論文の内容の要旨

Profiling of nascent peptide sequences that regulate peptidyl-tRNA drop-off and investigation on expression of proteins lacking the N-terminus due to the drop-off

（ペプチジル-tRNA 脱落を制御するペプチド新生鎖配列のプロファイリング

と脱落に伴う N 末端欠損タンパク質発現の検証）

氏名　田島　研也

Introduction (Chapter 1)

Ribosomal translation reaction elongates polypeptide chains by repeating accommodation of aminoacyl-tRNA delivered by EF-Tu into ribosomal A site, peptidyl transfer of a nascent peptide on P-site peptidyl-tRNA onto A-site aminoacyl-tRNA, and translocation mediated by EF-G in which A-site peptidyl-tRNA and P-site deacyl-tRNA move into P site and A site, respectively (**Figure**). Although short peptidyl-tRNA accidentally drops off from the translating ribosome, the actual mechanisms and molecules that induce peptidyl-tRNA drop-off have remained elusive. It is believed that mRNA contexts or translation factors such as EF-G are involved in the peptidyl-tRNA drop-off. Ribosome stalling is one of the plausible causes of peptidyl-tRNA drop-off. Certain nascent peptides could induce ribosome stalling via formation of inactive state for peptidyl transfer by interaction with the ribosome exit tunnel in which nascent peptides penetrate. A stretch of proline residues in nascent peptides also causes ribosome stalling due to its poor peptidyl donor and acceptor ability. The strength of stalling depends on the number of prolines and neighboring amino acid residues. In former studies, strength of ribosome stalling was investigated on up to 1600 kinds of native proteins containing consecutive proline motifs.

In this PhD study, I comprehensively profiled 8420 kinds of nascent peptide sequences containing consecutive prolines against regulation of peptidyl-tRNA drop-off utilizing mRNA display-based approach. By analyzing broader sequence coverage than the former studies, it was revealed that the peptidyl-tRNA drop-

off occurs depending on the nascent peptide sequences containing the proline stretch. Finally, in order to investigate the peptidyl-tRNA drop-off in *E. coli* endogenous translation system, I expressed and purified *E. coli* proteins containing consecutive prolines, which were sequenced by LC-ESI MS/MS after protease digestion. As a result, the evidence of peptidyl-tRNA drop-off was obtained.

