Transcription in Eukaryotes
&
Translation

Comparison of eukaryotic and prokaryotic promoter recognition

Eukaryotes: general transcription factors (GTFs). TFI factors for RNAP I, TFII factors for RNAP II and TFIII factors for RNAP III

Prokaryotes: σ factors

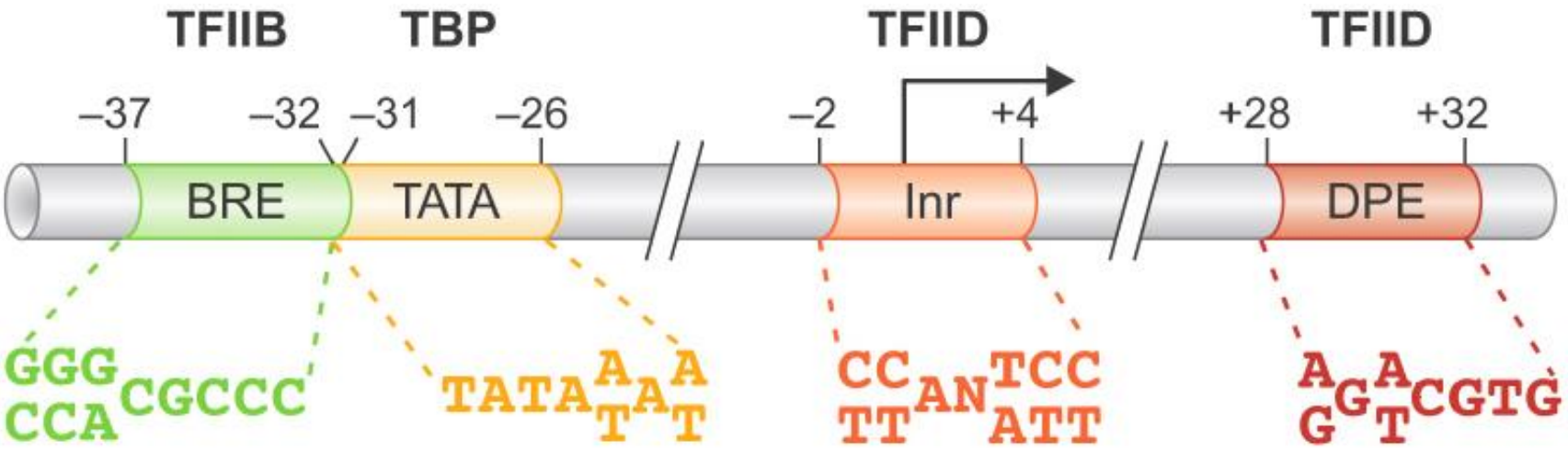
In addition to the RNAP and GTFs, *in vivo* transcription also requires

- Mediator complex
- DNA-binding regulatory proteins
- chromatin-modifying enzymes

RNA polymerase II core promoters are made up of combinations of 4 different sequence elements

Eukaryotic core promoter (~40 nt): the minimal set of sequence elements required for accurate transcription initiation by the Pol II machinery in vitro

Pol II core promoter



- TFIIB recognition element (BRE)
- The TATA element/box
- Initiator (Inr)
- The downstream promoter element (DPE)

Regulatory sequences

The sequence elements other than the core promoter that are required to regulate the transcription efficiency

Those increasing transcription:

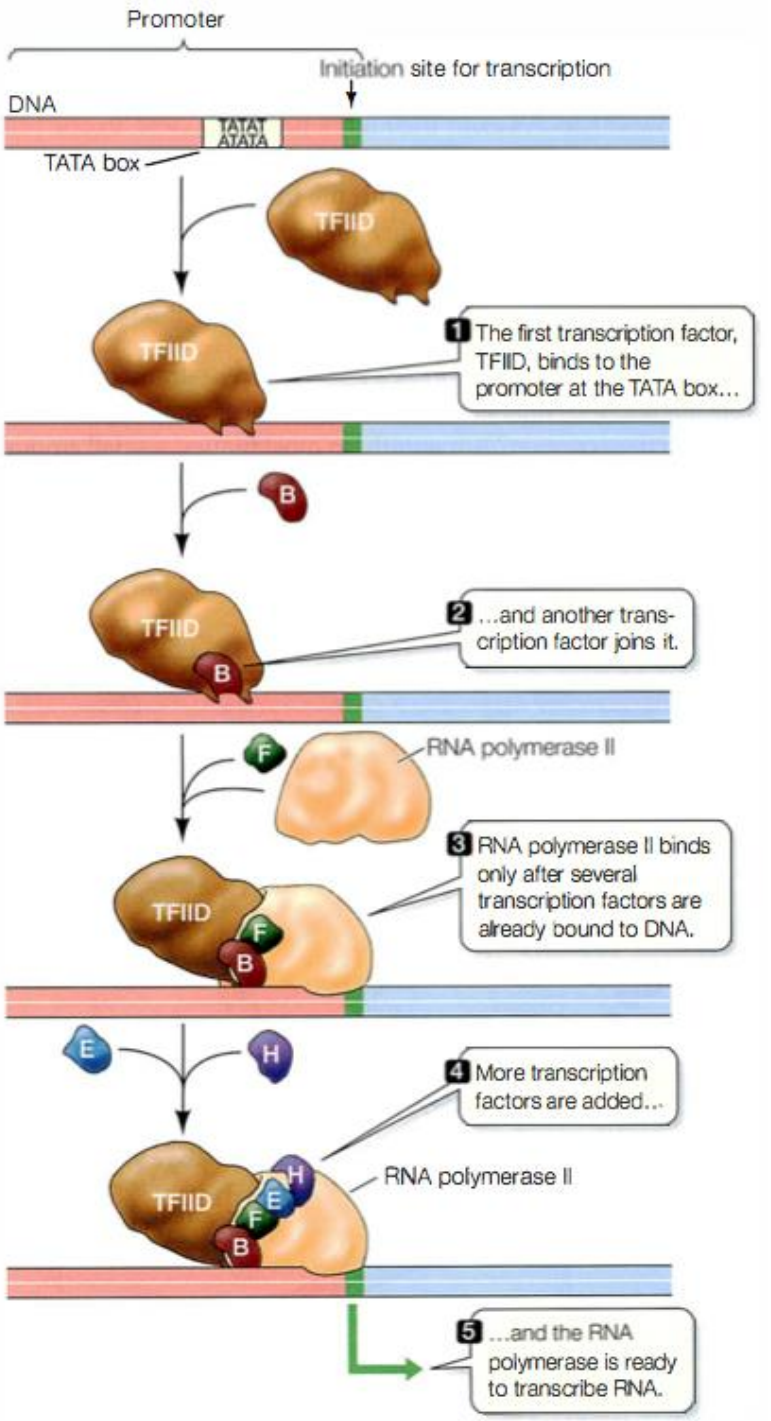
- Promoter proximal elements
- Upstream activator sequences (UASs)
- Enhancers

