

Lecture 5

1. INITIATION

- Assembly of active ribosome by placing the first mRNA codon (**AUG** or START codon) near the P site and pairing it with initiation tRNA, fMet-tRNA^{fMet}.
- Occurs once per polypeptide chain or molecule.

2. ELONGATION

- Involves three distinct steps for each mRNA codon or amino acid in the new polypeptide chain:
 1. Transfer of proper aminoacyl-tRNA from cytoplasm to A-site of ribosome.
 2. Covalent linkage of new amino acid to growing polypeptide chain - **peptidyl transfer**.
 3. Movement of tRNA from A-site to P-site and simultaneous movement of mRNA by 3 nucleotides - **translocation**.
- Occurs multiple times per polypeptide.

3. TERMINATION

- Reading of final mRNA codon (STOP codon) and dissociation of polypeptide from ribosome.
- Occurs once per polypeptide chain.

ELONGATION OF PROTEIN SYNTHESIS

- **3** distinct steps to add one amino acid to the growing polypeptide chain.
- Occurs many times per polypeptide, the number of which depends upon the number of mRNA codons or amino acids in the protein
- The Elongation Cycle is similar in prokaryotes and eukaryotes.
- Fast: 15-20 amino acids added per second
- Accurate: 1 mistake every ~10,000 amino acids

Transfer of Aminoacyl-tRNA

Non-initiator, aminoacyl-tRNA is placed in the ribosomal A-site over the mRNA codon in such a way that **base pairing** occurs between the anticodon loop of the tRNA and mRNA codon ([see lecture 1](#)).

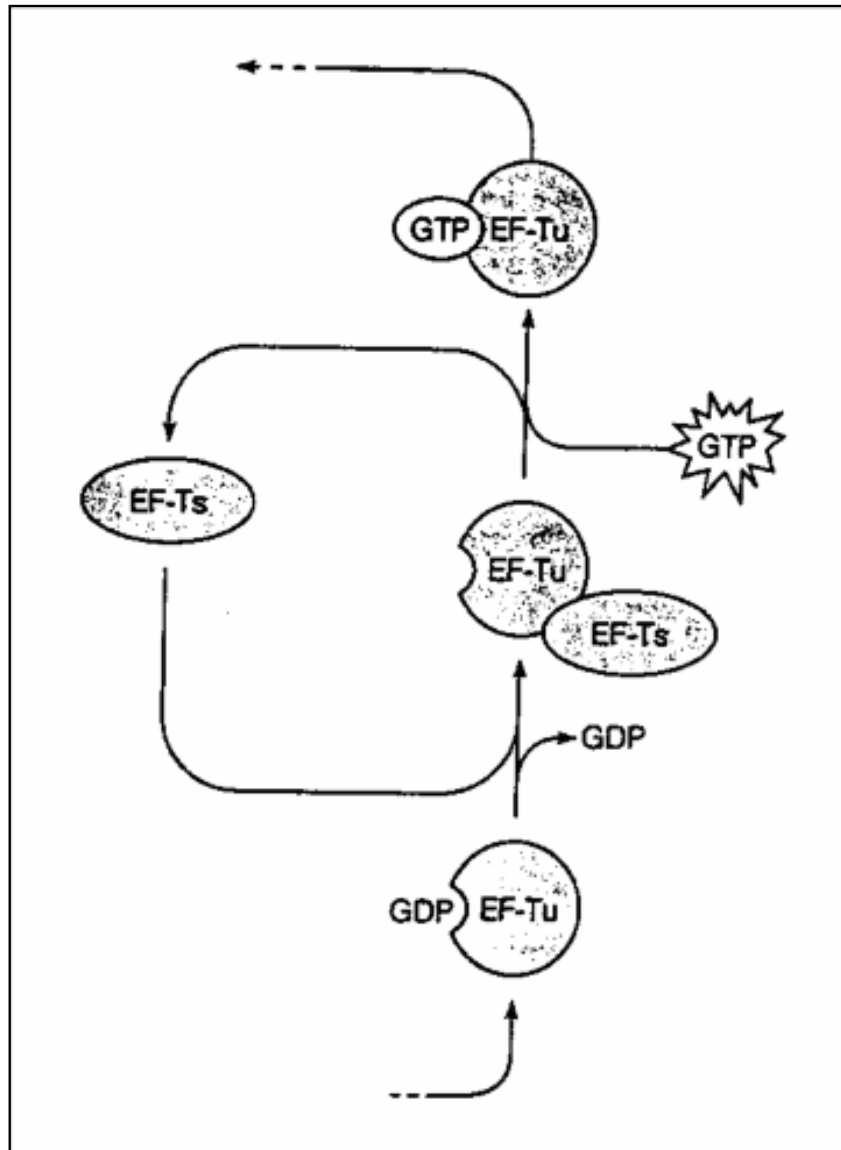
Aminoacyl-tRNA transfer is facilitated by two soluble protein transfer factors, called **elongation factors**, EF-Tu and EF-Ts in prokaryotes:

- **EF-Tu** binds GDP and GTP and is a model G protein.
- **EF-Ts** exchanges GDP for GTP on EF-Tu.

