Single-molecule imaging with a tagged ribosome to explore trans-translation

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Hiroaki Imataka

Department of Materials Science and Chemistry, Molecular Nanotechnology Research Center, Graduate School of Engineering, University of Hyogo, Himeji 671-2280, Japan. Tel: +81-79-267-4021, Fax: +81-79-267-4885, e-mail: imataka@eng.u-hyogo.ac.jp

Single-molecule imaging is a powerful technique to visualize molecular interactions and movements. Translation is one of the most interesting targets for researchers with the molecular-imaging skills, since mRNA, tRNA and translation factors interact with or move inside or on the ribosome in an ordered manner. Trans-translation is a bacterial quality control system to rescue the ribosomes stalled at the 3' end of the mRNA, and this phenomenon is recapitulated in vitro with defined factors including two trans-translation-specific entities tmRNA and SmpB. Zhou et al. (Single molecule imaging of the trans-translation entry process via anchoring of the tagged ribosome. J Biochem 2011;149:609-618.) successfully visualized the interaction of the tmRNA-SmpB complex with the ribosome by immobilizing the ribosome on the quartz surface with the HaloTag technology. This ribosome-anchoring system may be useful for the imaging analysis of other processes of translation.

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Abbreviations: EF-Tu, elongation factor Tu; MLD, mRNA-like domain; SmpB, small protein B; TLD, tRNA-like domain.

Single-molecule imaging enables visualization of interactions and movements of biological molecules. Translation is a considerably complex process that is operated in concert by ribosomes, mRNA, tRNAs and translation factors. The conventional biochemistry has been for decades giving insight into the molecular events that occur inside and on the ribosome during translation, and researchers with excellent skills in the single-molecular imaging have now begun to visualize them (1-3).

In 2001, Ueda et al. developed the PURESYSTEM, which recapitulates the bacterial translation in vitro with purified ribosomes, tRNAs and translation factors (4). The PURESYSTEM is a product that rightly materializes the conventional biochemistry, i.e. in vitro reconstitution of a biological event with purified molecules, and has been greatly contributing to the understanding of the mechanism of translation (5-7) and to the development of new technology (8, 9). Since the PURESYSTEM reproduces all the stages of translation: initiation, elongation, termination and recycling only with defined molecules, one can explore any stage of translation by removing or adding (a) factor(s) of interest. Shimizu and Ueda successfully reproduced trans-translation using the PURESYSTEM (10), and Zhou et al. subsequently visualized the initial phase of trans-translation in a recent paper (11).

Trans-translation is a bacterial quality control system to rescue ribosomes stalled at the 3' end of defective mRNAs that lack a stop codon. When degradation of mRNA from the 3' end goes into the coding region or the mRNA is cleaved somewhere within the coding region before the translating ribosome reaches the stop codon, the ribosome has no way but to stall at the 3' end of the mRNA with an incomplete polypeptide unreleased. Unless rescued, the stalled ribosomes would become dead-end machinery, and consequently the capacity of protein synthesis should be reduced: see Himeno et al. (12) for an excellent review of trans-translation.

Two components tmRNA and SmpB (small protein B) play principal roles in trans-translation. tmRNA is a bifunctional RNA acting as tRNA and mRNA through its TLD (tRNA-like domain) and MLD (mRNA-like domain), respectively. The 3' end of tmRNA is aminocylated with alanine by alanyl-tRNA synthetase. The alanyl-tmRNA forms a complex with SmpB and EF-Tu (elongation factor Tu), and this complex interacts with the stalled ribosome. The SmpB protein binding to the TLD of tmRNA mimics the tRNA anticodon stem-loop, which is absent from the TLD (13, 14). The TLD-SmpB module enters the A site (aminocylated tRNA-binding site) of the stalled ribosome. The incomplete polypeptide is then transferred to the alanyl-tmRNA, and the peptidyl TLD-SmpB module is translocated to the P site (peptidyl tRNA-binding site) of the ribosome. The template for translation is now switched to the MLD, which encodes a tag sequence (10 amino acids) plus a stop codon. Since translation terminates canonically on MLD, the ribosome dissociates into subunits for the next translation. The tagged polypeptide undergoes protease-dependent degradation, since the C-terminal tag represents a signal of an immature polypeptide (15).

