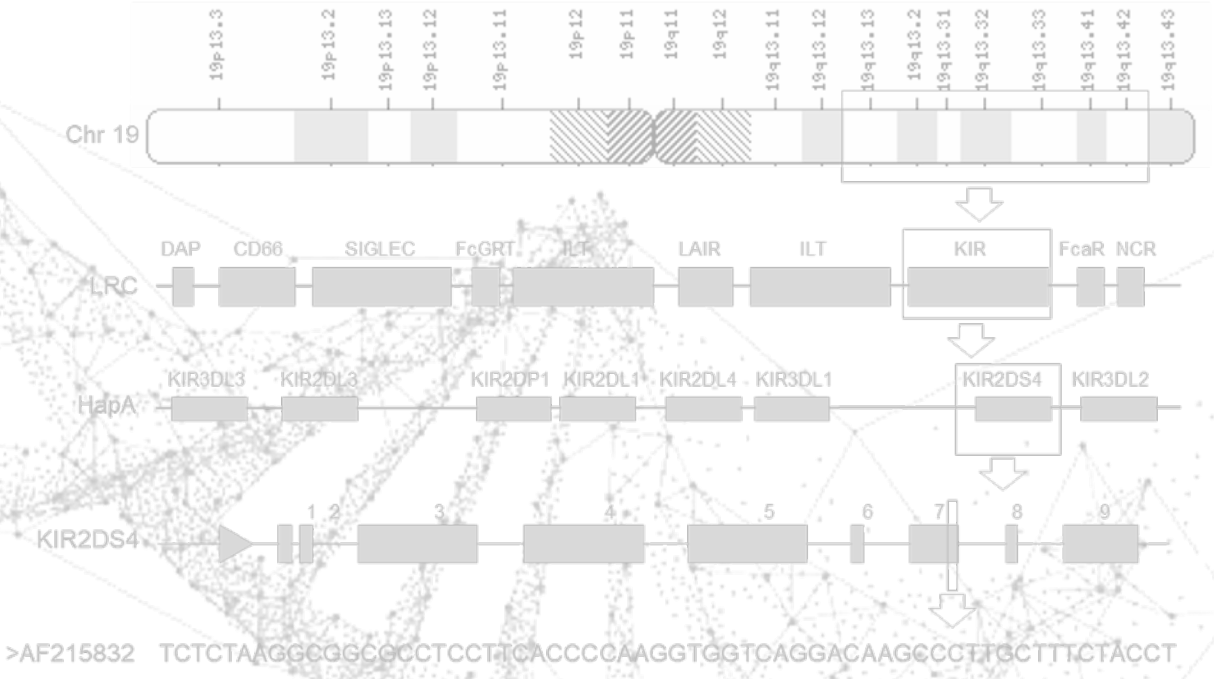


Prokaryote and Eukaryote Translation

San Luis Potosi State University (UASLP) Mexico
Molecular Biology Course, Faculty of Medicine post-graduate program

Dr. Christian A. García-Sepúlveda
Viral & Human Genomics BSL-3 Laboratory

Last updated November 19, 2024 v1

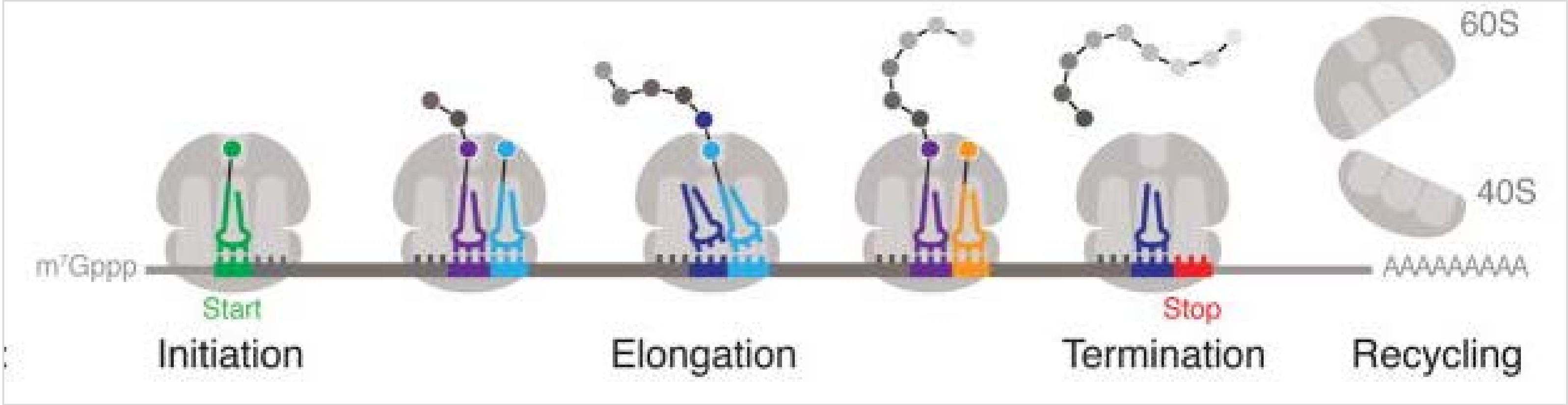
Introduction

Across all domains of life, translation of the information encoded in mRNA to protein is performed by a large macromolecular machine called the ribosome.

The ribosome reads the information one codon (three nucleotides) at a time, translating it into protein through tRNAs that recognize codons to insert specific amino acids.

The tRNAs are really the central ‘translators’ in the translation process.

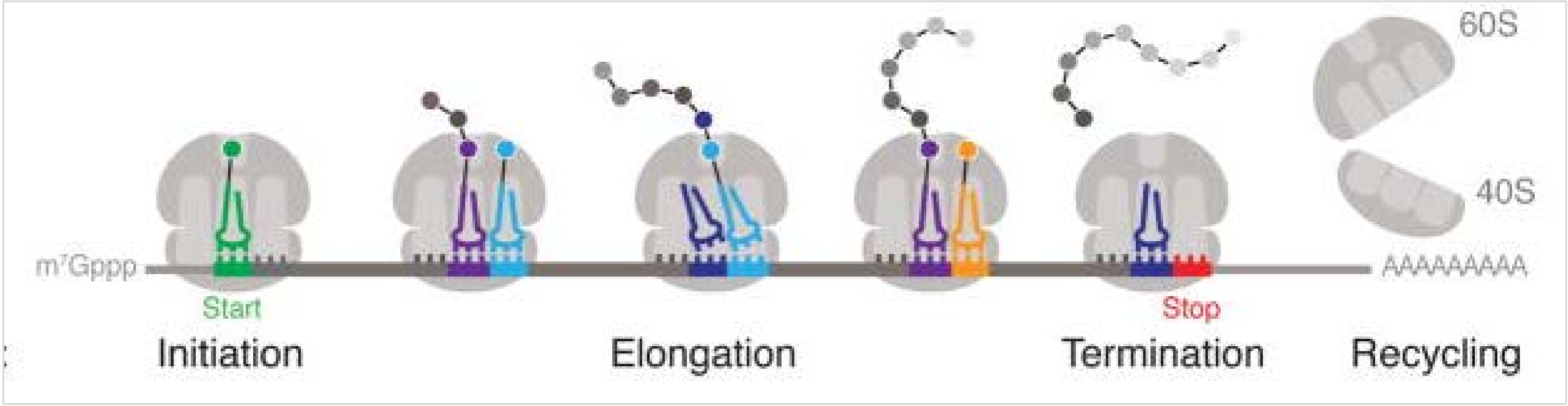
The process of translation can be broken into four main phases: initiation, elongation, termination and ribosome recycling