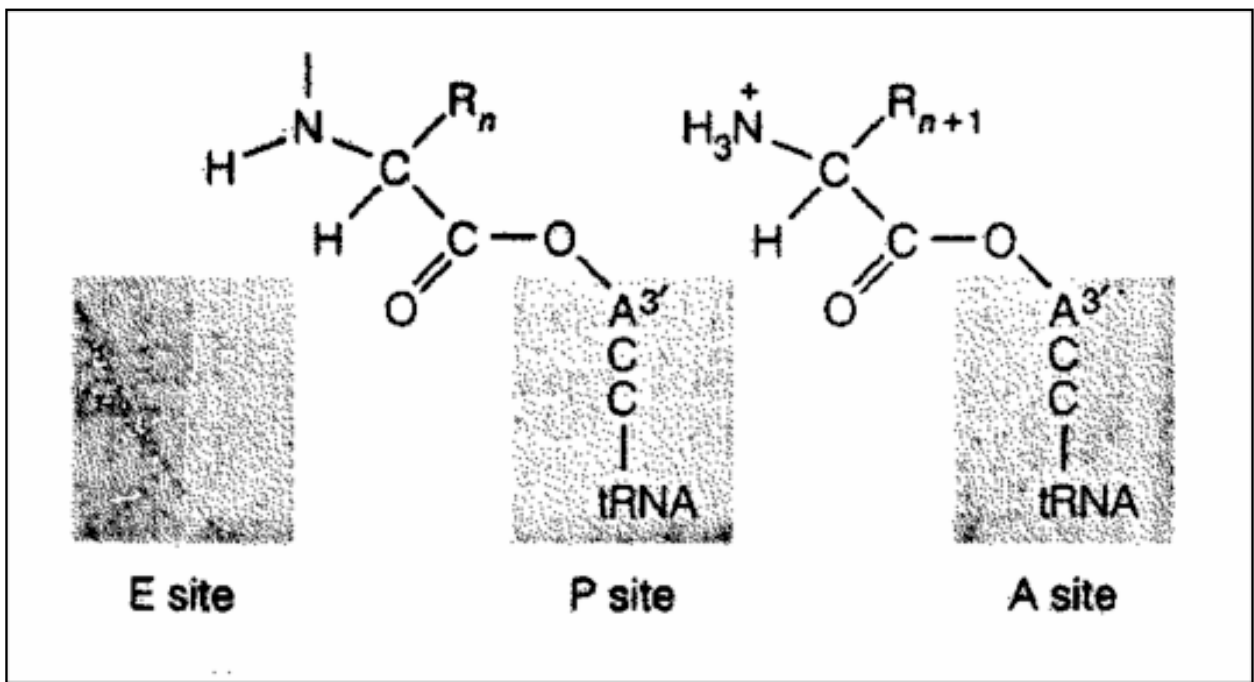
The elongation factors are similar in eukaryotes. Instead of two proteins, there is a stable trimer, eEF1-alpha-beta-gamma, which carries out the same function as EF-Tu and EF-Ts. eEF1-alpha is the eukaryotic equivalent of EF-Tu, and eEF1-beta-gamma the eukaryotic equivalent of EF-Ts.

EF-Tu-GDP is the inactive form. EF-Ts activates EF-Tu by catalyzing the exchange of GDP for GTP. EF-Tu-GTP is the active form which binds to non-initiator tRNAs to which the aminoacyl

group has been attached.

