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Letter to the Editor

Human SRP RNA and E. coli 4.5S RNA Contain a Highly Homologous Structural Domain

At present the most powerful tool available for determining the secondary structure of an RNA is the phylogenetic approach (Noller, 1984). This has been the primary method used to derive the secondary structure of the RNA component of the signal recognition particle (SRP), 7SL RNA (herein referred to as SRP RNA; Ullu et al., 1982). This analysis was carried out using the sequences of the human, frog, and fly SRP RNAs (Gundelfinger et al., 1984; Ullu and Tschudi, 1984; Zwieb, 1985; Zwieb and Ullu, 1986), all of which are functionally interchangeable in chimeric particles reconstituted in vitro (Walter and Blobel, 1983). More recently, RNAs from the yeasts Schizosaccharomyces pombe (Brennwald et al., 1988; Poritz et al., 1988; Ribes et al., 1988) and Yarrowia lipolytica (Poritz et al., 1988) and the archaebacterium Halobacterium halobium (Moritz et al., 1985) have been identified and suggested to be homologs of SRP RNA. The wide evolutionary diversity represented in this collection of sequences has allowed us to refine the phylogenetic description of the SRP RNA secondary structure and has revealed a unique and highly conserved structural domain. Surprisingly, we find this domain in the 4.5S RNA of Escherichia coli (Hsu et al., 1984) and in the small cytoplasmic RNA (scRNA) of Bacillus subtilis (Struck et al., 1988).

For the purposes of this analysis, the structure of SRP RNA can usefully be divided into four domains (using the human RNA as a prototype; these are labeled I–IV in Figure 1). Domain I is a variable structure comprising the 5′ end of the molecule. Domain II is the main stem, ending in a bifurcation into two stem–loop structures which define domains III and IV. Both domain II and domain III are characterized as highly base-paired helices that are frequently interrupted by bulged nucleotides and internal loops. However, the positions and sequences of the internal loops are not conserved in domain III and are only conserved in domain II when a subset of species, i.e., the higher eukaryotes, are examined (Zwieb, 1985). In marked contrast, we found that the 50 nucleotides constituting domain IV form a rigidly determined structure. It is composed of three short helices of defined length that are flanked by internal loops whose sequences are highly conserved across this set of RNAs. Figure 2 shows an alignment of the refined secondary structures of this domain which has been optimized, using the phylogenetic approach, starting from the published structures.

Figure 2 also demonstrates that a domain identical to domain IV is found in the 4.5S RNA of E. coli and in the scRNA of B. subtilis. The alignment of Figure 1 shows that these shorter prokaryotic RNAs can be viewed as portions of the "classical" bifurcated SRP RNA structure with individual domains deleted. The strong similarity of the domain IV structures suggests that these domains may serve similar functions in the diverse organisms and that the RNAs may be derived from a common evolutionary precursor. This argument is further supported by the structure of the archaebacterium H. halobium RNA, which closely resembles eukaryotic SRP RNA in every respect (Figures 1 and 2).

Biochemical assays have delineated a clear function for higher eukaryotic SRP in the process of protein secretion. According to the current model, SRP acts as an adaptor molecule that links translation to the translocation of secretory and membrane proteins across or into the endoplasmic reticulum membrane (Walter and Lingappa, 1986). During this process, SRP interacts intimately with the ribosome and assures the cotranslational targeting of nascent secretory proteins. The central portion of SRP

