Tema 11
Traducción

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Last updated Jan 2019

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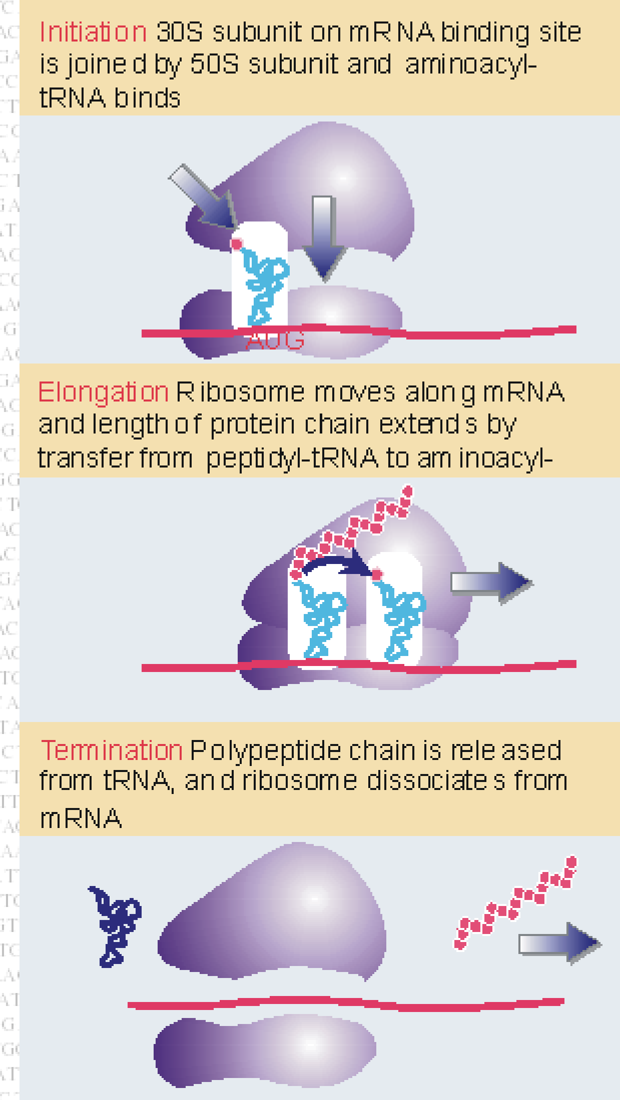
Etapas de la traducción

Protein synthesis divided into the three stages:

1.- Initiation

2.- Elongation

3.- Termination



Initiation 30S subunit on mRNA binding site is joined by 50S subunit and aminoacyl-tRNA binds

Elongation Ribosome moves along mRNA and length of protein chain extends by transfer from peptidyl-tRNA to aminoacyl-

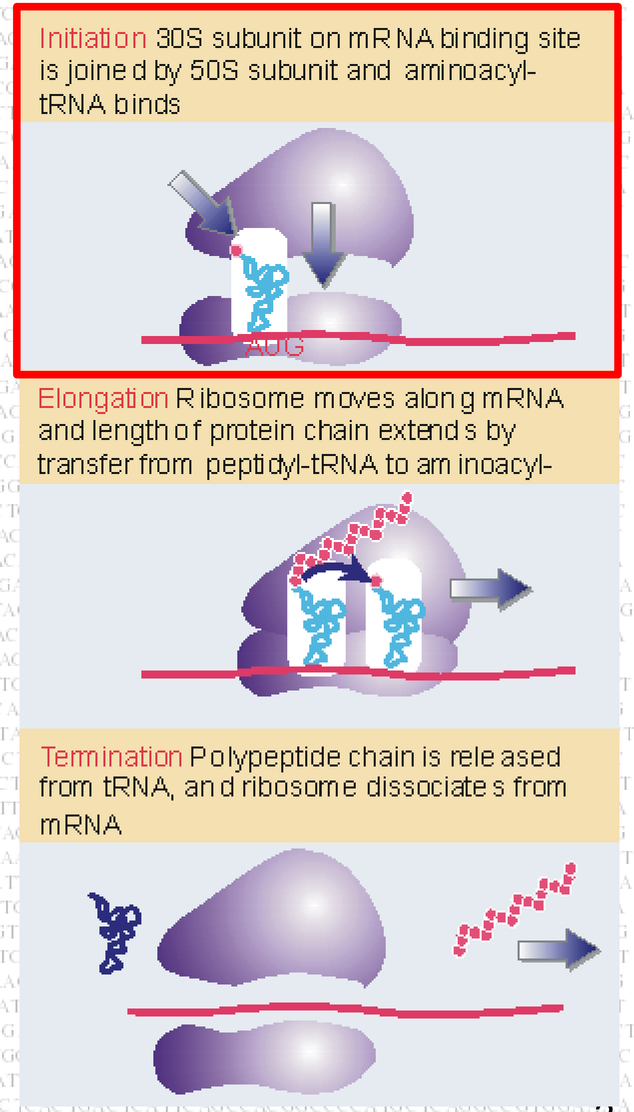
Termination Polypeptide chain is released from tRNA, and ribosome dissociates from mRNA

Etapas de la traducción

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.