Those repressing elements: silencers, boundary elements, insulators

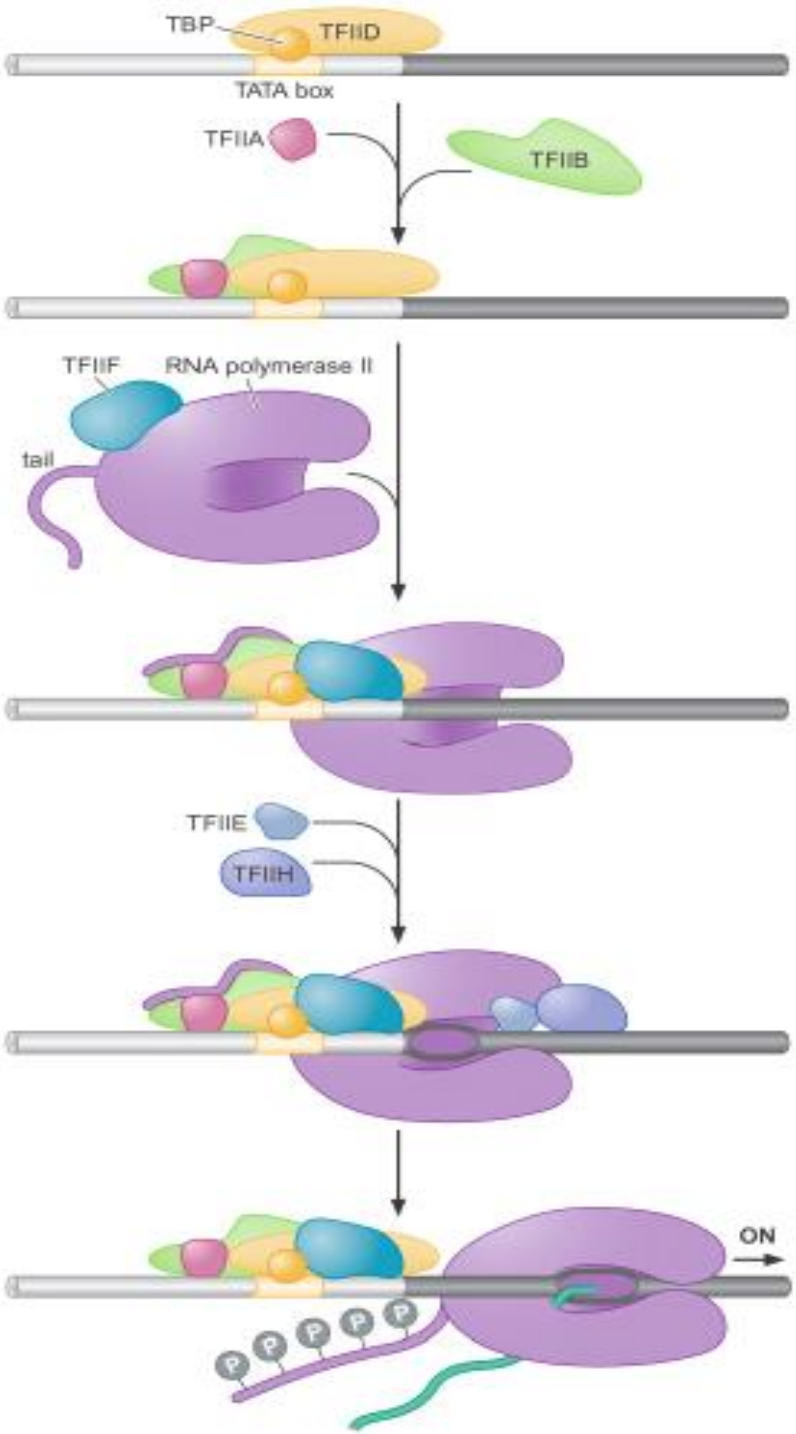
RNA Pol II forms a pre-initiation complex with GTFs at the promoter

The involved GTFIIs (general transcription factor for Pol II)

- TFIID=TBP (TATA box binding protein) + TAFs (TBP association factors)
- TFIIA, B, F, E, H



1. <u>TBP in TFIID</u> binds to the TATA box
2. <u>TFIIA and TFIIB</u> are recruited with TFIIB binding to the BRE
3. <u>RNA Pol II-TFIIF</u> complex is then recruited
4. <u>TFIIE and TFIIH</u> then bind upstream of Pol II to form the pre-initiation complex
5. Promoter melting using energy from ATP hydrolysis by TFIIH)
6. Promoter escapes after the phosphorylation of the CTD tail

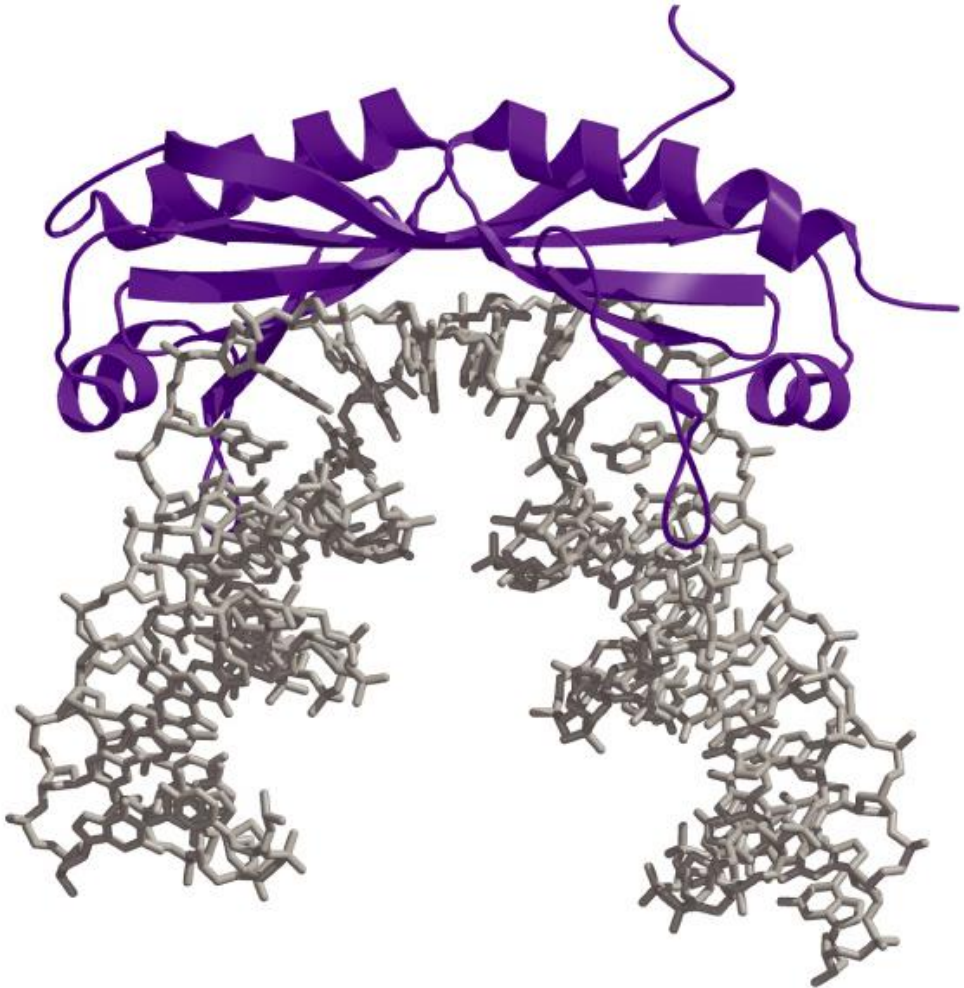


Promoter escape

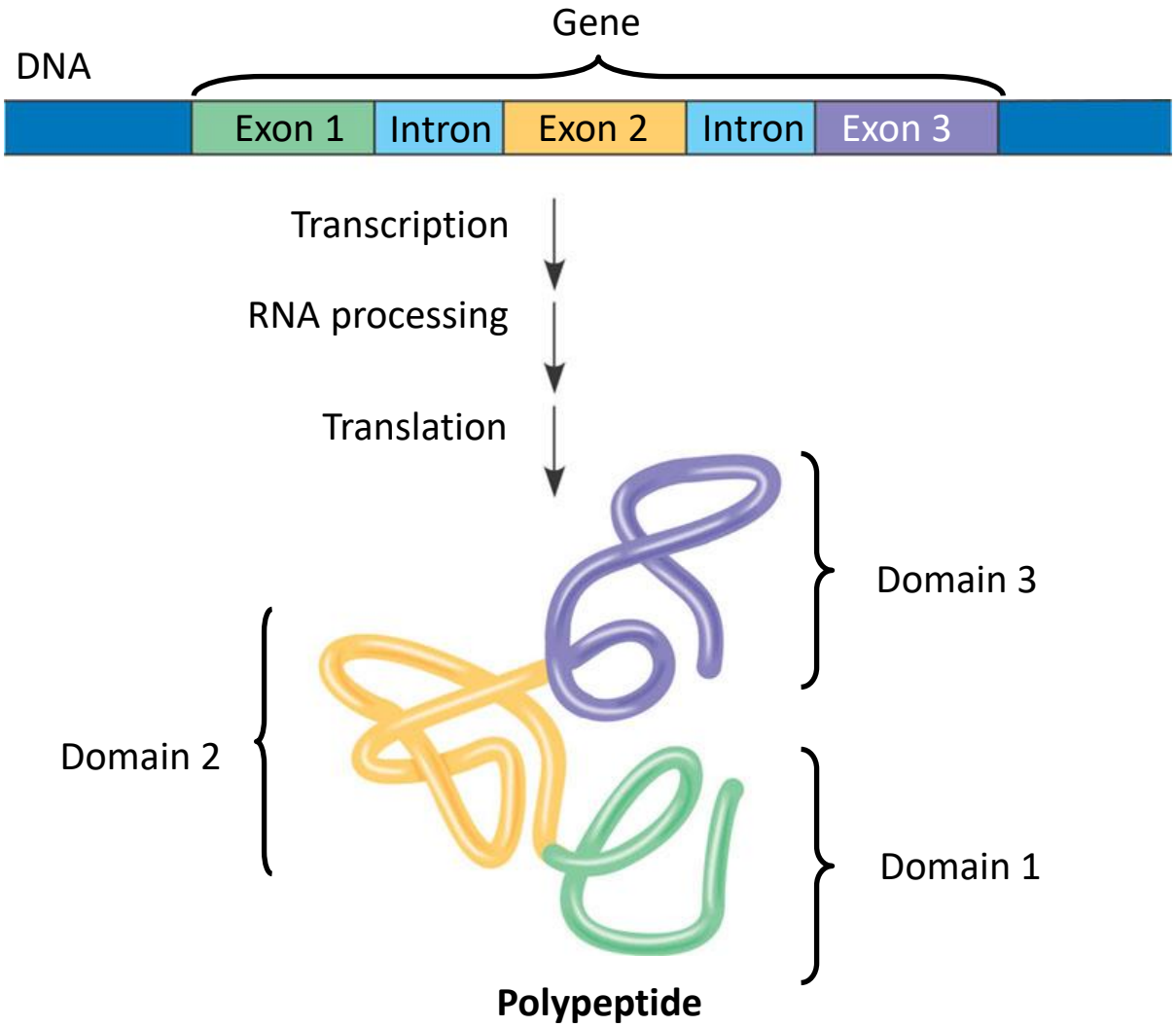
Stimulated by phosphorylation of the CTD (C-terminal domain) tail of the RNAP II

