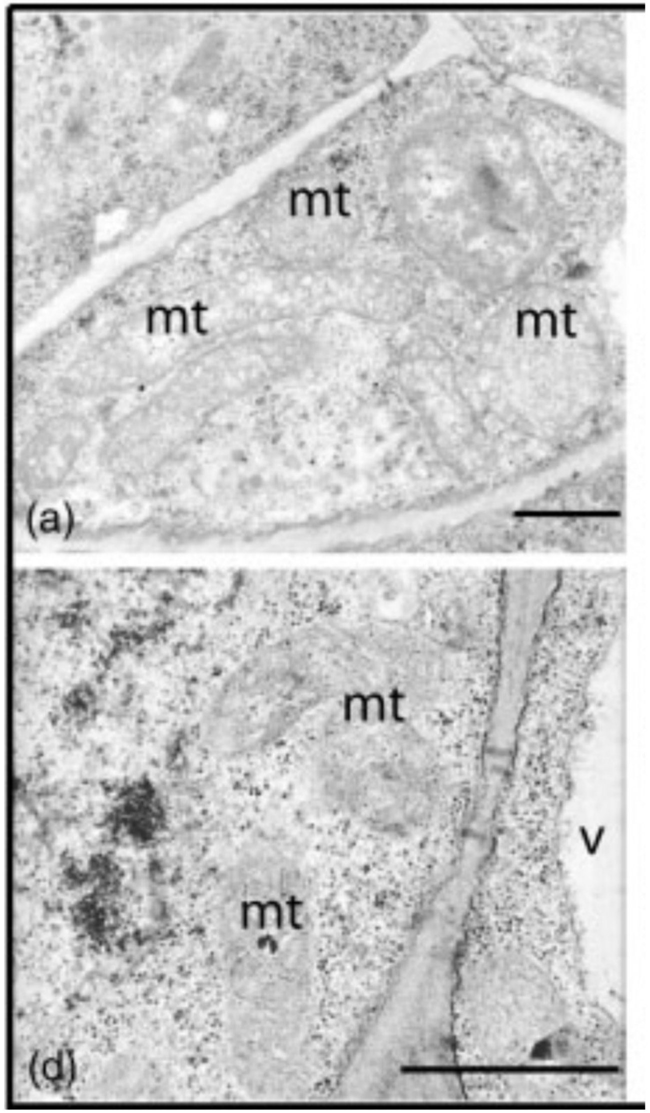
Ribosome

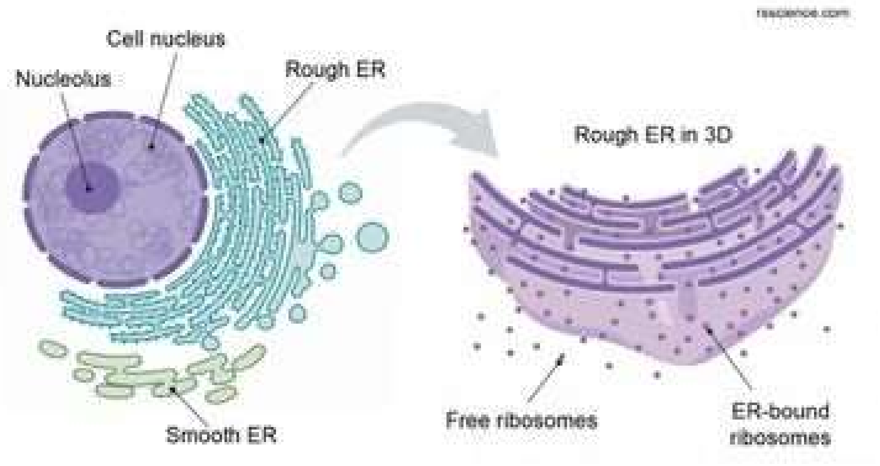


Bar: 1 µm

- Present in large Scale
- Site of protein synthesis

Example of meristematic cells packed with ribosomes and mitochondria

Types of Ribosomes

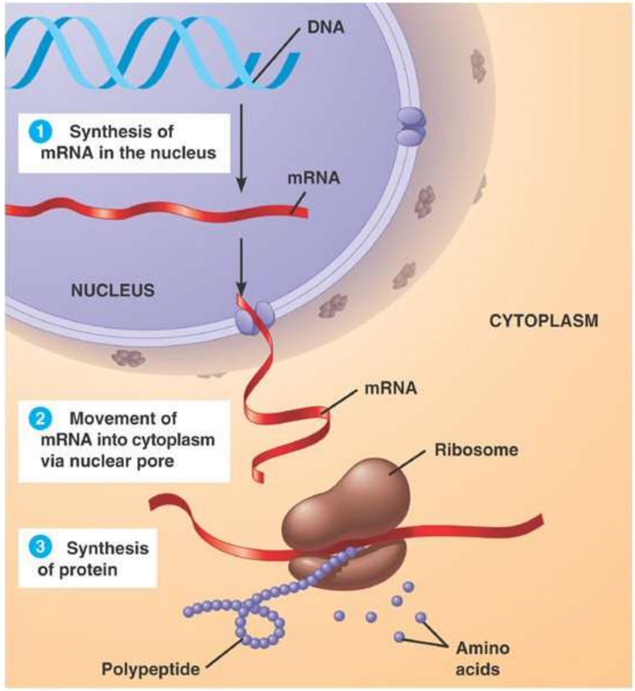


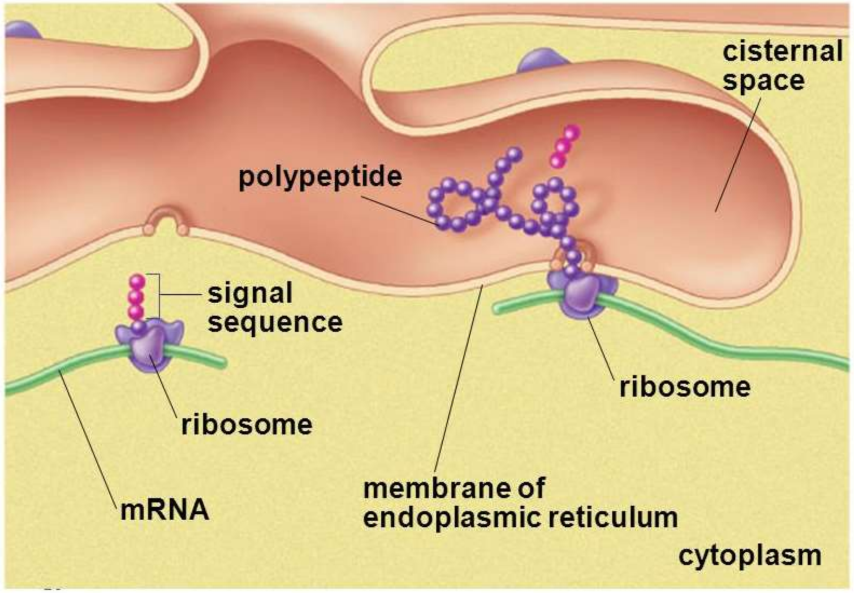
Free ribosomes

- Suspended in cytosol
- Synthesize proteins that function in cytosol

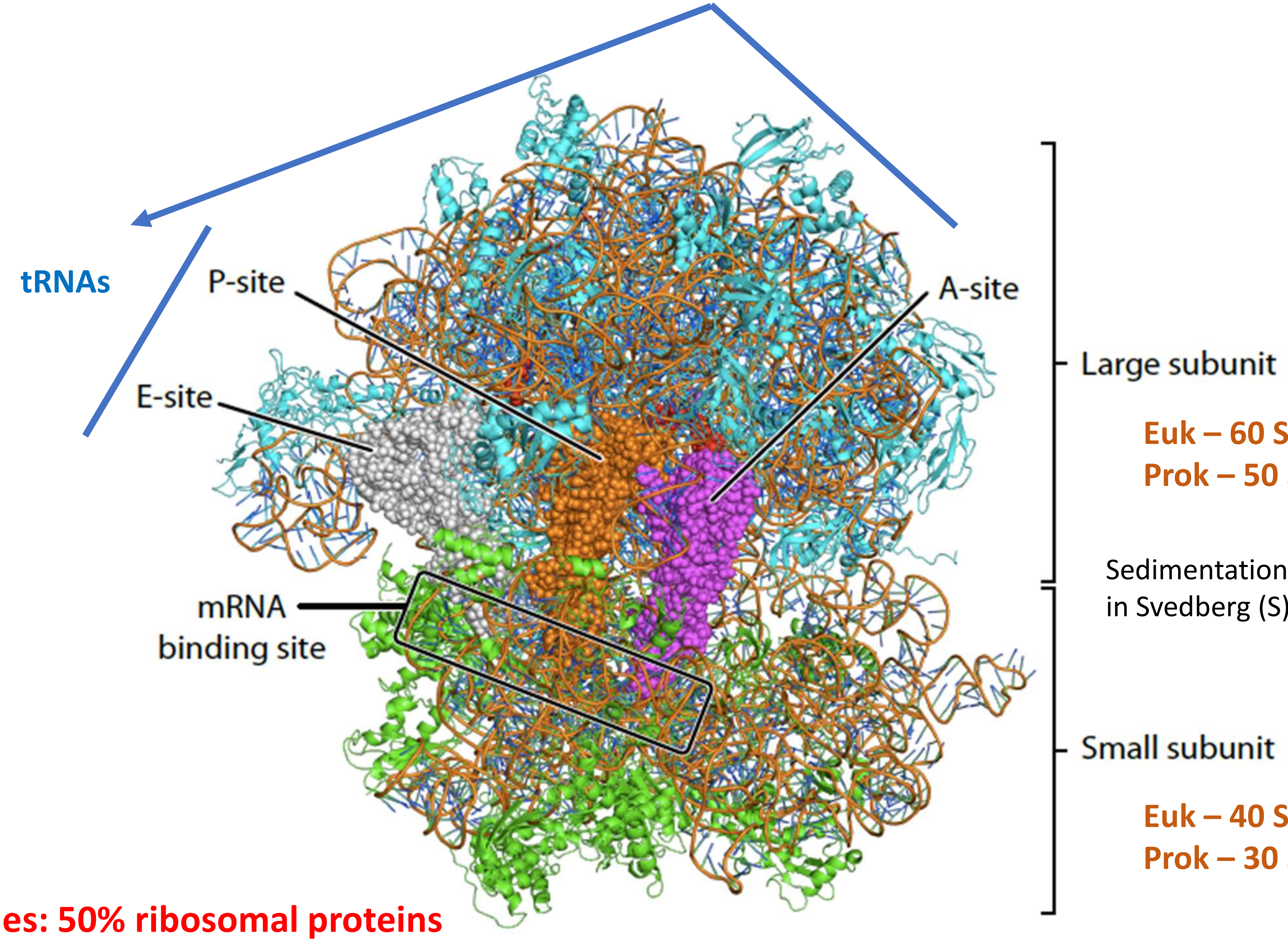
Bound Ribosome

- Attached to endoplasmic reticulum
- Synthesize proteins for export or for membranes





