

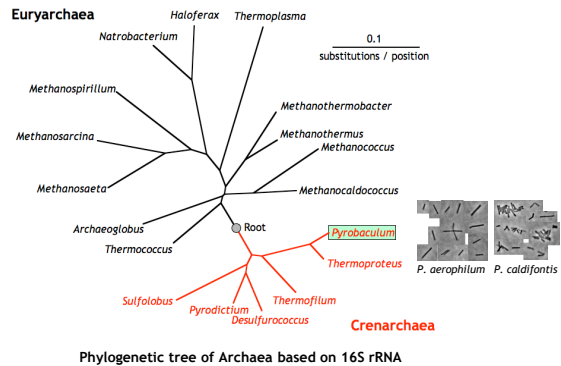
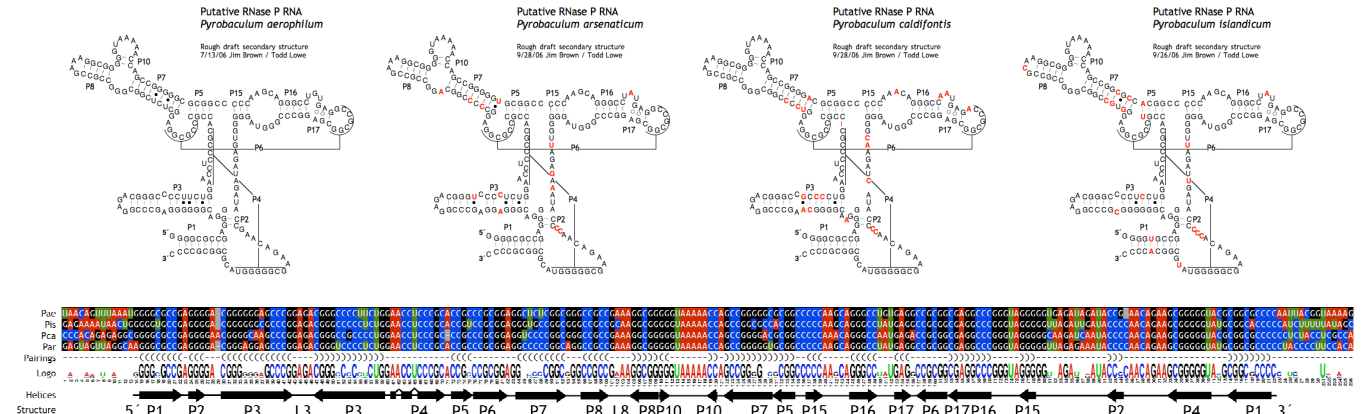
RNase P in *Pyrobaculum*?

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When the genome sequence of the hyperthermophilic archaea *Pyrobaculum aerophilum* was released in 2002, genes encoding neither the catalytically-active RNA subunit of the RNase P, nor any of the four highly-conserved protein subunits of the enzyme could be identified. Although essential for 5' processing in the biosynthetic pathway of transfer RNAs, the apparent lack of all of the subunits of this enzyme was rationalized by the observation that consensus promoters could be identified immediately upstream of most tRNA genes; it was thought that tRNAs must be transcribed without 5' leaders, and thus without the need for RNase P processing.

However, Todd Lowe's group at UC Santa Cruz have recently identified a conserved putative RNase P RNA gene in an "intergenic spacer" in all 4 currently available *Pyrobaculum* species genome sequences. This RNA contains most of the most highly conserved sequence and structural elements known to be directly involved in substrate binding and catalysis, but lacks the otherwise highly conserved second "S" domain involved in modulating substrate specificity and perhaps also containing the binding sites for the protein subunits.

We are currently testing the functional competence of this RNA, and cell extracts, using a series of potential substrates including tRNA precursors with very short leaders.



Putative RNase P RNA genes in *Pyrobaculum*

The complete genome sequences of 4 species of *Pyrobaculum* (*P. aerophilum*, *P. islandicum*, *P. arsenaticum* & *P. caldifontis*) contain a conserved sequence located in an intergenic region between hypothetical (unassigned) protein-coding sequences. The sequence, sequence variation, and potential secondary structure suggests that it encodes an RNase P RNA.

However, the encoded RNase P RNA would consist of only the catalytic domain of the RNA; the only remains of the "specificity domain" are P10 and P8 (or is this P9?), which together constitute the substrate T-loop binding elements. We can find no evidence for the presence of other genes encoding the S-domain of the RNA, nor for the 4 known archaeal RNase P protein subunits; however, it is believed that these proteins bind to the S-domain of the RNA.

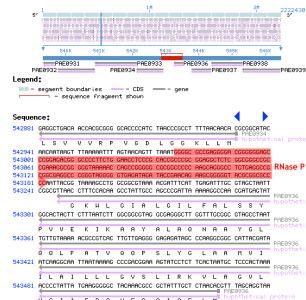
We are collaborating with the Todd Lowe lab at UC Santa Cruz to test whether or not this is a *bona fide* RNase P RNA gene. We are cultivating *Pyrobaculum aerophilum*, and *P. caldifontis*; these organisms are facultative microaerophilic heterotrophs that grow at 96 °C.