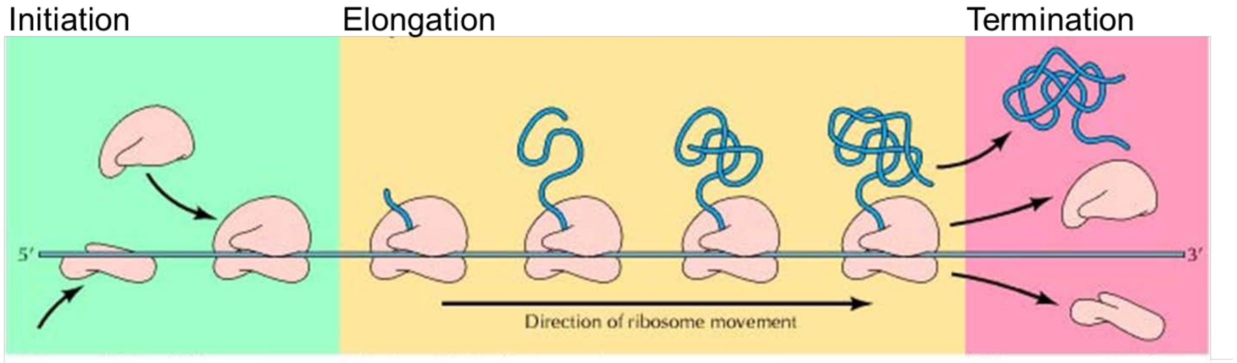


Reference here

Translation phases

Protein synthesis divided into the three stages:





rRNA's are recruited
to mRNA and start codon
identified

Transpeptidation of amino-acids successively
elongates polypeptide chain

Stop codon identified by
termination factors releases
peptide and frees rRNA's

Reference here

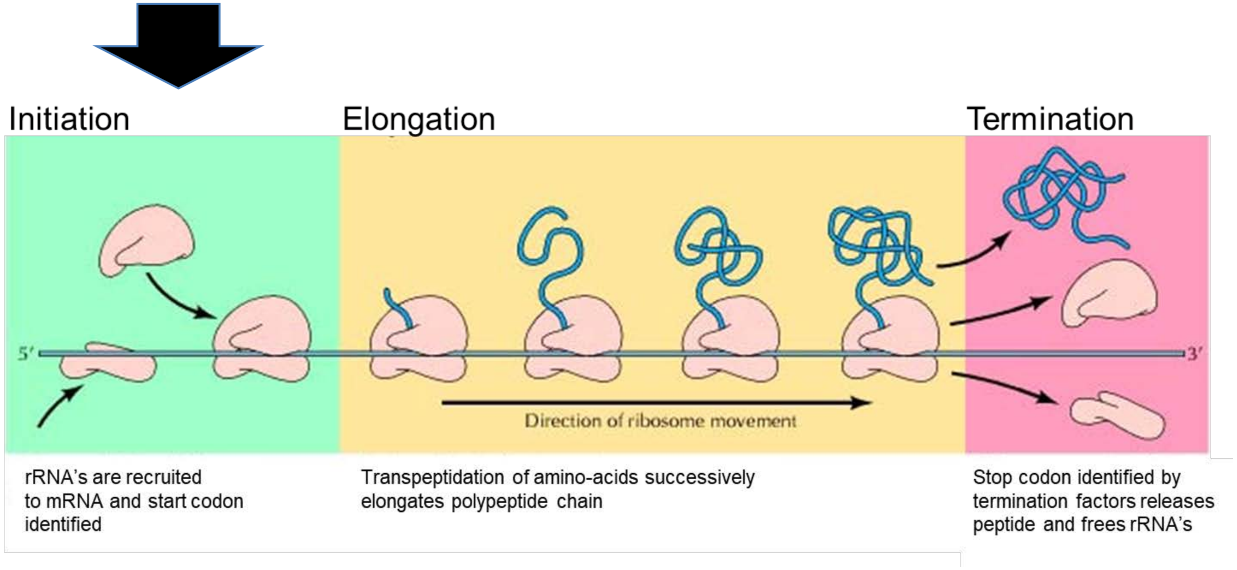
Translation phases

Initiation involves the reactions that precede formation of the peptide bond between the first two amino acids of the protein.

It requires the ribosome to bind to the mRNA, forming an initiation complex that contains the first aminoacyl-tRNA.

This is a relatively slow step in protein synthesis, and usually determines the rate at which an mRNA is translated.

Involves prokaryote (IF) or eukaryote (eIF) initiation factors.



rRNA’s are recruited to mRNA and start codon identified

Transpeptidation of amino-acids successively elongates polypeptide chain

Stop codon identified by termination factors releases peptide and frees rRNA’s

Reference here

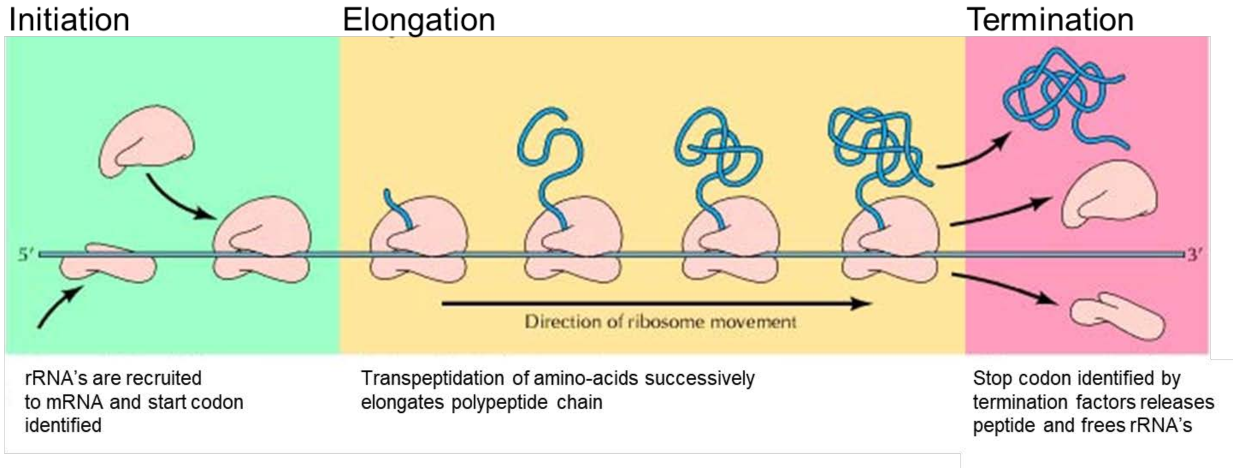
Translation phases

Elongation includes all the reactions from synthesis of the first peptide bond to addition of the last amino acid.

Amino acids are added to the chain one at a time.

This is the most rapid step in protein synthesis.

Involves prokaryote (EF) or eukaryote (eEF) elongation factors.



rRNA's are recruited to mRNA and start codon identified

Transpeptidation of amino-acids successively elongates polypeptide chain

Stop codon identified by termination factors releases peptide and frees rRNA's

Reference here

Translation phases

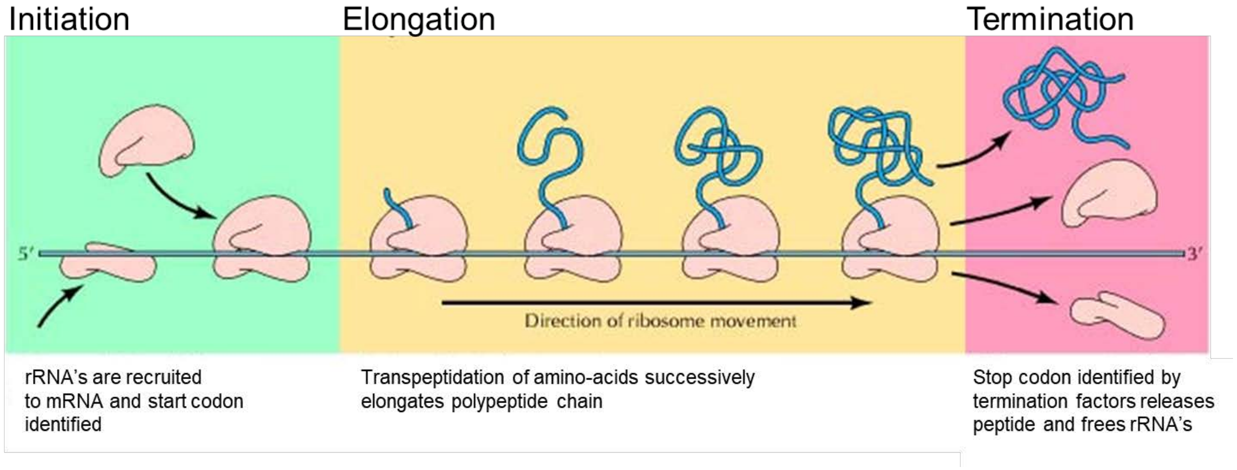
Termination includes polypeptide chain release and ribosomal dissociation from mRNA.

While start codons need nearby sequences to start translation, a stop codon alone is sufficient to terminate translation.

Stop codons signal the termination of this process by binding release factors.