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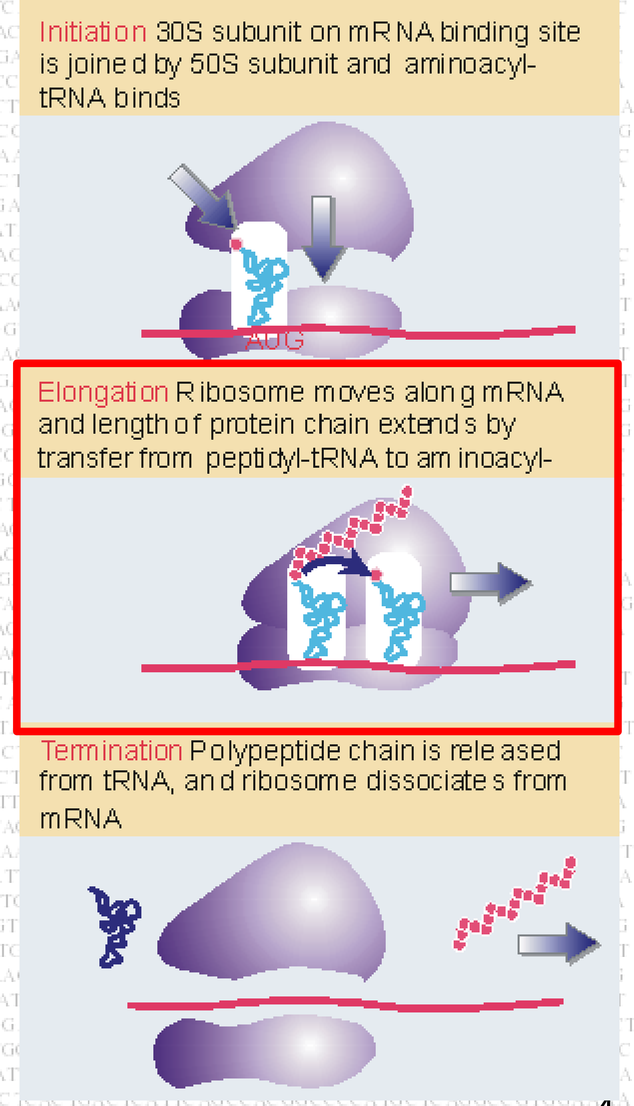
Etapas de la traducción

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.



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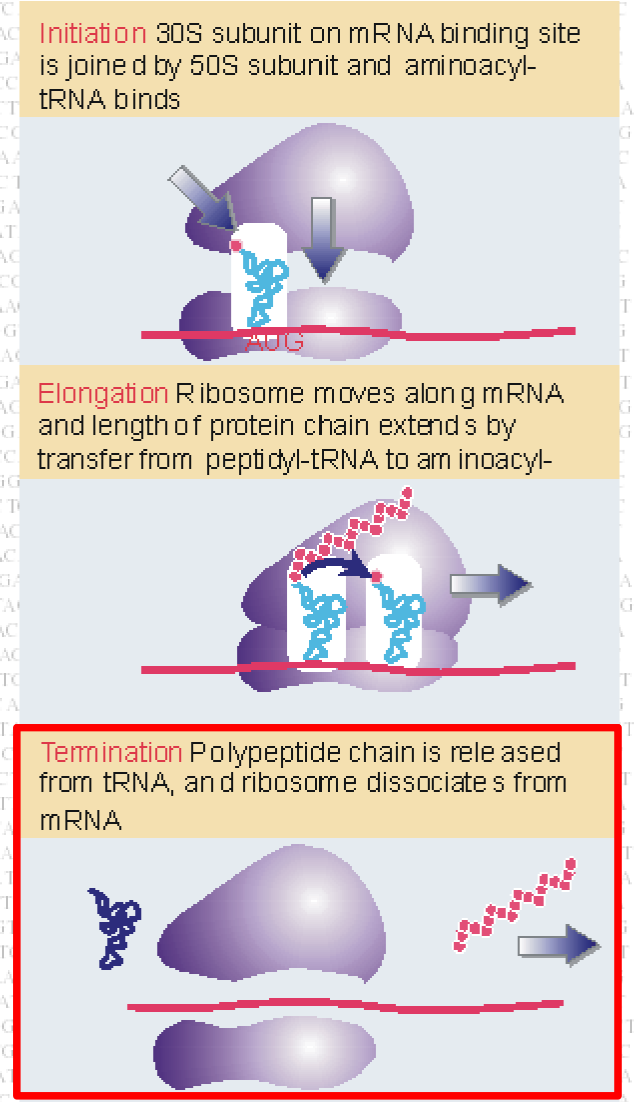
Etapas de la traducción

Termination encompasses the steps that are needed to release the completed polypeptide chain and dissociate the ribosome from the mRNA.