- CTD contains the heptapeptide repeat Tyr-Ser-Pro-Thr-Ser-Pro-Ser
- Phosphorylation of the CTD "tail" is conducted by a number of specific kinases including a subunit of TFIIH

TBP binds to and distorts DNA using a β sheet inserted into the minor groove



Correspondence between exons and protein domains



Translation Initiation in mRNAs

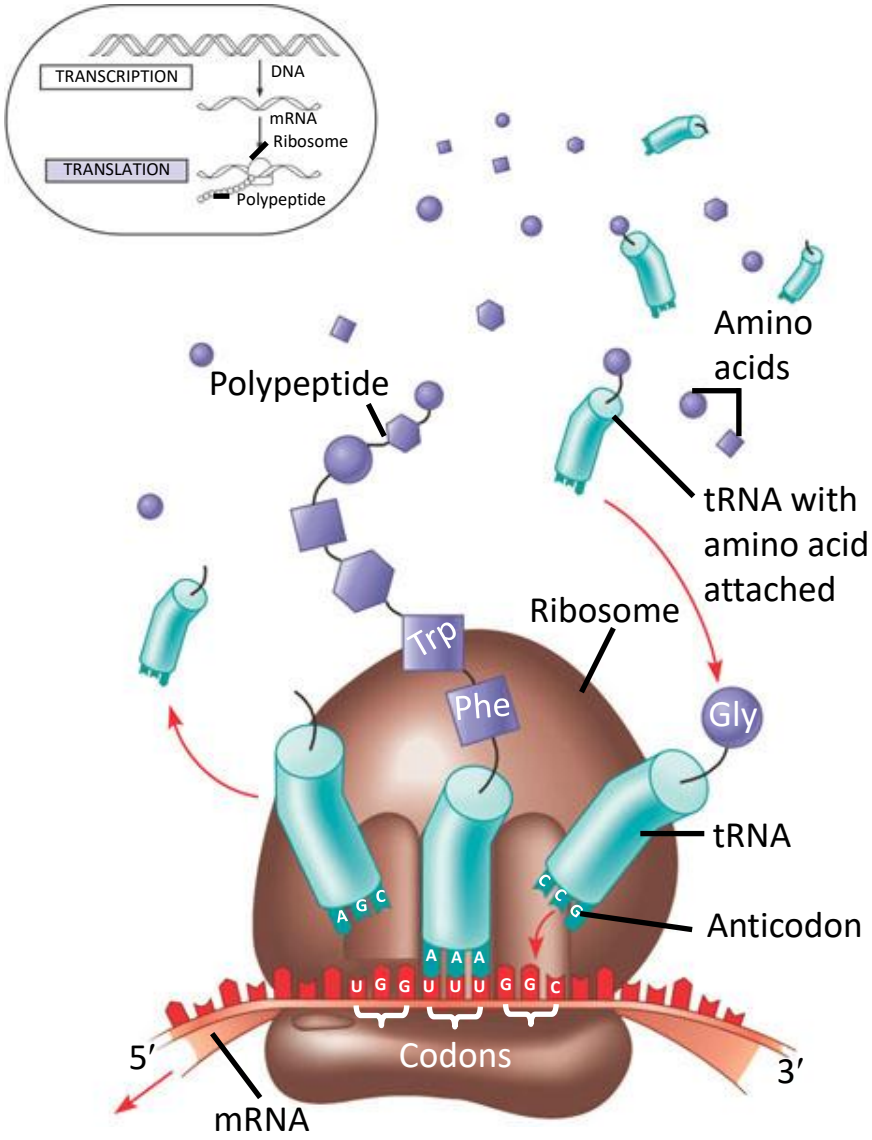
Shine Dalgarno Sequence in Prokaryotes

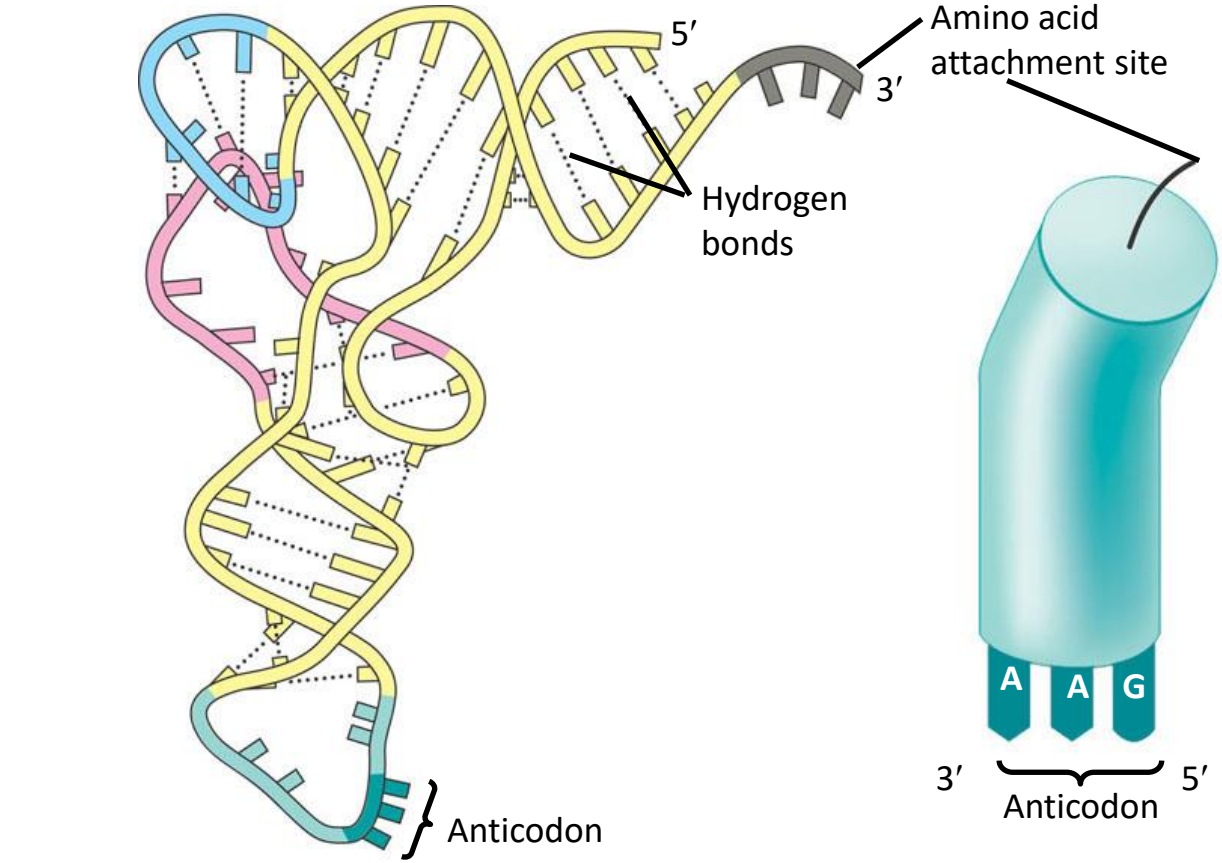
