Elongation factors in translation and beyond

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Background:
During translation, the genetic information of a cell is converted into proteins. This process is managed by ribosomes, which are assisted by protein factors. One of these, bacterial elongation factor EF-Tu, transports aminoacylated tRNAs to the ribosome in a GTP-dependent manner (Fig. 1A). During this task, EF-Tu undergoes a large conformational change, which is believed to be of functional importance. Our aim is to describe the dynamic aspects of the structural transitions of EF-Tu in time and space during translation. During infection of E. coli by the RNA virus Qβ, a RNA-dependent RNA polymerase complex is formed with the purpose of replicating the viral genome (Fig. 1B). Apart from the virus-encoded β-subunit, the complex consists of three host proteins, EF-Tu, EF-Ts and ribosomal protein S1. We want to deduce the role of the host proteins during Qβ genome replication. The translation apparatus is the target of a number of bacterial toxins, which bring the cell into a dormant state during stress (Fig. 1D). This allows the bacterium to survive e.g. antibiotic treatment. The are neutralized by antitoxins, which are targets of the Lon protease (Fig. 1C). We aim at reducing the action of bacterial toxins to increase the succresate of antibiotic treatments by inhibiting the Lon protease.

Techniques:
General molecular biology and biochemistry methods e.g. cloning, site-directed mutagenesis, PCR, RT-PCR and Western blotting.

Protein expression and purification
Labeling of proteins for FRET-based assays (Fig. 2 D and E)
Infectivity assays (Fig. 2A)

Detection and study of protein-protein interactions:
- yeast two-hybrid system (Fig. 2B)
- FRET-based assays
- pull-down assays
- co-immunoprecipitation

Protein characterization e.g.
- protein-RNA binding
- activity assays

Projects:
Single-molecule studies of EF-Tu dynamics
- design and characterization of mutants for labeling
- labeling and FRET studies

Structure-function studies (Fig. 3B) of EF-Tu and EF-Ts e.g.
- GTpase activity
- guanine-nucleotide exchange

Studies of RNA replication by the Qβ replicase complex
- template recognition and binding
- separation of product and template
- role of host proteins during replication (Fig. 3C)

Inactivation of the Lon protease (Fig. 3A)
- establishment of a selection system
- establishment of a peptide library
- library screening to identify lon inhibitors
- characterization of lon inhibitors

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