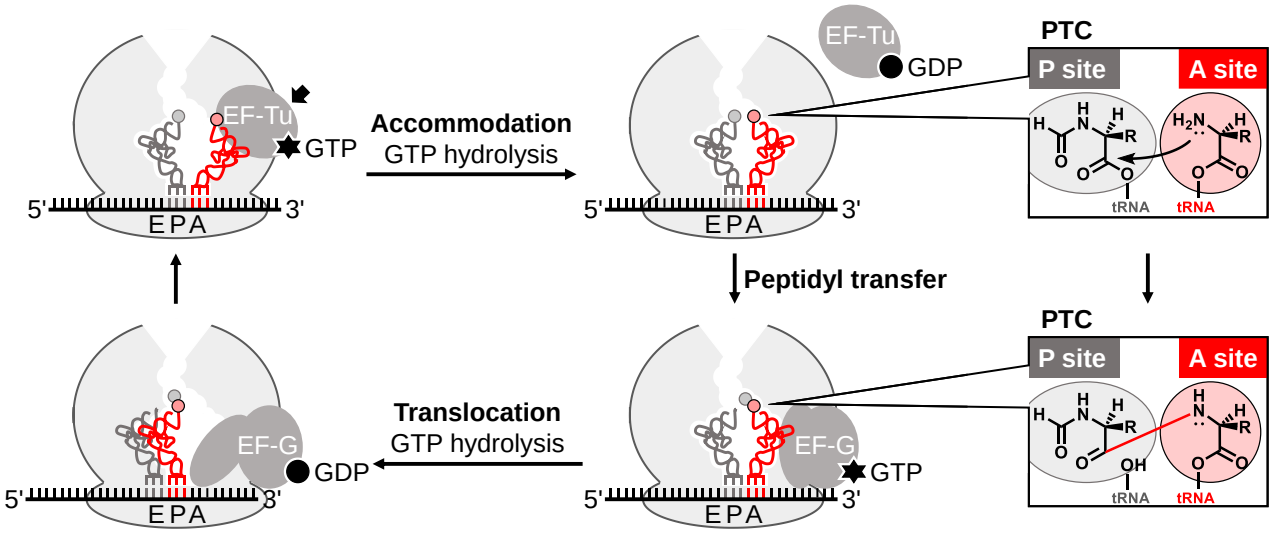

Figure Elongation of a nascent peptide in translation.

In elongation phase of translation, accommodation, peptidyl transfer, and translocation take place in this order. aa-tRNAs are supplied by aaRS.

Results and Discussion

Comprehensive profiling of nascent peptide sequence on peptidyl-tRNA drop-off (chapter 2)

In order to comprehensively analyze the effect of nascent peptide sequences containing the proline stretch on peptidyl-tRNA drop-off, I performed saturation mutagenesis in nascent peptides utilizing Random non-standard Peptide Integrated Discovery (RaPID) system. I constructed an mRNA library having randomized NNN codons to express a library of randomized peptide sequences. Here shows the profiling strategy. The mRNA library was ligated with a puromycin linker, translated into a N-terminally biotinylated peptide library in reconstituted cell-free translation system combined with genetic code reprogramming, conjugated to the corresponding mRNA via puromycin, and reverse-transcribed to generate peptide-mRNA-cDNA conjugate. The peptide-mRNA-cDNA conjugates induced peptidyl-tRNA drop-off was selectively removed, other N-terminally biotinylated peptide-mRNA-cDNA were recovered by streptavidin, and analyzed by next-generation sequencing. Sequencing data were converted to the frequency of a peptide sequence in the input library or in a library recovered, which were interpreted as enrichment of the frequency of a peptide sequence, indicating strength of peptidyl-tRNA drop-off. As a result, the peptide sequence-dependent regulation of peptidyl-tRNA drop-off was observed. In order to confirm reliability of the profiling system, 17 kinds of representative peptides were individually expressed *in vitro* and quantified by LC-ESI MS. Good correlation was obtained between deep sequencing results and LC-ESI MS quantification, which demonstrated the reliability of the system. It was concluded that strength of peptidyl-tRNA drop-off was regulated by nascent peptide sequence.

Investigation of peptidyl-tRNA drop-off in *in vitro* synthesis of *E. coli* protein derivatives and *in vivo* expression of *E. coli* native proteins *(*Chapter 3)

Finally, I investigated the peptidyl-tRNA drop-off in *in vitro* and *in vivo* protein synthesis. I chose 16 *E. coli* proteins or putative proteins for expression. Preliminary, the short peptide fragments derived from the proteins were expressed *in vitro*, analyzed by tricine-SDS PAGE and LC-ESI MS. Peptidyl-tRNA drop-off was observed in expression of 6 peptide fragments among above 16 peptide fragments, 6 proteins were cloned into a plasmid, expressed in *E. coli*, purified, digested by proteases, and sequenced by LC-ESI MS/MS. As a result, the evidence of peptidyl-tRNA drop-off in the proteins synthesis was obtained.

Conclusion

Utilizing RaPID display, 8420 kinds of nascent peptide sequences were comprehensively profiled on strength of peptidyl-tRNA drop-off. The reliability of developed system was supported by quantification of expressed peptides by the independent method. To the best of my knowledge, this work covered the widest sequences for quantification of frequency of peptidyl-tRNA drop-off, which provide insight into regulation of peptidyl-tRNA drop-off depending on the peptide nascent sequences. In *in vitro* synthesis of protein derivatives and *in vivo* synthesis of a protein, peptidyl-tRNA drop-off was revealed as a new mode of abnormal translation event.