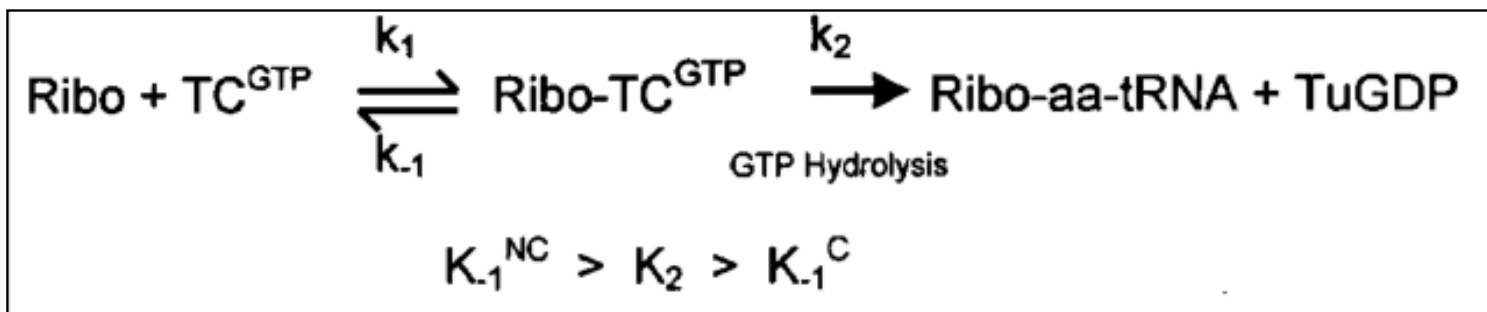


EF-Tu-GTP-aminoacyl-tRNA is then carried to the ribosome. The complex binds to the ribosome, with the aminoacyl-tRNA in the A-site. Ribosome binding stimulates GTP hydrolysis and EF-Tu-GDP dissociates from the ribosome, free to recycle through the step multiple times.



EF-Tu is partially responsible for the high degree of accuracy of protein synthesis via a mechanism called kinetic proofreading. The error rate of protein synthesis is only 1 wrong amino acid for every 10,000 amino acids added to polypeptides.

When a charged tRNA is positioned into the A-site, the anticodon must base pair with the mRNA codon. If there is an incorrect match, the incorrect aa-tRNA dissociates from the ribosome before GTP hydrolysis occurs. If there is a correct match, GTP hydrolysis occurs and EF-Tu-GDP leaves the ribosome before the cognate aminoacyl-tRNA can dissociate and EF-Tu-GDP dissociates instead, leaving the correct tRNA on the ribosome.



EF-Tu is so important to cellular function that it is one of the most abundant cytoplasmic proteins (>5%). There is one copy of the EF-Tu protein for each tRNA molecule in the cell.

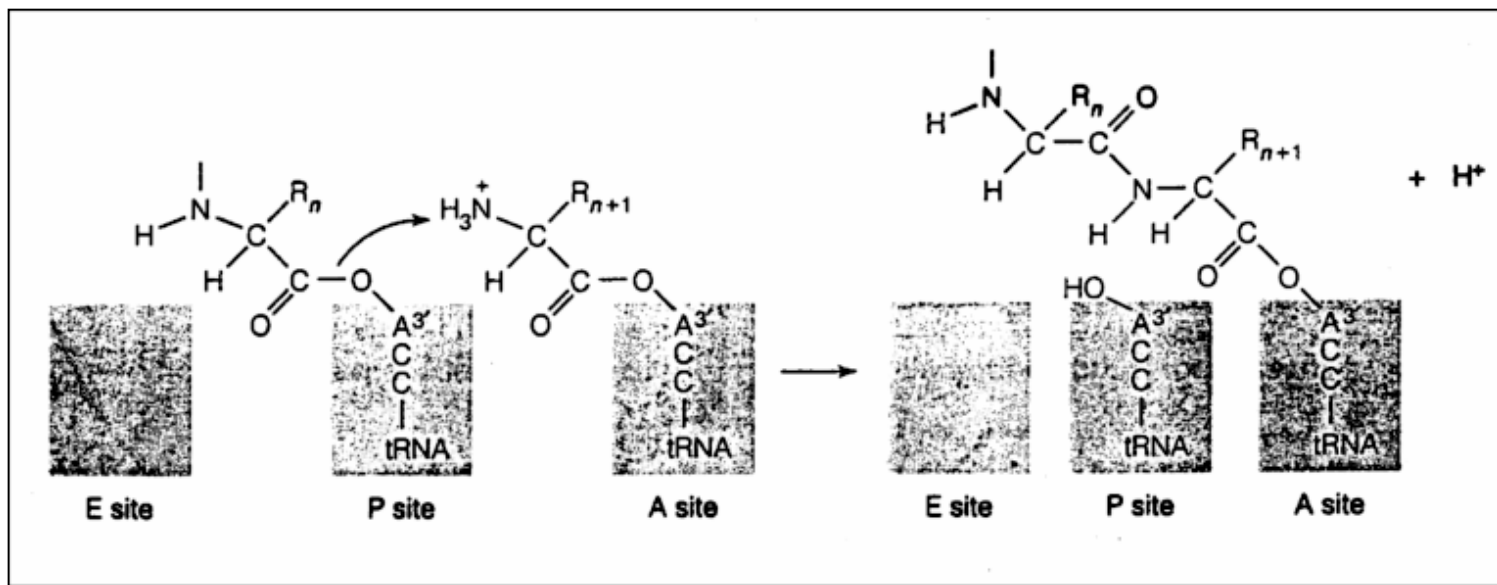
[Next: Peptidyl Transfer](#)

Please report typos, errors etc. by [EMAIL](#) (mention the title of this page).

