Note

Escherichia coli tRNAs Are Resistant to the Hyperprocessing Reaction of Homologous E. coli Ribonuclease P Ribozyme

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Bacterial ribonuclease P RNA ribozyme can do the hyperprocessing reaction, the internal cleavage reaction of some floppy eukaryotic tRNAs. The hyperprocessing reaction can be used as a detection tool to examine the stability of the cloverleaf shape of tRNA. Until now, the hyperprocessing reaction has been observed in the heterologous combination of eukaryotic tRNAs and bacterial RNase P enzymes. In this paper, we examined the hyperprocessing reaction of Escherichia coli tRNAs by homologous E. coli RNase P, to find that these homologous tRNAs were resistant to the toxic hyperprocessing reaction. Our results display the evidence for molecular co-evolution between homologous tRNAs and RNase P in the bacterium E. coli.

Key words: tRNA; hyperprocessing; ribonuclease P; evolution; Escherichia coli

Many biomolecules exist and function in the cell, and most of them work together in harmony. If unexpected interactions between some molecules might occur, the biological system of the cell should be disrupted. All or most molecules in the cell, therefore, should have co-evolved through their evolutionary process. Especially, in the case of functional basic molecules, the co-evolutionary processes should be very important. Through studies on transfer tRNA (tRNA) and ribonuclease P (RNase P) molecules, we found some experimental evidence for co-evolution between the tRNAs and RNase P on the molecular level.

Transfer RNA molecules are basic, ubiquitous molecules, and are important in the protein synthesis system. In most cases, the tRNA molecule has a common cloverleaf structure, consisting of four major stems, three major loops, and one extra variable loop. The cloverleaf structure of tRNA molecules are highly conserved beyond the species.7

RNase P is also a ubiquitous molecule, and is one of the tRNA-processing enzymes that produce mature tRNA molecules by cleaving the tRNA precursor at the 5'-end.2,3 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, with RNase P activity when without the protein component.2,4 Among many RNase P enzymes, two bacterial enzymes from Escherichia coli and Bacillus subtilis have been focused on. The bacterial P ribozymes accept and cleave hairpin RNAs with the CCA-3' tag sequence as well as the tRNA precursor as substrates (see Fig. 1A). The former activity of the bacterial RNase P enzymes sometimes results in an unusual cleavage reaction of mature tRNA molecules. If the mature tRNA molecule contains unexpected self-complementary regions that contribute to disruption of the cloverleaf structure and to formation of another hairpin structure with the 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 and/or B. subtilis RNase P RNA(s).8,10 We named this unusual cleavage reaction hyperprocessing (see Fig. 1B).10 Until now, four hyperprocessable eukaryotic tRNAs have been found experimentally: fly alanine, histidine,9 initiator methionine,10 and human tyrosine tRNAs.10 In the cases of fly alanine and histidine tRNAs, natural molecules containing the full base modifications are also hyperprocessed.10 Through experimental studies, we have extracted the criteria for the hyperprocessing reaction as the common features of the hyperprocessed tRNAs (see Fig. 1C). All these hyperprocessing reactions are observed in the heterologous combination of tRNA and RNase P. Why do such unusual cleavage reactions of tRNA molecules by bacterial RNase P occur? We think that is the result of diversity of evolution of tRNA and RNase P molecules in

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Abbreviations: RNase P, ribonuclease P; tRNA, transfer RNA
Hyperprocessing Reaction in Homologous Combination

(A) The bacterial RNase P cleaves the 5'-leader sequence in the tRNA precursor (left) as well as 5'-region in the hairpin RNA with a CCA-3' tag sequence (right). (B) In the case of Drosophila tRNA\textsuperscript{\textsubscript{1173}}, the indicated sequences (G\textsuperscript{17}-G\textsuperscript{40} and U\textsuperscript{64}-C\textsuperscript{67}) contribute to the conformational change of the tRNA to the denatured hairpin structure, the hyperprocessible folding, to be internally cleaved at the indicated site as a 'hypr' (G\textsuperscript{50}-G\textsuperscript{77} bond). (C) The criteria for the hyperprocessible tRNAs extracted from the experimentally hyperprocessed tRNAs and their base-replaced mutants. In every case, the 3'-half of the tRNA molecule has the ability to form another large hairpin, containing the T-arm as a core hairpin. In the region N\textsuperscript{64}-N\textsuperscript{67}, consisting of the anticodon-stem strand and the extra variable loop region, the complementary bases to the region N\textsuperscript{64}-N\textsuperscript{67} should be present to form a new helix adjacent to the T-arm. In the newly formed helix (right), a one-base mismatch (only one base mismatch) can be permitted. If a tRNA fits these criteria, the 3'-half of the tRNA has the power to form a new helix, which should be recognized as a substrate by the bacterial RNase P, and is expected to be hyperprocessed.

different species: the eukaryotic tRNAs have co-evolved with the eukaryotic RNase P, not with the bacterial enzyme, while, the bacterial tRNAs have co-evolved with the bacterial RNase P. Unexpected heterologous encounter between the eukaryotic tRNAs and the bacterial RNase P in vitro, we think, may trigger the hyperprocessing reaction of tRNAs by the RNase P.