TAG (UAG in RNA) “Amber”
TAA (UAA in RNA) “Ochre”s
TGA (UGA in RNA) “Opal (or Umber)

Involves prokaryote or eukaryote termination factors (TF)



rRNA’s are recruited to mRNA and start codon identified

Transpeptidation of amino-acids successively elongates polypeptide chain

Stop codon identified by termination factors releases peptide and frees rRNA’s

Reference here

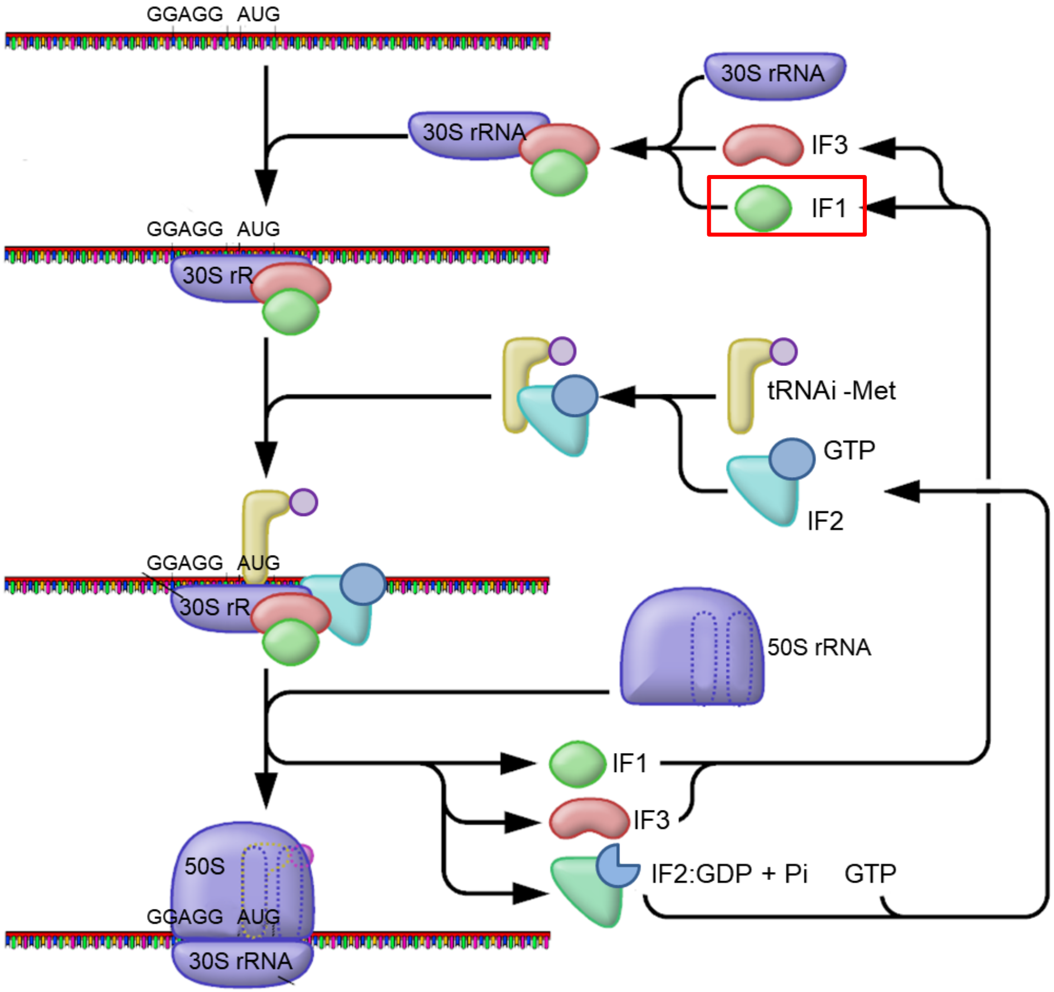
Prokaryote translation initiation events

IF1

1. Prevents an aminoacyl-tRNA from entering A site.

2. Recruits IF2:tRNA:GTP to the 30S subunit.

3. Prevents the 50S rRNA from binding to 30S rRNA (and thus of 70S rRNA).



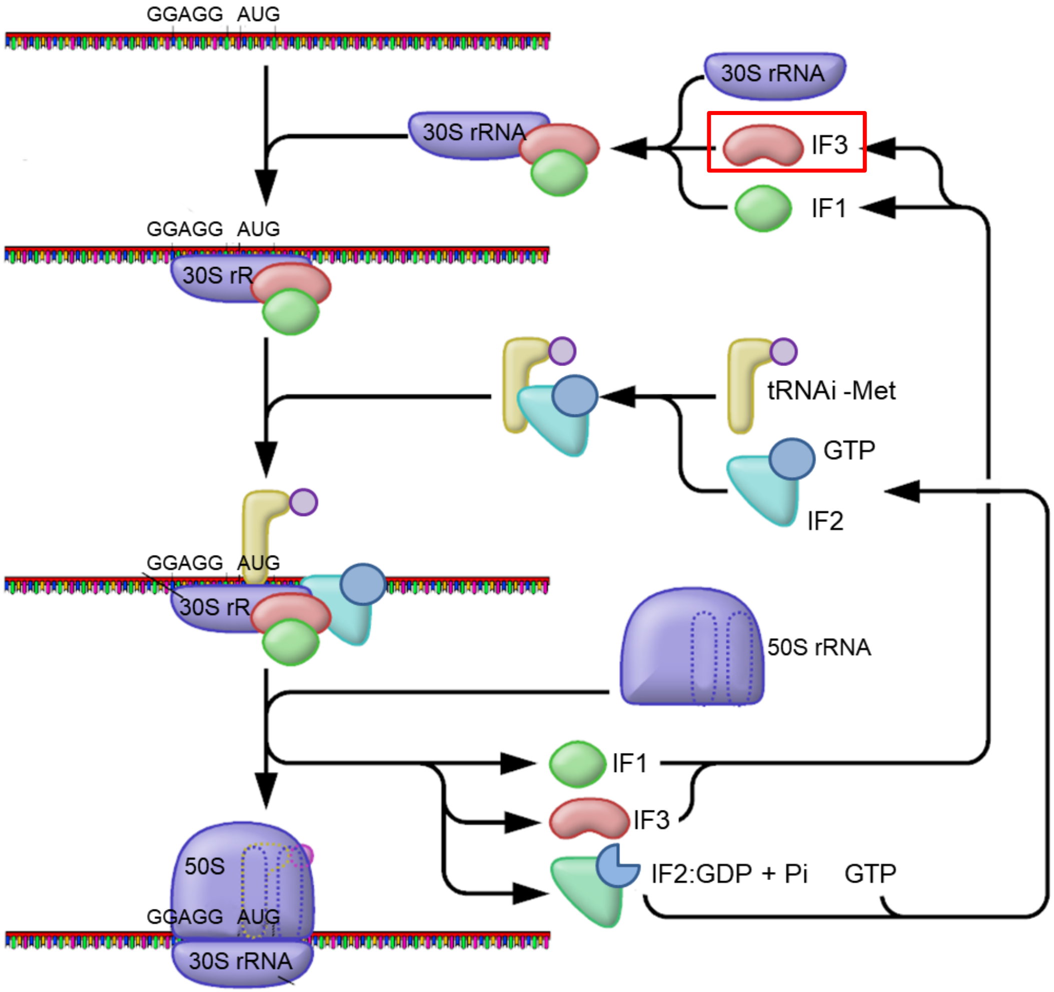
Reference here

Prokaryote translation initiation events

IF3

1. Stabilization of free 30SrRNA.
2. Functions as fidelity factor during assembly of ternary initiation complex.
3. Recruits 30S rRNA to mRNA.
4. Not universally found in all bacterial species.
5. Allows rapid codon-anticodon pairing of tRNAi to start codon.

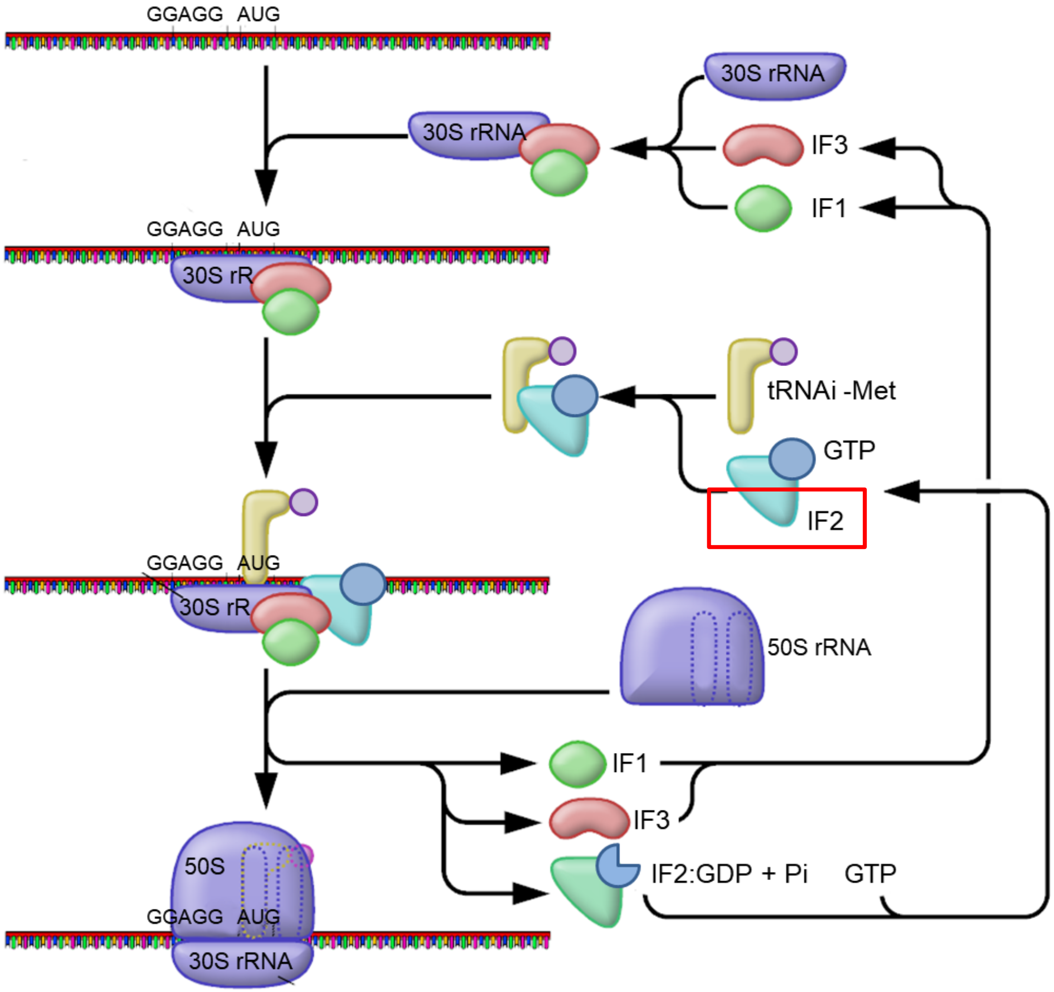
Ternary initiation complex is 30S + tRNA + mRNA.



