bind SRP19 (12). Binding was fully restored in a mutant RNA (Δ35) that included helices 7, 8, and the conserved portion of helix 5 (Δ35 is equivalent to the portion of the RNA shown in Fig. 5, left). Analyses of RNAs that disrupted a limited number of base pairs in helices 5, 7, and 8 demonstrate that helices 5 and 7 and the proximal two-thirds of helix 8 do not contribute significantly to the formation of the SRP19-binding site. In contrast, two other mutations, 8C and 8D, abolished binding to SRP19. Since these two mutants are expected to interfere with the formation of a stable tetraloop 198, SRP19 could recognize tetraloop 198 in a sequence-independent manner. However, examination of the data obtained from RNA footprinting experiments with α-sarcin (11) supports the view that SRP19 contacts the distal part of helix 8 and not tetraloop 198 itself. At a slight excess of protein over RNA, α-sarcin reported an enhanced accessibility in the tetraloop 198 that was probably due to the binding of SRP19 to the neighboring distal segment of helix 8. Protection of tetraloop 198 was seen only at a 10-fold excess of SRP19 or at increased levels of RNA degradation. Since, as shown above, SRP19 binds to a GAAA tetraloop, the protection could be explained by the interaction of a second SRP19 molecule with tetraloop 198 at an increased protein-RNA ratio. It should also be noted that, in contrast to tetraloop 147, tetraloop 198 is not universally conserved; in the plant SRP RNAs, it is replaced by a hexanucleotide loop (10). However, this binding site might be missing in the plant particle.

The conservation pattern of SRP RNA in the SRP19-binding site agreed closely with the experimentally determined binding requirements. For example, bases in tetraloop 147 that could be altered without affecting protein binding are variable; bases that were essential for binding are conserved. Thus, it is concluded that SRP19-like polypeptides are likely to be part of the repertoire of the SRPs from a wide variety of members of the eucarya and archaea. An exception might be found in the bacterial SRP RNA equivalent that is represented by the 4.5 S RNA and misses helix 6 (26-28, 10). However, the universally conserved helix 8 is part of the SRP19-binding site.

SRP RNA must be folded to a high degree so that its size and shape agree with the dimensions (240 × 60 Å) determined in the electron microscope (29, 30). Circumstantial evidence from nuclease accessibility (31–33), comparative sequence analysis (10), and cross-linking (34) indicates a high degree of folding. Molecular modeling (12, 34)3 shows that the sim-
pliest way to account for the data is to place the conserved part of helix 5 and helices 6 and 8 approximately parallel to each other. Fig. 5 represents a portion of a hypothetical model of the SRP RNA near SRP19. The protein is assumed to be globular and sandwiched between helix 6 and helix 8, effectively creating a tightly folded domain. The major binding site involves tetraloop 147 and a large portion of helix 6; significant contacts are made with the distal segment of helix 8. If the angles between the axes of the protruding helices 5, 6, and 8 are altered, this could account for the minor effects on SRP19 binding by mutant RNAs 5C, C1, and 8F. Since SRP19 is a primary RNA-binding protein, in the sense that it does not require other proteins for its interaction with SRP RNA (35), it might stabilize or even actively alter the RNA conformation during SRP assembly (14, 30, 36).

Relation of Human SRP19 to Ribosomal Protein S15—The predominant function of RNA tetraloops appears to be the formation of a stable hairpin (3–5, 7). I demonstrate that tetraloops can have a more specific function in the sequence-specific recognition of proteins. Comparative sequence analysis shows that tetraloop 147 conforms to a motif (GNAR) that is somewhat different from GNRA found in many tetraloops in ribosomal RNA. Interestingly, only the tetraloop at position 727 of 16 S ribosomal RNA alternates like tetraloop 147. This led me to the discovery of another tetraloop-binding protein, ribosomal protein S15. Both proteins bind to their respective RNA independently of other proteins. SRP19 was shown to promote binding of SRP54, probably by stabilizing or inducing an alternative RNA conformation (36, 37). Similarly, ribosomal proteins S6 and S18 depend on S15 for binding to ribosomal RNA, and there is evidence that an S15-induced conformational change in 16 S RNA is required for the interaction with S6 and S18 (21, 22, 24). Sequence comparison revealed a significant similarity between human SRP19, the bacterial ribosomal protein S15, and an S15 homolog, the archaeal ribosomal protein H-S11 (25). The similarity covers nearly all of S15 and about two-thirds of SRP19, excluding 25 amino acids from each terminus. 24 amino acids from the C terminus of SRP19 were not required for binding to SRP RNA (13, 14), which is in excellent agreement with the proposed alignment.

