Background and aims:
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 aminoaclylated tRNAs to the ribosome in a GTP-dependent manner (Fig. 1A). During this task, EF-Tu undergoes a 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 [1, 2].

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 [3]. Nonsense mutations, which result in in-frame premature termination codons (PTCs), account for approx. 11% of all inherited diseases in humans. Thus, therapeutic strategies that suppress nonsense mutations have the potential to provide benefits for a broad range of patients. Our goal is to establish reporter constructs, which allows the selection of DNA-encoded peptide drugs that suppress PTCs in specific genetic contexts without gross effects on termination at normal termination codons (Figure 1C).

Techniques:
- General molecular biology and biochemistry methods e.g. cloning, mutagenesis, (RT)-PCR, and Western blotting.
- Establishment of reporter-based selection systems
- Selection of cyclic peptide inhibitors (Fig. 3C)
- Protein expression and purification
- Labeling of proteins for FRET-based assays (Fig. 2A and B)
- Infectivity assays (Fig. 2C)
- Detection and study of protein-protein interactions e.g..
  - yeast two-hybrid system
  - FRET-based assays (Fig. 2B)
  - pull-down and co-IP
- Protein characterization e.g.
  - protein-RNA binding (Fig. 2D)
  - activity assays (Fig. 2E)

Projects:
- Single-molecule studies of EF-Tu dynamics
  - design and characterization of mutants for labeling
  - labeling and FRET studies
- Structure-function studies (Fig. 3A) 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. 3B)
- Selection of circular peptide inhibitors from DNA library
  - inhibition of viral replication
  - inhibition of premature termination


The protein synthesis machinery in health and disease
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