Prokaryote translation initiation events

IF2

1. Binds to tRNAi.
2. Controls entry of tRNAi onto the ribosome.
3. Transfers tRNAi into the partial P site.
4. When the 50S subunit joins, it hydrolyzes GTP causing a conformational change which releases IF2 and allows the 70S ribosome to form.



Prokaryote Shine-Dalgarno sequence

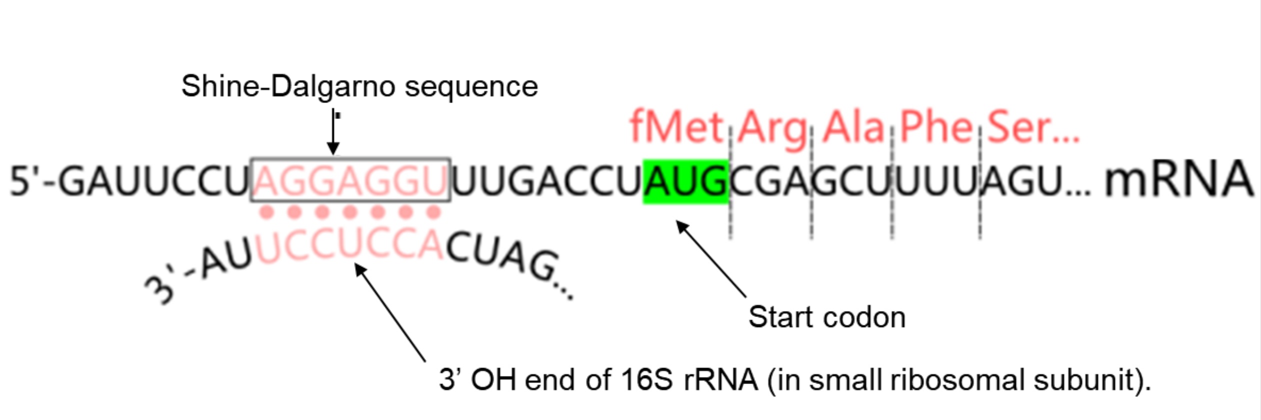
Ribosomal binding site in bacterial (common) and archaeal (rare) messenger RNA.

Generally located around 8 bases upstream of the start codon AUG.

Recruits the ribosome to the mRNA to initiate protein synthesis in the start codon.

Also present in some chloroplast and mitochondrial transcripts.

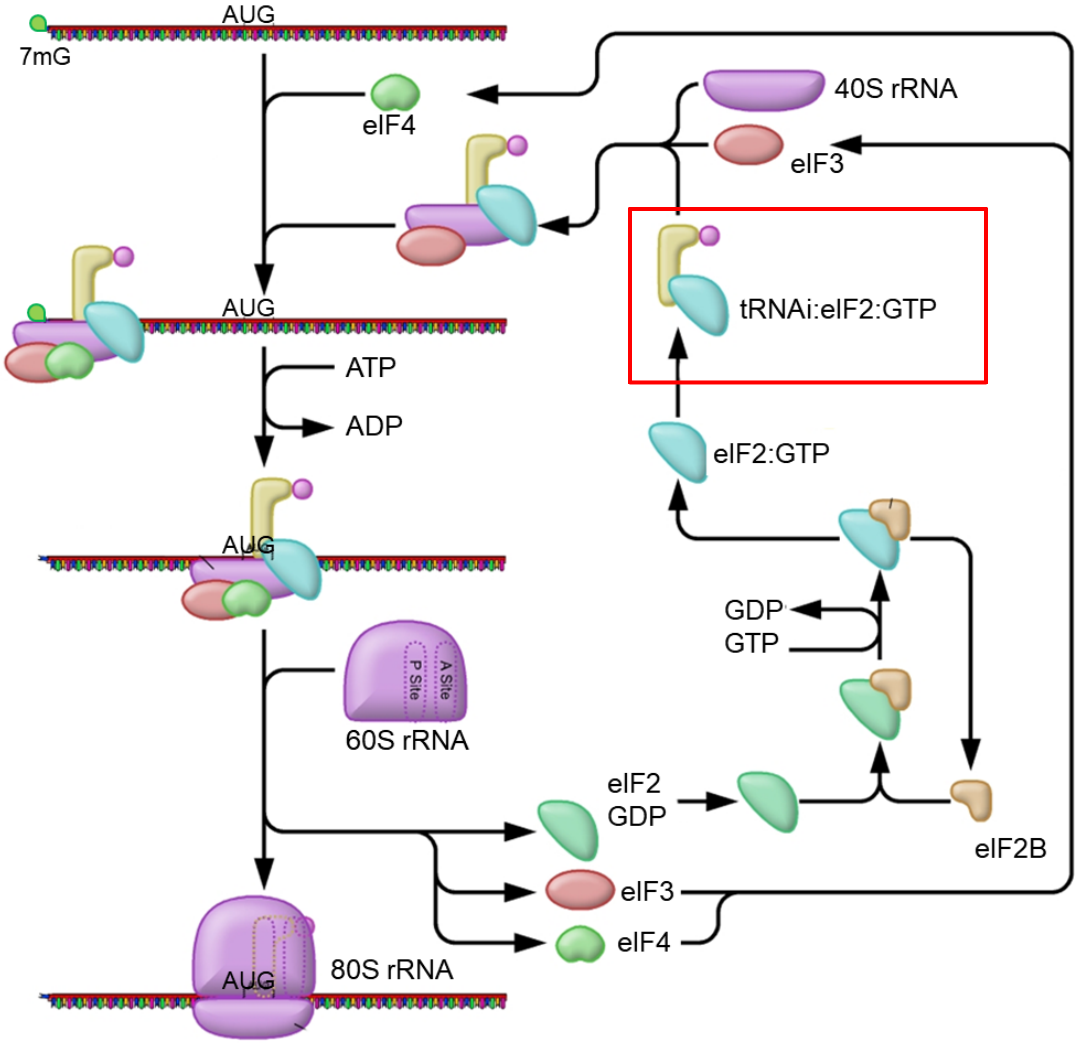
Proposed by Australian scientists John Shine and Lynn Dalgarno in 1973.