ELONGATION OF PROTEIN SYNTHESIS (cont.)

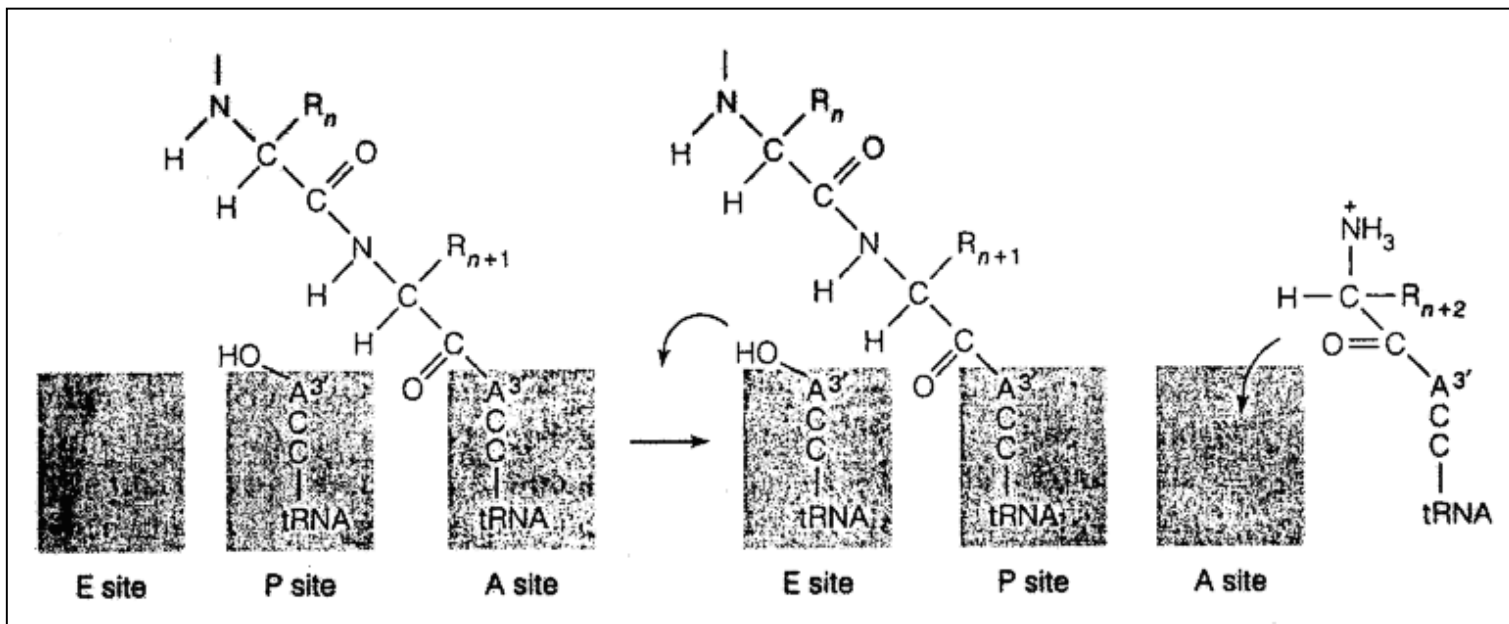
Peptidyl Transfer or Transpeptidation

Next, the growing polypeptide chain on the P-site tRNA (peptidyl tRNA) is *covalently* linked to the amino acid attached to the A-site tRNA, that is *a peptide bond is formed*. The P-site tRNA is now unacylated and the A-site tRNA is covalently linked to the growing polypeptide chain. This step is somewhat of a mystery but is believed to be catalyzed by a region of the 50S subunit, called the peptidyltransferase complex. The complex utilizes both proteins and 23S rRNA. There is growing evidence that 23S rRNA actively catalyzes the peptidyl transfer step but the mode of action is yet unknown.



Translocation

The A-site tRNA with the growing polypeptide chain is then moved within the ribosome to the P-site while the deacylated P-site tRNA is moved to the E-site. Simultaneously, the mRNA is shifted by *exactly* 3 ribonucleotides or 1 codon.



The process requires a soluble protein factor, called elongation factor EF-G. EF-G is similar, but much larger than EF-Tu. EF-G is also active as the GTP complex.

