Ribonuclease P structure and function in Archaea

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Abstract

An important approach to understanding RNA-based catalytic function by ribonuclease P is the investigation of its evolutionary diversity in structure and function. Because RNase P enzymes from all organisms are thought to share common ancestry, the fundamental features of structure and biochemistry should be conserved in all of its modern forms. In contrast to the bacterial enzyme, the RNase P enzymes from Eucarya, organelles, and Archaea are poorly understood. This review describes our nascent understanding of the structure and function of RNase P in Archaea, and how this enzyme compares to its homologs in the other evolutionary Domains.

Abbreviations: RNase P – ribonuclease P; tRNA – transfer RNA; pre-tRNA – 5′-unprocessed precursor transfer RNA; Archaea – a.k.a. archaeobacteria; Bacteria – a.k.a. eubacteria; Eucarya – a.k.a. euukaryotic nucleus/cytoplasm.

Introduction

Although RNAs are central to many of the most fundamental cellular processes, our understanding of RNA structure and function is rudimentary compared to that of protein or DNA. As a notable example of only a small number of catalytically-active RNAs (a.k.a. ribozymes), RNase P is an excellent experimental model system for the examination of RNA structure and function, and the interaction of RNA with other cellular components.

RNase P is the endoribonuclease responsible for the removal of 5′ (leader) sequences from pre-tRNAs and at least a few other small RNAs such as the 4.5S RNA [for review, see 1]. RNase P is required by, and present in, all cells and those subcellular organelles that carry out tRNA biosynthesis (i.e. mitochondria and chloroplasts); however, only the bacterial enzymes, especially those of Escherichia coli and Bacillus subtilis, have been studied in detail. In Bacteria, RNase P is composed of a large RNA of ca. 400 nucleotides and a small polypeptide of ca. 120 amino acids [2]. The RNA is the catalytic subunit of the bacterial enzyme; under appropriate conditions in vitro the RNA alone is capable of accurate and efficient 5′-processsing of pre-tRNAs [3]. The RNA subunit of bacterial RNase P RNA is one of only a few known catalytically-active RNAs, and is the only such RNA that acts in a truly catalytic fashion in its naturally evolved form.

In contrast to the situation in Bacteria, the RNase P enzymes from Eucarya, Archaea, and organelles are poorly understood. With the possible exception of those from chloroplasts [4], and HeLa cell mitochondria [4a] all RNase P enzymes are ribonucleoproteins, but catalysis by the RNA component alone has not been demonstrated in vitro for any non-bacterial RNase P [1]. Because archaeal RNase P RNAs are not capable of catalysis, and yet are sufficiently similar to those of Bacteria for the identification of homologous sequences, they have the potential to provide needed insight into the essential functionality of the enzyme.

Biochemical properties

Archaeal RNase P has been characterized biochemically in only two species: Haloferax volcanii (previously known as Halobacterium volcanii) and Sulfolobus acidocaldarius (previously misclassified as S. solfatarius). The RNase P from H. volcanii in many respects
resembles the bacterial enzyme [5]. The buoyant density of the enzyme in cesium sulfate is 1.61, indicating that it is composed predominantly of RNA. Enzymatic activity is sensitive to micrococcal nuclease, as are the activities of the enzymes from Bacteria, Eucarya, and mitochondria (but not chloroplasts), suggesting that the RNA fraction is essential for activity. However, RNA prepared from the holoenzyme by deproteinization, or RNA synthesized in vitro, entirely lacks catalytic activity; the RNA alone is apparently not capable of catalysis in the absence of other (presumably protein) components of the enzyme [5, 6]. It has been reported that activity in the H. volcanii RNase P RNA could be reconstituted with the protein subunit of bacterial RNase P enzymes [6] and that a crude H. volcanii protein fraction could reconstitute holoenzyme-like activity in E. coli RNA, but these results have not been substantiated in more recent experiments [7, 8]. The gene encoding an RNA that both copurifies with RNase P activity and is recognizably similar to bacterial RNase P RNAs (see below) has been cloned and sequenced [6].