Eukaryote translation initiation events

eIF2

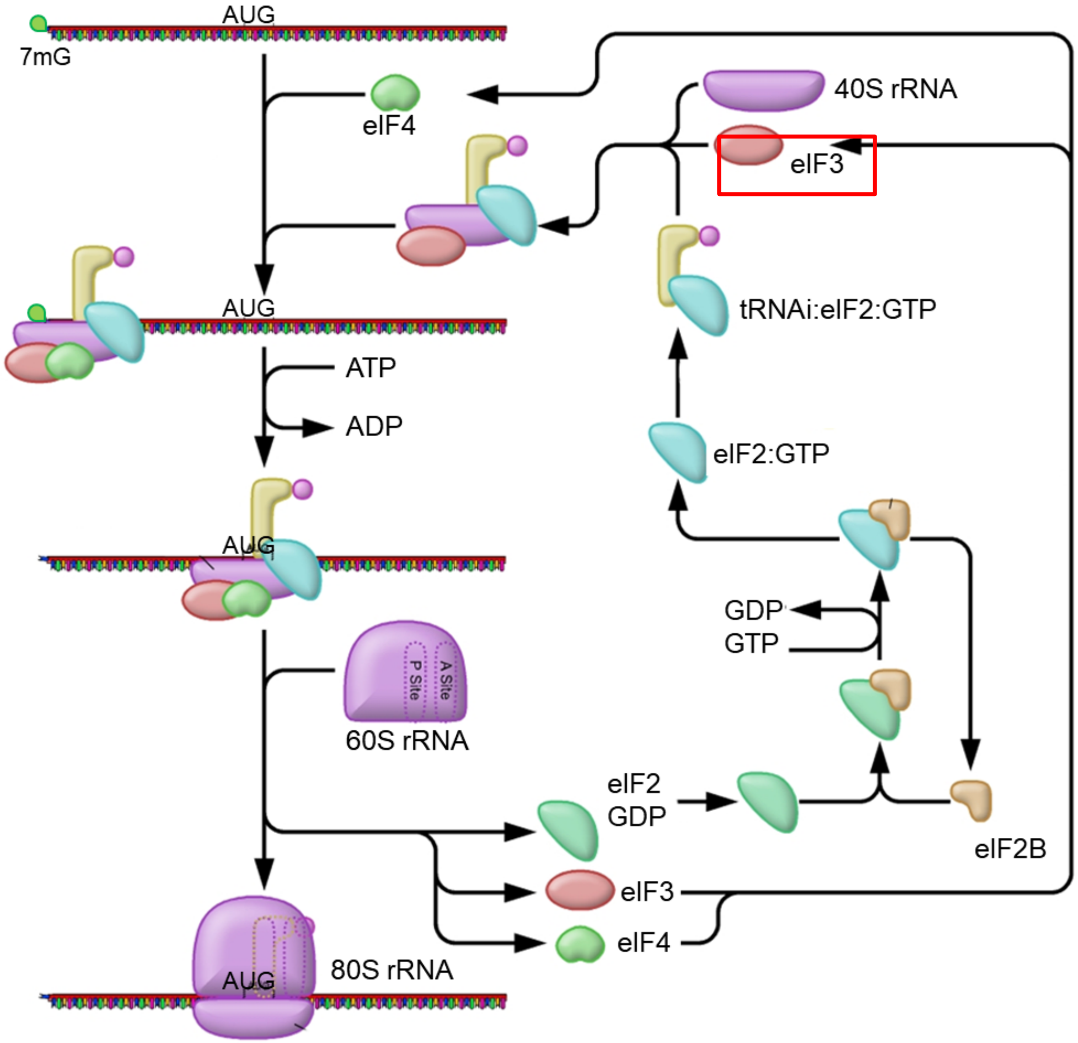
1. The Met-charged initiator tRNA (Met-tRNAiMet) is brought to the P-site of the small ribosomal subunit by eukaryotic initiation factor 2 (eIF2).
2. Hydrolyzes GTP, and dissociates several factors from the small ribosomal subunit to assemble 80S ribosome.
3. Recruits the large (60S) subunit.
4. Needs to have it's GTP rephosphorylated by eIF2B before being recycled for tRNAi use.



Eukaryote translation initiation events

eIF3

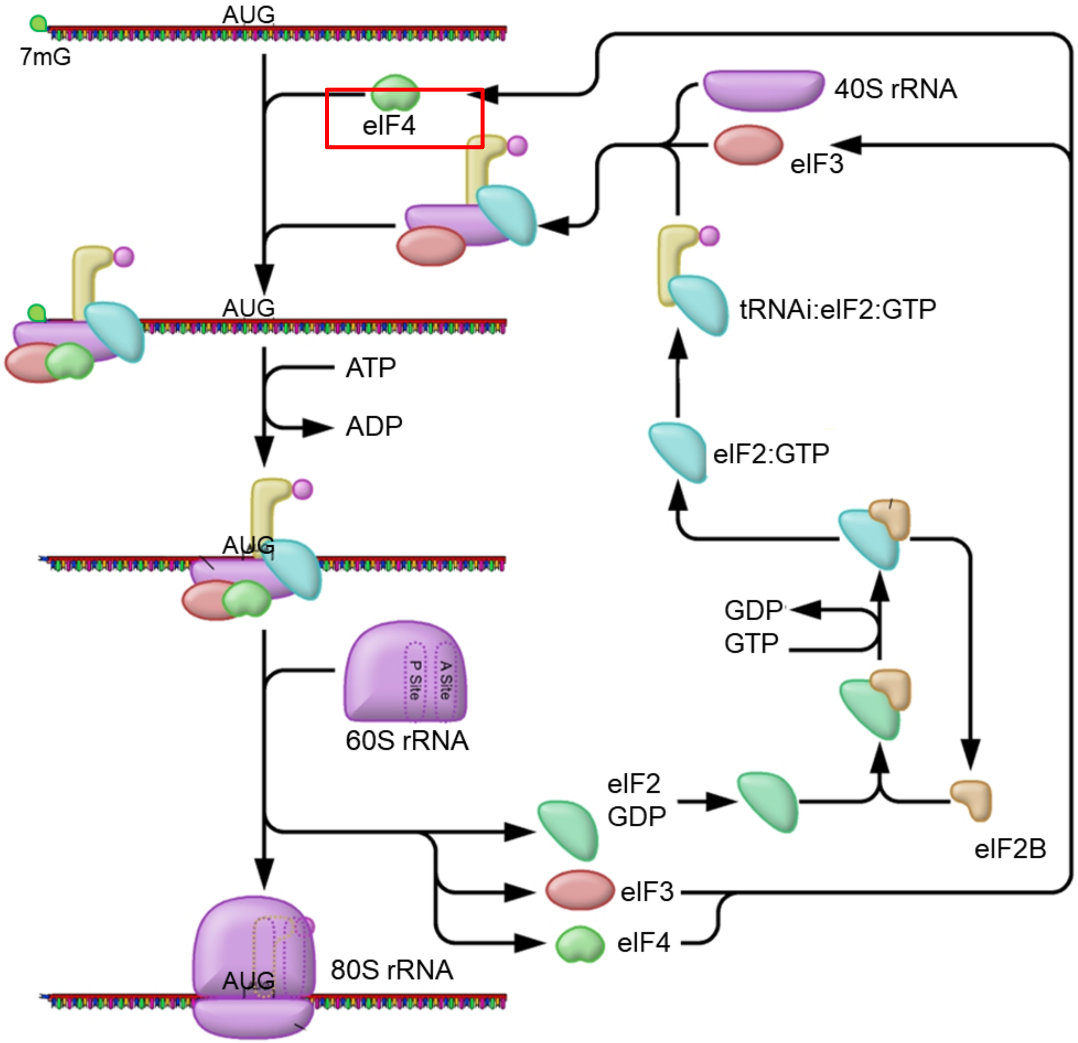
1. Largest translation initiation factor - consists of 13 subunits (eIF3a-m) with a combined molecular weight of ~800 kDa.
2. Conserved across eukaryotes.
3. Stimulates nearly all steps of translation initiation.
4. Binds the small ribosomal subunit (40S).
5. Directly interacts with eIF4F.
6. Contains RNA recognition motifs (RRMs) which allow IRES (internal ribosome entry site) cap-independent translation.



Eukaryote translation initiation events

eIF4

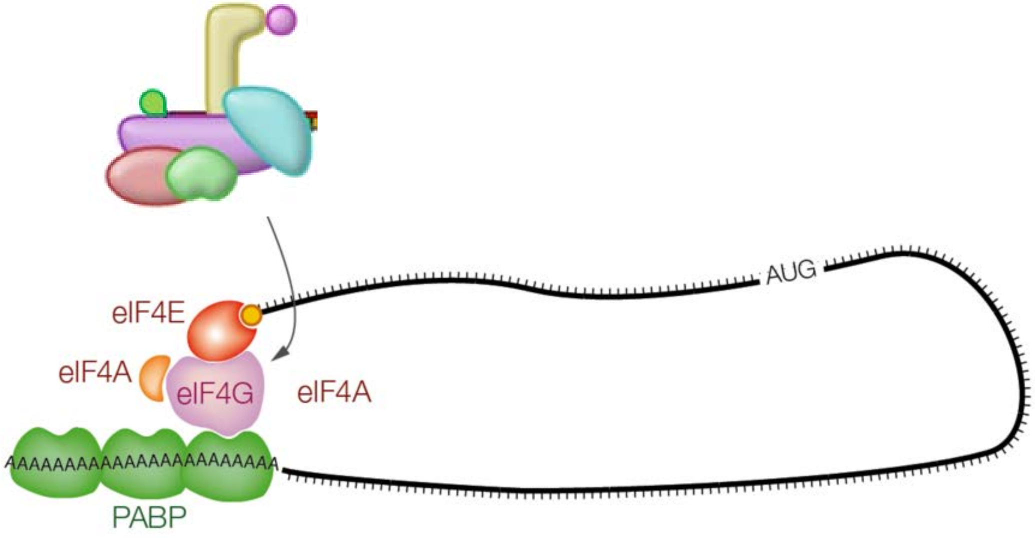
1. Heterotrimeric protein complex that binds the 5' cap of mRNAs.
2. Three subunits:
   DEAD-box RNA helicase (eIF4A)
   Cap-binding (eIF4E), and
   Large "scaffold" (eIF4G).
3. Recruits small ribosomal subunit (40S) to the 5' cap of mRNAs.
4. Responsible for cap-dependent translation initiation.
5. Binding of the cap by eIF4E is often considered the rate-limiting step of cap-dependent initiation



Recognition of eukaryote 5’cap & Poly-A tail.

