論文内容の要旨

論文題目：Functional analysis of the C-terminus region of SmpB in the ribosomal A site entry of tmRNA
(tmRNA のリボソーム A サイトエントリーにおける SmpB の C 末端領域の機能解析)

氏名：周　站平　(Zhou Zhan-ping)

Transfer-messenger RNA (tmRNA) combines the dual functions of tRNA and mRNA. It rescues stalled ribosomes caused by defective mRNAs in bacteria cells, via the trans-translation process. Trans-translation is initiated by the alanyl-tmRNA entry into the A site of the ribosome. SmpB is a tmRNA-specific binding protein and enhances the alanylation efficiency of tmRNA and facilitates tmRNA entry into the ribosome. There is no codon-anticodon reaction in trans-translation and Studies demonstrate that SmpB that binds to the A site on the 30S subunit and mimics an anticodon arm of tRNA plays a crucial role for the "decoding" process of tmRNA.

SmpB has a 30-residues C-tail with random structure. The c-tail of SmpB is near the decoding center in trans-translation. Although many studies demonstrate that mutations of åresidues on c-tail severely damage the function of SmpB, it still remains unclear how SmpB compensates for the role of the anticodon of tRNA and how the c-tail of SmpB interacting with ribosome in trans-translation. Here we studied the interaction of tmRNA/SmpB complex and ribosome using biochemical methods and biophysical techniques like single-molecule imaging. The movement of tmRNA/SmpB complex on ribosome can be traced in real time.

Results:

1 SmpB stimulates GTP hydrolysis and only one SmpB molecule is needed in trans-translation for one 70s ribosome

SmpB is also essential for the GTP hydrolysis by EF-Tu on a ribosome. In the presence of limited concentration of a ribosome, the increase of a GTP hydrolysis rate was linearly dependent upon the ribosome concentration. The turnover rates were calculated as 2.4 s^{-1} in the case of tmRNA and 5.3 s^{-1} in the case of TLD, respectively. These values are in same order of magnitude as compared to the case of that carried out by a ribosome and EF-G (reported as 4.3 s-1 by Seo et al.19).

Fixed the SmpB concentration at 0.125 mM, which was not saturating, and addressed the effect of tmRNA or TLD concentration on the GTP hydrolysis rate. In the case of TLD, the GTP hydrolysis rate was increased in proportion to the TLD concentration and was saturated when the concentration reached equivalent to SmpB. In the case of tmRNA, GTP hydrolysis was also increased in proportion to tmRNA concentration and was nearly saturated when the concentration reached equivalent to SmpB. These results suggested that only one molecule of SmpB is necessary for the GTP hydrolysis per one 70S ribosome.

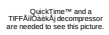

Figure 1. Kinetic studies of GTPase stimulation activity of SmpB

2 The C-tail mutants of SmpB cannot support the alanyl-tmRNA accommodation into the A site of the ribosome

The residues on the disorder c-terminus tail of SmpB were conserved. SmpB153 that lacks C-terminal seven amino acids residues is reported incapable of facilitating TLD-derived template-independent polyalanine synthesis. We prepared a series of truncated variants that lack C-tail residues stepwisely (SmpB153, SmpB149, SmpB139, SmpB136, SmpB130) and introduced single or double alanine substitutions to the residues of D137, K138 and R139. All the variants possessed the alanylation enhancing activity and indicate that the residues in the C-terminal region of SmpB are not involved in the enhancement of alanylation nor binding to the TLD.

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Figure 2. Aminoacylation efficiency of SmpB variants

The series of truncated SmpB variants (SmpB153, SmpB149, SmpB139) and SmpB^{DE} could not support the attachment of SsrA tag to the DHFR while the single mutants (R139A, K138A, and D137A) or double mutants (R139A/K138A, R139A/D137A, K138A/D137A) did. The results strongly indicate that the trans-translation deficient SmpB mutants cannot facilitate tmRNA accommodation in the A site of the ribosome

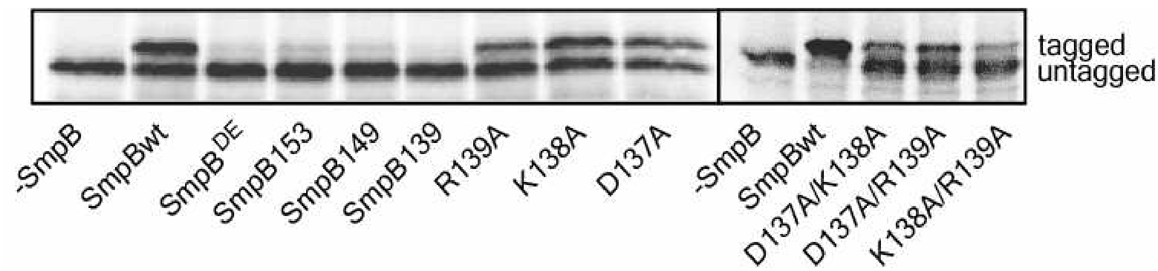


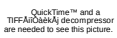
Figure 3. SsrA tagging supporting activity of SmpB variants.

3 Trans-translaiton deficient SmpB still triggers GTP hydrolysis of EF-Tu

Using the series of mutant SmpB, GTPase stimulating activity was monitored. Interestingly, all of the prepared mutants still triggered GTP hydrolysis of EF-Tu. The decrease of GTPase stimulating activity correlated with the numbers of deleted C-tail residues, suggesting large part of the C-tail region of SmpB is involved in the GTPase stimulation. The mutation of D137 and R139 also reduced the rate of GTP hydrolysis, suggesting these residues are also involved in the GTPase stimulation.

TLD

tmRNA



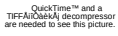


Figure 4. Activities of SmpB variants on the GTP hydrolysis stimulation by EF-Tu on the ribosome

4 PMB80 can block the non-specific binding of SmpB

Single-molecule imaging can follow the movement of tmRNA/SmpB movement on ribosome in real time. But the non-specific binding of SmpB is a main barrier. 0.1% of PMB80 can block the non-specific binding of SmpB. Omission of EF-Tu and AlaRS strengthened the non-specific binding of SmpB and cannot be the control of the experiment.

Figure 5. Block of non-specific binding of SmpB by PMB80

Conclusion

SmpB binds on the tRNA-like domain of tmRNA and compensates for the lack of codon-anticodon of trans-translation. Although studies suggest that two molecules of SmpB bind to both subunits and necessary for the trans-translation in bulk, in the present study, the results suggest that only one molecule of SmpB is necessary to cause the structural rearrangement of the ribosome to trigger GTP hydrolysis of EF-Tu. The removal of the C-tail region of SmpB results as trans-translation deficient SmpB but still stimulates the GTP hydrolysis of EF-Tu, suggesting that the C-tail of SmpB is essential for the alanyl-tmRNA accommodation but not for the stimulation of GTP hydrolysis. Non-specific binding of SmpB to the glass seemed to be blocked by 0.1% of PMB80. The interaction of SmpB and ribosome may be elucidated in detail and in real time using single-molecule imaging.