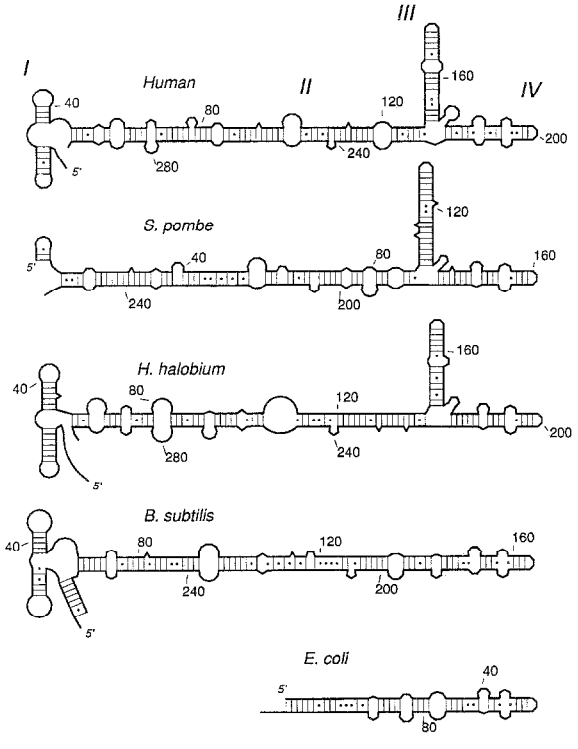

Figure 1. Alignment of Secondary Structures for the SRP RNAs and Their Prokaryotic Homologs

The sequences are taken from references cited in the text. The S. pombe sequence (Poritz et al., 1988) was corrected by the deletion of a C residue at the 5′ end as determined by Brennwald et al. (1988) and Ribes et al. (1988). Xenopus laevis and Drosophila melanogaster structures are virtually identical to the human RNA structure and have not been presented. The Y. lipolytica RNA structure has been published in this format recently (Poritz et al., 1988), but see Figure 2 for a minor revision of domain IV. Domains I–IV on the human RNA structure are defined in the text. The line drawings were generated using the RNA structure-editing computer program STRED, written by Bryn Weiser in the laboratory of Harry Noller.

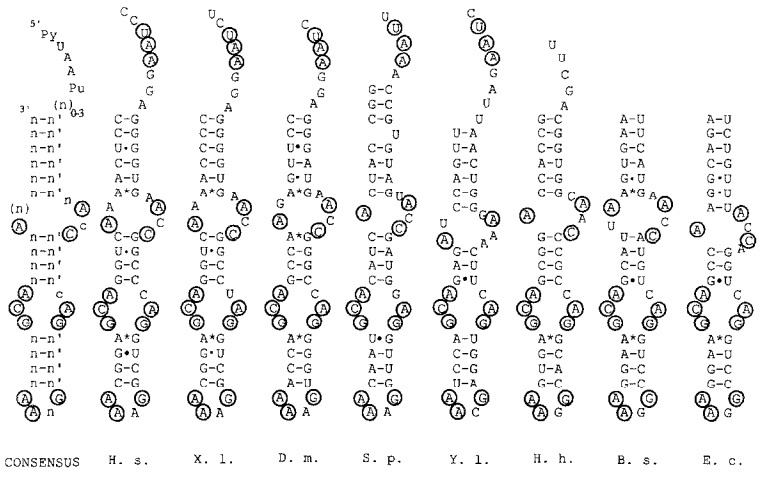

Figure 2. Conservation of the Domain IV Motif

The primary and secondary structures of the domain IV regions of the eukaryotic and archaebacterial SRP RNAs are aligned with the homologous domains of the prokaryotic 4.5S RNA of E. coli and the scRNA of B. subtilis. H. s.: Homo sapiens, bases 169–220. X. l.: X. laevis, bases 168–219. D. m.: D. melanogaster, bases 170–220. S. p.: S. pombe, bases 132–182. Y. l.: Y. lipolytica, bases 153–202. H. h.: H. halobium, bases 172–219; B. s.: B. subtilis, bases 141–184; E. c.: E. coli, bases 32–74. To facilitate comparison with Figure 1, the sequences are oriented 5′ to 3′, reading down on the right and up on the left as indicated for the consensus. Conserved nucleotides in single-stranded regions are circled. Py and Pu in the consensus structure are pyrimidine and purine residues, respectively.