The RNase P enzyme from Sulfolobus acidocaldarius, on the other hand, resembles the eucaryal enzyme in several aspects [9]. The molecular weight of the enzyme, estimated by gel filtration, is ca. 400 kDa, similar to that of the eucaryal enzyme from Schizosaccharomyces pombe (450 kDa) measured in similar fashion. The density of the S. acidocaldarius enzyme is only slightly greater than that of protein in cesium sulfate (1.28 g cm⁻³), and activity is resistant to treatment with micrococcal nuclease. Although these observations suggested that the enzyme lacks an RNA component, an RNA does copurify with enzymatic activity – this RNA is not degraded by nuclease treatment. The gene encoding this nuclease-resistant, copurifying RNA has been cloned and sequenced, and contains clear similarity to both the H. volcanii and bacterial RNase P RNAs [8]. As in H. volcanii, catalytic activity has not been detected in RNA extracted from the holoenzyme or RNA synthesized in vitro from the cloned gene, nor has enzymatic activity been reconstituted from the synthetic RNA by the addition of RNase P protein from Bacteria. The additional component(s) of the archaean enzymes that are required for catalytic function have yet to be identified.

RNA structure

Despite the observation that the RNA components of archaean RNase P enzymes by themselves lack activity, they contain obvious similarity in sequence to their catalytically-proficient homologs from Bacteria, but not to those of Eukarya or organelles. The secondary structures of archaean RNase P RNA (see Figure 1) has been analyzed by comparative analysis of 11 complete and partial gene sequences [7]. Although not as well-defined as the model of the bacterial RNA (which is based on more than 100 sequences), all but one of the helices that make up the secondary structure (P10) are well-supported by the occurrence of evolutionary covariation in two or more base pairs.

The similarity of the archaean RNase P RNA secondary structure with that of the Bacteria is striking, especially in the phylogenetically conserved 'core' of the catalytically-active bacterial RNA. In fact, the archaean RNA structures seem qualitatively more like the common form of the bacterial RNA (e.g. that found in E. coli) than do some of the highly modified bacterial RNAs (e.g. those of Bacillus). The archaean structures lack P13 and P14 and the flanking nucleotides, but this domain is also absent in the RNAs of the low G+C Gram-positive Bacteria (e.g. Bacillus) and the α-purple Bacteria (e.g. Alcaligenes eutrophus); these bacterial RNAs are nevertheless catalytically active [10, 11]. The archaean RNAs also lack P18, which is absent in the catalytically-active RNA of the green sulfur Bacteria (Chlorobium) [12]. The P13–P14 and P18 domains are not essential for activity in the E. coli RNA [12, 13], and both are involved in tertiary interactions with P8, which in turn apparently plays a role in recognition of the T-loop of the pre-tRNA substrate [14]. The structure of the P15/P16/P17 region varies in the archaean RNAs; the loop between P15 and P16 in the bacterial RNA is involved in binding the 3' terminus of the substrate [15, 16]. Although many of the archaean RNAs are quite unlike the bacterial RNAs in this loop, those of the genus Methanobacterium conform to the bacterial consensus, but nevertheless are not catalytically active [7]. In fact, the archaean structures lack only one helix present in all bacterial RNase P RNAs, the 2bp helix P11, and contain most (88%) of the invariant nucleotides of the bacterial RNAs. Given the similarities between the secondary structures of the archaean and bacterial RNase P RNAs, the structural basis for the inability of the archaean RNAs to carry out catalysis is not obvious. Nevertheless, this catalyt-
Figure 1. Archaeal RNase P RNA secondary structure. The secondary structures of the *H. volcanii* and *S. acidocaldarius* RNase P RNAs are shown. Helix designations (P1–P18) are based on homology to the bacterial RNA [12], e.g., that of *E. coli*, which is shown for comparison. In the archaeal consensus, only those nucleotides present in all of the available archaeal sequences [7] are included; invariant nucleotides are shown as G, A, U or C, variable nucleotides as dots, and lines between bases indicate base-pairings supported by evolutionary covariation.
ic ‘defect’ in the archaeal RNAs must reside in the differences between these RNAs.

**Conclusion**

The unique feature of the archaeal RNase P enzymes are that their RNA components are not by themselves capable of catalysis despite their striking resemblance to their catalytically-active bacterial homologs. The step in the catalytic cycle that fails, the structural basis for this failure, and the means by which the presumed protein component overcomes this failure, are as yet unknown. Comparison of the structural and biochemical properties of catalytic vs non-catalytic RNase P RNAs will provide needed insight into the essential functionality of the enzyme.

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**References**