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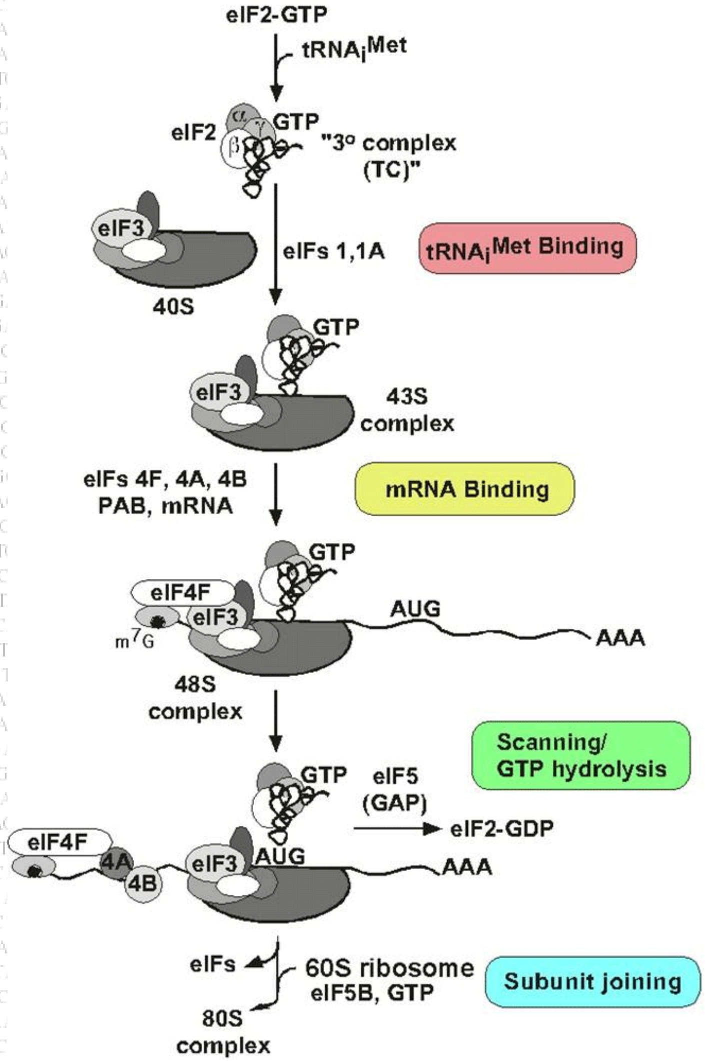
Factores accesorios

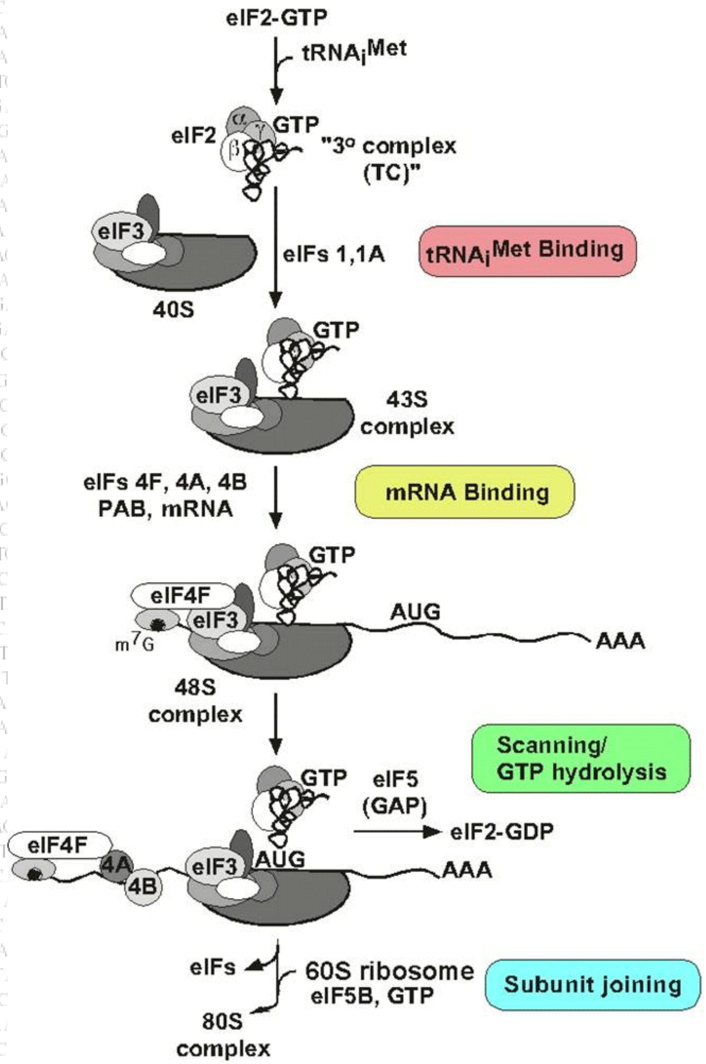
- Different sets of accessory factors assist the ribosome at each stage.
- Energy is provided at various stages by the hydrolysis of GTP.
- Accessory factors are not covalently linked to Ribosome (proteins or RNA).



Factores accesorios

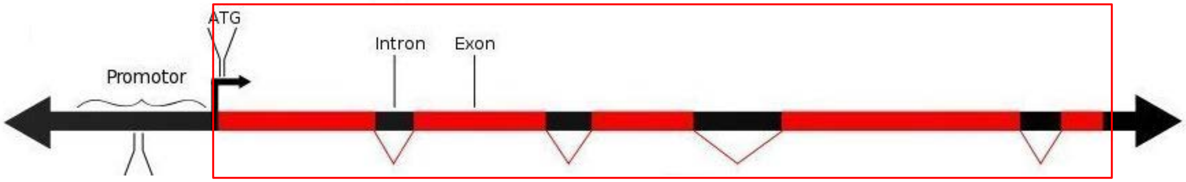
Initiation in bacteria needs 30S subunits and accessory factors

- Initiation factors (IF in prokaryotes, eIF in eukaryotes) are proteins that associate with the small subunit of the ribosome during initiation.
- Initiation of protein synthesis is not a function of intact ribosomes, but is undertaken by the separate subunits, which reassociate during the initiation reaction.

