Noda Institute for Scientific Research GRANT
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Outline of Research Result

Construction of a highly efficient protein synthesis system by using circular mRNAs and permuted/intronic tRNA processing

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Research aims

Circular mRNAs are expected to be a powerful tool for use in the production of recombinant proteins because they show high exonuclease resistance and efficient translation initiation. Some reports have shown that circular mRNAs can be used as a template via internal ribosome entry during bacterial translation.[1), 2)] On the other hand, the translational activity of circular mRNAs is generally inefficient, suggesting that the mRNA terminus is still required for efficient translation, although the details of the mechanism remain unclear. To facilitate the recombinant production of desired proteins and further understanding of the translation mechanism in bacteria, the construction and analysis of the translation system using circular mRNAs are required.

Circularly permuted tRNAs found in the nuclear genome of some unicellular algae (including *Cyanidioschyzon merolae* 10D that inhabits an extreme environment) and in the genome of some archaea are matured via a characteristic circular RNA intermediate during post-transcriptional processing.[3)] In permuted tRNA genes, the sequences encoding the 5′-half and 3′-half of the specific tRNA are separated and inverted on the genome (Fig. 1). Biochemical analyses have defined a processing pathway in which the termini of tRNA precursors are ligated (probably depending on the tRNA-intron splicing machinery) to form a circular tRNA, which is then cleaved to generate the typical clover-leaf structure. In the present study, we tried to construct mRNA circularization systems via either the tRNA-intron splicing system or the self-splicing group I intron of bacterial tRNA. In addition, we assessed the translational activity of *Escherichia coli* and *Bacillus subtilis* mutant cells that lacked tRNA and tRNA modification enzymes, to identify the mutants with increased protein production ability.

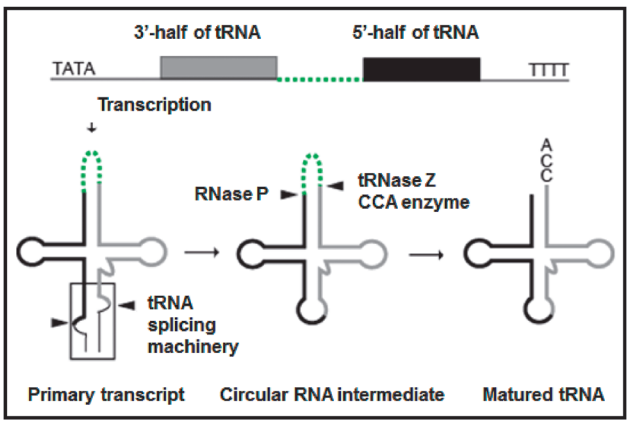

Fig. 1. Processing model of a pre-tRNA of permuted tRNA genes via a circular RNA intermediate, probably depending on tRNA processing enzymes.

Methods

We prepared a marker gene that could produce a functional protein only when circularization of mRNA had occurred via either (1) the tRNA-intron splicing system or (2) the self-splicing group I intron. (1) The tRNA-intron splicing endonuclease from organisms harboring permuted tRNA genes is supposed to recognize substrate RNAs depending only on the specific motif, which is originally found in the exon-intron junction of tRNA. Plasmids encoding a marker gene flanked by this RNA motif (Fig. 2A) and the *C. merolae* tRNA-intron splicing endonuclease genes were introduced into *E. coli*. (2) A plasmid containing a marker gene with inverted group I intron splicing sites derived from bacterial tRNA (Fig. 2B) was introduced into *E. coli* cells and an *E. coli* cell-free system. The circularization of mRNA and expression of the marker protein were assessed by RT-PCR and western blotting.

The *β*-galactosidase activity of *E. coli* and *B. subtilis* mutant strains defective in tRNA genes and anticodon modification enzyme genes was analyzed to identify the genes that affect translational activity. Then, some strains with a combination of the mutations for further increase of translational activity were constructed.

