論文の内容の要旨

論文題目

Ribozyme-based synthesis of acyl-tRNAs in translation system
（翻訳系内におけるリボザイムを基礎としたアシルtRNAの合成）

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Flexizymes are artificial RNA catalysts that facilitate the acylation of tRNAs with highly flexible to both structure of its substrates and kinds of tRNAs. The *de novo* acylation system based on flexizymes allow us to synthesize a wide variety of mis-charged tRNAs with virtually no limitation. On the other hand, assignment of amino acid substrates to tRNA *in situ*, in translation apparatus have not been demonstrated because of no specificity for tRNAs. Here we construct *in situ* generation system of aminoacyl-tRNAs based on ribonuclease P (RNase P) and catalytic precursor tRNAs that have flexizyme sequences in 5' reader regions. The self-aminoacylation of catalytic precursor tRNA and its maturation by RNase P enable to specifically assign amino acid substrate to a tRNA in E.coli reconstituted cell-free translation system. The *de novo* assignment system described here demonstrated to assign 11 kinds of non-proteinogenic substrates to an anticodon in translation apparatus, indicating this system can express various non-proteinogenic peptide with one series of biocatalyst. Additionally aminoacyl-tRNAs could be generated by using the DNA template of catalytic precursor tRNA. Thus, *de novo* assignment system described here opens a new avenue for the application of flexizyme *in vivo*. Furthermore, I improved the environmental problem of flexizyme system. Previous flexizyme systems have been required relatively high Mg^{2+} concentrations (>50 mM) for the full function. For instance, lowering the Mg^{2+} concentration to ~1 mM, where the *in vivo* translation system generally functions, resulted in significant decrease in activity. To overcome this limitation, we implanted a new random domain into a part of flexizyme to aim at selecting a new functional domain that would exhibit acylation at lower Mg^{2+} environment such as *in vivo* translation apparatus. After the selection of new accessory domain, mutations were further introduced for selected ribozyme to optimize the extra sequences of putative catalytic core. Indeed, *in vitro* selection of active species from such an RNA pool afforded a new flexizyme, called mdFx showing 10 fold improvement of Mg^{2+} dependency. Thus, this new flexizyme opens a new avenue to construct a *de novo* aminoacylation system of tRNAs *in vivo*.