NNNNNNNAGGAGG---8bp----AUGNNNNNNNNNN

Kozak's Consensus Sequence in Eukaryotes

NNNNNNA/GNNAUGGNNNNNNNNNN

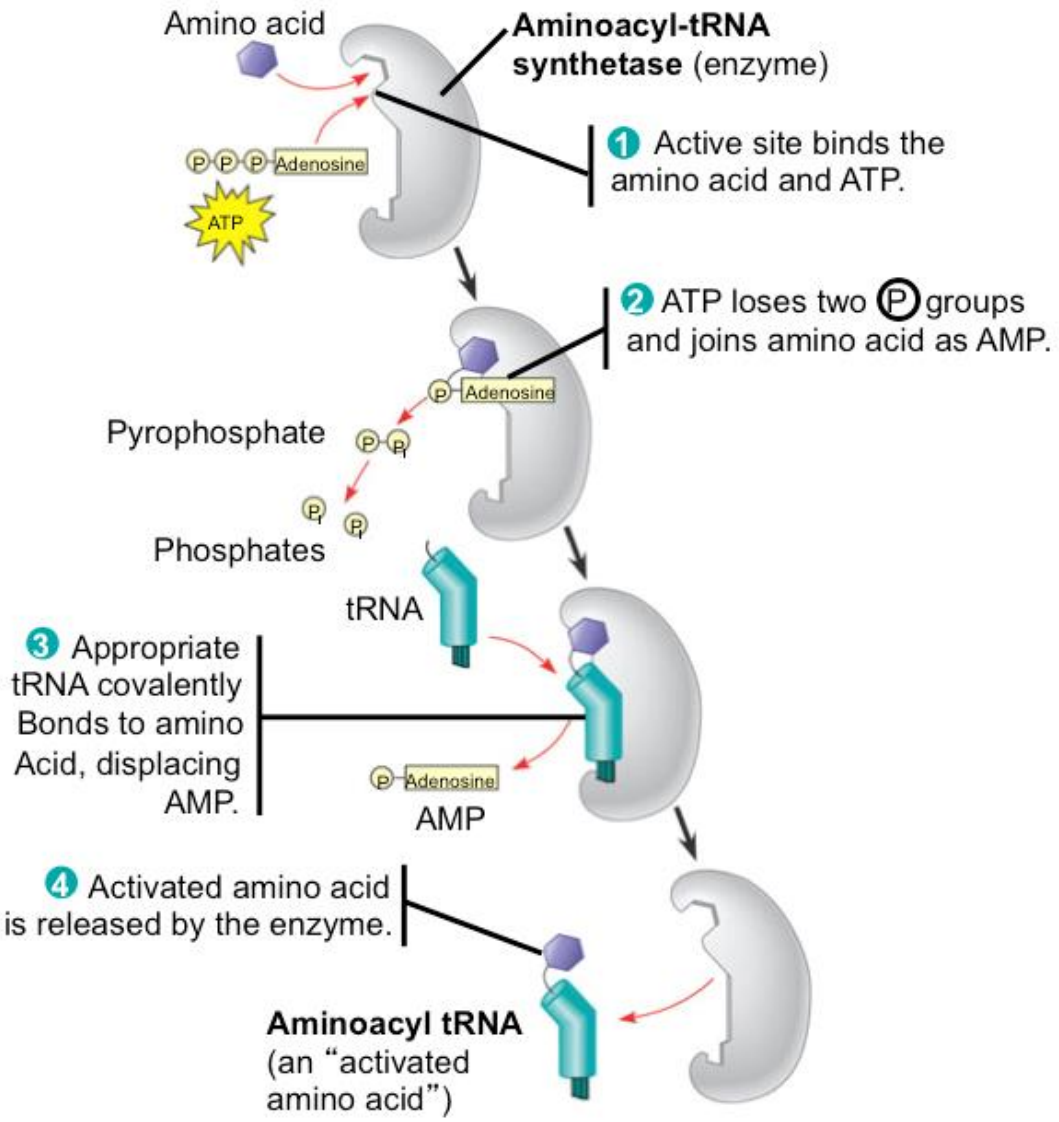
Translation: the basic concept



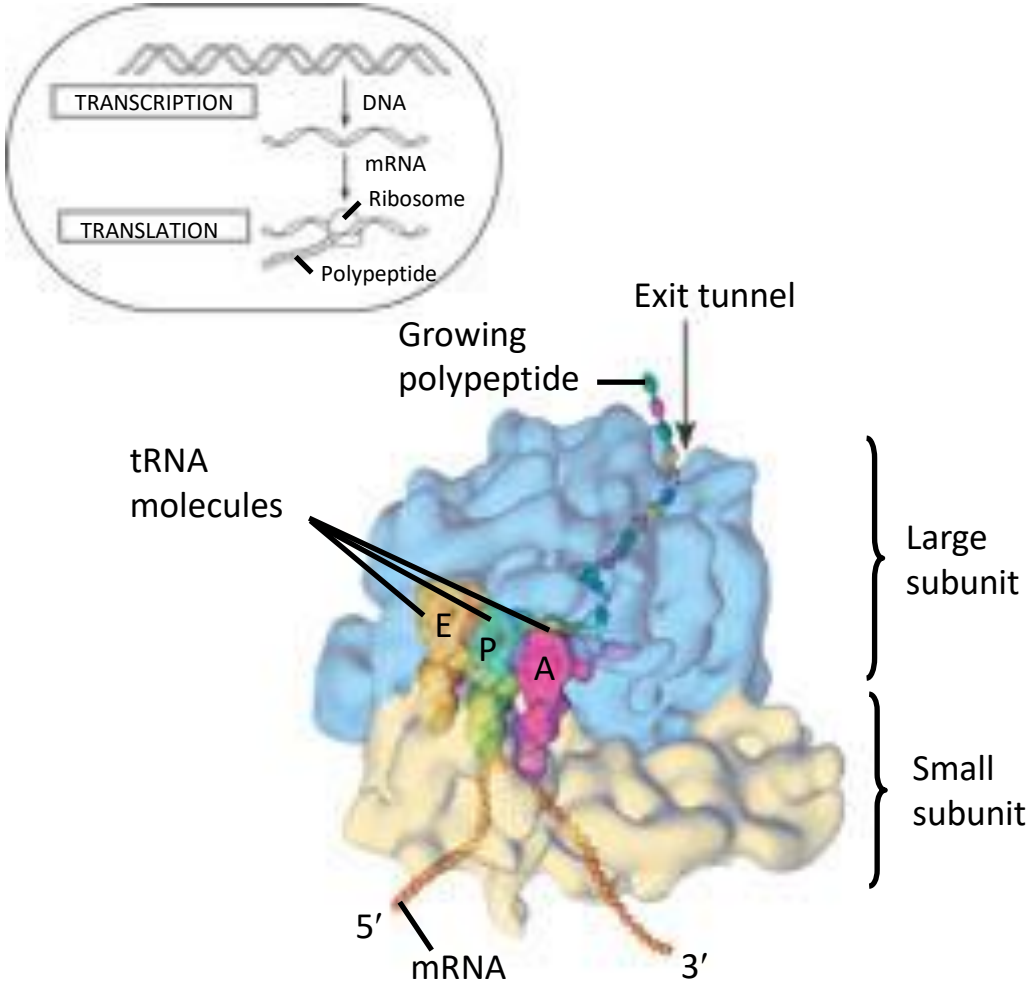


(b) Three-dimensional structure

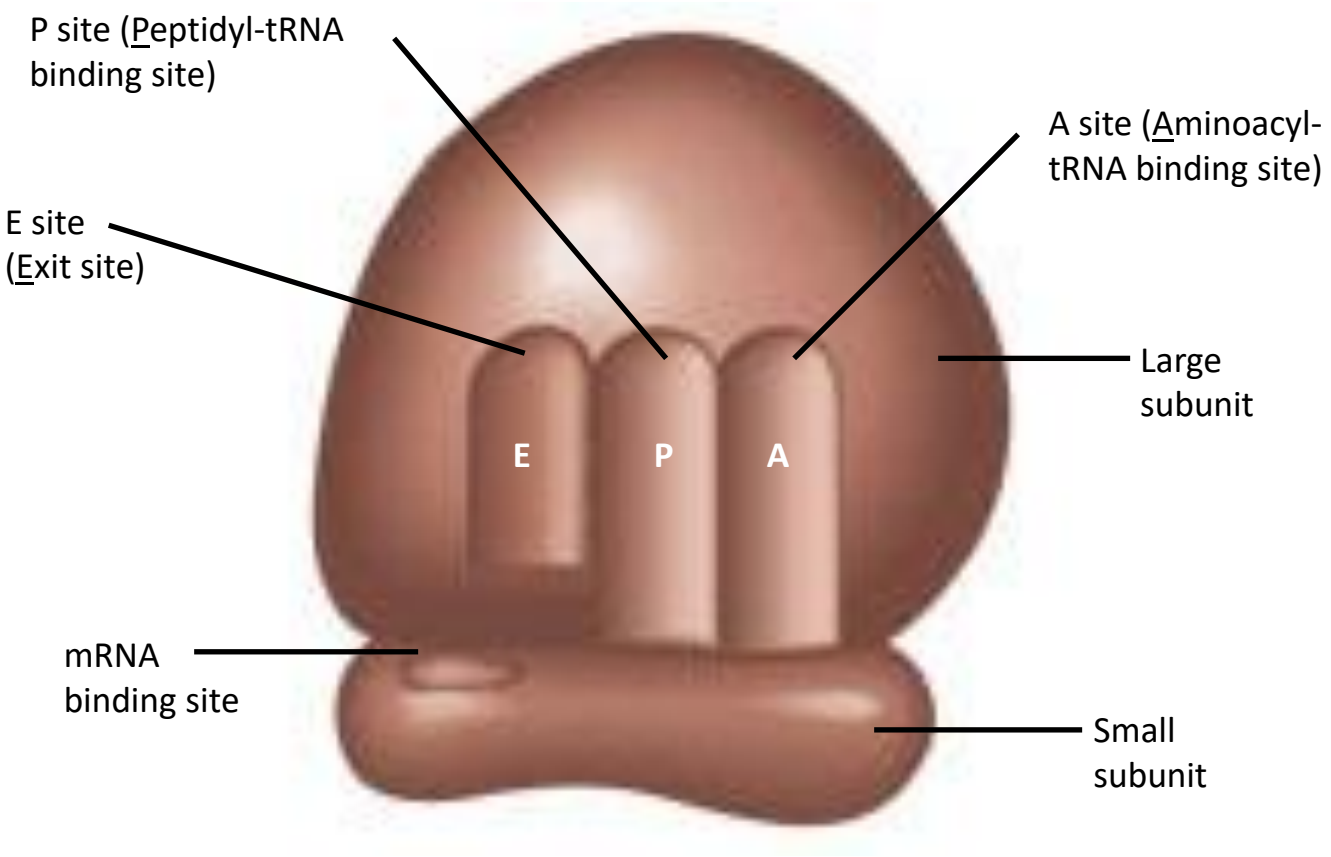
An aminoacyl-tRNA synthetase joins a specific amino acid to a tRNA



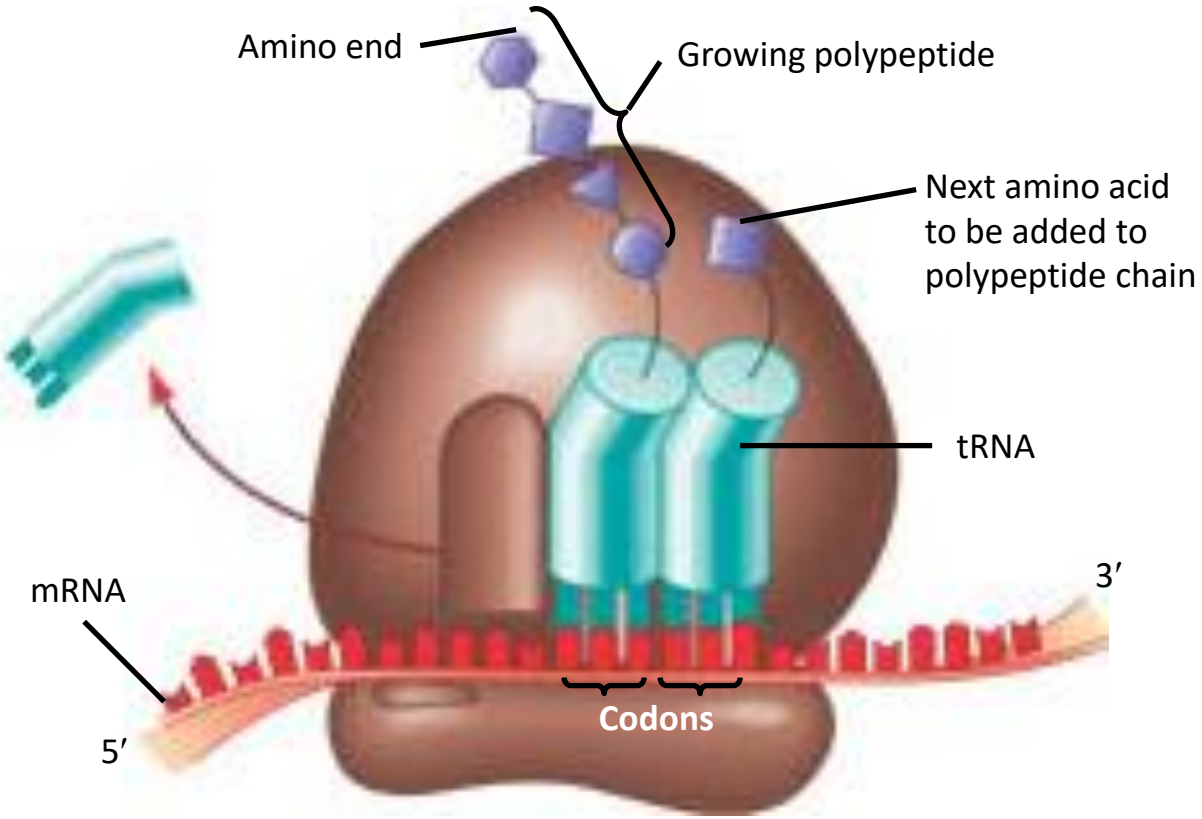
The anatomy of a functioning ribosome



