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t-Riboregulator: Regulation of Nonsense Suppression by Modulating 3’ Processing of Suppressor tRNA

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Recently, we rationally evolved in-vitro-transcribed, unmodified, and non-aminoacylated amber suppressor tRNAs toward higher suppression efficiency in wheat germ extract (WGE).[1] The artificial evolution was conducted in the following four steps: [1st] anticodon alteration of natural tRNAs; [2nd] chimerization of efficient suppressors in the first generation; [3rd] investigation and optimization of the effective parts in the second generation; [4th] combination of the optimized parts in the third generation. The suppression efficiency of the suppressor obtained in the last generation was approximately 60% (85% in the presence of an eRF1 aptamer), which was 2.4-fold higher than that of the best suppressor in the first generation.

In this study, we employed this highly evolved suppressor and an amber-mutated gene to evaluate tRNA processing, especially terminal (5’ and 3’) processing, in WGE. Specifically, we prepared various kinds of terminal-premature tRNAs based on the evolved suppressor and compared their suppression efficiencies to that of the mother tRNA. As a result, 3’ processing including CCA addition was found to rapidly proceed in WGE in contrast to extremely slow 5’ processing. However, 3’ processing was effectively inhibited by distorting the tRNA structure with a distortion-inducing sequence in the 3’ end. We therefore used the structure-distorted suppressor to develop a novel gene regulation system, named “t-riboregulator“, wherein nonsense suppression (i.e., expression of full-length protein) is controlled by a modulator (nucleic acids) that restores the tRNA structure and the 3’ processing activity of the structure-distorted suppressor. The t-riboregulator system is expected to be used as an alternative of or in combination with normal mRNA-based riboregulators or other gene regulators such as riboswitches to construct artificial gene circuits in synthetic biology.

[1] Ogawa, A., *et al*., Improvement of *in vitro*-transcribed amber suppressor tRNAs toward higher suppression efficiency in wheat germ extract, *Organic & Biomolecular Chemistry*, 9 : 8495-8503, 2011.