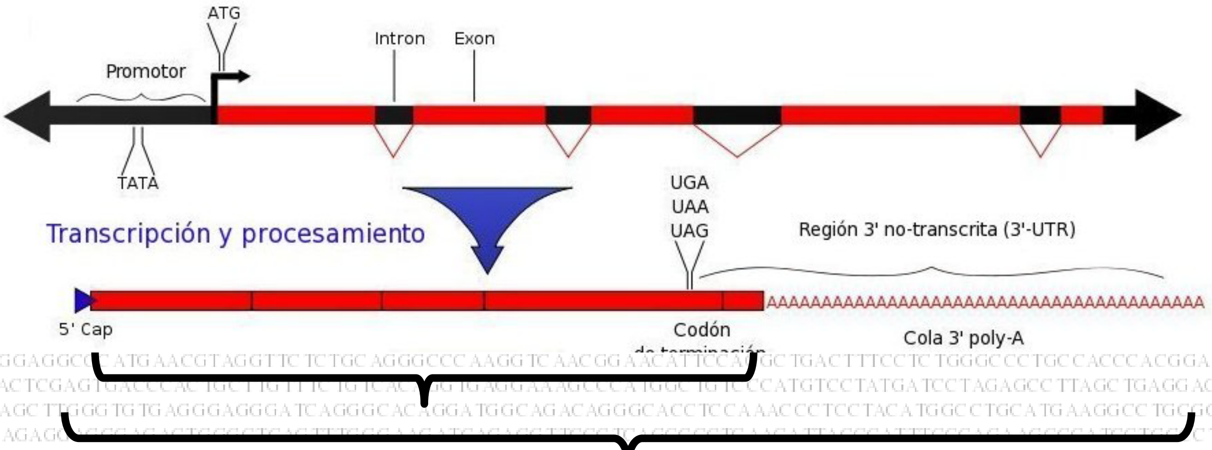


Iniciación

Virtually all eukaryotic mRNAs are monocistronic.



Iniciación



mRNA is usually longer than coding region.