(a) Computer model of functioning ribosome. This is a model of a bacterial ribosome, showing its overall shape. The eukaryotic ribosome is roughly similar. A ribosomal subunit is an aggregate of ribosomal RNA molecules and proteins.

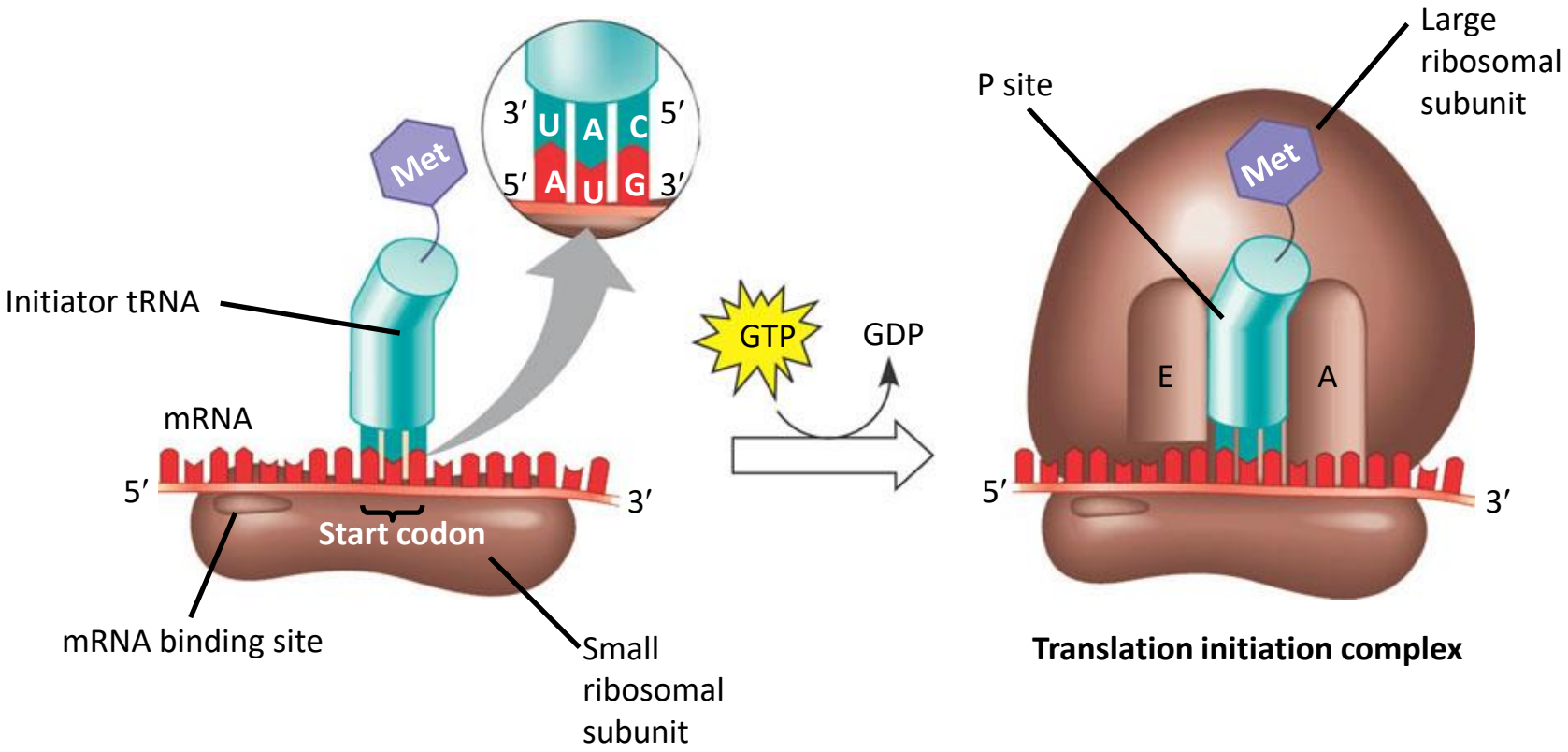


(b) Schematic model showing binding sites. A ribosome has an mRNA binding site and three tRNA binding sites, known as the A, P, and E sites. This schematic ribosome will appear in later diagrams.



