We have developed the hyperprocessing technique to evaluate the stability of the cloverleaf shape of pre-transfer RNA (pre-tRNA). Application of this strategy to hyperprocessible human tyrosine pre-tRNA indicated that the natural intron sequence did not contribute to stabilization of the cloverleaf shape of this pre-tRNA, while the artificial intron with elongated anticodon-stem completely inhibited hyperprocessing of it. Our data suggested that the contemporary intron sequence may be a vestige of the ancient pre-biotic world, but not has been retained as a temporal stabilizer of the pre-tRNA before the base modifications.

Key words: tyrosine transfer RNA (tRNA); hyperprocessing; ribonuclease P; Escherichia coli

What is the role of the tRNA intron sequence? To tackle this unsolved problem, we applied the hyperprocessing technique to the intron-containing human tyrosine pre-tRNA. The hyperprocessing technique is a shape monitoring tool of pre-tRNA utilizing the substrate shape dependence of the bacterial ribonuclease P (RNase P) ribozyme on magnesium ion concentration. At low concentrations of magnesium ions below 10 mM, the RNase P ribozyme accepts only canonical cloverleaf shaped pre-tRNA as a substrate. At higher concentrations of magnesium ions of 10 mM or more, the ribozyme also accepts a hairpin RNA with a CCA-3’ tag sequence as well as cloverleaf pre-tRNA as a substrate.1–4) The presence of higher concentrations of magnesium ions also affects the stability of the cloverleaf (pre-)tRNAs to raise the content of misfolded or denatured RNAs to other conformations. Sometimes the 3’-half region of the denatured (pre-)tRNA forms a hairpin shape, and the newly formed hairpin derived from (pre-)tRNA is recognized as a substrate by the ribozyme and is cleaved internally. So the combination of the presence of high concentration of magnesium ion for reaction conditions and the presence of the bacterial ribozyme can be applied to examine the stability of the cloverleaf shape of (pre-)tRNA under crucial conditions.3–13)

The results indicated that human tyrosine pre-tRNA and the A44U mutant lacking the intron sequence were hyperprocessible (Fig. 2B, lanes 3 and 8). Comparison of the contents of the products indicated that the helix formation of regions U39–G46 and U66–A73 contributed to the occurrence of hyperprocessing of these RNAs. The results showed that the natural intron-containing pre-tRNA ‘(+)-intron’ was also hyperprocessed at the same sites with the ‘(−)-intron’ pre-tRNA (Fig. 2B, lanes 3 and 13). These results indicated that the presence of

Note

The Natural Intron Sequence of Human Tyrosine Pre-transfer RNA Is Not a Temporal Stabilizer for Cloverleaf Structure

Tomoaki ANDO, Terumichi TANAKA,† and Yo KIKUCHI

Division of Bioscience and Biotechnology, Department of Ecological Engineering, Toyohashi University of Technology, Tempakuchō, Toyohashi, Aichi 441-8580, Japan

Received December 26, 2003; Accepted May 11, 2004

We have reported several examples of hyperprocessible tRNAs, the criteria for the occurrence of the hyperprocessing reactions, and some applications of this technique to evaluate the stability of the cloverleaf structure of tRNA.3,11) Human tyrosine (pre-)tRNA is a hyperprocessible tRNA.3,7,10) The complementary regions U39–G46 and U66–A73 contribute to form an alternative hairpin at high concentrations of magnesium ions, and the newly formed hairpin is a substrate for the ribozyme and is cleaved (Fig. 1). In this study, we focused on the role of the tRNA intron and examined the contribution of the extra sequence of this pre-tRNA to the stabilization of the cloverleaf shape of hyperprocessible pre-tRNA.