DNA from these cultures will be used to generate PCR products from which both putative RNase P RNAs and pre-tRNA substrates can be generated by *in vitro* transcription. The activity of these RNase P RNAs (if any) will be compared to bacterial and other archaeal RNase P RNAs in RNA-alone reactions.

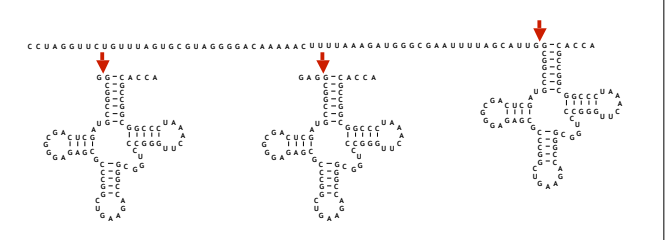
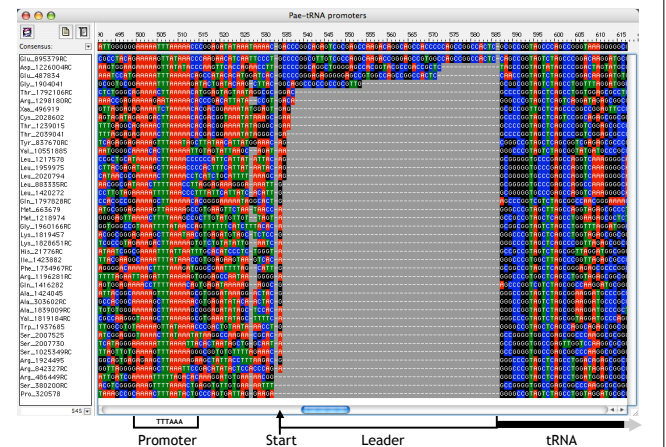
In addition, cell extracts will be fractionated by DEAE chromatography and assayed for RNase P activity. The properties of this activity (if any) will be compared to that of the RNA alone. Of particular interest will be the relative activities of the RNA alone vs the holoenzyme, the buoyant density of the holoenzyme (which reflects protein composition), and the comparison of substrate specificity in *Pyrobaculum* vs traditional RNase P enzymes.

Why is this so interesting?

All bacterial and most archaeal RNase P RNAs are catalytically active *in vitro* in the absence of protein. However, we have been studying the enzyme from *Methanococcus* and relatives; these archaeal RNase P RNAs lack essential elements of structure in the C-domain required for substrate recognition. We believe that these structural and functional roles of the RNA have been replaced by additional proteins. This serves as a model for understanding the evolutionary processes resulting in the takeover of the "RNA World" by the modern "DNA/RNA/Protein World". The *Pyrobaculum* system may represent either an even more dramatic replacement of RNA by protein, or a "de-volution" replacement of an RNA/protein RNase P with a specialized RNA-only enzyme.



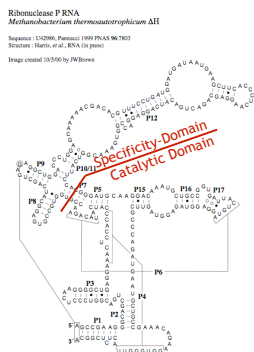
Are most tRNAs in *Pyrobaculum* transcribed with only 1- or 2-nt leaders?



Because most tRNAs in *Pyrobaculum* are predicted to have very short leaders, we have designed substrates based on the *P. aerophilum* tRNA^{Phe} that have 1-ny, 3-nt, or long leaders. These will be tested with RNase P RNAs and partially-purified enzyme preps from *P. aerophilum*, *P. caldifontis*, and *E. coli*.

What is RNase P?

Ribonuclease P (RNase P) is a ribonuclease, present in all cells, that removes 5' leader sequences from tRNA precursor transcripts. RNase P is composed of an RNA of ca. 400 nucleotides and a single protein in Bacteria or 9-12 proteins in the eukaryotic nucleus. The RNA is the catalytic subunit; RNase P is an 'ribozyme'. RNase P and other ribozymes are thought to be remnants of the 'RNA World', a stage in the emergence of life before the evolutionary invention of protein or DNA.



This is a typical "type A" bacterial or archaeal RNase P RNA, comprised of two independently folding domains, the "Specificity domain", and the "Catalytic domain".

RNase P in Archaea has properties of both the bacterial and eukaryotic forms of this enzyme. We previously found that the RNA subunit of archaeal RNase P is much like that of Bacteria, and quite different than that of eukaryotes. However, there are at least 4 proteins associated with this RNA, unlike the single small protein in Bacteria, and these proteins are homologs of 4 of the eukaryotic nuclear RNase P proteins, without similarity to the bacterial protein.