The average mRNA is 1000-2000 bases long

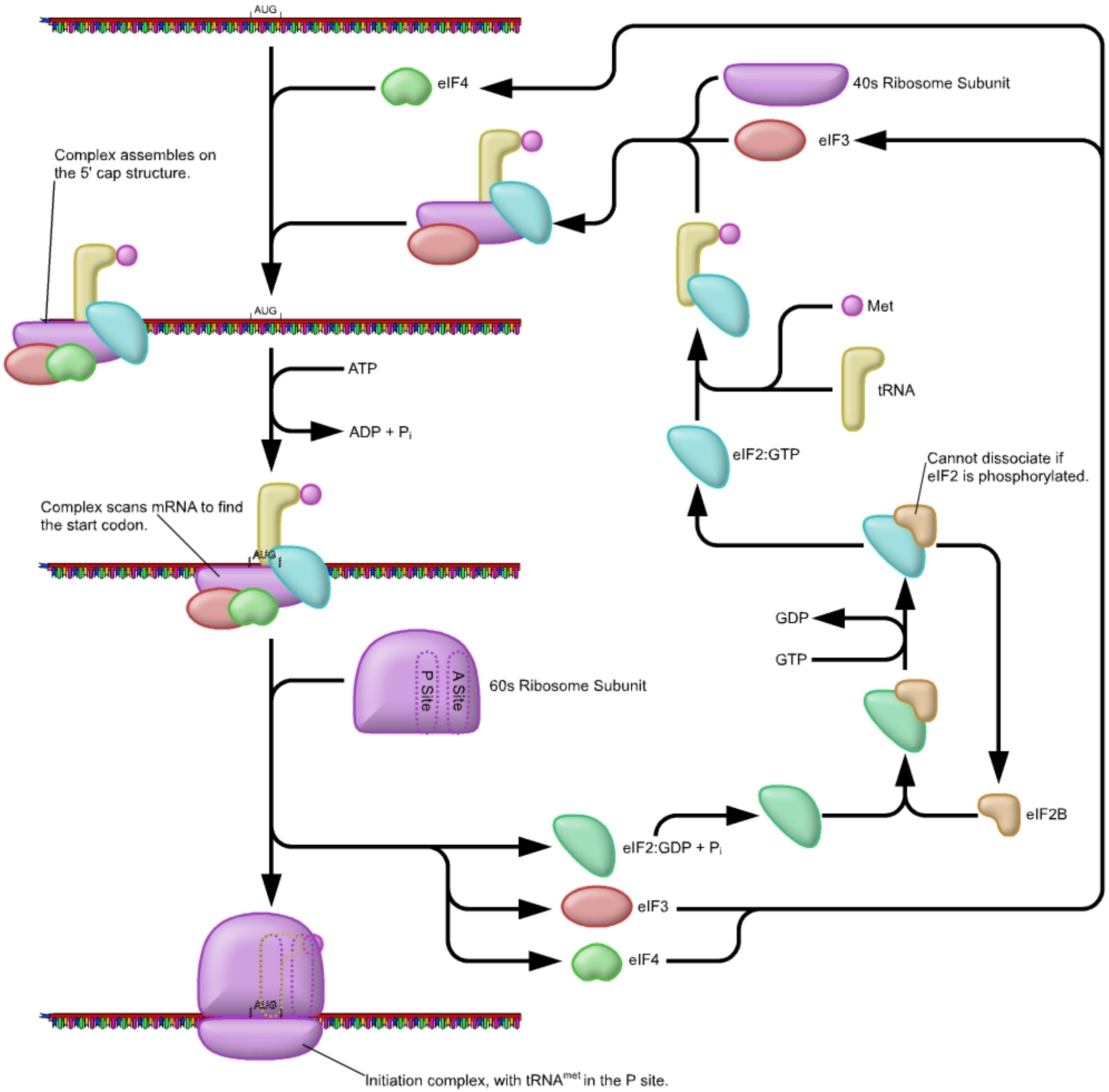
Methylated cap at the 5’ terminus

100-250 poly-A at the 3’ terminus.

Iniciación

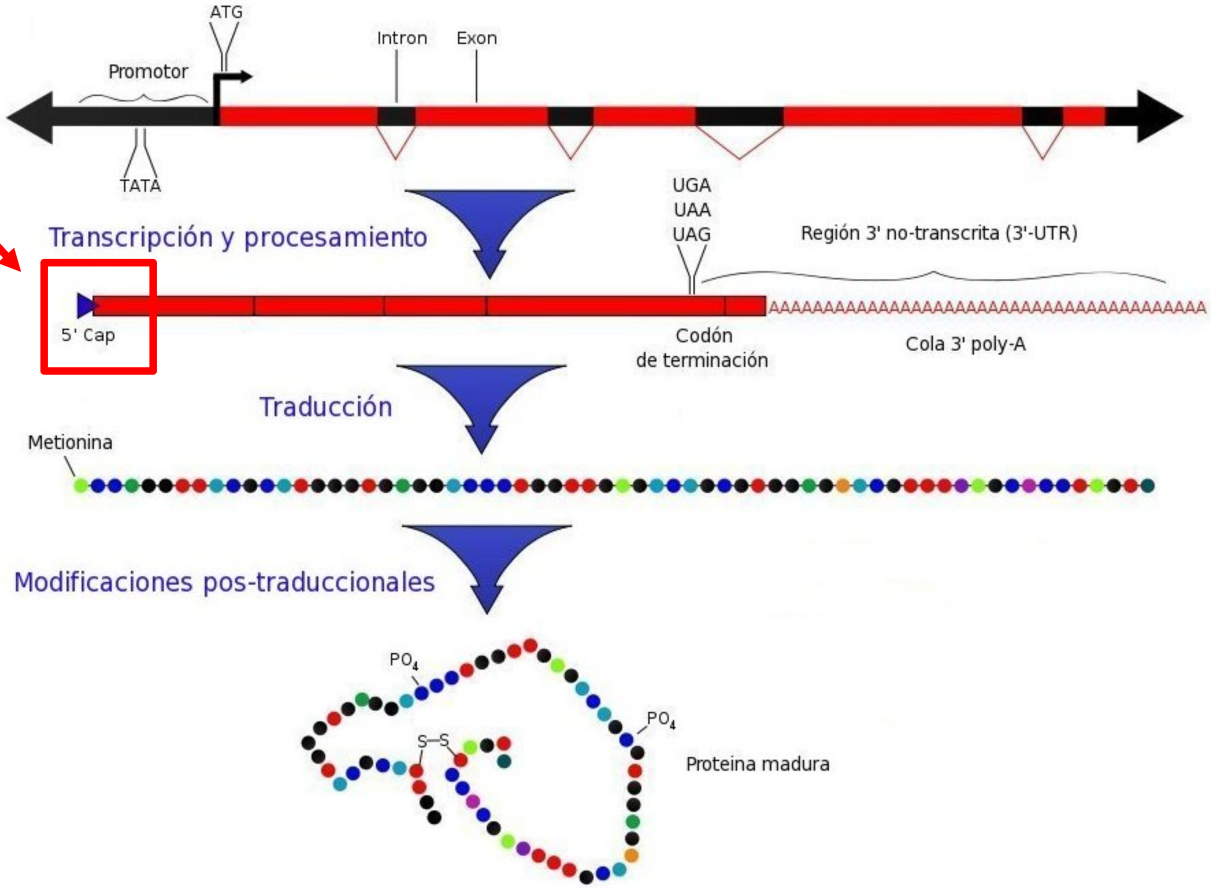
Initiation of protein synthesis in eukaryotes is similar to that in prokaryotes.

The order of events is different, and the number of accessory factors is greater.



