RNase P in Archaea

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Looking to a warm place for P
RNase P is present in all cells, and in mitochondria and plastids.

RNase P is the pre-tRNA 5´ endonuclease.

RNase P assay

RNase P is present in all cells, and in mitochondria and plastids.
Why study RNase P?

• RNase P is apparently a leftover of the ‘RNA World’, and so can teach us about the emergence of life and the origin of Bacteria, Archaea and Eukaryotes.

• RNase P is a ribozyme (RNA enzyme), and so can teach us about RNA structure and function.

• RNase P is highly conserved in Bacteria, and very different in eukaryotes, and like the ribosome is a large essential RNP, and so has great potential as a target for novel antimicrobials.

• RNases I, II, III, A, B, E, G, H, P1, P2, PH, S, T1, T2, U1, U2, V1, and Z were already taken.
RNase P in Bacteria

The RNA by itself is catalytically proficient \textit{in vitro}.

1 large RNA \textit{rnpB}
377nt/140kDa (blue & purple)

1 small protein \textit{rnpA}
119aa/14kDa (red)

Product mature tRNA
~76nt/24kDa (green)
RNase P in Eukarya (yeast nucleus)

One large RNA
Rpr1 - 120kDa
This RNA is only generally similar to the bacterial RNA

Nine assorted proteins
Pop1p - 100kD
Pop3p - 23kD
Pop4p - 33kD
Pop5p - 20kD
Pop6p - 18kD
Pop7p - 16kD
Pop8p - 15kD
Rpp1p - 32kD
Rpr2p - 16kD
None of these are at all similar to the bacterial protein

The RNA is absolutely dependent on the proteins for activity
Archaea are an ideal “missing link” for understanding the evolutionary differences between Bacteria and eukaryotes.
RNase P in Archaea - a Preview

A bacterial-like RNA...

rnpB - 96kDa (293nt)

A catalytically-active “type A” RNA in most cases, only distantly related to the eukaryotic RNA

but 4 eukaryotic-like proteins.

MTH687p - 15kDa = Pop5p
MTH688p - 28kDa = Rpp1p
MTH11p - 11kDa = Pop4p (C-terminal half)
MTH1618p - 17kDa = Rpr2p

Not at all like the single bacterial protein

RNase P in Archaea is an evolutionary chimera
The Archaea

Phylogenetic tree of the Archaea based on ssu-rRNA sequence analysis
### RNase P in Archaea: Early Information

<table>
<thead>
<tr>
<th><strong>Halofexx volcanii</strong></th>
<th><strong>Sulfolobus acidocaldarius</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.61 g/cm³ in Cs₂SO₄ (but also reported to be inactivated)</td>
<td>1.27 g/cm³ in Cs₂SO₄ (suggests protein alone)</td>
</tr>
<tr>
<td>rapid pelleting in glycerol</td>
<td>large exclusion radius (400kDa)</td>
</tr>
<tr>
<td>nuclease sensitive</td>
<td>nuclease resistant</td>
</tr>
<tr>
<td>copurifying 435nt RNA (degraded by nuclease treatment) only vaguely similar to other P RNAs</td>
<td>copurifying 315nt RNA (not degraded by nuclease treatment) only vaguely similar to other P RNAs</td>
</tr>
<tr>
<td>RNA not catalytically active</td>
<td>RNA not catalytically active</td>
</tr>
</tbody>
</table>

Early reports of catalytic activity by the RNA alone and reconstitution with bacterial protein could not be reproduced and were discounted.

The RNase P RNAs of *Methansarcina barkeri* and *Methanocaldococcus jannaschii* are also not catalytically active, leading to the conclusion that archaeal RNase P RNAs, like those of eukaryotes, absolutely depend on protein for function.

Our first task, then was to identify the differences in bacteria and archaeal RNase P RNAs that might be responsible for this difference in activity.
Archaenal RNase P RNA 2° structures

**Ribonuclease P RNA**

*Haloferax volcanii* DS-2


Structure: Harris, et al., RNA (in press)

Image created 10/5/00 by JW Brown

**Ribonuclease P RNA**

*Sulfolobus acidocaldarius*(solfataricus) P1


Structure: Harris, et al., RNA (in press)

Image created 10/5/00 by JW Brown

Determined to base-pair resolution by comparative sequence analysis
The type A structure is common to most Archaea and Bacteria
Some archaeal RNase P RNAs are active!

Assays contain 4M NH₄OAc, 300mM MgCl₂, a 200:1 ratio of enzyme to substrate, incubated for 3.5 hr at 45°C. *In vivo* or *in vitro* RNA.
Properties on archaeal RNase P enzymes

*Methanothermobacter thermoautotrophicus*

<table>
<thead>
<tr>
<th>Property</th>
<th>RNA-alone</th>
<th>Holoenzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$</td>
<td>$&gt;50,000\text{nM}$</td>
<td>34.5±3.5\text{nM}</td>
</tr>
<tr>
<td>$k_{\text{cat}}$</td>
<td>$&gt;2.2/\text{min}$</td>
<td>52.6±4.3/\text{min}</td>
</tr>
<tr>
<td>density</td>
<td>1.65</td>
<td>1.42</td>
</tr>
<tr>
<td>$T_{\text{opt}}$</td>
<td>~50°C</td>
<td>≥80°C</td>
</tr>
<tr>
<td>$[\text{NH}<em>4\text{OAc}]</em>{\text{opt}}$</td>
<td>3.0M</td>
<td>0.8M</td>
</tr>
<tr>
<td>$[\text{MgCl}<em>2]</em>{\text{opt}}$</td>
<td>300mM</td>
<td>5mM</td>
</tr>
<tr>
<td>exclusion volume</td>
<td>(nd)</td>
<td>450kDa</td>
</tr>
</tbody>
</table>