Ribosome molecular structure



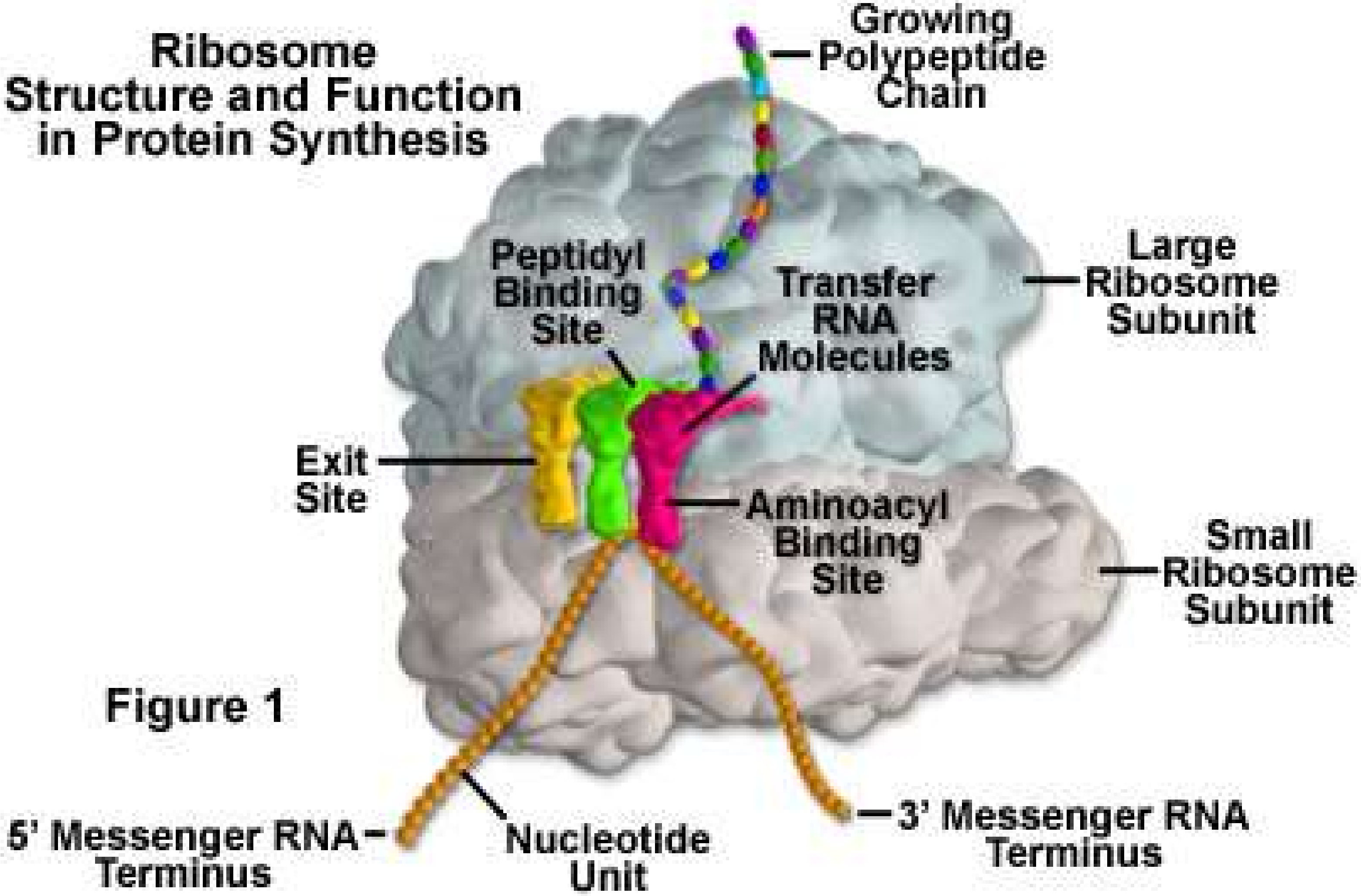
Euk – 60 S
Prok – 50 S

Sedimentation rate
in Svedberg (S) units

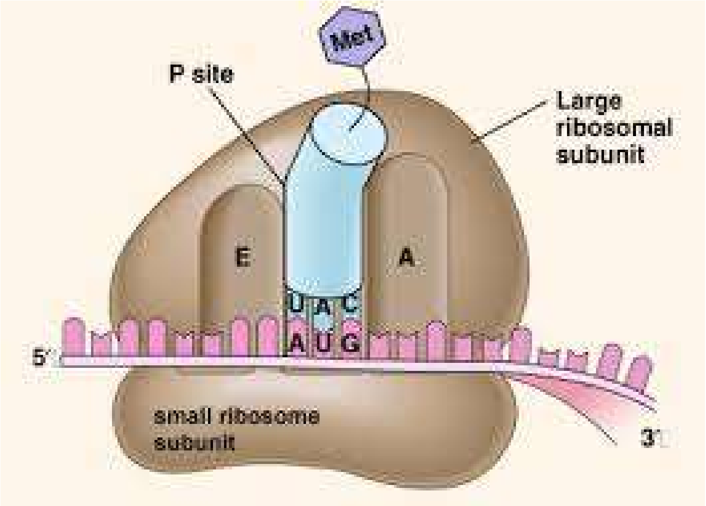
Euk – 40 S
Prok – 30 S

Ribosomes: 50% ribosomal proteins
50% ribosomal RNA

Ribosome molecular structure



Recognition of start codon



