# The protein synthesis machinery in health and disease

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**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 aminoacylated tRNAs to the ribosome in a GTP-dependent manner (Fig. 1A). During this task, EF-Tu undergoes conformational changes, and our aim is to deduce their functional importance [1, 2].

Nonsense mutations result in in-frame premature termination codons (PTCs), which 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 select DNA-encoded peptide drugs that suppress PTCs in specific genetic contexts without gross effects on termination at normal termination codons (Figure 1C).

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. One goal is to deduce the molecular details of Qβ genome replication [3]. Another goal is to study, if Qβ RNA can be taken up by intestinal cells and potentially affect their phenotype (Fig. 3B).

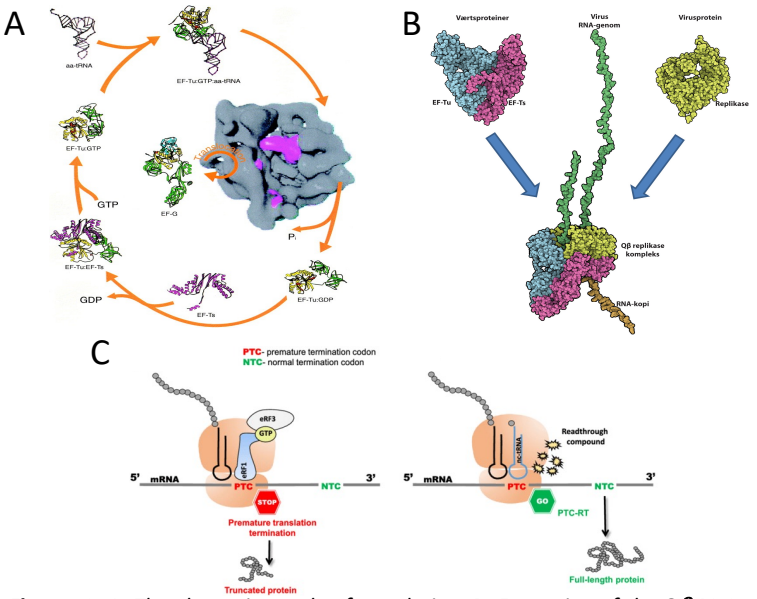


***Figure 1. A.*** *The elongation cycle of translation.* ***B.*** *Formation of the Qβ RNA polymerase complex, which copies the viral RNA genome.* ***C.*** *The readthrough drug (stars in right-hand panel) allows translation of a full-length protein.*

**Techniques:**

- General molecular biology and biochemistry methods e.g. cloning, mutagenesis, (RT)-PCR, and Western blotting.
- Protein expression and purification
- Fluorscence labeling of protein (Fig. 2A)
- Protein characterization in activity assays
- RNA production and manipulation (Fig. 2B)
- RNA tracking by microscopy
- Infectivity assays (Fig. 2C)
- Protein-RNA interaction assays (Fig. 2D)
- Construction of DNA-encoded peptide libraries
- Establishment of selection strategies
- Characterization of selected circular peptides
  - Alanine scanning mutagenesis
  - Effect in patient cell lines

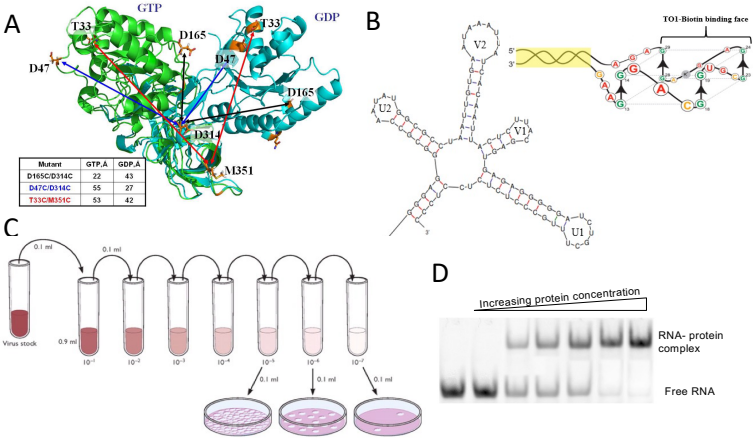


***Figure 2. A.*** *Protein labelling.* ***B.*** *Insertion of fluorescent RNA aptamer into phage genome* ***C.*** *Infectivity assay.* ***D.*** *Electrophoretic mobility shift assay.*

**Projects:**

- Single-molecule studies of EF-Tu dynamics (Fig. 3A)
  - structure-based design of mutants
  - expression and purification of mutants
  - characterization of mutant activity
  - labeling of mutants and single-molecule studies
- Cross-kingdom RNA communication (Fig. 3B)
  - labeling of phage RNA genome with RNA aptamer
  - tracking of phage RNA genome in various cell types
  - study phenotypic effect of phage RNA in eukaryotes
- Selection of circular peptide inhibitors from DNA library (Fig. 3C)
  - inhibition of viral replication
  - inhibition of premature termination
- Studies of RNA replication by the Qβ replicase complex
  - template recognition and binding
  - separation of product and template
  - role of host proteins during replication

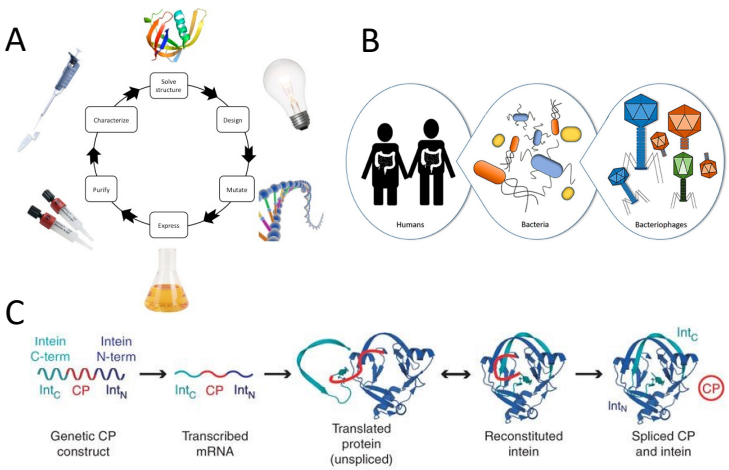


***Figure 3. A.*** *Structure-function study cycle.* ***B.*** *Cross-kingdom RNA transmission and signaling.* ***C.*** *Formation of circular peptides by intein splicing.*

1. Kavaliauskas et al. Structural dynamics of EF-Tu during aa-tRNA delivery to the ribosome (2018) *Nucl. Acids Res.* Aug 11. doi: 10.1093/nar/gky651
2. Johansen et al. *E. coli* EF-Tu bound to a GTP analogue displays an open conformation (2018) *Nucl. Acids Res.* Aug 11. doi: 10.1093/nar/gky697
3. Gytz et al. Structural basis for RNA-genome recognition during bacteriophage Qβ replication (2015) *Nucl. Acids Res.* 43:10893