The archaeal RNA mimics the properties, including general lead cleavage sensitivity, of bacterial RNAs lacking peripheral stabilizing elements, but the fact that the RNA contains any activity implies that they have all of the sequences and structures required for substrate recognition and catalysis.
Chimeric holoenzymes are active

Functional reconstitution is reflected in the enhancement of activity in the presence of protein in assays performed at low ionic strength

Archaeal RNase P RNAs can bind the Bacillus subtilis RNase P protein, and the chimeric holoenzymes are functional!
Archaea ought to have a bacterial-like protein

...but, they don’t!

LOCUS  B.aphidocola       114 bp
DEFINITION B.aphidocola       114 b, 114 bases, 58069AB0 checksum.
ORIGIN
  1  MLNYFFKKS KLLKSTNFQY VFSNPCNKNT FHINILGRSN LLGHFRLGLS
  51  ISRKNIKHAY RNRKIKRLIR ETFRLQHRL ISMDFVVIAK KNIVYLNKK
 101  IVNILEYIWS NYQR
//
LOCUS  B.burgdorferi       119 bp
DEFINITION B.burgdorferi       , 119 bases, E73BF549 checksum.
ORIGIN
  1  MRKRNISLKS KIEIQKIFKE GKLIRFSNLN LKMFYKSNHL VYSRILVTFS
  51  KGFGRGSVKN RIRRFLKFEEF RKRLELELGI ALDIIFVVSY GKLTLTYFSI
 101  ESLMKGLVLR CERIGESK
//
LOCUS  B.halodurans       118 bp
DEFINITION B.halodurans       , 118 bases, FC638DB3 checksum.
ORIGIN
  1  MKKEHRIKRS DEFSRVFNEG FSVANQRFVI YVLPKEGQDF FRVLSVSikki
  51  GNATNVRNVRK LRLRFFQPHE EQAISGERDY VIARKPAAD MTEQVKGSL
 101  WHVCKKAKII QPKVRAHK
//
LOCUS  B.subtilis       119 bp
DEFINITION B.subtilis       119 bp, 119 bases, 724961C checksum.
ORIGIN
  1  MSHLKKRNRL KKNEDFQKFVF KHGTSVANQF FYLPTLDQPE NDELRVGLSV
  51  SKKIGNAVMR NRKRLFQKA FLEEKERLKE KDIYIIARKP ASQLYEEETK
 101  KSLQHFLRKS SLYKSSSSK
//
RNase P in Eukarya (yeast nucleus)

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- Rpr2p - 16kD
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Proteins identified in Dave Engelke’s lab
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Archaea encode a protein with vague similarity to yeast nuclear P protein Pop5p!
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Archaea encode proteins with vague similarity to 4 yeast nuclear P proteins!
Westerns using antisera specific to each protein reveal that all 4 proteins copurify exactly with RNase P activity in fractions from glycerol gradients of partially-purified RNase P.
Activity can be reconstituted from the archaeal RNA and these 4 proteins

Kouzuma, et al., (Makoto Kimura lab) 2003 BBRC 306:666-673

This is the *Pyroccoccus horikoshi* system, but it also works in *Methanothermobacter thermoautotrophicus*. 
The interactions between homologous components are conserved.
The archaeal proteins are not similar in structure to the bacterial P protein. This argues against any underlying homology between the bacterial and any of the archaeal proteins.

- **Mth11p/Pop4p** (Archaeoglobus fulgidus) - an oligonucleotide-binding β-barrel
- **Mth688p/Rpp1p** (Pyrococcus horikoshii) - a TIM metallohydrolase α/β-barrel
- **Mth1618p/Rpr2p** (Pyrococcus horikoshi) - an EF-G α/β-sandwich
- **Mth687p/Pop5p** (Pyrococcus furiosus) - an RNR α/β-sandwich

**bacterial rnpA** (Thermotoga maritima) - an EF-G α/β-sandwich
Possible evolutionary scenarios: RNA

This scenario is uncontroversial
In this scenario, the single small bacterial protein represents evolutionary **streamlining** from a more complex archaeal-like primitive state.
Possible evolutionary scenarios: Protein

In this scenario, the LCA is closer to the RNA World, with an RNA-only RNase P, and the 2 emerging branches recruited proteins independently.
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Our hypothesis is that in type M enzymes, protein sequences directly replace the structure and functions lost in the RNA.
**Pyrobaculum** RNase P lacks essential structure

All 4 RNase P protein-encoding genes are also absent

Putative RNase P RNA

*Pyrobaculum aerophilum*

Rough draft secondary structure

7/13/06 Jim Brown / Todd Lowe

But each tRNA gene is directly preceded by a promoter; so what is the substrate?
RNA Ontology and the RNase P Database
Acknowledgements

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Fall 2006 Meeting
North Carolina Branch of the American Society for Microbiology
The JC Ralston Arboretum
NC State University, Raleigh, NC
Thursday, October 12, 2006

Abstract deadline : September 28th
Registration deadline : Oct 5 (or at the door)
Registration fee + 2006 NC ASM membership

The featured speaker this year will be Dr. William Shafer from the Emory University School of Medicine

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