This is the first report of a protein that recognizes a distinct subset of RNA tetraloops. The striking similarity between SRP19 and ribosomal protein S15 on the structural, as well as functional levels, supports the idea that the proteins are phylogenetically related and likely to be the first recognized members of a new family of RNA-binding proteins. However, because of the prevalence of RNA tetraloops, similarities may have developed independently. In the future, the study of tetraloop-binding proteins is expected to provide an excellent opportunity for understanding the molecular interactions between protein and RNA.

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REFERENCES
1. Woese, C. R., Gutek, R. R., Gupta, R., and Noller, H. F. (1983) Microbiol. Rev. 47, 621-669
2. Gutek, R. R., Weisser, B., Woese, C. R., and Noller, H. F. (1985) Prog. Nucleic Acids Res. Mol. Biol. 32, 155-216
3. Woese, C. R., Winker, S., and Gutek, R. R. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 847-857
4. Cheong, C., Varani, G., and Tinoco, I., Jr. (1990) Nature 346, 680-682
5. Heiz, A. A., and Pardi, A. (1991) Science 253, 191-194
6. Antao, V. F., Lai, S. Y., and Tinoco, I., Jr. (1991) Nucleic Acids Res. 19, 5901-5905
7. Uhlenbeck, O. C. (1990) Nature 346, 613-614
8. Walter, P., and Lingappa, V. (1986) Annu. Rev. Cell Biol. 2, 499-516
9. Walter, P., and Blobel, G. (1986) Cell 34, 525-533
10. Larsen, N., and Zwieh, C. (1991) Nucleic Acids Res. 19, 209-216
11. Siegel, V., and Walter, P. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 1801-1805
12. Zwieh, C. (1991) Nucleic Acids Res. 19, 2955-2960
13. Zwieh, C. (1991) Biochem. Cell Biol. 69, 649-654
14. Kümisch, K., Webb, J., Lingelbach, K., Gnaigele, H., and Dobberstein, B. (1990) J. Cell Biol. 111, 1793-1802
15. Nelson, R. M., and Long, L. G. (1989) Annu. Rev. Biochem. 58, 147-157
16. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) in Molecular Cloning: A Laboratory Manual, pp. 173-185, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Maxam, A. M., and Gilbert, E. (1960) Methods Enzymol. 65, 499-560
18. Hisao, K. (1991) Nucleic Acids Res. 19, 2787
19. Zwieh, C., Kim, J., and Adron, S. (1986) in BioMethods 5, 245-257
20. Lingelbach, K., Zwieh, C., Webb, J. R., Marshallay, C., Hoben, J. P., Walter, P., and Dobberstein, B. (1988) Nucleic Acids Res. 16, 9431-9442
21. Minshawa, S., and Nomura, M. (1970) Nature 226, 1214-1218
22. Held, W. A., Ballou, B., Minshawa, S., and Nomura, M. (1974) J. Biol. Chem. 249, 3109-3111
23. Serstov, P., Changhien, L., Craven, G. R., and Noller, H. F. (1988) J. Mol. Biol. 200, 301-308
24. Stern, S., Powers, T., Changhien, L., and Noller, H. F. (1989) Science 246, 783-790
25. Arndt, E., Breitbahr, G., and Kimura, M. (1986) FEBS Lett. 184, 227-232
26. Strack, J. C., Vogel, D. W., Ulbrich, N., and Erdmann, V. A. (1988) Nucleic Acids Res. 16, 2719
27. Zwieh, C. (1989) Enzyme Microb. Technol. 11, 327-336
28. Poritz, M. A., Strub, K., and Walter, P. (1988) Cell 55, 4-6
29. Andrews, D., Walter, P., and Ottenanowey, P. F. (1987) EMBO J. 6, 3471-3477
30. Zwieh, C. (1988) Prog. Nucleic Acids Res. Mol. Biol. 37, 207-234
31. Gundelfinger, E. D., Di Carlo, M., Zepf, D., and Melli, M. (1984) EMBO J. 3, 2335-2339
32. Gundelfinger, E. D., Kruse, E., Melli, M., and Dobberstein, B. (1983) Nucleic Acids Res. 11, 7063-7074
33. Undrezzoni, M., and Gerbi, S. A. (1991) EMBO J. 10, 677-777
34. Zwieh, C., and Schlier, D. (1989) Biochem. Cell Biol. 67, 434-442
35. Zwieh, C., and Ullu, E. (1986) Nucleic Acids Res. 14, 4659-4667
36. Prehn, S., Wiedmann, M., Rappoport, T. A., and Zwieh, C. (1987) EMBO J. 6, 2003-2007
37. Morris, T., Funatsu, G., Funatsu, M., and Wittmann, H. G. (1976) FEBS Lett. 54, 307-309
38. Devereux, J., Haeberli, P., and Smithies, 0. (1984) Nucleic Acids Res. 12, 387-388
39. Griakos, M., and Burgess, R. R. (1986) Nucleic Acids Res. 14, 6755-6763