The poly(A)-binding protein (PABP) also associates with the eIF4F complex via eIF4G, and binds the poly-A tail of most eukaryotic mRNA molecules.

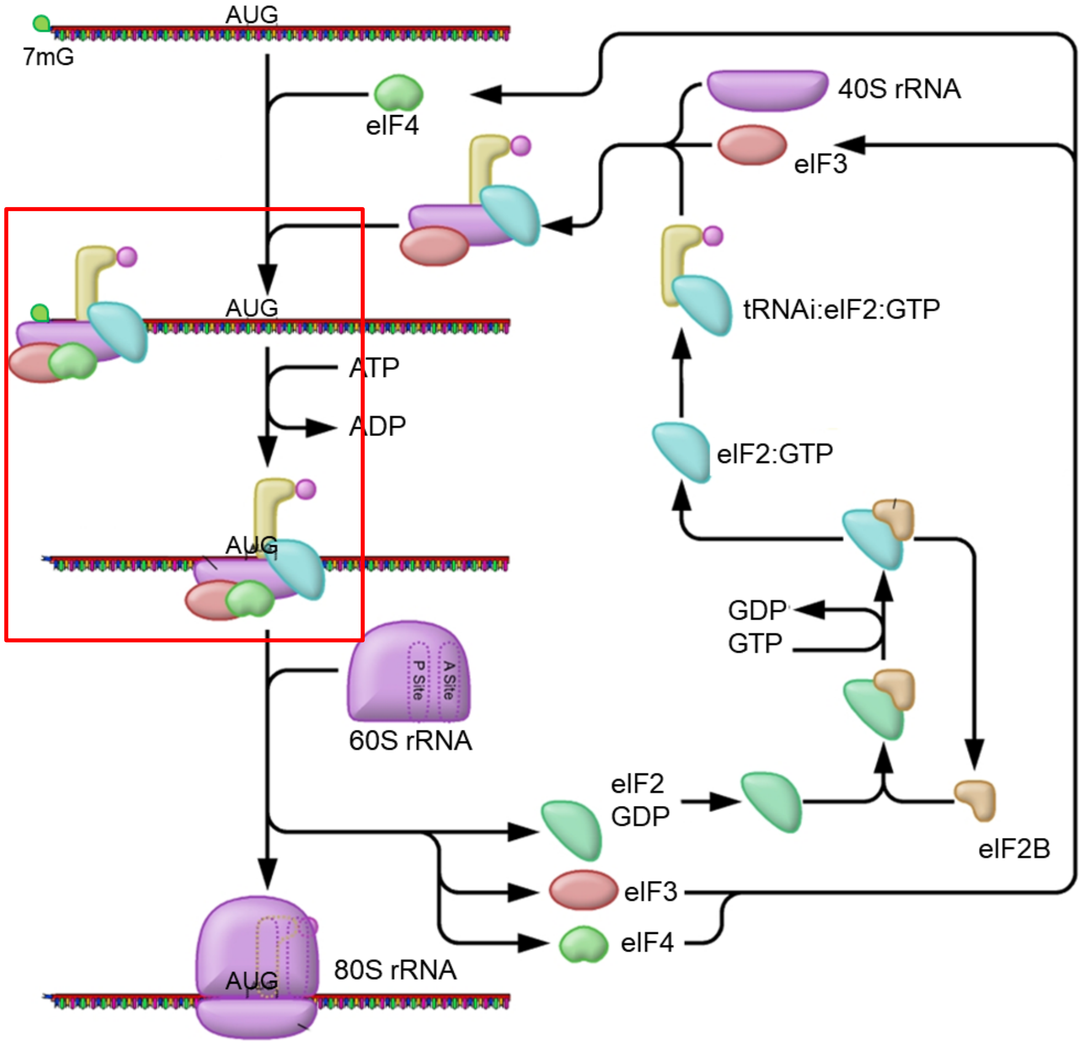
eIF4G has been implicated in playing a role in circularization of the mRNA during translation.



Eukaryote translation initiation events

5’-Cap recruitment and scanning

1. Eukaryote translation requires binding at 5’ Cap.
2. ATP hydrolysis allos for 40S unit to scan 5’ UTR (untranslated leader).
3. Scanning stops at start codon in Kozak context.
4. Choosing the correct start codon is critical as it determines the reading frame and thus the polypeptide sequence!
5. In other words, context matters.



Kozak consensus sequence (Eukaryotes)

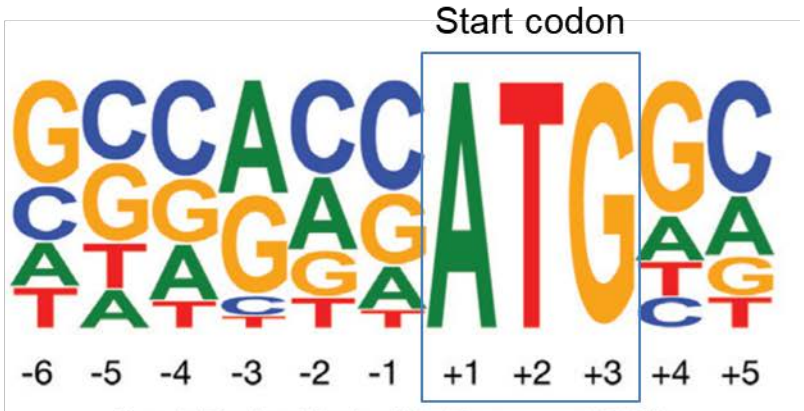
Regarded as the optimum sequence for initiating translation in eukaryotes.

Named after the scientist who discovered it, Marilyn Kozak.

Not to be confused with the ribosomal binding site (RBS), that being either the 5′ cap of mRNA.

Not needed for internal ribosome entry site (IRES).

	-6	-5	-4	-3	-2	-1	+4
A	17%	18%	25%	61%	27%	15%	23%
C	19%	39%	53%	2%	49%	55%	16%
G	44%	23%	15%	36%	13%	21%	46%
T	20%	20%	7%	1%	11%	9%	15%



Kozak Region Nucleotide Frequency Matrix

Prokaryote elongation

Once initiator tRNA is in P site the ribosome is ready for the elongation phase.

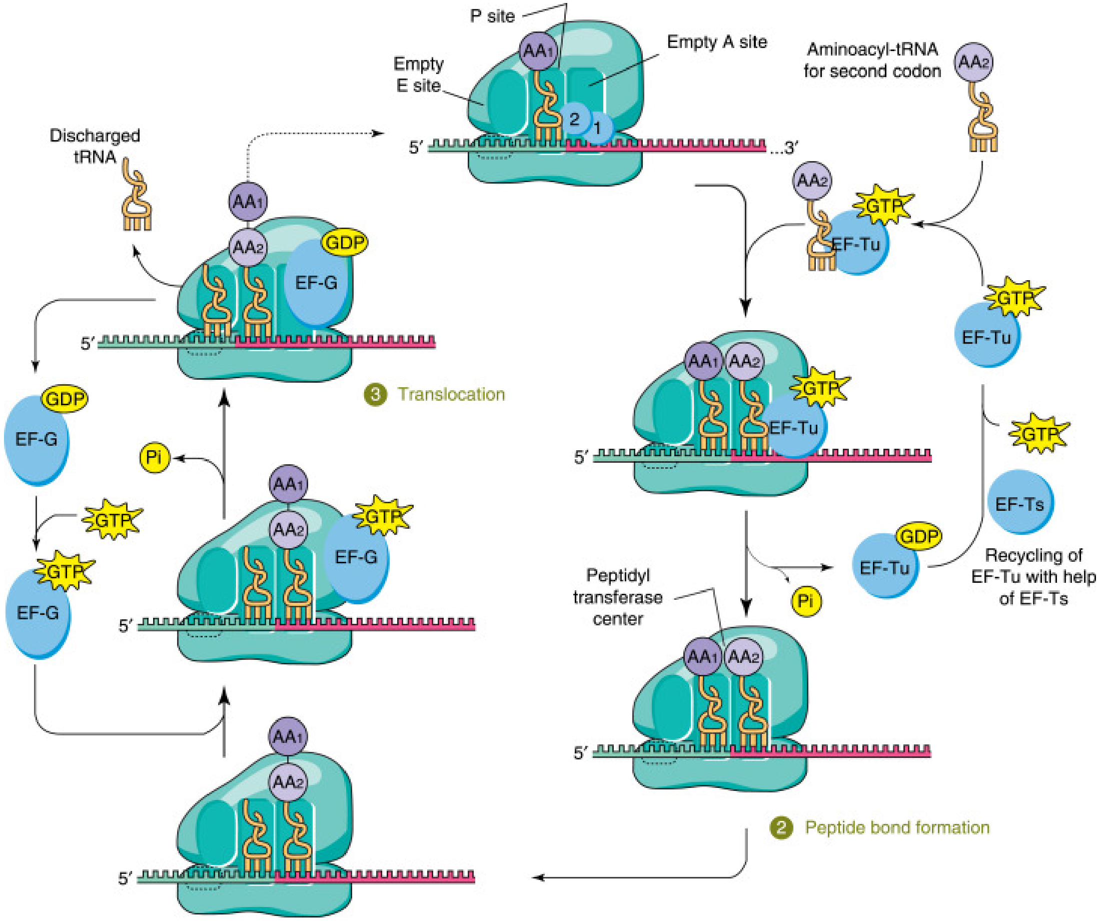
Elongator tRNA:EF-Tu:GTP challenge codon.

