Pfu RNase P cleavage and the function of protein subunits

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Ribonuclease (RNase) is an enzyme that catalyzes the breakdown of RNA into smaller components. RNase P is responsible for cleaving the phosphate backbone at the 5' leader sequence of precursor tRNAs and leaving matured tRNAs with phosphate at their 5’ ends. It is unique from other RNase because RNase P is a ribozyme whose RNA molecule can act as an enzyme. RNase P is composed of one RNA and a varying number of protein subunits depending on the source. Irrespective of the source, the RNA is the catalytic subunit and it can mediate cleavage at the correct position in the absence of protein. The function of RNase P RNA is highly dependent on the divalent metal ions that bind to RNase P RNA, for example Mg^{2+}, which plays vital roles in RNase P RNA structure and catalysis. *In vivo*, both RNase P RNA and protein subunits are necessary, because RNase P protein is important to enhance the substrate binding affinity and increase the metal ion affinity in the active site.

Available data suggest that RNase P recognizes the L-shape structure of all precursor tRNAs. The interaction between the precursor tRNA and RNase P RNA has been demonstrated to include: the interaction between the T-stem-loop region (TSL-region) of the precursor tRNA and its binding site of RNase P RNA; the -1 position of precursor tRNA (residue immediately upstream of the RNase P cleavage site) interacting with the conserved A_{248} (M1 RNA numbering) in RNase P RNA; and the RCCA-motif at the 3’ end of the precursor tRNA pairing with a conserved GGU-motif in the P15-loop region in RNase P RNA.

On the basis of studies of bacterial RNase P RNA (M1 RNA) cleaving short model substrates which have been shown to be RNase P substrate, we used *Pyrococcus furiosus* RNase P as an archaeal model and demonstrated that *Pfu* RNase P RNA (*Pfu* RPR) can also cleave model hairpin loop substrates and compared the cleavage site recognition with M1 RNA. We have also indicated that *Pfu* RNase P proteins (*Pfu* RPPs) are able to enhance the cleavage of the model substrates by *Pfu* RPR and investigated the influence of *Pfu* RPPs on cleavage site selection during processing of model hairpin substrates by *Pfu* RPR. Finally, we also introduced different A_{248} point mutations (M1 RNA numbering, in *Pfu* RPR A_{228}) to *Pfu* RPR and discussed the interaction between the residue at the -1 position of the substrate and the conserved A_{248} in archaeal RNase P RNA.

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