Recently, we have found that a tRNA derived from \textit{Acholeplasma laidlawii}, a Firmicutes-group bacterium, was also hyperprocessed by the \textit{E. coli} and \textit{B. subtilis} RNase P enzymes.\textsuperscript{13} The results indicated that not only eukaryotic tRNAs are hyperprocessible.

Here, we have another question: are the bacterial tRNAs are cleavable by the homologous bacterial RNase P? In this paper, we examined the bacterial RNase P reaction of the bacterial tRNAs, in the homologous combination of \textit{E. coli} tRNAs and \textit{E. coli} RNase P. Our results will show some experimental evidence for the molecular co-evolution between the homologous RNase P and tRNAs.

The occurrence of the hyperprocessing reaction of tRNA depends on the stability of the cloverleaf shape of tRNA and also on the interaction between tRNA and RNase P molecules. The hyperprocessing reaction requires a higher magnesium ion concentration than the normal processing reaction.\textsuperscript{9,13} The higher concentration of the magnesium ion contributes to the tRNA-RNase P interaction,\textsuperscript{15} which allows the unusual hyperprocessing reaction. The hyperprocessing reaction of tRNA by \textit{E. coli} RNase P was reported first, and then the reaction by the \textit{B. subtilis} enzyme followed.\textsuperscript{9} The comparison of the \textit{E. coli} and \textit{B. subtilis} enzymes indicates that the \textit{E. coli} enzyme is more active in the hyperprocessing reaction as well as in the normal processing reaction.\textsuperscript{9,13} For example, in the case of fly initiator methionine tRNA, three cleavage sites are observed in the reaction by the \textit{E. coli} enzyme, while only one cleavage site is slightly observed in the reaction by the \textit{B. subtilis} enzyme.\textsuperscript{15} In every hyperprocessing reaction examined, the \textit{E. coli} enzyme displayed more effective hyperprocessing activity. In other words, the \textit{E. coli} RNase P is a sharper 'cleaver' in the RNase P reaction. For these reasons, we have decided to examine the RNase P reaction of the \textit{E. coli} tRNAs in the homologous combination.

If the \textit{E. coli} RNase P is an absolute cleaver, the \textit{E. coli} cells shall not have any floppy tRNA molecules. According to the criteria, the extracted common features from the hyperprocessed tRNAs, we have searched over \textit{E. coli} tRNA sequences to find that six tRNAs fit the criteria (not shown). Among them, we have prepared three candidate tRNAs to examine the hyperprocessing reaction: tRNA\textsuperscript{\textsubscript{3s}} (Fig. 2A), tRNA\textsuperscript{\textsubscript{3e}} (Fig. 2B), and tRNA\textsuperscript{\textsubscript{3o}} (Fig. 2C). In tRNA\textsuperscript{\textsubscript{3s}}, the regions G\textsuperscript{42}-G\textsuperscript{70} and U\textsuperscript{69}-C\textsuperscript{75} are complementary, in tRNA\textsuperscript{\textsubscript{3e}}, the regions U\textsuperscript{59}-G\textsuperscript{46} and U\textsuperscript{68}-A\textsuperscript{73} are complementary, and in tRNA\textsuperscript{\textsubscript{3o}}, the regions C\textsuperscript{50}-G\textsuperscript{46} and C\textsuperscript{56}-G\textsuperscript{72} are complementary. The three suspicious tRNAs are expected to be hyperprocessed by the \textit{E. coli} RNase P at around the 5'-edge of the newly formed 3'-hairpin.
Fig. 2. RNase P Reactions of E. coli tRNAs.

The suspected E. coli tRNAs used in this study: (A) tRNA\textsuperscript{5\prime} (the regions G\textsuperscript{5\prime}-G\textsuperscript{3\prime} and U\textsuperscript{5\prime}-C\textsuperscript{3\prime} are expected to have the power to form a helix), (B) tRNA\textsuperscript{U2} (the regions U\textsuperscript{5\prime}-G\textsuperscript{3\prime} and U\textsuperscript{6\prime}-A\textsuperscript{3\prime} are for a helix), and (C) tRNA\textsuperscript{Eco} (the regions C\textsuperscript{5\prime}-G\textsuperscript{3\prime} and C\textsuperscript{6\prime}-G\textsuperscript{3\prime} are for a helix). The numbers for base position are according to Sprinzl et al.\textsuperscript{11}