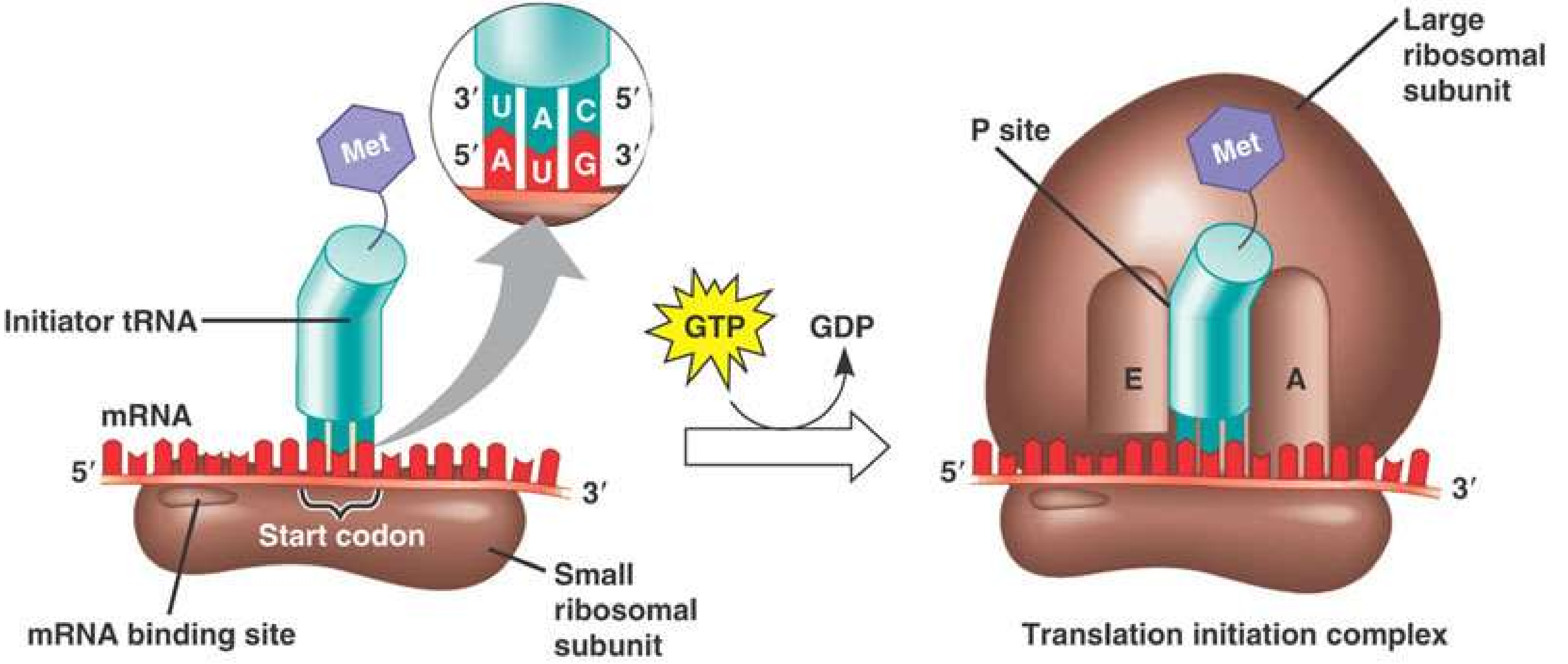
tRNA + a.a. methionine encounters an mRNA

↓

Attaches and starts to scan for a start signal.

↓

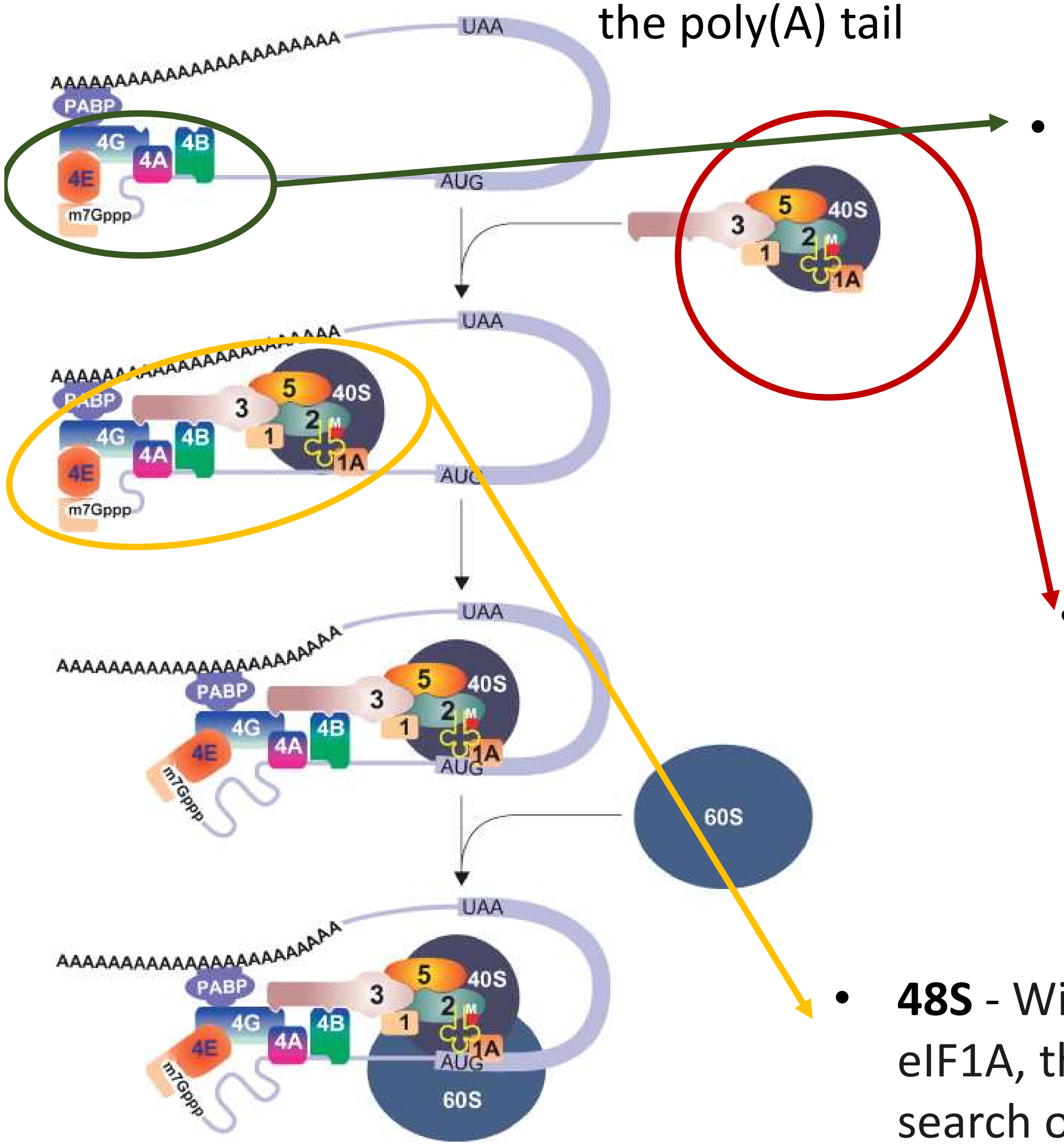
When it finds the start sequence AUG, the large subunit joins the small one to form a complete ribosome and the protein synthesis is initiated.