Zhou et al. observed the association of tmRNA-SmpB-EF-Tu with the ribosome by the single-molecule imaging (11). An important technical point for the imaging analysis of translation is how to anchor the ribosomal complex on the quartz plate. Different approaches have been reported for this purpose. One method is that mRNA is labelled with biotin and immobilized on the streptavidin-derivatized quartz plate; thereby the ribosome is indirectly anchored via
mRNA on the plate (3, 16, 17). Obviously, this method is not appropriate for the study of trans-translation, because the stalled mRNA dissociates during the process of trans-translation. Thus, the ribosome should be directly anchored on the plate, entailing either rRNA or a ribosomal protein to be modified. For a successful example of the rRNA-modification, the 16S rRNA is genetically engineered to have a 23 nt extension in the helix 44, and hybridization with a biotinylated oligonucleotide that is antisense to the extended helix can immobilize the ribosome on the streptavidin-derivatized plate. Since the oligonucleotide is also labelled with Cy3, the position of the ribosome is fluorescently monitored (18). On the other hand, Zhou et al. undertook a different approach: modification of a ribosomal protein (S2) by inserting the HaloTag (11).

The HaloTag (297 amino acids, Promega) is a mutant of the bacterial haloalkane dehalogenase (19). The haloalkane dehalogenase removes a halide from an aliphatic hydrocarbon. During the reaction of this enzyme, a covalent ester bond is formed between the enzyme and the substrate. The intermediate ester bond is subsequently hydrolyzed, and the substrate is released from the enzyme as an alcohol (20). Due to the mutation (H272F), the HaloTag fails to hydrolyze the ester bond and thereby the ligand remains attached to the enzyme. Thus, a HaloTag fusion protein can be labelled with a chloroalkane linker fused with useful molecules. Zhou et al. synthesized a biotin-Cy3-HaloTag ligand from the HaloTag amine(O4) ligand (Promega), and the biotin-Cy3-HaloTag ligand was conjugated with the recombinant HaloTag-S2 fusion protein. The biotin-Cy3-conjugated HaloTag-S2 proteins were then incorporated into S2-less ribosomes (11). Remarkably, the reconstituted ribosomes exhibited a 70% translation activity as compared with the wild-type ribosomes, despite a large insertion (297 amino acids plus biotin-Cy3-HaloTag ligand) into the S2 subunit (11). The modified ribosome was then immobilized on the quartz surface through the biotin and Streptavidin interaction (Fig. 1).

To monitor the binding of the tmRNA complex (tmRNA/SmpB/EF-Tu) to the ribosome, tmRNA is labelled with Cy5. Consequently, the tmRNA complex is detected by the fluorescence of Cy5, whereas the ribosome on the plate is localized by the Cy3 fluorescence. The authors considerably reduced non-specific binding of Cy5-tmRNA/SmpB/EF-Tu to the plate by using a detergent, and thereby successfully visualized the Cy5 signal (tmRNA complex) co-localized with the Cy3 signal (ribosome) (11). The fluorescence spots of Cy5 appeared and disappeared with intervals of seconds, indicating that multiple rounds of interaction between tmRNA and the ribosome occur (11). The conventional biochemistry, which measures an average of the signals in the bulk, cannot show such a dynamic of molecules.

Although the article describes only the initial phase of trans-translation, the authors surely aim to depict trans-translation as a whole at the single-molecule level, and they can doubtless accomplish this task. Furthermore, the HaloTag-dependent anchoring of the ribosome may be utilized for the imaging analysis of other translational processes.

Conflict of interest
None declared.

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References