Iniciación

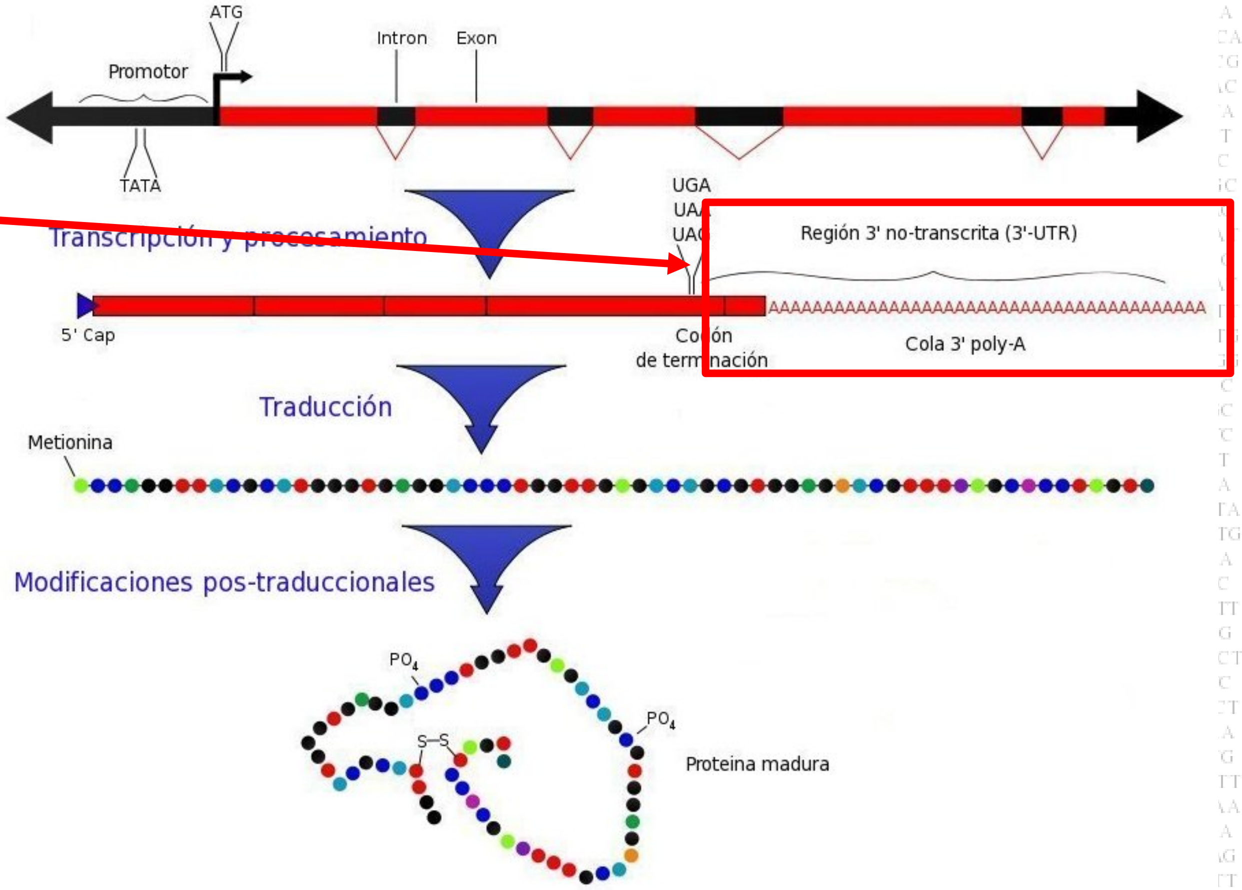
The nontranslated 5’ leader is relatively short, usually <100 bases.



Iniciación

The nontranslated 3’ trailer is often rather long (100 - 1000 b).

By virtue of its location, the leader cannot be ignored during initiation, but the function for the trailer is less obvious.