(c) Schematic model with mRNA and tRNA. A tRNA fits into a binding site when its anticodon base-pairs with an mRNA codon. The P site holds the tRNA attached to the growing polypeptide. The A site holds the tRNA carrying the next amino acid to be added to the polypeptide chain. Discharged tRNA leaves via the E site.

The initiation of translation

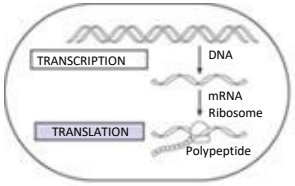


Translation initiation complex

1 A small ribosomal subunit binds to a molecule of mRNA. In a prokaryotic cell, the mRNA binding site on this subunit recognizes a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine (Met).

2 The arrival of a large ribosomal subunit completes the initiation complex. Proteins called initiation factors (not shown) are required to bring all the translation components together. GTP provides the energy for the assembly. The initiator tRNA is in the P site; the A site is available to the tRNA bearing the next amino acid.

The elongation cycle of translation

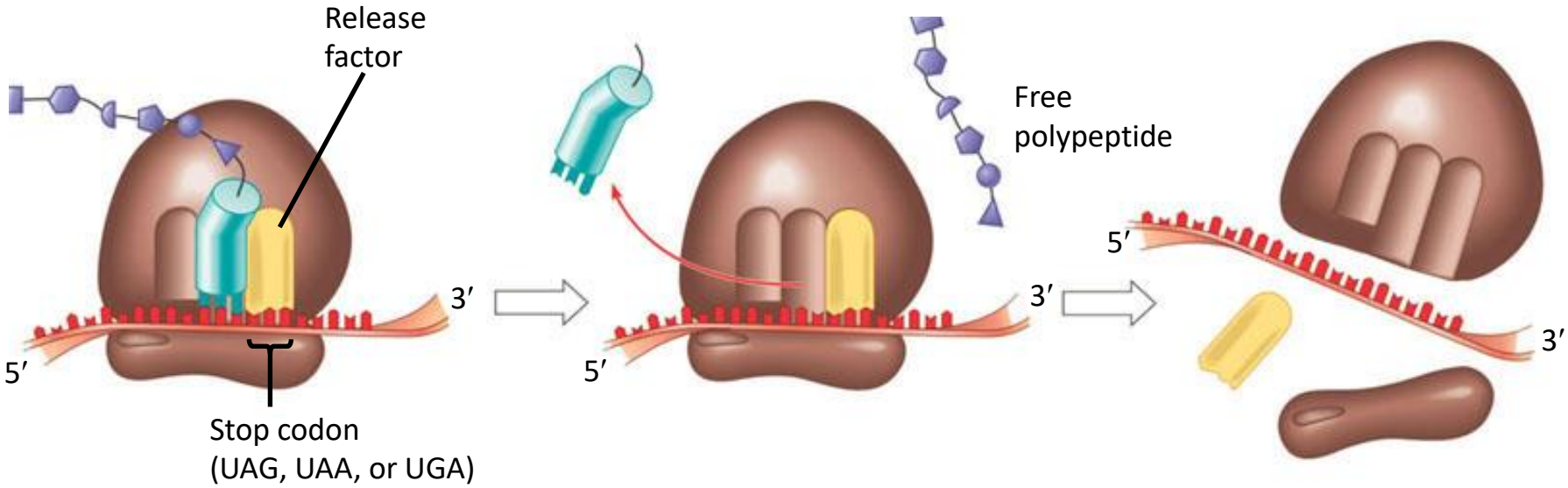


1 Codon recognition. The anticodon of an incoming aminoacyl tRNA base-pairs with the complementary mRNA codon in the A site. Hydrolysis of GTP increases the accuracy and efficiency of this step.

2 Peptide bond formation. An rRNA molecule of the large Subunit catalyzes the formation of a peptide bond between the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site. This step attaches the polypeptide to the tRNA in the A site.

3 Translocation. The ribosome translocates the tRNA in the A site to the P site. The empty tRNA in the P site is moved to the E site, where it is released. The mRNA moves along with its bound tRNAs, bringing the next codon to be translated into the A site.

The termination of translation

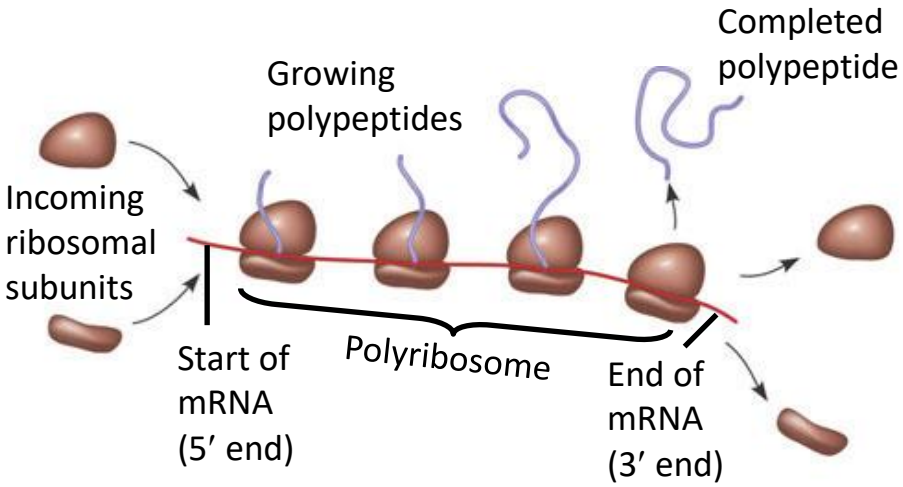


1. When a ribosome reaches a stop codon on mRNA, the A site of the ribosome accepts a protein called a release factor instead of tRNA.

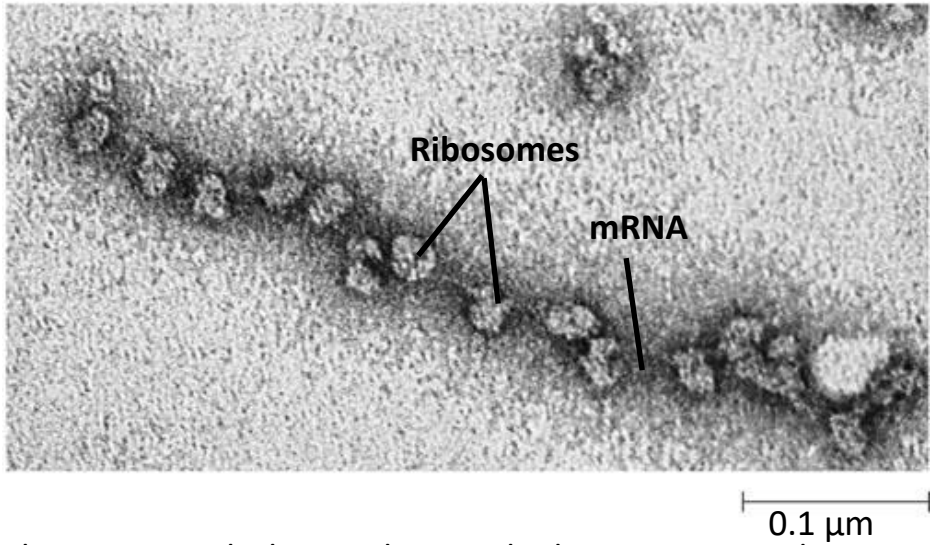
2. The release factor hydrolyzes the bond between the tRNA in the P site and the last amino acid of the polypeptide chain. The polypeptide is thus freed from the ribosome.