If challenge is met (complementarity exists) EF-Tu hydrolyses GTP to GDP.

Conformational change in EF-Tu pushes elongator tRNA into A site.

EF-Tu is freed.

EF-G:GTP is then recruited, accesses A site, hydrolyses GTP, changes conformation, pushes tRNA back to P site and frees A site.



Eukaryote elongation

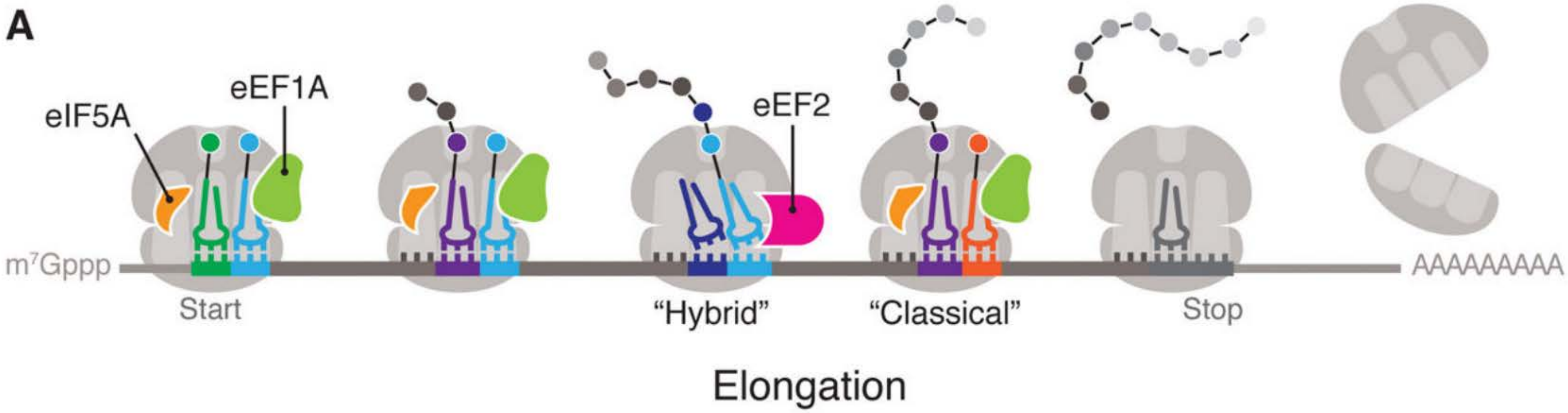
A process that begins after translation initiation has taken place and an 80S ribosome is positioned at an AUG start codon with a methionyl-tRNAiMet in the P site.

Extends from the loading of the first aminoacyl-tRNA at the start of the ORF (after the initiation codon) until the ribosome reaches the termination codon at the end of the ORF.

Is thought to be mostly conserved relative to bacterial elongation.

Translation elongation comprises three basic steps:

1. tRNA selection (or decoding)
2. Peptidebond formation, and
3. Translocation of the mRNA–tRNA complex



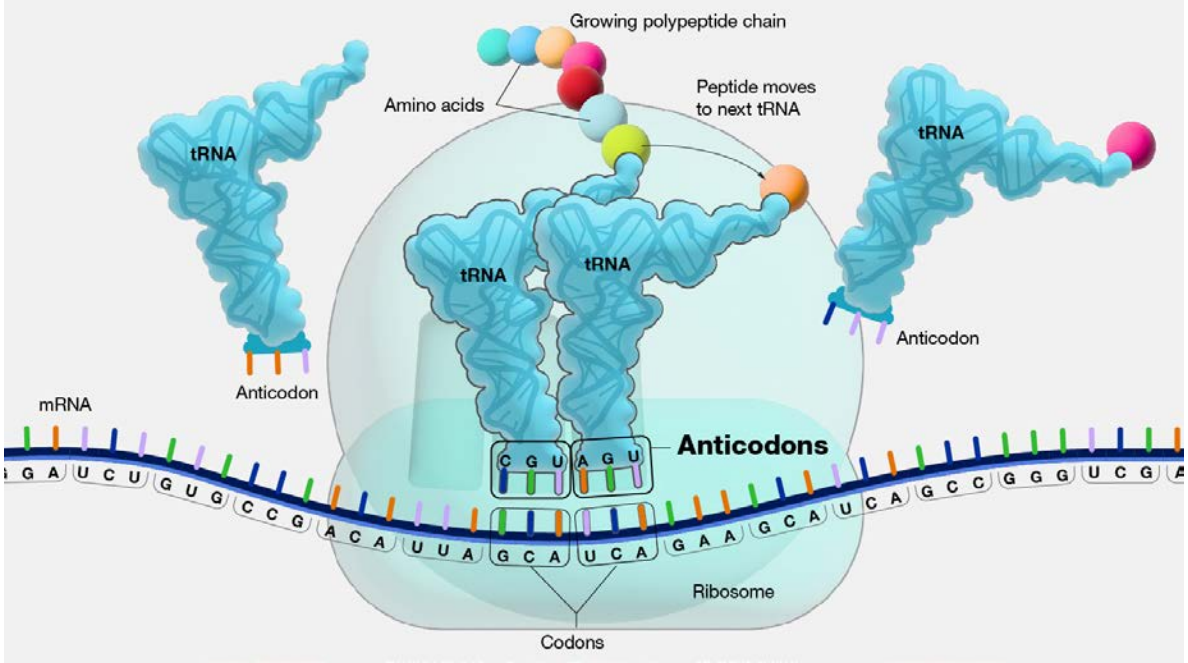
[Roadblocks and resolutions in eukaryotic translation. Schuller AP Nature reviews. Molecular Cell Biology, 2018]

Elongation: tRNA selection

tRNA selection is the process wherein the aminoacyl-tRNA with the proper anticodon to match the mRNA codon is loaded into the A site of the ribosome.

Aminoacyl-tRNAs are delivered to ribosomal site A by eEF1A (elongation factor Tu (EFTu) in bacteria) in the form of a ternary complex with GTP.

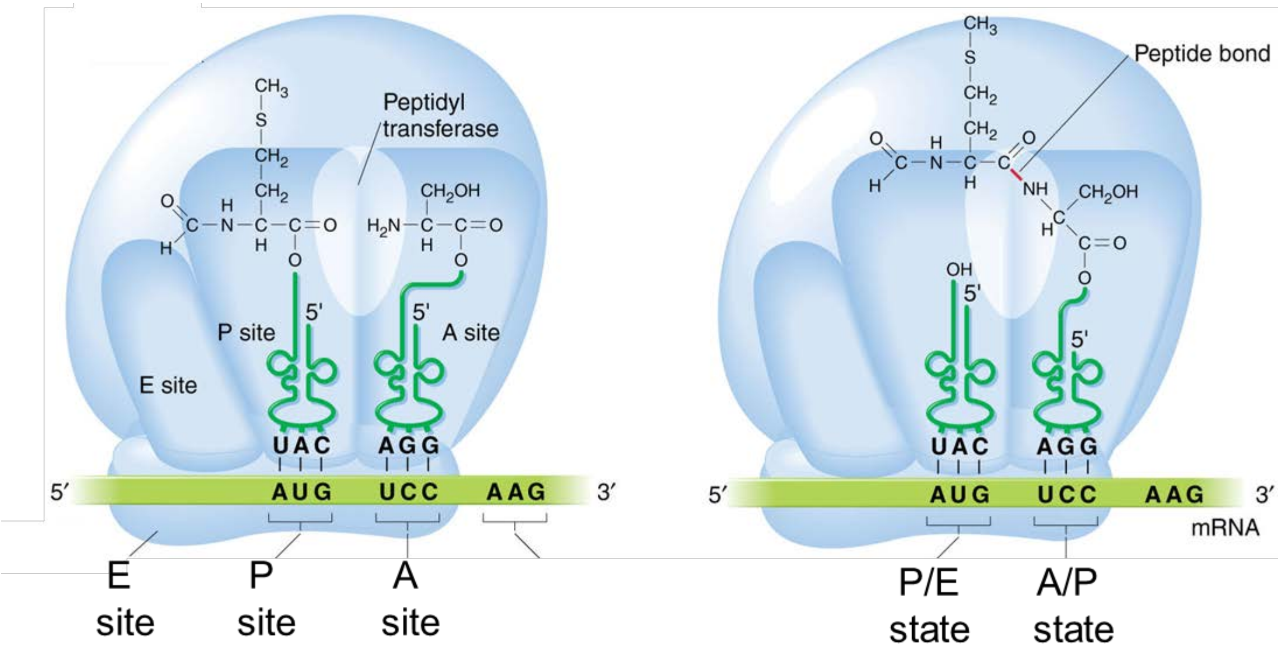
If cognate interactions between codon and anticodon are sensed, eEF1A hydrolyzes GTP to enable the tRNA to be fully accommodated into the A site.