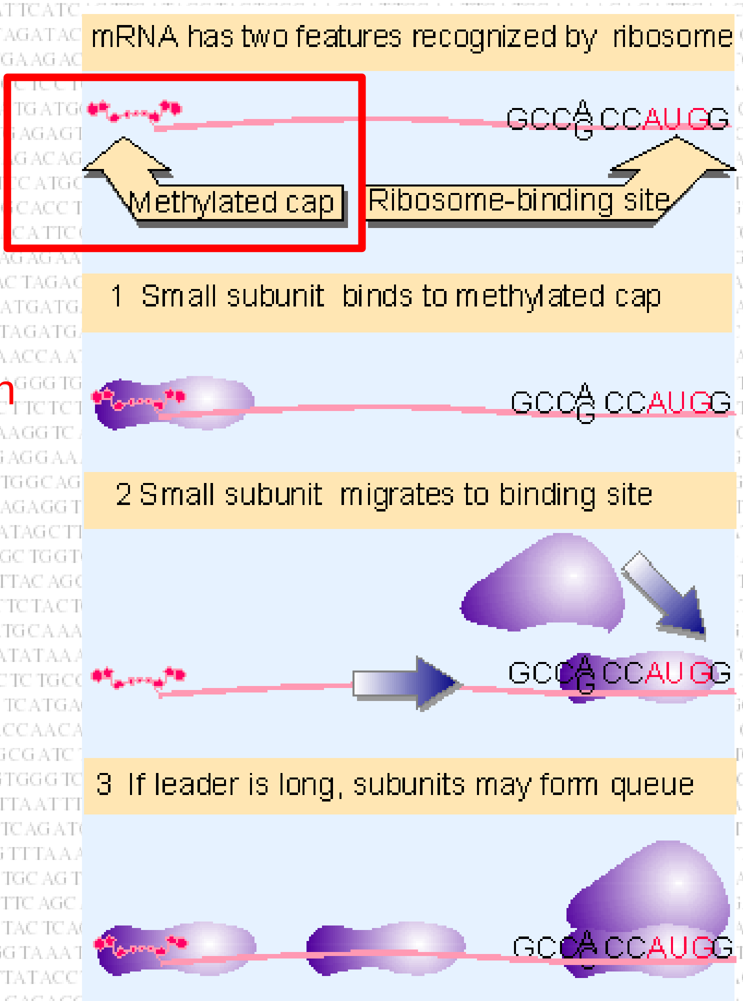


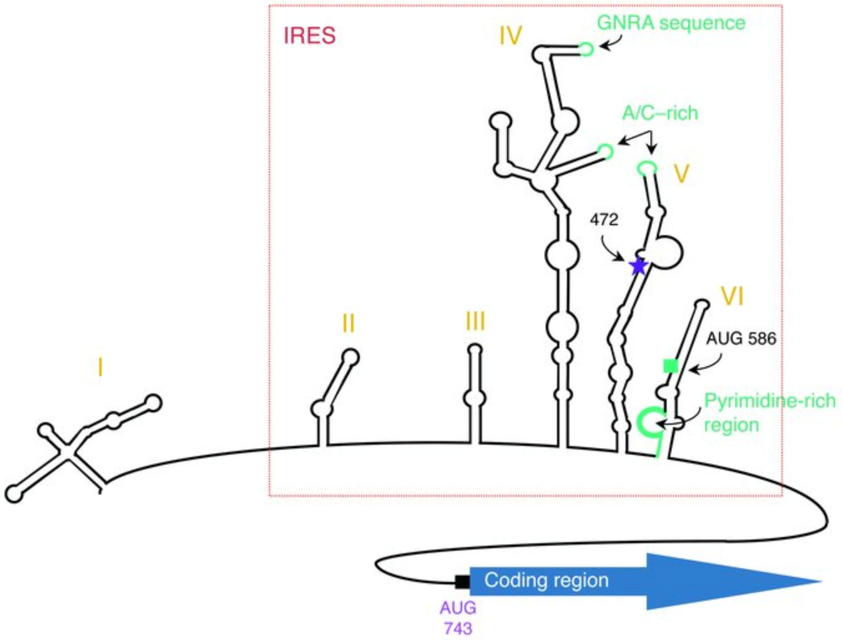
Scanning

The first feature to be recognized during translation of a eukaryotic mRNA is the methylated cap that marks the 5' end.

Messengers whose caps have been removed are not translated efficiently.

Binding of 40S subunits to mRNA requires several initiation factors, including proteins that recognize the structure of the cap.





