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Hyperprocessing reaction of tRNA by *Bacillus subtilis* ribonuclease P ribozyme

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Ribonuclease P (RNase P) is one of tRNA-processing enzymes to produce mature tRNA molecules by cleaving the tRNA precursor at the 5′-end [1]. RNase P is one of the ribonucleoprotein enzymes, consisting of one RNA subunit and one or more protein subunit(s). The RNA subunit of bacterial and of some archaeal RNase P is a ribozyme, being able to perform the RNase P activity without the protein component [1].

Among many RNase P enzymes, two bacterial enzymes from *Escherichia coli* and *Bacillus subtilis* have been focused. The bacterial P ribozymes accept and cleave hairpin RNAs with CCA-3′ tag sequence as well as tRNA precursor as substrate. If the mature tRNA molecule contains unexpected self-complementary regions which contribute to disruption of the cloverleaf structure and to formation of another hairpin structure with CCA-3′ tag, the newly formed hairpin can be a substrate for RNase P reaction. Recently, we found that some eukaryotic tRNAs, containing such self-complementary regions, are internally cleaved at around the anticodon stem region by *E. coli* RNase P RNA (see Fig. 1; [2–4]). We denoted this unusual cleavage reaction as hyperprocessing [2]. Until now, three hyperprocessible tRNAs are experimentally found: *Drosophila* alanine, histidine, and initiator methionine tRNAs [2,4]. All these hyperprocessing reactions are performed by *E. coli* RNase P RNA. Here, we have a question that 'an RNase P ribozyme from other species can catalyze the hyperprocessing reaction?'

In this paper, we examined the hyperprocessing activity of *B. subtilis* RNase P ribozyme with hyperprocessible tRNAs in vitro, and found that the *B. subtilis* ribozyme also performed the cleavage activity. We think that the study of the hyperprocessing reaction of tRNAs will reveal the hidden features of bacterial RNase P ribozymes, especially of the profound substrate recognition mechanism.

We cloned *B. subtilis* RNase P RNA gene onto a plasmid pGEM-3Z from *B. subtilis* cells according to the sequence obtained on the web site. We also prepared the *E. coli* RNase P RNA for control experiments. These RNase P RNAs were prepared by in vivo transcription.

Two tRNA precursors, pre-tRNA^{Ala} and pre-tRNA^{His}, were prepared to examine the enzyme activities. Two tRNAs, tRNA^{Ala} and tRNA^{His}, are hyperprocessible tRNAs: the internal complementary regions (G^{43}–U^{47} and A^{67}–C^{71} in tRNA^{Ala}; G^{37}–G^{43} and U^{66}–C^{72} in tRNA^{His}) contribute to the tRNA formation change. In case of *E. coli* RNase P RNA reactions, disruption of the cloverleaf structure and newly formation of the double-hairpin structure of tRNA^{Ala} and tRNA^{His} occur in vitro to be detected as internally cleaved products [4]. These tRNA precursors contain natural mature sequences and additional 19 or 20 nucleotides at the 5′-flanking region. These tRNAs were also prepared by in vitro transcription from plasmid DNAs.

The results are shown in Fig. 1. The reactions were done under magnesium ion-containing conditions (5 or 60 mM). The *B. subtilis* enzyme as well as *E. coli* enzyme were catalytically active to produce mature tRNAs from the precursor substrates in the presence of 60 mM magnesium ion in every case (see the band 'mat' in lanes 3, 5, 11, and 13). Under 5 mM magnesium-containing conditions, the *E. coli* enzyme was slightly more active than the *B. subtilis* enzyme (see the band 'mat' in lanes 2, 4, 10, and 12). The results also indicated that the *B. subtilis* enzyme also performed the hyperprocessing activity (see the band 'hyp' in lanes 5 and 13). Compared with the cleavage sites by *E. coli* enzyme, the *B. subtilis* enzyme demonstrated almost the same cleavage pattern. These results strongly suggest that the substrate recognition mechanisms of the *B. subtilis* enzyme and the *E. coli* enzyme are quite similar to each other, although the secondary structures of these enzymes are not the same. Both enzymes accepted and catalyzed the unusual hairpin RNAs with CCA-3′ tag sequence as well as natural tRNA precursors.