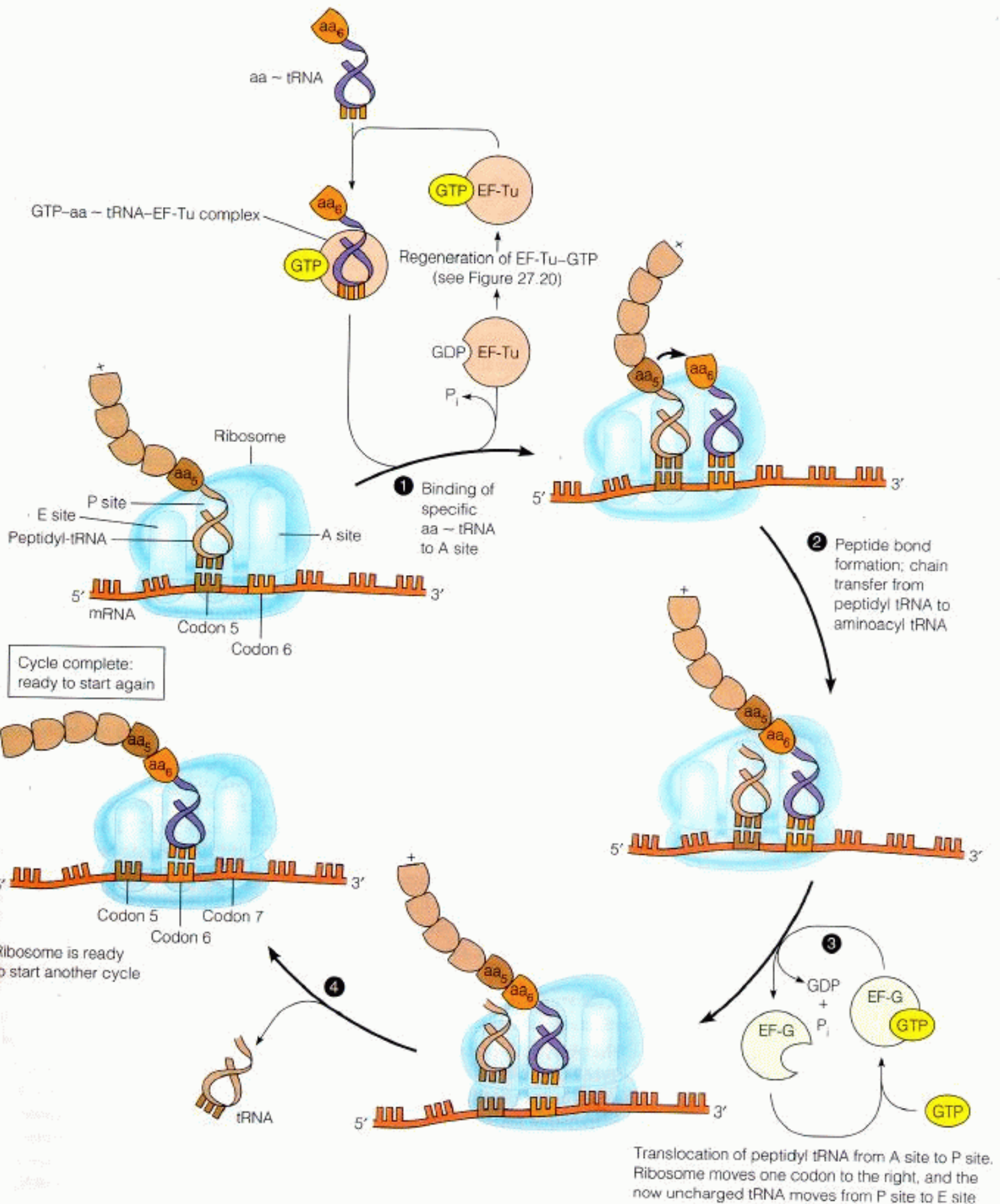
Although many older texts report that the binding of EF-G-GTP to the ribosome provides the energy for translocation, it is now known to be wrong. The energy derived from GTP hydrolysis drives translocation.

After GTP hydrolysis, EF-G-GDP is released from the ribosome, the tRNA carrying the polypeptide chain is in the P site, and the next mRNA codon is in the A site. The ribosome is now ready to start the elongation cycle over again to add a new amino acid, until a termination signal is reached. It appears that EF-G catalyzes its own exchange of GDP and GTP. No soluble guanine nucleotide exchange factor has yet been found. The equivalent of EF-G in eukaryotes is eEF2.

[Next: Schematic of Elongation](#)

Please report typos, errors etc. by [EMAIL](#) (mention the title of this page).