Figure 2A shows the cloverleaf shape of human pre-tRNA37‘ (+)-intron’) with the natural intron sequence ‘(−)-intron’) and the artificial intron sequence ‘art-intron’). The natural intron sequence is inserted between the bond G37–A38 to form a bulged hairpin in the anticodon-loop of tRNA. The artificial intron-containing pre-tRNA has a long anticodon stem without bulges. Additionally we prepared another RNA, ‘A44U’, to confirm the hyperprocessible hairpin formation of this pre-tRNA. In this study, we used the E. coli RNase P ribozyme as a cleaver. The results of the RNase P reactions are summarized in Fig. 2B. The reactions at 0 or 5 mM magnesium ions are for control reactions, and those at 60 mM are for hyperprocessing reactions. We chose these concentrations because higher concentrations of magnesium ions promote the occurrence of hyperprocessing reactions.

The results indicated that human tyrosine pre-tRNA and the A44U mutant lacking the intron sequence were hyperprocessible (Fig. 2B, lanes 3 and 8). Comparison of the contents of the products indicated that the helix formation of regions U39–G46 and U66–A73 contributed to the occurrence of hyperprocessing of these RNAs. The results showed that the natural intron-containing pre-tRNA ‘(+)-intron’ was also hyperprocessed at the same sites with the ‘(−)-intron’ pre-tRNA (Fig. 2B, lanes 3 and 13). These results indicated that the presence of

† To whom correspondence should be addressed. Fax: +81-532-44-6929; E-mail: tanakat@eco.tut.ac.jp

Abbreviations: RNase P, ribonuclease P; tRNA, transfer RNA
the natural intron sequence did not prevent the occurrence of hyperprocessing reaction of this pre-tRNA, but slightly enhanced the occurrence of the hyperprocessing. On the other hand, the results for the artificial intron-containing pre-tRNA showed that this RNA was completely resistant to hyperprocessing (Fig. 2B, lane 18). These data indicated that the elongated anticodon-stem can stabilize the cloverleaf structure of this pre-tRNA.

The data showed, then, that the natural intron sequence of human tyrosine pre-tRNA made no contribution to the stabilization of the cloverleaf shape, at least in this case. Considering the results for the artificial intron-containing pre-tRNA, the contemporary tRNA intron sequence is not a temporal stabilizer of pre-tRNA before base modifications, but may be a vestige of stabilizing sequences in the pre-biotic world. Our hyperprocessing technique might indicate the roles of extra sequences of pre-tRNA molecules.

Acknowledgments

We are thankful to Ms. Etsuko Sakai for radioisotope operations. This work was supported by a Grant-in-Aid for Scientific Research in Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1) Pace, N. R., and Brown, J. W., Evolutionary perspective on the structure and function of ribonuclease P, a
Fig. 2. RNase P Reactions of Human Tyrosine Pre-tRNA Derivatives.
(A) Pre-tRNA and its derivatives used in this study. The genes coding for tRNA precursors and tRNA-derived RNAs were constructed using synthetic DNAs with restriction sites onto a commercial plasmid DNA, pGEM-3Z (Promega). The nucleotide sequences were confirmed by DNA sequencing. These RNAs were prepared by in vitro transcription using T7 RNA polymerase (‘TT7 RNA polymerase’, TOYOBO) after cleavage of the template DNA by the restriction enzyme.3) The RNAs were labeled at the 3’-end with [$\alpha$-$^32$P]CTP as described previously.5) (B) PAGE analyses of the RNase P ribozyme reactions of the pre-tRNAs. The RNase P reactions were done under the usual conditions (10 nM of each human pre-tRNA$^{39}$ and its derivatives, 0.36 μM E. coli RNase P RNA, 0 or 5 or 60 mM MgCl₂, 100 mM NH₄Cl, 5% [v/v] polyethylene glycol 6000, 50 mM Tris–HCl; pH 7.8, 37°C; 120 minutes; in 10 μl reaction volume). The products were developed on 10% PAGE containing 8 M urea, and were quantitatively analyzed by PhosphorImager (Molecular Dynamics). ‘OH’+, ‘T1’, and ‘U2’ represent partially alkaline hydrolyzed, nuclease T1 hydrolyzed, or nuclease U2 hydrolyzed size markers respectively. (C) Schematic representation of the newly formed hairpins with the cleavage sites.

10) Ando, T., Tanaka, T., Hori, Y., and Kikuchi, Y., Kinetic analysis on hyperprocessing reaction of human tyrosine