The similarity of results by the *E. coli* enzyme and the *B. subtilis* enzyme also indicates that the cloverleaf structures of *Drosophila* tRNA^{Ala} and tRNA^{His} are not stable under the condition of high magnesium concentration. We suppose that magnesium ion can be toxic to the stability of tRNA molecules when its concentration is high.

The above results strongly suggest that every bacterial RNase P ribozyme contains the hyperprocessing activity. We think that the hyperprocessible activity may be a common feature of bacterial RNase P ribozyme and a useful tool for analysis of catalytic features of RNase P enzymes.

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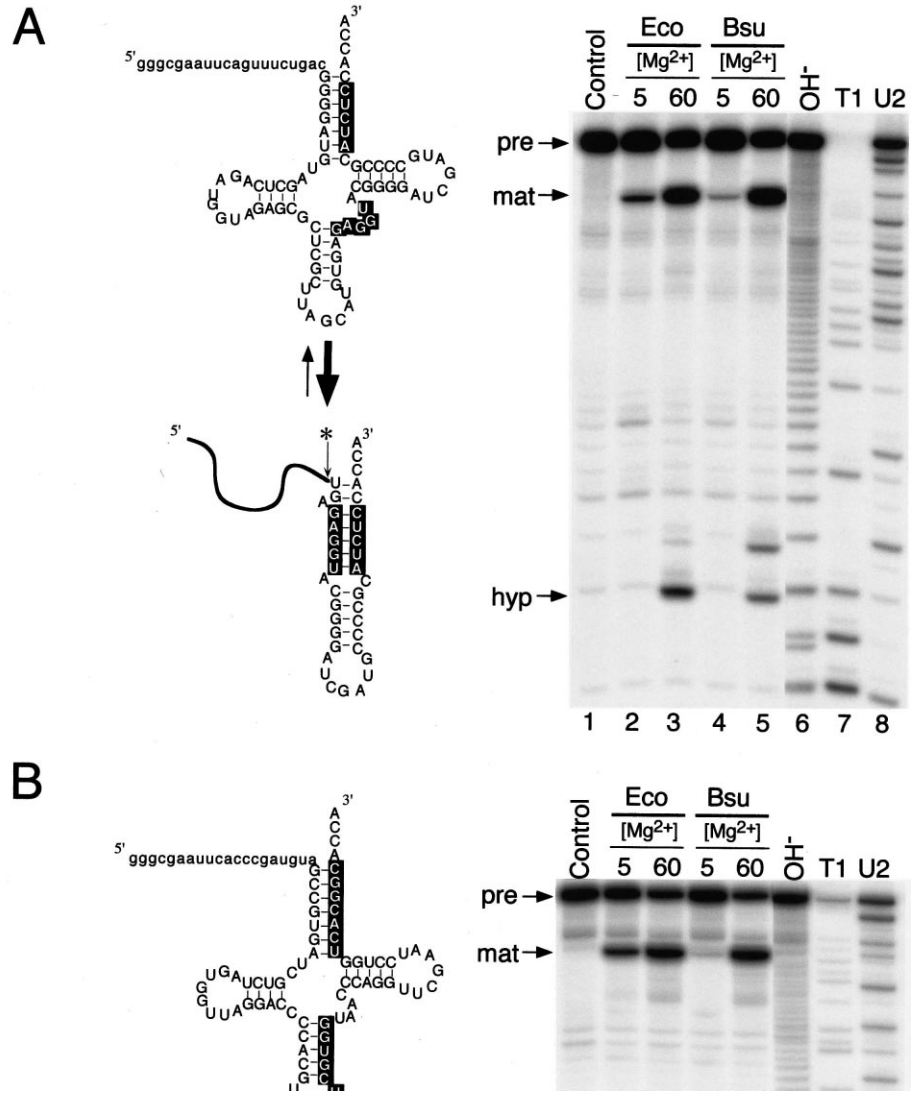

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