Chain elongation in prokaryotic translation

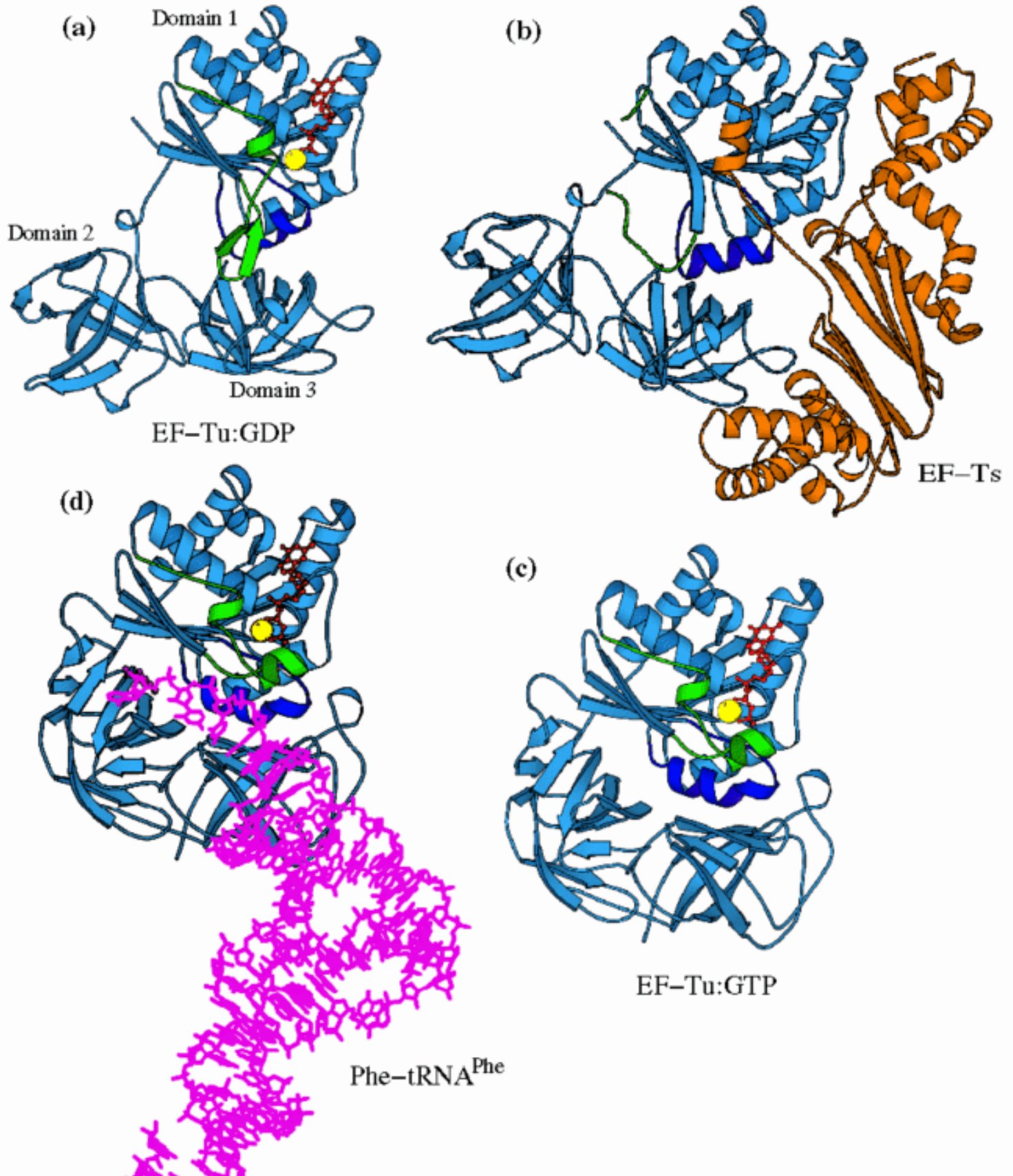


The elongation process is depicted as a cycle. Following translocation (step 3) and empty tRNA release (step 4), the ribosome is ready to accept the next aminoacyl tRNA (aa-tRNA) and repeat the cycle. This cycle will continue until a termination codon is reached.

Next: Atomic Structures of EF-Tu Complexes

Please report typos, errors etc. by [EMAIL](#) (mention the title of this page).

ATOMIC STRUCTURES OF EF-Tu COMPLEXES





[Next: Termination of Protein Synthesis](#)

Please report typos, errors etc. by [EMAIL](#) (mention the title of this page).

TERMINATION OF PROTEIN SYNTHESIS

1. The mRNA Signal

STOP Codons: **UAA**, **UAG**, or **UGA**

There are no tRNAs that recognize the STOP codons **UAA**, **UAG**, or **UGA**.