Description of the consensus: Starting from the loop end, domain IV is characterized by a 4 base hairpin, 5′-GnAA. This sequence is highly conserved; however, since it is common among 4 base hairpin loops in rRNA (Woese et al., 1984), its conservation may reflect a particularly favorable RNA structure rather than a functionally significant feature of this set of RNAs. (We note that domain III in all of the RNAs that contain this domain ends in the closely related sequence 5′-GnAG.) The hairpin loop is followed by a 4 bp stem that often ends in a nonstandard G-A base pair. This stem is succeeded by an internal loop ("nAG/GCA loop") that is the most conserved loop in the whole molecule; it has the sequence 5′-nAG on the 5′ side, opposite the sequence 5′-GCA on the 3′ side. In this loop, "n" is usually a C residue. Following the loop is a second 4 bp stem (shortened to 3 bp in Y. lipolytica and E. coli), which ends at a second internal loop ("AC-rich loop") with a 4 nucleotide AC-rich sequence on the 5′ side opposite a 1 or 2 base sequence that always contains an A residue on the 3′ side. The next stem is 6 or 7 bp long (with one bulged U in S. pombe) and frequently starts with a G-A base pair. At the end of this stem is a single-stranded region ("PyUAAPu loop") forming the junction between domains III and IV. It contains the conserved sequence 5′-PyUAAPu within 3 nucleotides of the 5′ end. However, this consensus sequence is only found in eukaryotes.

RNA (the right-hand half of domain II and domains III and IV) and its associated proteins are sufficient for the signal recognition and targeting functions of SRP (Siegel and Walter, 1986), while additional translational-control functions of the particle map to domain I. The site(s) on SRP that interacts with the ribosome has not been assigned.

Nothing is yet known about the function of the B. subtilis scRNA, but several reports have suggested that the 4.5S RNA of E. coli is involved in the process of translation. E. coli 4.5S RNA is a stable RNA that is essential for viability (Brown and Fournier, 1984). Its abundance in E. coli, about one molecule for every ten ribosomes, resembles that of SRP RNA in eukaryotic cells (Hsu et al., 1984; Poritz et al., 1988). Using a conditionally expressed allele of the 4.5S RNA gene, it was shown that one of the first identifiable defects in an E. coli mutant that has ceased making 4.5S RNA is a general inhibition of protein synthesis (Brown and Fournier, 1984; Bourgaize and Fournier, 1987). This defect was reproduced in an in vitro translation extract made from these cells and could be traced specifically to the ribosomes. In an elegant genetic experiment, Brown (1987) isolated extragenic suppressors of the conditionally expressed 4.5S RNA allele and mapped them to the gene for the elongation factor EF-G. Taken together, these data have been interpreted to imply a role for 4.5S RNA in the maintenance of the protein synthetic capacity.

How can we reconcile our present understanding of SRP and 4.5S RNA function with the presence of a common structural domain? Under the assumption that these structures evolved from a common precursor, we consider two models equally tenable given the data available.

According to the first model, 4.5S RNA may play a role in the secretion or membrane insertion of E. coli proteins, i.e., it may be part of a prokaryotic SRP analog. There is at present no positive biochemical or genetic evidence for a ribonucleoprotein or RNA involved in this process (Müller and Blobel, 1984; Randall and Hardy, 1987). Since E. coli is clearly capable of secreting many proteins post-translationally, only a few secreted or membrane proteins may need to use an SRP-dependent cotranslational pathway. But as long as a subset of these is essential for viability, 4.5S RNA will be essential for viability. Hence, if more than one targeting pathway exists, it would be difficult to identify those proteins that might require SRP-dependent targeting obligatorily. This, together with the small target size of the gene, may explain why the 4.5S RNA has never been isolated in screens for secretory mutants.

According to the second model, the conserved RNA motif performs an evolutionarily ancient function, involved in an essential aspect of cellular physiology which is in some way linked to translation. 4.5S RNA would represent a minimal example of such an entity. In eukaryotes, this structure has become part of a larger molecule with additional (or alternative) secretion-promoting activities. In this model, domain IV could confer upon SRP the specific ribosome-binding properties required for its signal recognition function. This model opens the intriguing possibility that SRP in eukaryotic cells could be essential for functions other than those that have been defined to date.

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