Translation – Initiation step

mRNAs need to be capped and polyadenylated

- Multiple subunits of the poly(A) binding proteins (PABPs) bind to the poly(A) tail

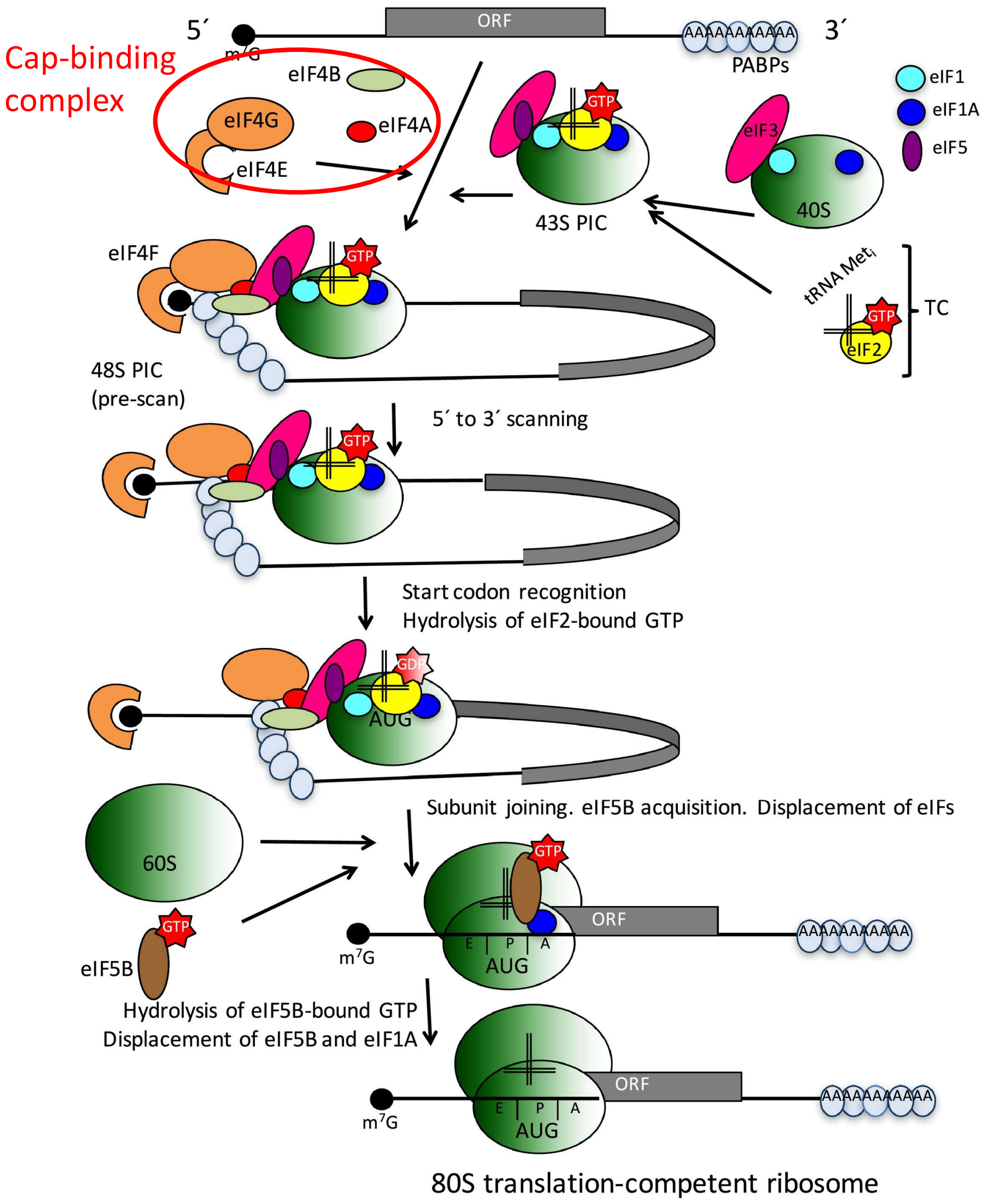


- **eIF4F complex or CAP-binding complex**: cap-binding protein eIF4E and the scaffolding protein eIF4G, eIF4A (ATP-dependent helicase activity). Helicase eIF4A, assisted by eIF4B, unwinds the secondary structures of the mRNA to allow binding of the 43S pre-initiation complex (43S PIC)

- formation of the **43S pre-initiation complex (PIC)**: ternary complex (TC) (initiation factor 2-methionine transfer RNA (eIF2-Met-tRNAi-GTP)), the 40S ribosomal subunit, eIF3, eIF1A and eIF1A.

- **48S** - With the assistance of eIF4A, eIF4B, eIF1 and eIF1A, the 40S scans the 5′ untranslated regions in search of the initiation codon.

Translation – Initiation step



With the assistance of eIF4A, eIF4B, eIF1 and eIF1A, the 40S scans the 5’ untranslated regions in search of the initiation codon.

Upon AUG recognition, eIF5 promotes hydrolysis of the eIF2-bound GTP to GDP (faded red star) catalyzed by eIF2. The eIF2-GDP is replaced by eIF5B, that along with eIF1A, assists in the 60S ribosome subunit recruitment.

