Development of amber suppressor tRNAs appropriate for incorporation of nonnatural amino acids

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ABSTRACT

An amber suppression method has been used for incorporation of nonnatural amino acids into proteins. However, the incorporation efficiency of nonnatural amino acids through an amber codon has been low, which restricts the application of the proteins containing nonnatural amino acids. In this study, we screened a wide variety of amber suppressor tRNAs to discover tRNAs capable to incorporate nonnatural amino acids with high efficiency. To this purpose, synthetic amber suppressor tRNAs of E. coli and Mycoplasma capricolum were screened for the incorporation of a fluorescently labeled nonnatural amino acid in an E. coli cell-free translation system. tRNAs that showed high capability for the incorporation were then mutated not to be aminoaacylated by any of endogenous aminoaacyl-tRNA synthetases of E. coli and to enhance the incorporation capability. As a result of these investigations, we successfully obtained several amber suppressor tRNAs with high ability for the incorporation of nonnatural amino acids.

RESULTS AND DISCUSSION

Experiments were carried out as shown in Figure 1. All nucleotide sequences of tRNAs were obtained from GenBank and The Genomic tRNA Database (http://lowelab.ucsc.edu/GtRNAdb/). Three oligo DNAs were connected by primer extension and PCR to generate template DNAs, which were transcribed to tRNA(-CA)s by in vitro transcription using T7 RNA polymerase. The resulting tRNA(-CA)s were then ligated with BFLAF-pdCpA by using T4 RNA ligase. A streptavidin gene containing a UAG codon at the Tyr83 position was used to evaluate the incorporation of BFLAF. The BFLAF-tRNAs and the streptavidin mRNA were added to an E. coli cell-free translation system. The reaction mixtures were applied to SDS-PAGE and the gel was visualized by a fluorescence image analyzer and Western blotting using anti-T7tag antibody. The aminoaacylation of the tRNAs by endogenous aaRS was evaluated by the expression of full-length streptavidin in the presence of non-aminoaacylated tRNA, which was analyzed by Western blotting using anti-T7 tag antibody. In the screening of E. coli tRNAs, tRNA^{trp} and tRNA^{trp} gave a distinct band of the full-length streptavidin on both fluorescence image and Western blotting, whereas yeast tRNA^{trp} gave no obvious band. These results suggest that the tRNA^{trp} and tRNA^{trp} have high ability for the incorporation of nonnatural amino acids. However, these tRNAs were found to be aminoaacylated by some endogenous aaRS by Western blotting for non-aminoaacylated tRNAs. To eliminate the aminoaacylation for the tRNA^{trp}, A1-U72 and G73, which had been identified as identity determinants, were substituted to G1-C72 and N73 (N indicates A, C, G or U). Fluorescence image of SDS-PAGE showed that G1-C72; A73 mutant and G1-C72; G73 mutant had higher ability for the incorporation than the original tRNA^{trp}. Western blot analysis for the non-aminoaacylated tRNAs, however, suggested that the G1-C72; A73 was not aminoaacylated, whereas the G1-C72, G73 mutant was slightly aminoaacylated.
In the screening of *Mycoplasma capricolum* tRNAs, tRNA\(^{TP}_{1}\) gave a distinct band on fluorescence image. Western blot analysis of translation product in the presence of non-aminocylated tRNA showed that the *Mycoplasma capricolum* tRNA\(^{TP}_{1}\) was aminocylated as well as *E. coli* tRNA\(^{TP}_{1}\). Therefore, the tRNA\(^{TP}_{1}\) was mutated to contain G1-C72; A73 mutation. Fluorescence image and Western blotting analysis indicate that the G1-C72; A73 mutant is the most efficient amber suppressor tRNA without suffering from aminocyclation.

![Diagram](https://example.com/diagram.png)

**Figure 2.** Secondary structure of amber suppressor tRNAs. (A) Yeast tRNA\(^{TP}\), (B) *E. coli* tRNA\(^{TP}\) and G1-C72; A73 mutant. (C) *Mycoplasma capricolum* tRNA\(^{TP}\) and G1-C72; A73 mutant.

**CONCLUSION**

We successfully obtained an amber suppressor tRNA showing high ability and specificity of incorporating nonnatural amino acid by the screening of synthetic amber suppressor tRNAs and the introduction of mutations. The amber suppressor tRNA will be useful for introducing various types of nonnatural amino acids into proteins.

**REFERENCES**