Results

The active form of the *C. merolae* tRNA-intron splicing endonuclease composed of multiple subunits could not be produced in *E. coli*, and attempts to introduce expression of the enzyme caused severe growth defects. On the other hand, a sufficient volume of marker protein was detected from the circularization of mRNA derived from the marker gene containing the inversed group I intron splicing sequence.

The *β*-galactosidase analysis using *E. coli* and *B. subtilis* mutant strains identified genes for tRNA and tRNA-modi-

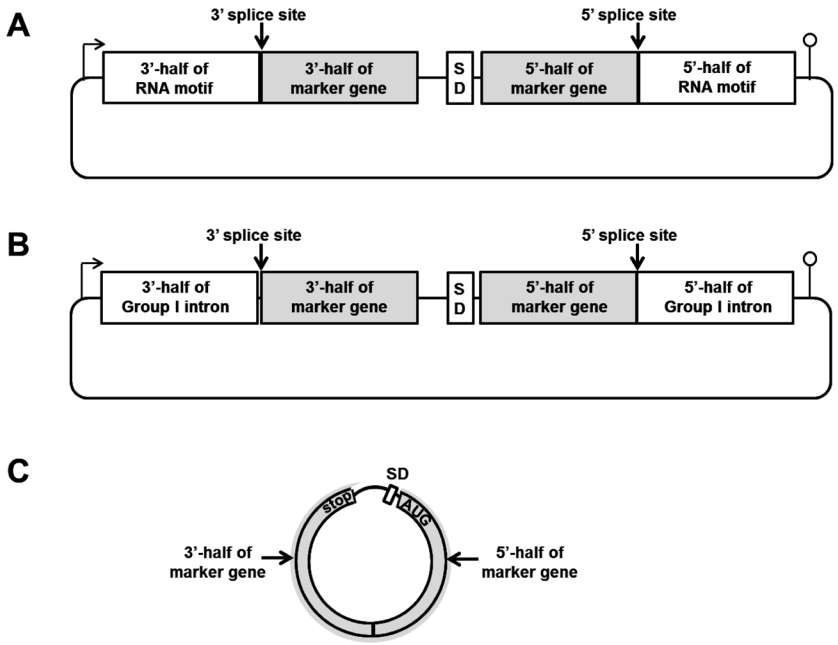

Fig. 2. Plasmids containing marker genes that can be expressed by circularization of mRNA via either (A) the tRNA-intron splicing machinery and (B) the self-splicing group I intron. (C) A circularized form of mRNA.

fying enzymes that affected the translational activity. In *B. subtilis*, the lack of tRNA or the enzyme modifying the anticodon corresponding to the four-codon boxes sometimes increased the *β*-galactosidase activity. Some combinations of these mutations further increased the translational activity, albeit only slightly. On the other hand, in *E. coli*, most of the genes for tRNA and the tRNA-modifying enzyme appeared to be essential for the bacterium's viability and efficient translation.

Conclusion

We attempted to construct a translation system using mRNA circularized via either the tRNA-intron splicing machinery or the self-splicing group I intron. The active form of *C. merolae* tRNA-intron splicing endonuclease could not be expressed in *E. coli*. This was probably due to a failure of appropriate protein folding, or to nonspecific cleavage of intrinsic RNA molecules in *E. coli* cells. On the other hand, mRNA circularized via the group I intron produced a sufficient volume of marker protein, showing the utility of this system for further analysis. The introduction of *cis*-elements involved in ribosome binding may improve the efficiency of the internal translation initiation. In *E. coli*, most tRNAs are essential for efficient translation, and the lack of tRNA genes generally decreases the translation of codons. This tendency is observed in other bacteria.[4] The results in the present study showed the characteristic features of codon recognition in *B. subtilis* cells: the less the anticodon repertoire exists, the more the translational activity increases. *B. subtilis* is a useful model for investigating the codon recognition mechanism, and it could provide clues for the construction of an efficient translation system using bacterial ribosomes.

References

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