The GTP of eIF5B is hydrolyzed, eIF5B-GDP and eIF1A leave the complex, yielding a translation-competent 80S ribosome.

eIF – eukaryotic Initiation Factor

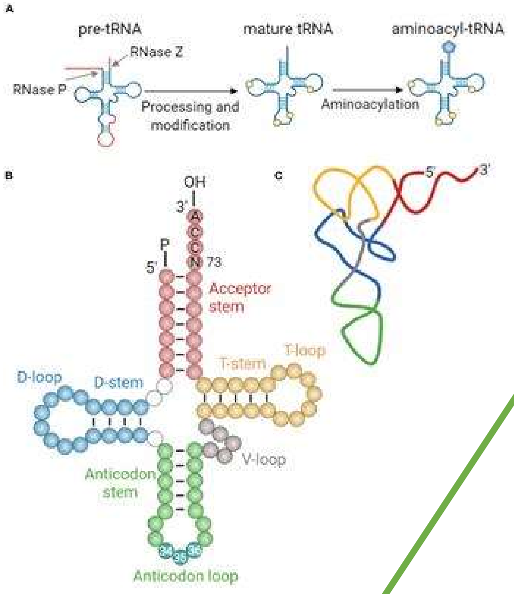
DOI: (10.1111/tpj.13520)

Translation – Elongation step

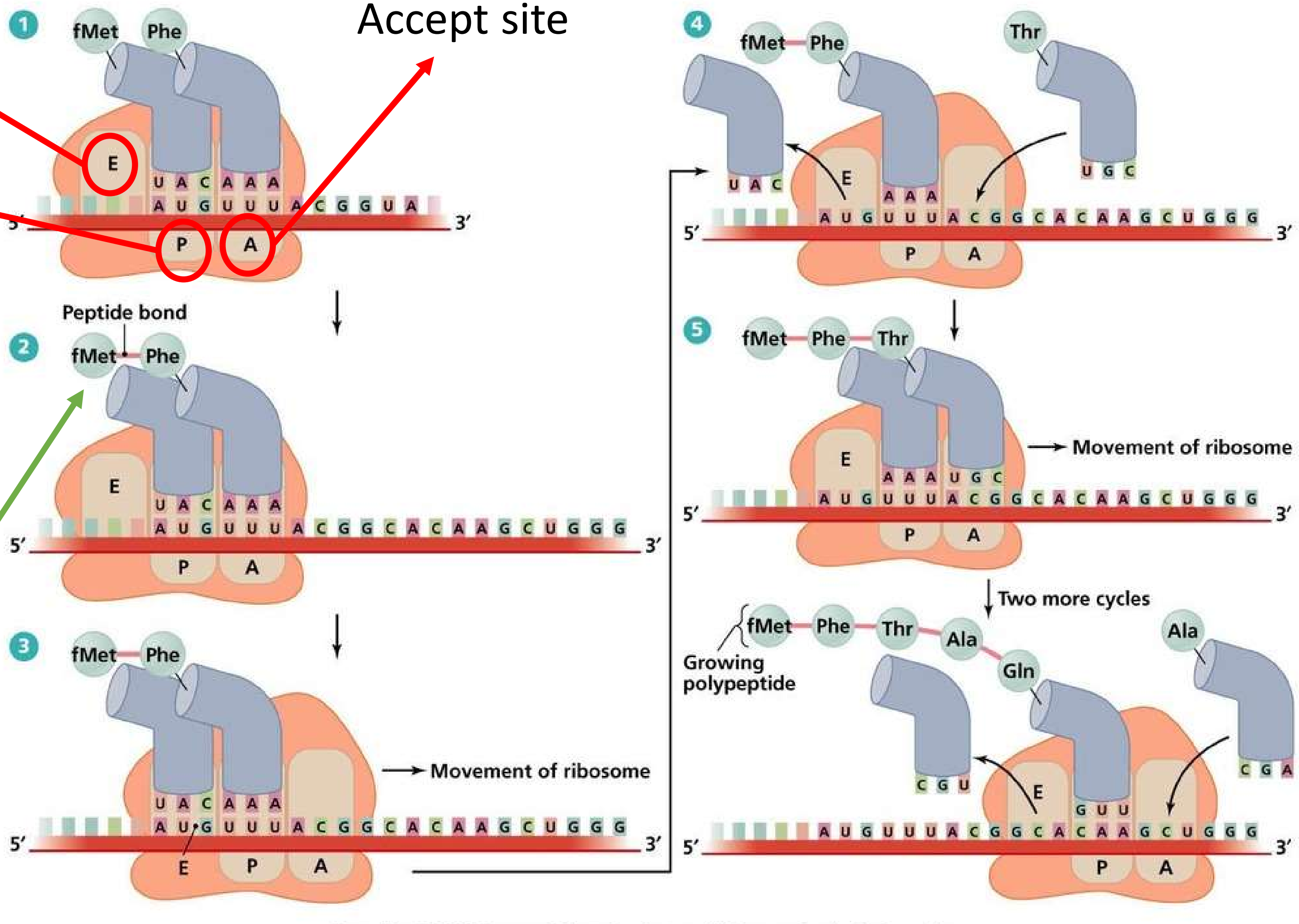
Exit site

Peptide site

Accept site



tRNA



Copyright © 2006 Pearson Education, Inc., publishing as Benjamin Cummings.

rRNA catalyze the peptidyl transferase reaction which forms peptides bounds between a.a.

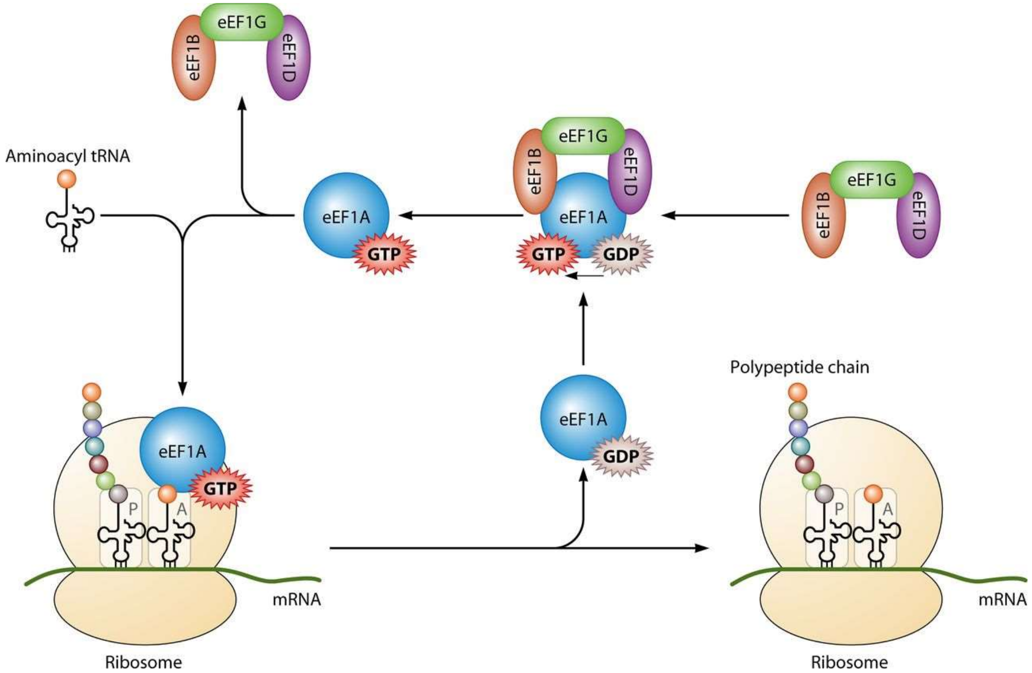
Elongation

During elongation, 80S ribosomes move along the mRNA three nucleotides per step.