2. Soluble Protein Release Factors

RF1 responds to **UAA** or **UAG**

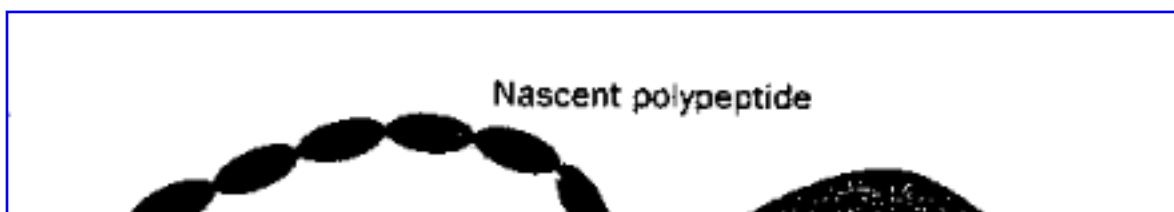
RF2 responds to **UAA** or **UGA**

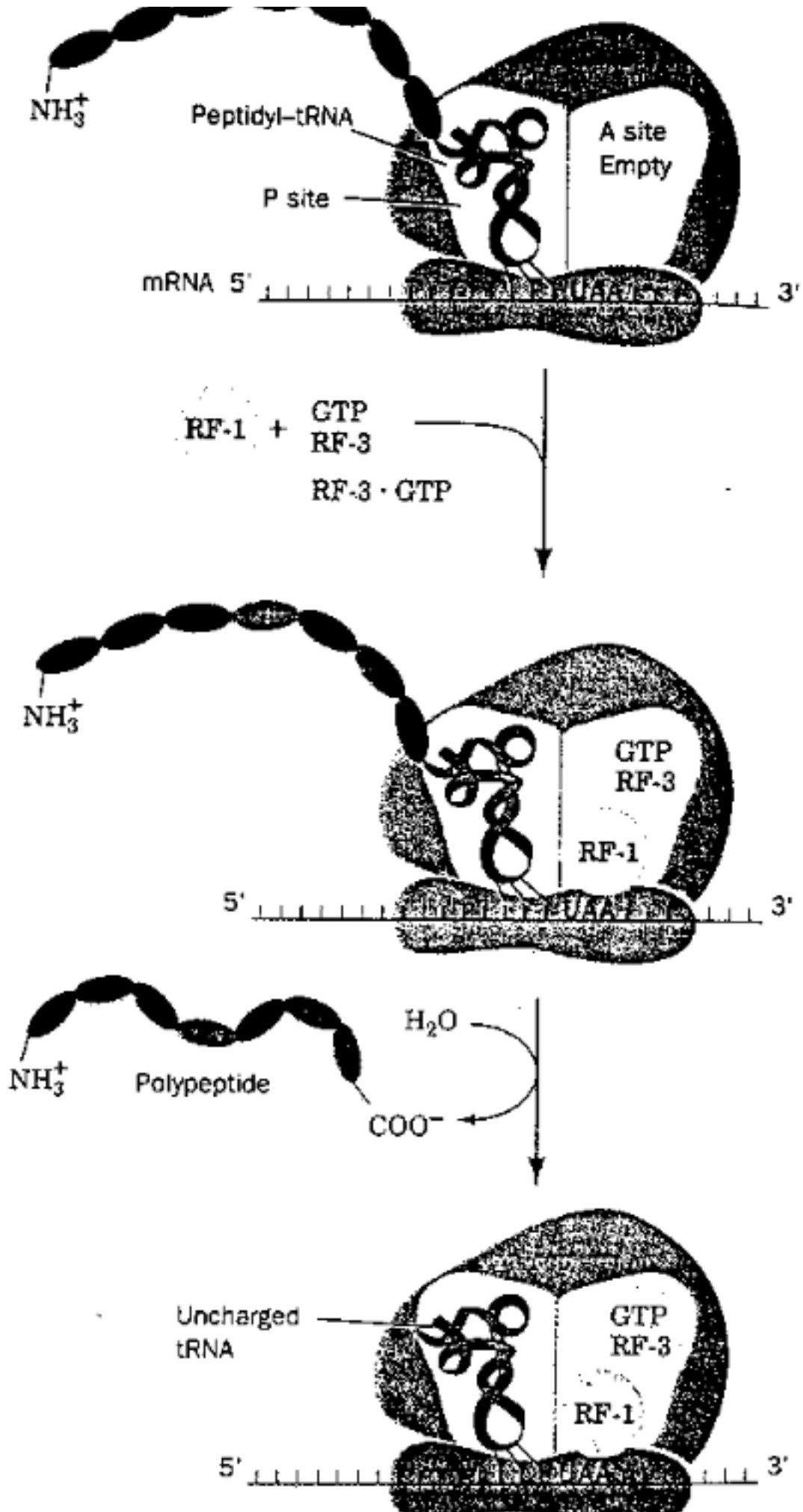
RF3, a GTPase (like EF-Tu and binds in a similar A-site location)

