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Single-molecule FRET studies on interactions between elongation factor 4 (LepA) and ribosomes

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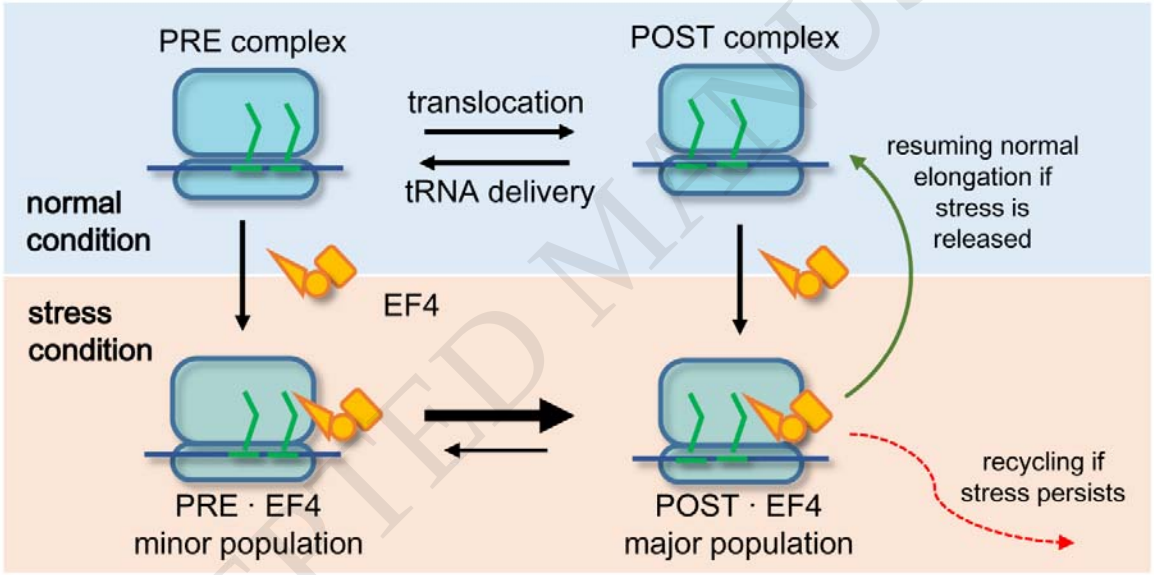
Graphical abstract



Abstract:

Elongation factor 4 (EF4) is one of the highly conserved translational GTPases, whose functions are largely unknown. Structures of EF4 bound ribosomal PRE-translocation and POST-translocation complexes have both been visualized. On top of cellular, structural, and biochemical studies, several controversial models have been raised to rationalize functions of EF4. However, how EF4 modulates elongation through its interactions with ribosomes has not been revealed. Here, using single-molecule fluorescence resonance energy transfer assays, we directly captured short-lived EF4·GTP bound ribosomal PRE and POST translocation complexes, which may adopt slightly different conformations from structures prepared using GDP, GDPNP, or GDPCP. Furthermore, we revealed that EF4·GTP severely impairs delivery of aminoacyl-tRNA into the A-site of the ribosome and moderately accelerates