The 80S ribosome decodes the ORF of the mRNA into proteins in three steps:

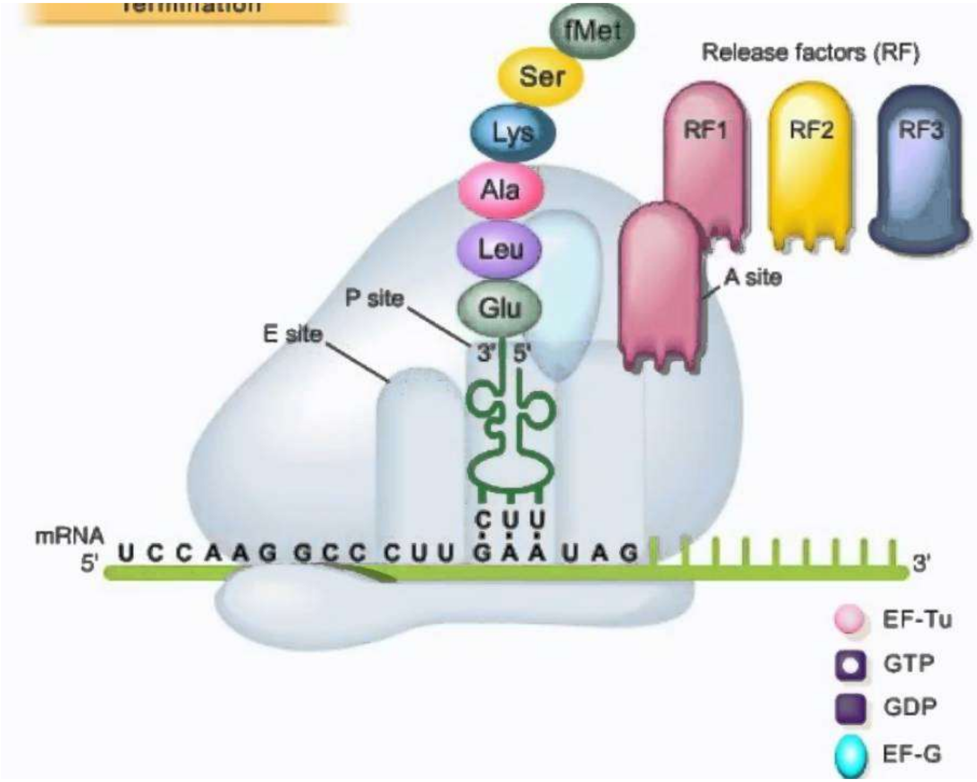
- decoding of the codon
- peptide bond formation
- translocation of the reading frame

Protein is synthesized one a.a. at the time through the coordinate action of aminoacyl-tRNA and eEFs (eukaryotic elongation factors).



Eukaryotic elongation factor 1A (eEF1A) in complex with GTP delivers an aminoacylated tRNA to the A site of the ribosome. GTP is hydrolyzed when codon-anticodon recognition occurs, and eEF1A-GDP is released from the ribosome.

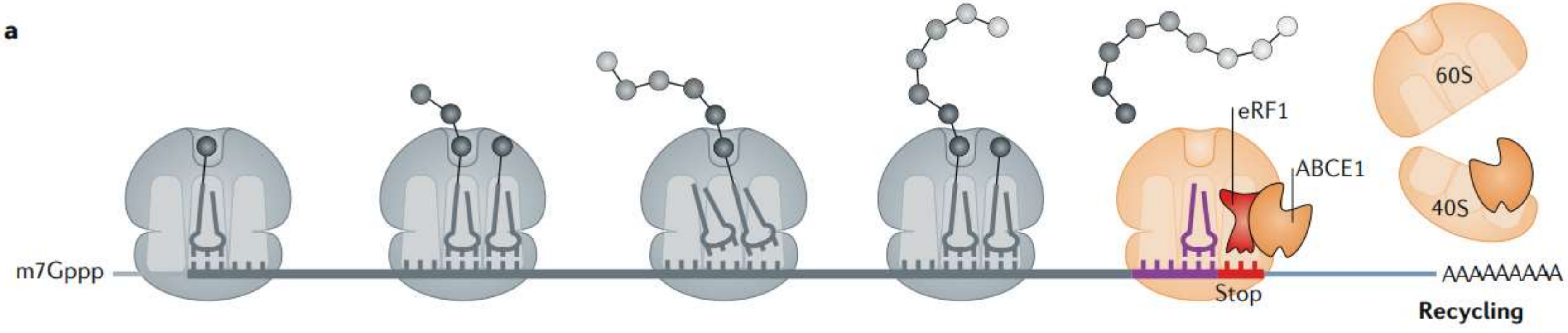
Translation – Termination step



Stop codons are not recognized by tRNAs. Instead, stop codons are recognized by **release factors**, which catalyze the release of the completed polypeptide chain from the peptidyl-tRNA and the dissociation and release of the ribosome from the mRNA

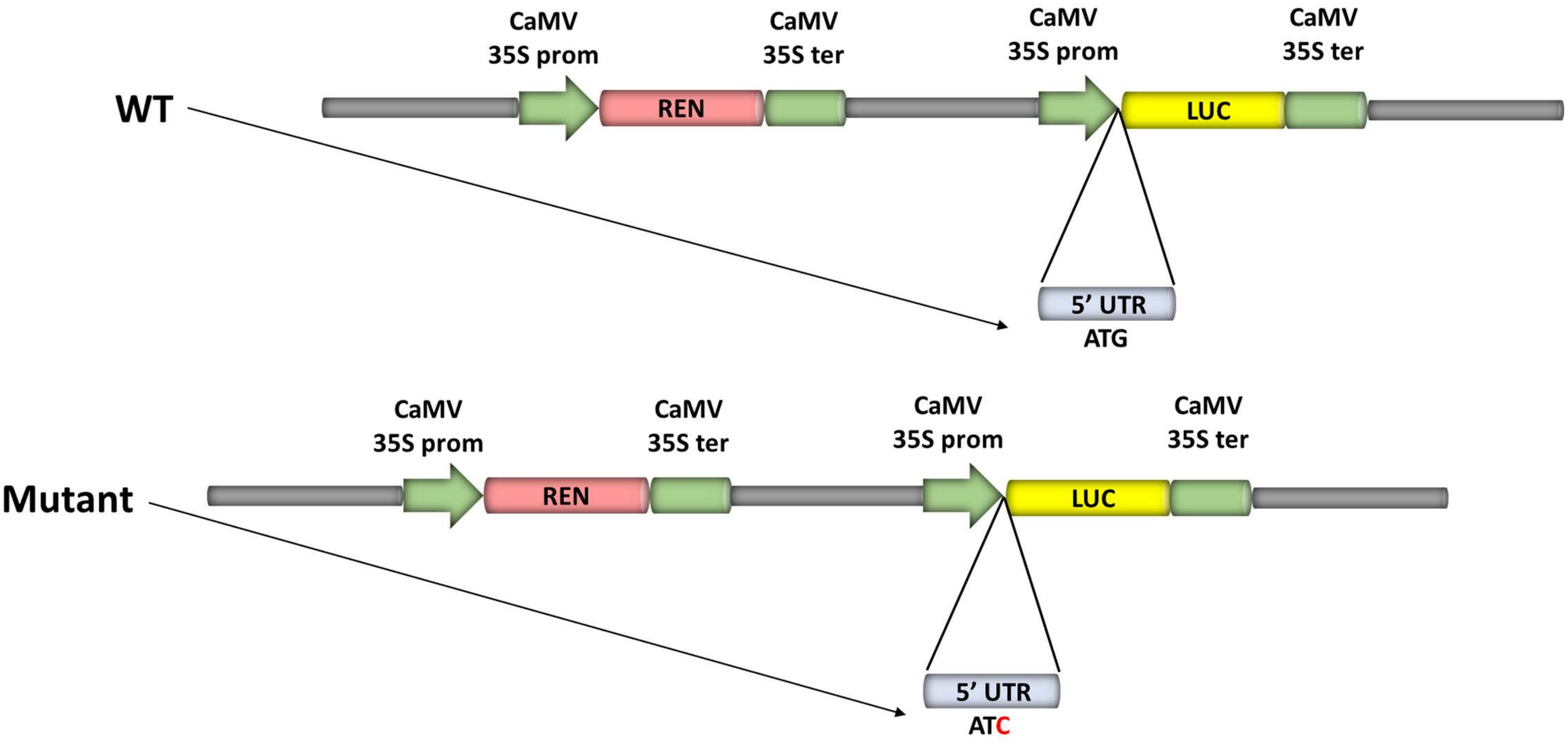
Translation termination in eukaryotes occurs in response to a stop codon (UAG, UAA, or UGA) in the A site and requires release factors (RFs), which bind to the A-site as an eRFs/GTP complex. After GTP hydrolysis by eRF3, eRF1 triggers hydrolysis of the polypeptidyl-tRNA, releasing the completed protein product. This leaves an 80S ribosome still bound to the mRNA.