3. The two ribosomal subunits and the other components of the assembly dissociate.

Polyribosomes

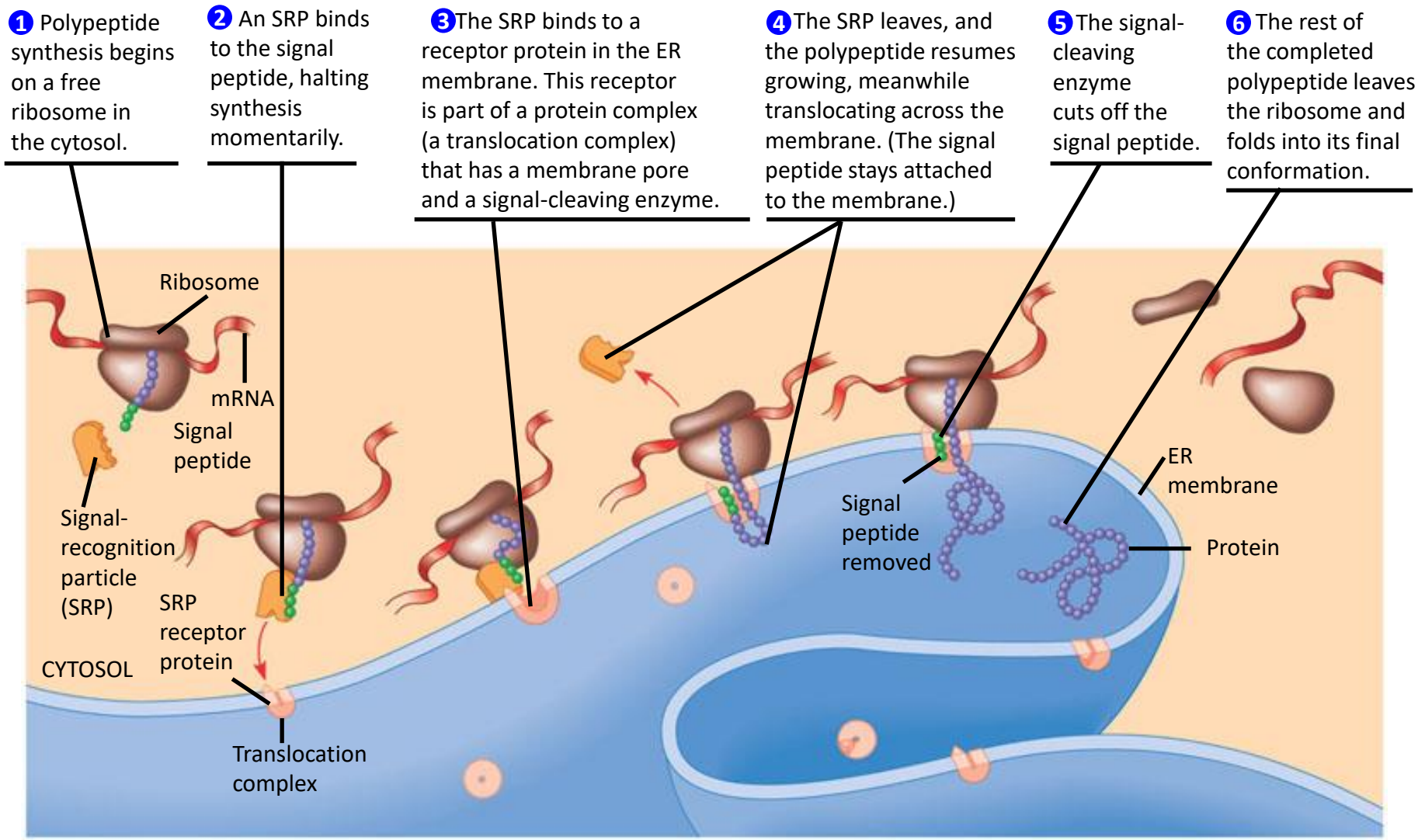


(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.

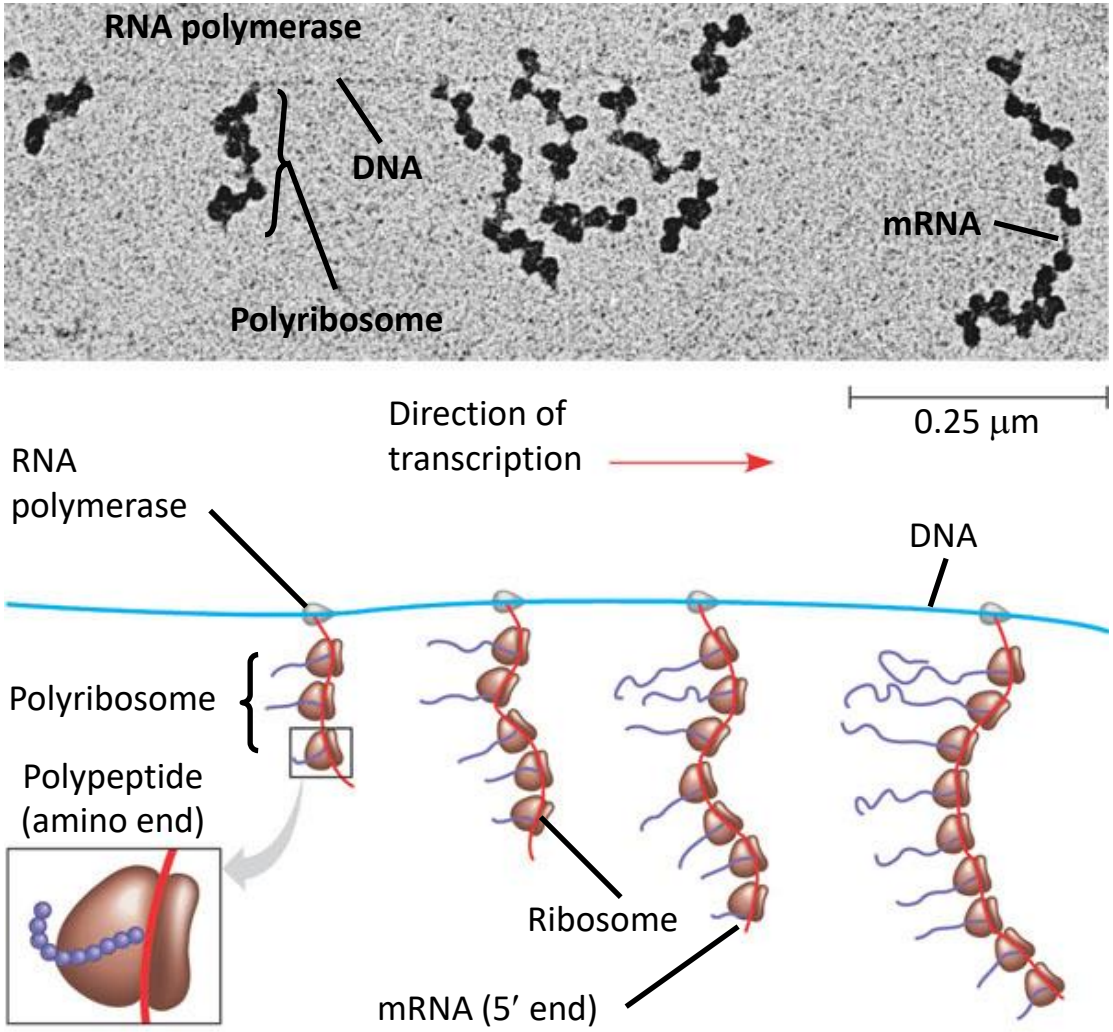


(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

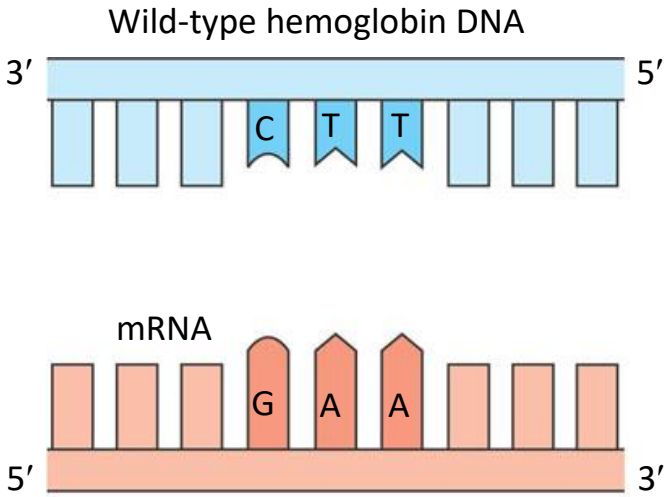
The signal mechanism for targeting proteins to the ER