Elongation: transpeptidation

The amino group of the incoming amino acid attacks the ester linkage on the peptidyl-tRNA in the ribosomal P site and the growing peptide chain is transferred to the tRNA in the A site.

tRNAs adopt an altered ‘hybrid’ state, in which the anticodon end of the tRNAs remain positioned essentially in the P and A sites of the small ribosomal subunit, while the acceptor ends of the tRNA are positioned in the E site and P sites of the large subunit (P/E and A/P states, respectively)

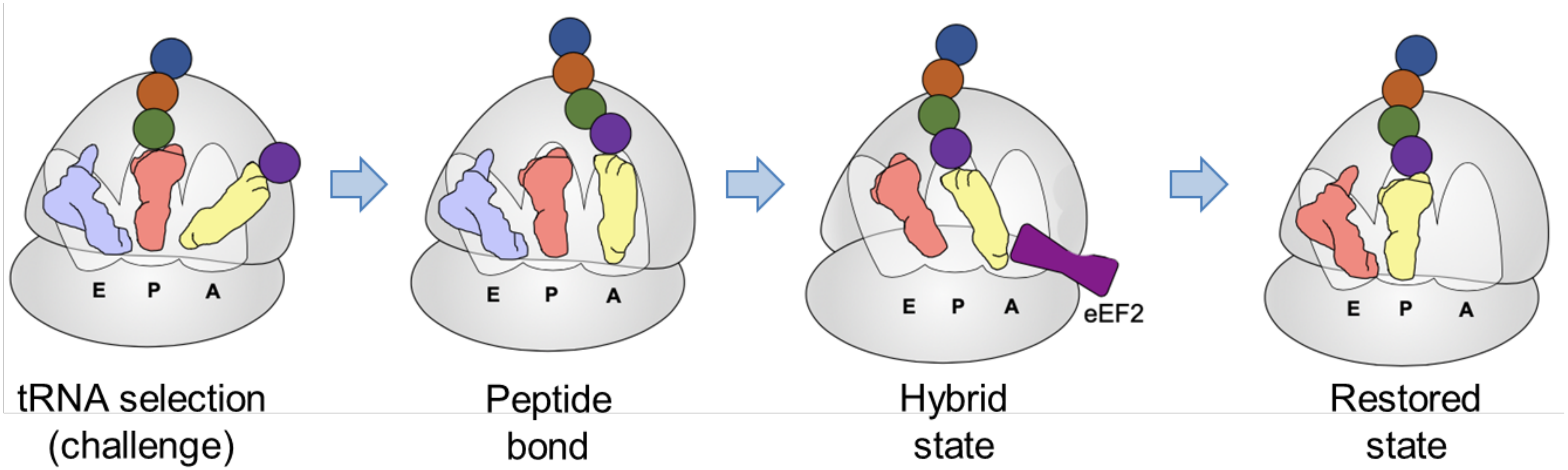


Elongation: transpeptidation

eEF2 (EFG in bacteria) returns the tRNAs to their ‘classical’ states (E/E and P/P).

In addition to eEF1A and eEF2, a third factor, eEF3, is essential for elongation in fungi, potentially by promoting tRNA release from the E site after translocation.

This elongation cycle is repeated until each codon has been translated and a complete protein synthesized.



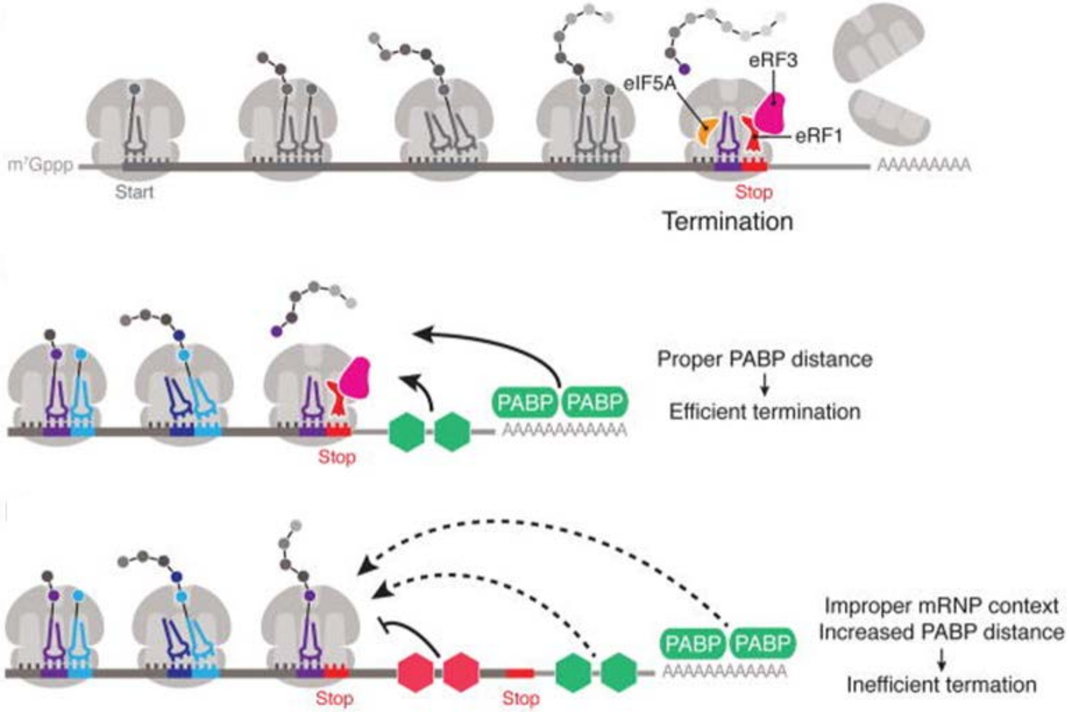
Translation termination

Ribosome encounters a stop codon in the ribosomal A site.

In eukaryotes, all 3 stop codons (UAA, UAG, & UGA) recognized by single release factor, eRF1.

Overall shape and size of eRF1 strikingly similar to that of a tRNA (molecular mimicry).

eRF1 affects peptidyl-transferase center & hydrolyzes the nascent peptide from peptidyl-tRNA.



[Roadblocks and resolutions in eukaryotic translation. Schuller AP Nature reviews. Molecular Cell Biology, 2018](#)

Laboratorio de Genómica Viral y Humana

Instalaciones de Alta Contención Biológica Nivel de Bioseguridad 3 (BSL-3) CDC-certificadas

Facultad de Medicina UASLP
San Luis Potosí, México

www.genomica.uaslp.mx

GenomicaUASLP

YouTube GenomicaUASLP

Content copyright and license

The Viral and Human Genomics Laboratory is committed to promoting the human rights of free access to knowledge and to receiving the benefits of scientific progress and its applications by providing universal access to all the resources and publications it produces. This is in agreement with article 15 of the United Nations International Covenant on Economic, Social and Cultural Rights published on April 30, 2020.

All information included in this document is in the public domain, was compiled by the licensor and is distributed under a Creative Commons Attribution 4.0 International (CC BY 4.0 DEED) license which grants the licensee (you) the right to copy, remix, transform, develop and redistribute the material in any medium or format for any purpose, including commercial purposes provided that:

1) Corresponding credit is given to the licensor as “CA García-Sepúlveda, Laboratory of Viral and Human Genomics UASLP”,

2) Any changes to the original document are indicated and,

3) In no way suggest that the licensor endorses the derivative work.

(Last updated: August 23, © 2024.)