Translation – Recycling step



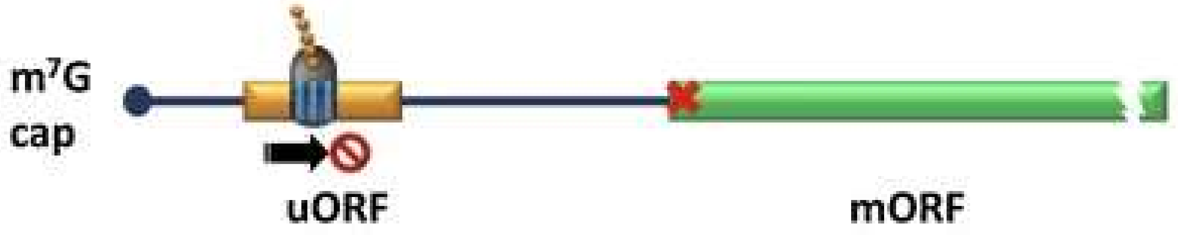
ATP-binding cassete sub-family E member 1 (**ABCE1**) binds to 80S ribosomes loaded with eukaryotic peptide chain release factor subunit 1 (eRF1) and uses the power generated from ATP binding and hydrolysis to dissociate the ribosomal subunits.

Analysis of the minimal uORFs on mORF translation – Dual Luciferase Assay

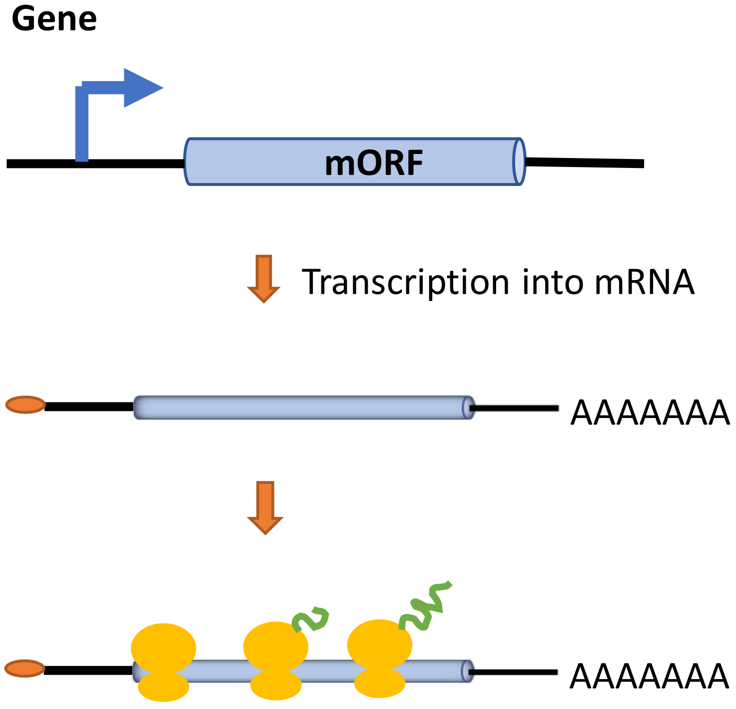


uORFs

- Protein-coding seq. distinct from main ORF (mORF) but present in the 5’ leaders of the same polycistronic mRNA
- Act as repressors of translation of mORFs.
- uORFs, themselves, can be translated into short peptides – can go up to 100 a.a. length.
- Some uORFs starts with non-AUG initiation codons.
- Even minimum uORFs comprising AUG-stop inhibit translation of mRNAs by stalling ribosomes on the uORFs.



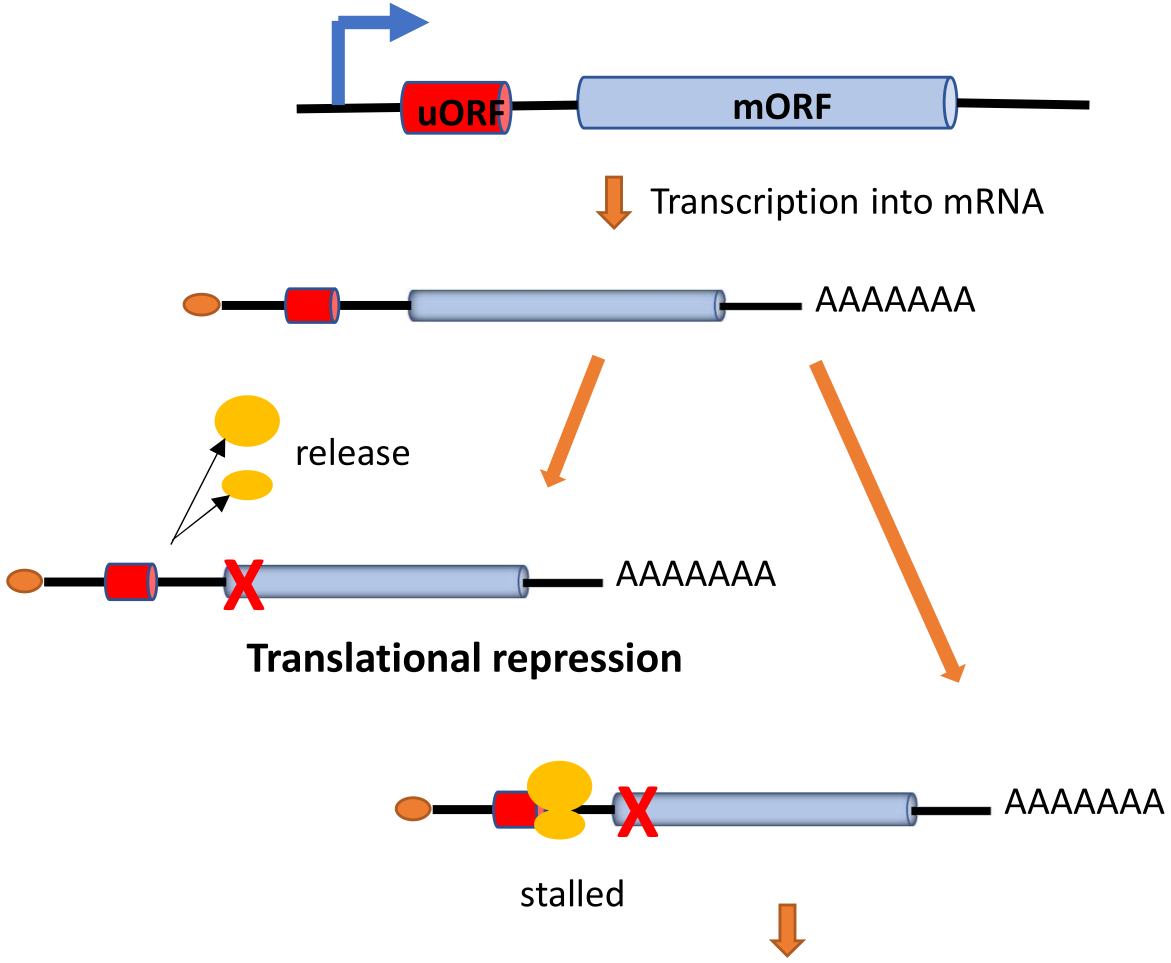
Canonical cap-dependent translation initiation.
Gene expression without uORFs