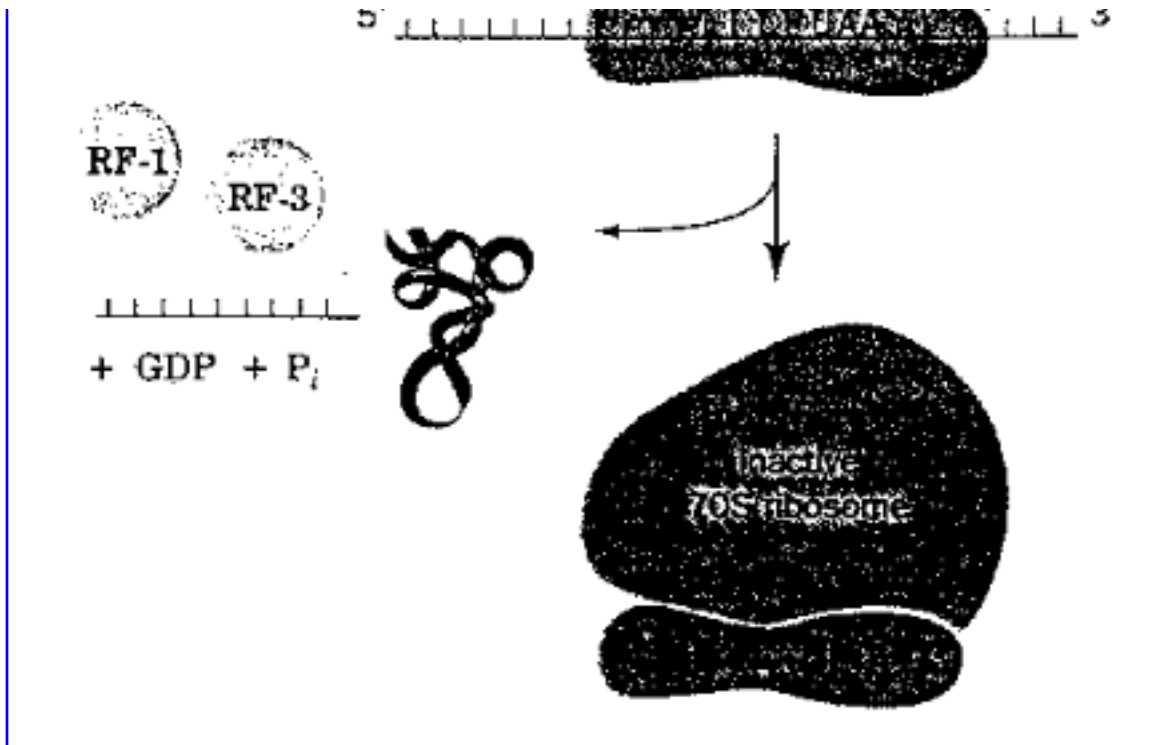
RF1/RF2 interact with RF3-GTP, have a similar shape as EF-Tu-GTP-aa-tRNA or EF-G, and bind in a similar ribosomal site (A-site). In a manner similar to [EF-G](#), GTP hydrolysis drives the movement of the terminal mRNA codon into the P-site, moving the last tRNA into the E-site and off. At the same time, the polypeptide chain is released after hydrolysis of the tRNA-peptide bond.

In eukaryotes, only a single release factor, eRF, is necessary. It recognizes all three STOP codons and interacts with GTP.

A mutation resulting in a premature STOP codon is called a [nonsense mutation](#).







The termination pathway in *E. coli* ribosomes: RF-1 recognizes the termination codons **UAA** and **UAG**, whereas RF-2 recognizes **UAA** and **UGA**. Eukaryotic termination follows an analogous pathway but requires only a single release factor, eRF, that recognizes all three termination codons.

[Next: Summary](#)

Please report typos, errors etc. by [EMAIL](#) (mention the title of this page).

Summary

- **Elongation** consists of **three** distinct steps to add one amino acid
- Requires three elongation factors: EF-Tu/EF-Ts and EF-G
- Requires two GTPs per cycle
- Occurs many times per polypeptide
- The elongation cycle is similar in prokaryotes and eukaryotes.
- Fast: 15-20 amino acids added per second
- Accurate: 1 mistake every ~10,000 amino acids

- **Termination** results in the release of the polypeptide chain
- Requires one of the three STOP codons: **UAA**, **UAG**, or **UGA**.
- Requires RF1 or RF2, and RF3 in prokaryotes (eRF in eukaryotes)
- Requires one GTP

- Each step of protein synthesis (initiation, elongation and termination) requires GTP

Next lecture: Regulation of Protein Synthesis at the Translational Level

And now: Movie time!

Please report typos, errors etc. by [EMAIL](#) (mention the title of this page).