RNase P in Archaea: Bacterial-type RNA, eukaryal-type proteins.

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RNase P is a ribonucleoprotein, and the RNA subunits of RNase P enzymes from all organisms are recognizably similar in sequence and secondary structure (for review, see 1). The RNA is the catalytic subunit of the enzyme, and in Bacteria and some Archaea, the RNA is capable of catalyzing the cleavage of tRNA precursors in the absence of protein; these RNAs are 'ribozymes' (2,3). Although the eukaryotic RNase P RNAs are not by themselves catalytically active, it seems likely nevertheless that the RNA is the catalytic subunit in the enzyme (4).

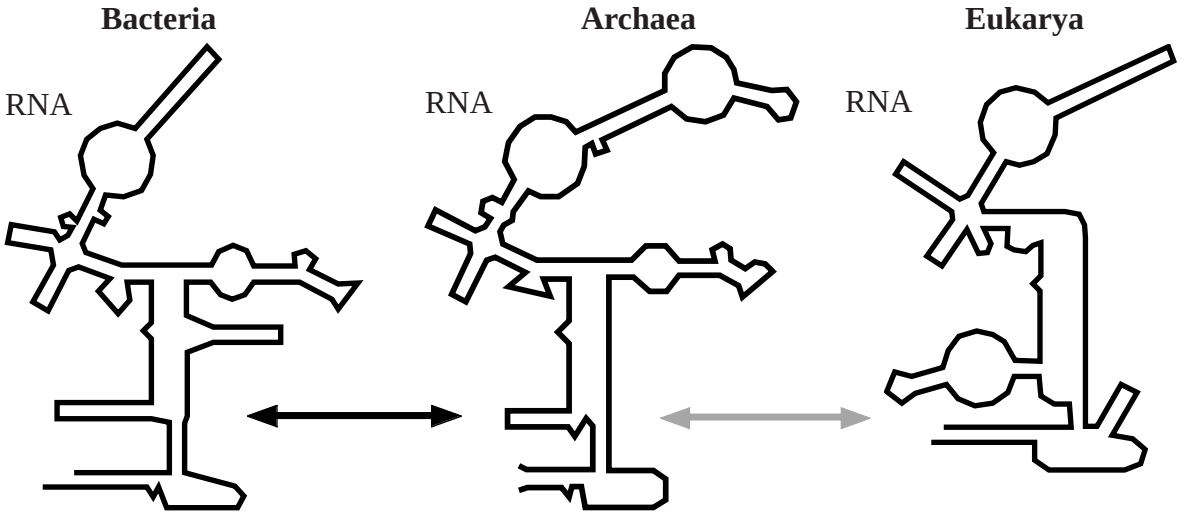
Archaeal RNase P enzymes have previously been characterized in only two species: *Haloferax volcanii* (5,6) and *Sulfolobus acidocaldarius*(7,8). Both RNase P enzymes contain RNAs, and these RNAs and those of other Archaea contain striking similarity in both sequence and secondary structure to bacterial RNase P RNAs, but not those of Eukarya. No proteins were identified as RNase P subunits in these enzymes.

The RNA component of the archaeal RNase P is much better understood than is the holoenzyme or protein component(s). We have determined the secondary structure of the RNA by comparative analysis; the resolution of the structure is similar to that of the model for the bacterial RNA secondary structure (9). The archaeal RNase P RNAs contain the sequences and structures of the bacterial, phylogenetically conserved catalytic core. Although the archaeal RNase P RNAs, like those of eukaryotes, were previously thought to depend absolutely on protein for activity, we have recently shown that those of the methanobacteria, thermococci, and halobacteria are catalytically active, in the absence of protein, in ionically extreme conditions: 3-4M ammonium acetate and 300-400mM $MgCl_2$ (3). The RNase P RNAs of the methanobacteria are the most active, and these are the archaeal RNAs that most closely resemble bacterial RNase P RNAs in sequence and secondary structure.

Although archaeal RNase P RNAs are similar in both sequence and structure to those of Bacteria rather than eukaryotes, and heterologous reconstitution between the *Bacillus subtilis* RNase P protein and some archaeal RNase P RNAs has been demonstrated (3), no archaeal protein sequences with similarity to any known bacterial RNase P protein subunit have been previously identified, and the density of *Methanothermobacter thermoautotrophicus* RNase P in Cs_2SO_4 (1.42 g/ml) is inconsistent with a single small bacterial-like protein subunit (10). Four hypothetical open reading frames (MTH11, MTH687, MTH688 and MTH1618) were identified in the genome of *M. thermoautotrophicus* that have sequence similarity to four of the nine *Saccharomyces cerevisiae* RNase P protein subunits: Pop4p, Pop5p, Rpp1p and Rpr2p, respectively (11). Polyclonal antisera generated to recombinant MTH11, MTH687, MTH688 and MTH1618 each recognized a protein of the predicted molecular weight in western blots of partially-purified *M. thermoautotrophicus* RNase P, and immunoprecipitated RNase P activity from the same partially-purified preparation. RNase P in Archaea is therefore composed of an RNA subunit similar to bacterial RNase P RNA and multiple protein subunits similar to those in the eukaryotic nucleus.

Current thought has it that RNase P in Bacteria is a primative form of the enzyme; the role of the RNA is predominant, minimally improved from the pre-protein "RNA World". RNase P in the

nucleus of eukaryotes, then, is thought of as more ‘advanced’; more complex, with the proteins having acquired more important roles in the enzyme. However, the presence of nuclear-like proteins in the archaeal RNase P, associated with a bacterial-like RNA, suggest that the bacterial protein is a simplification from the more primitive archaeal/nuclear protein complement. An understanding of the proteins and their role in RNase P function in Archaea will provide insight into the give-and-take between the modern RNA and Protein “worlds”.



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