The E. coli and B. subtilis ribonuclease P RNAs were prepared by \textit{in vitro} transcription with T7 RNA polymerase (\textit{T7 RNA polymerase}, Toyobo) using cleaved DNA templates as described previously.\textsuperscript{16} Each of the tRNA precursors was also prepared by \textit{in vitro} transcription using a cleaved DNA template derived from a commercial plasmid DNA, pGEM-3Z (Promega), on which the synthetic DNA fragment was cloned with EcoRI and HindIII restriction sites: 5\textsuperscript{\prime}-AATTC ACCCG ATGTA CGCGG AGGGG CGGAG GCCG ACCG ACTGG CCTCA AACA GTTGCC GTATC CAAAG TGGCC TACTC GCCGT ACATC GGGTG-3\textsuperscript{\prime} (for E. coli pre-tRNA\textsuperscript{5\prime}), 5\textsuperscript{\prime}-AATTC ACCCG ATGTA GGAGA GATGC GCTAC GCCGG GTATC CAAAG TGGCC TACTC GCCGT ACATC GGGTG-3\textsuperscript{\prime} (for B. subtilis pre-tRNA\textsuperscript{U2}), and 5\textsuperscript{\prime}-AATTC ACCCG ATGTA GGAGA GATGC GCTAC GCCGG GTATC CAAAG TGGCC TACTC GCCGT ACATC GGGTG-3\textsuperscript{\prime} (for B. subtilis pre-tRNA\textsuperscript{Eco}). The cloned sequences were confirmed by DNA sequencing. tRNA precursors were labeled at the 5\textsuperscript{\prime}-end with [\textsuperscript{\gamma}\textsuperscript{32}P]ATP and T4 RNA ligase as described according to the methods described previously. The hyperprocessing reaction was done at 37\textdegree C, for 120 minutes in a 10\textmu M reaction scale, under the standard conditions containing 5 or 60 mM Mg\textsuperscript{++} (0.36 \textmu M E. coli or B. subtilis RNase P RNA, 10-12 \textmu M RNA substrates, 100 mM NH\textsubscript{4}Cl, 5 or 60 mM MgCl\textsubscript{2}, 5% [w/v] polyethylene glycol, 50 mM Tris-HCl; pH 7.6), and the reaction products were developed on 20% PAGE containing 8 M urea, and were analyzed as described previously.\textsuperscript{16}

‘Pre’ and ‘mat’ on the left side of the photos represent the tRNA precursor and the mature tRNA, respectively. ‘Eco’, ‘Bsu’, ‘OH’-, ‘T1’, and ‘U2’ above the photos represent the products by E. coli RNase P, the products by B. subtilis RNase P, partially alkaline hydrolyzed size marker, the RNase T1 hydrolyzed size marker, and the RNase U2 hydrolyzed size marker, respectively. The ‘G’-mapping was done according to Gross et al.\textsuperscript{16} The numbers on the right side of the photos represent the base positions of the tRNA molecules. Triangles on the left side of the photo indicate the putative cleavage site by the bacterial RNase P in the reaction.
The results of the RNase P reactions of these E. coli tRNAs are shown in Fig. 2. The RNase P reactions were done by both E. coli and B. subtilis RNase P RNAs. The normal processing reaction, the cleavage of the 5'-leader sequence in the tRNA precursor, was efficiently done in every case by both E. coli and B. subtilis RNase P RNAs. These results indicate that the RNase P RNAs were 'active', while no hyperprocessed product of E. coli tRNA was detected. These results showed that the E. coli tRNAs were resistant to the hyperprocessing reactions, and showed that none of three tRNAs were by the hyperprocessible folding. These results are consistent with our model in which the E. coli tRNAs should be resistant to the hyperprocessing reaction by the homologous RNase P. Considering that the B. subtilis enzyme is less active in the RNase P reaction than the E. coli enzyme, these E. coli tRNAs being resistant to the hyperprocessing reaction by the B. subtilis enzyme is understandable.

The bacterial RNase P can accept a hairpin RNA with a CCA-3' tag sequence as a substrate. This feature provides the bacterial RNase P the hyperprocessing activity, an unusual internal cleavage of tRNA. The hyperprocessing activity of the RNase P must be toxic if all homologous tRNAs were hyperprocessible. So, the bacterial tRNAs must be resistant to the hyperprocessing reaction by the homologous RNase P. Our results indicated that the E. coli tRNAs were resistant to the hyperprocessing reaction by the E. coli RNase P. Our data suggest that a bacterium which has a sharp clearer (RNase P), tends to have tight and rigid tRNAs. We think that it is the result of co-evolution between the tRNAs and RNase P molecules: the strong 'shield' protects tRNA against the sharp 'sword' of RNase P activity. This model can also explain the reason of the hyperprocessing reaction of some eukaryotic tRNAs by bacterial RNase P enzymes. The eukaryotic RNase P enzymes recognize only tRNA precursors, not a hairpin RNA, and therefore do not perform hyperprocessing activity. Therefore, the eukaryotic RNase P permits the presence of some floppy, hyperprocessible tRNAs in the cells. As a result, the eukaryotic cells contain some floppy tRNAs which will be hyperprocessed by the bacterial RNase P, when they encounter the heterologous combination in vitro.

In this time, we showed the phenomenon that E. coli tRNAs were resistant to the internal cleavage reaction by the homologous RNase P, the criteria for the occurrence of the hyperprocessing reaction were updated: tRNA should not be homologous with the RNase P. We think there must be molecular strategies for the stabilization of the cloverleaf shape of the E. coli tRNAs. According to our 'double-hairpin intermediate' model, the presence of the long extra loop should be one answer. Moreover, some base-specific interactions might occur between E. coli tRNAs and the E. coli RNase P RNA. The studies using the hyperprocessing strategy will reveal the answer in the future.

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