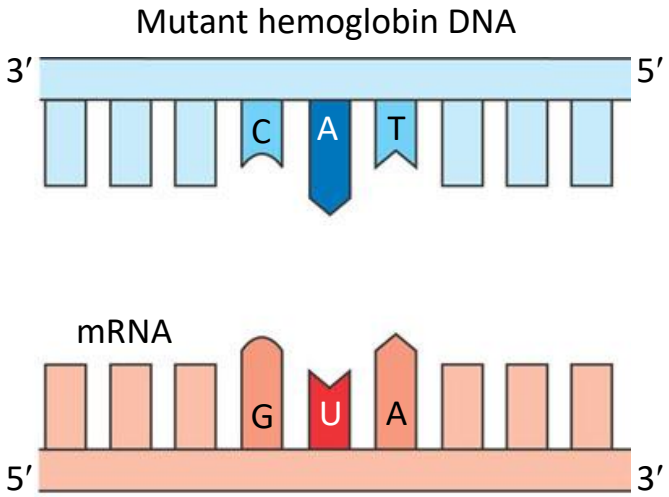


Coupled transcription and translation in bacteria



The molecular basis of sickle-cell disease: a point mutation



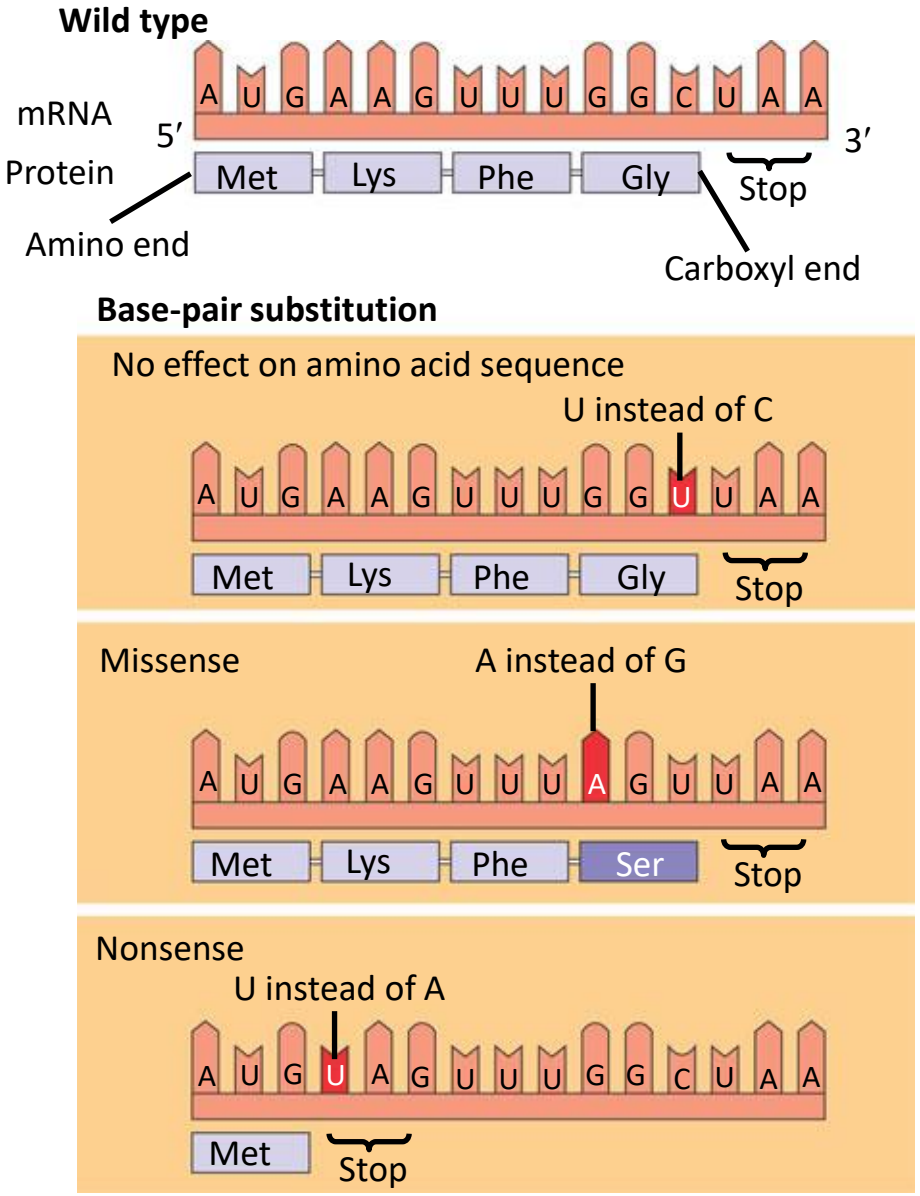


In the DNA, the mutant template strand has an A where the wild-type template has a T.

The mutant mRNA has a U instead of an A in one codon.

The mutant (sickle-cell) hemoglobin has a valine (Val) instead of a glutamic acid (Glu).

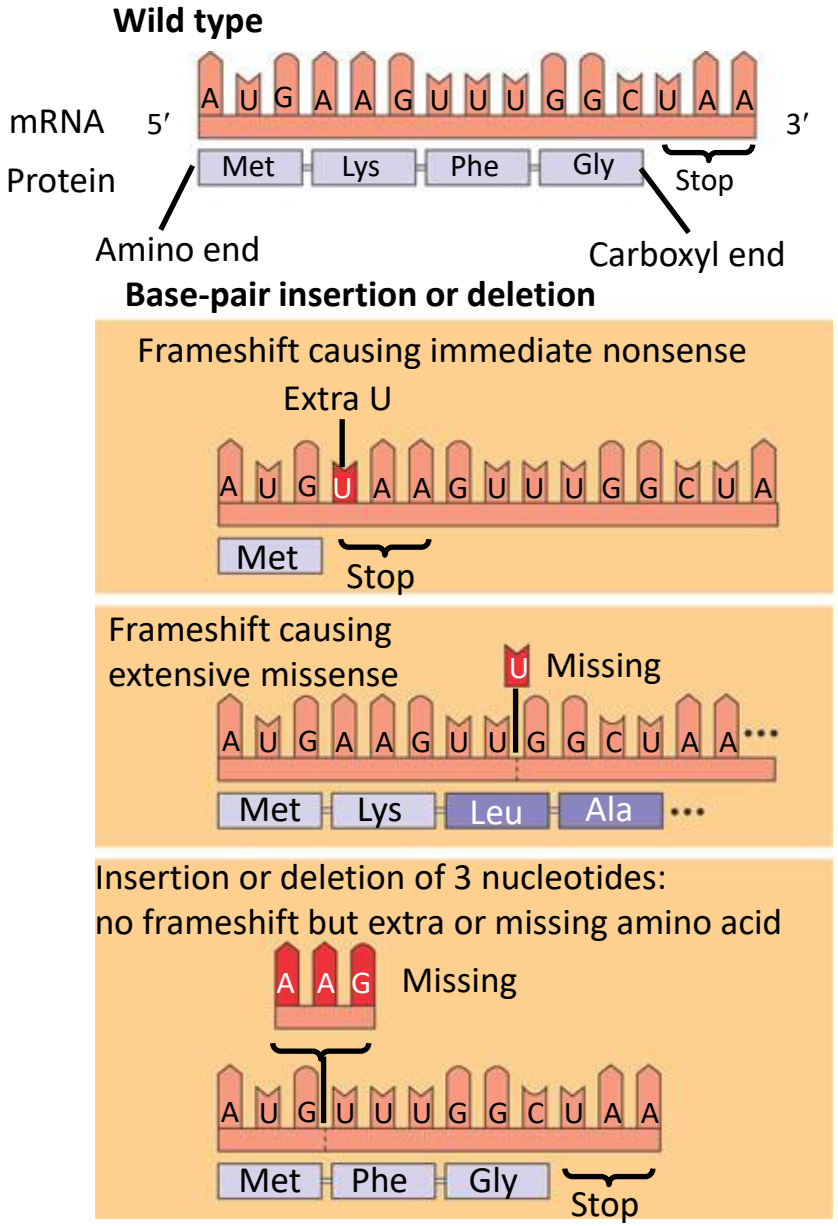
Base-pair substitution



Transition
Purine to Purine
And
Pyrimidine to pyrimidine

Transversion
Purine to pyrimidine
OR
Pyrimidine to Purine

Base-pair insertion or deletion



A summary of transcription and translation in a eukaryotic cell