Transcription into mRNA

Translation into protein

Gene expression in the presence of uORF



Transcription into mRNA

release

Translational repression

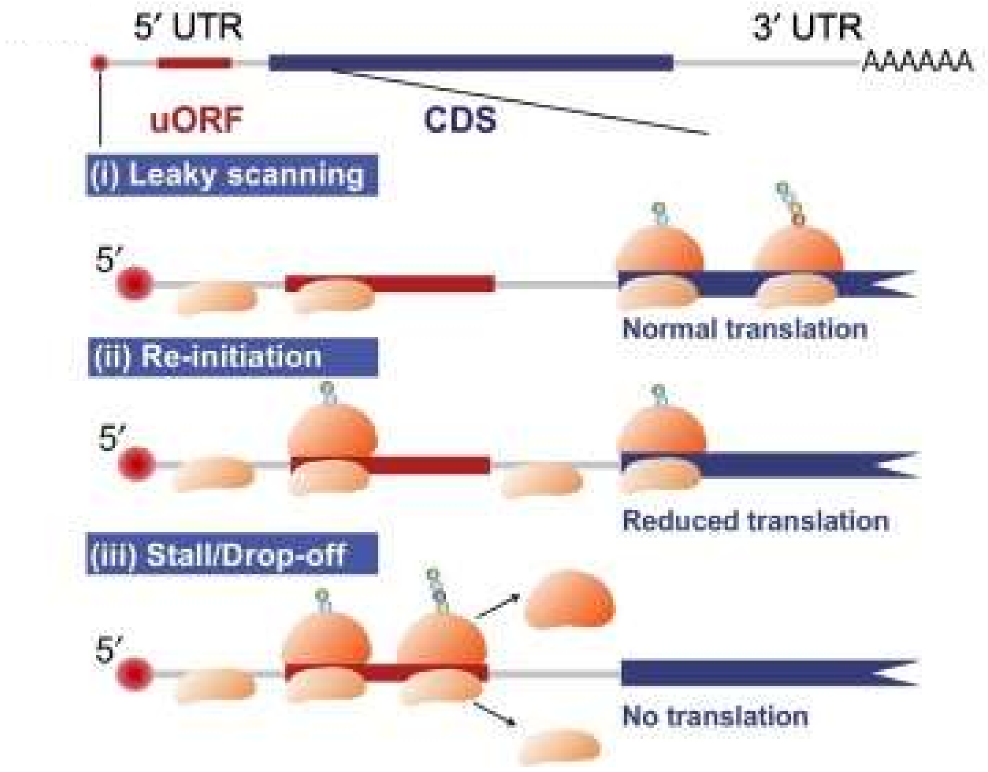
stalled

Eliminated by RNA decay pathways

Evading uORF-mediated regulation

- **Leaky Scanning** – The 40S scanning ribosomal passes over the initiation codon of the uORF avoiding uORF translation. When 40S reaches the mORF start codon, the 60S sub-unit is recruited.

- **Reinitiation** – After translation of the uORF, ribosome is disassembled and the 60S sub-unit is released. The remaining 40S sub-units continues scanning the downstream initiation codon and an 80S-translating ribosome is formed again at the initiation codon of the mORF.



Trends Biochemical Sciences (2019) 44:782-794

Notes

The process of translation initiation (slide 6).

To be translatable, mRNAs need to be capped and polyadenylated.

Multiple subunits of the poly(A) binding proteins (PAPBs) bind to the poly(A) tail.

The cap-binding complex, eIF4F, composed of the cap-binding protein eIF4E and the scaffolding protein eIF4G, is recruited to the mRNA cap.

The DEAD-box helicase eIF4A, assisted by eIF4B, unwinds the secondary structures of the mRNA to allow binding of the 43S pre-initiation complex (43S PIC).

The 43S PIC is composed of the 40S ribosome subunit (40S), eIF3, eIF1, eIF1A, the ternary complex (TC) and possibly eIF5. The TC is formed by the GTP-binding protein eIF2, GTP and Met-$\text{tRNA}^{\text{Met}}_{\text{i}}$.

The complex formed by the mRNA, eIF4F, eIF4B and the 43S PIC is called 48S. With the assistance of eIF4A, eIF4B, eIF1 and eIF1A, the 40S scans the 5' untranslated regions in search of the initiation codon. Upon AUG recognition, eIF5 promotes hydrolysis of the eIF2-bound GTP to GDP catalyzed by eIF2.

The eIF2-GDP is replaced by eIF5B, that along with eIF1A, assists in the 60S ribosome subunit recruitment.
eIF5B-GDP and eIF1A leave the complex, yielding a translation-competent 80S ribosome.

Notes

mRNA cap structure

- In eukaryotes, the 5′ cap (cap-0), found on the 5′ end of an mRNA molecule, consists of a guanosine (G) nucleotide connected to mRNA via an unusual 5′ to 5′ triphosphate linkage (ppp).

- This guanosine is methylated on the 7 position directly after capping *in vivo* by a methyltransferase. It is referred to as a 7-methylguanylate cap, abbreviated m^7Gppp.

Notes

uORFs

- Short, upstream ORFs (uORFs) located in the 5´-leader of the mRNA can be selected for translation.

- Typically, uORFs are considered to be inhibitors of down-stream translation initiation at the CDSs (coding sequences).

- The inhibitory effect of uORFs is attributed to the fact that in eukaryotes, 43S pre-initiation complex binds to the 5’-cap structure of the mRNA, then scans progressively 5’ to 3’ and initiates translation at the first encountered initiation codon that is in optimal context.