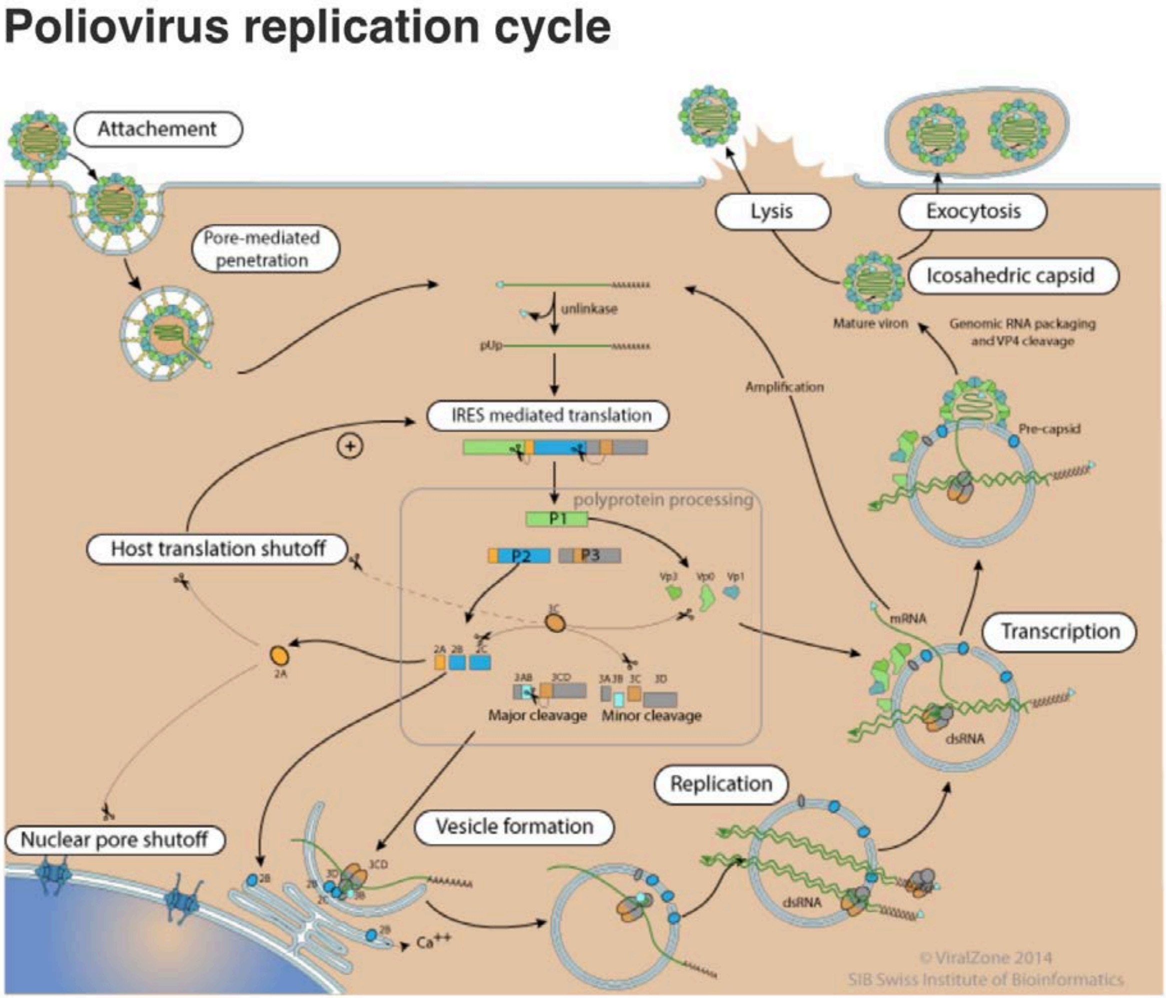
Cap detection

5'-caps are placed on all cellular or viral mRNAs and are essential for their translation in eukaryotic cytoplasm (but not for mitochondria).

The sole exception: poliovirus are not capped; only these exceptional viral mRNAs can be translated without caps.

Poliovirus infection inhibits the translation of host mRNAs.

This is accomplished by interfering with the cap binding proteins that are needed for initiation of cellular mRNAs, but that are superfluous for the noncapped poliovirus mRNA.



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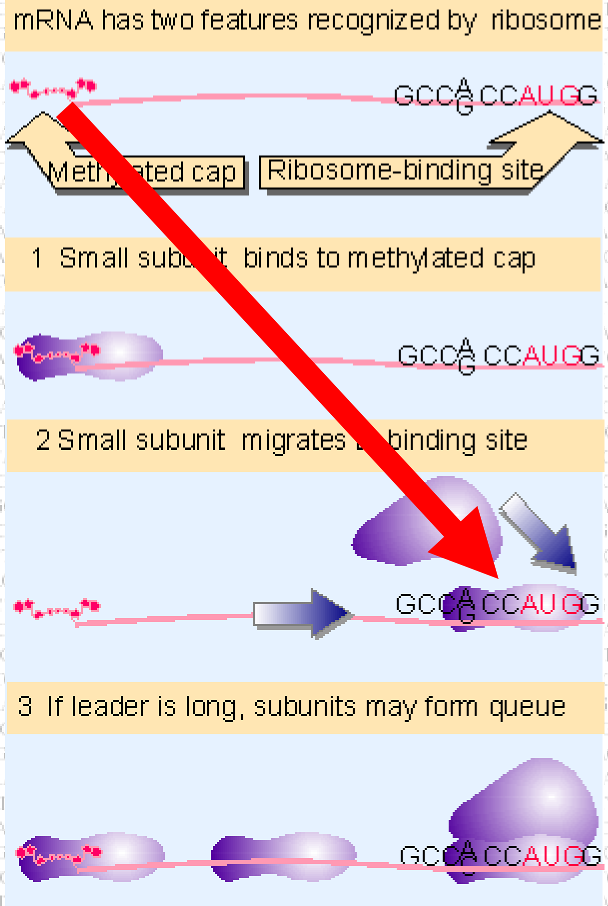
Iniciación

"scanning" model supposes that the 40S subunit initially recognizes the 5' cap and then "migrates" along the mRNA.

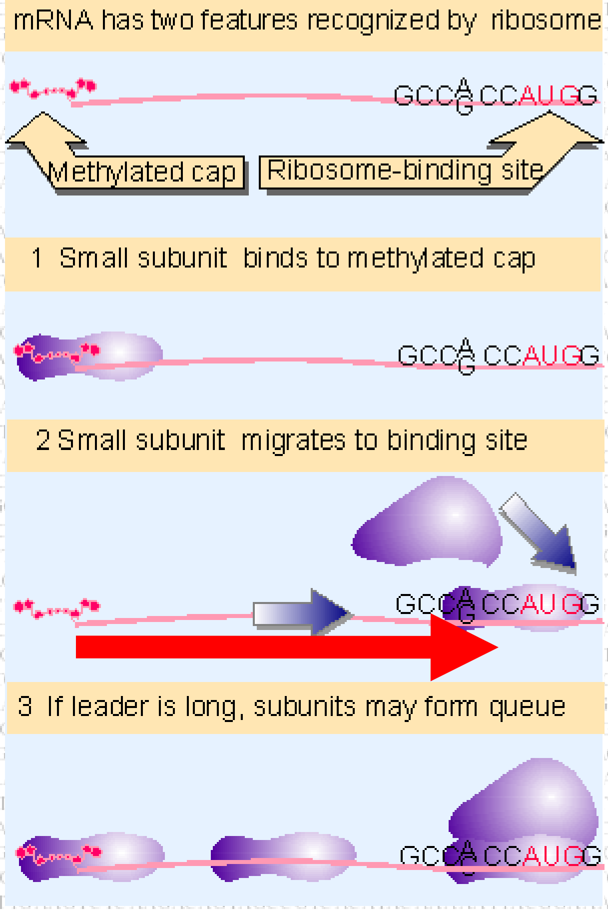
In many mRNAs the cap and AUG are farther apart, in extreme cases ~1000 bases distant.

Yet the presence of the cap is still necessary for a stable complex to be formed at the initiation codon.



Iniciación

Scanning from the 5’ end is a linear process.

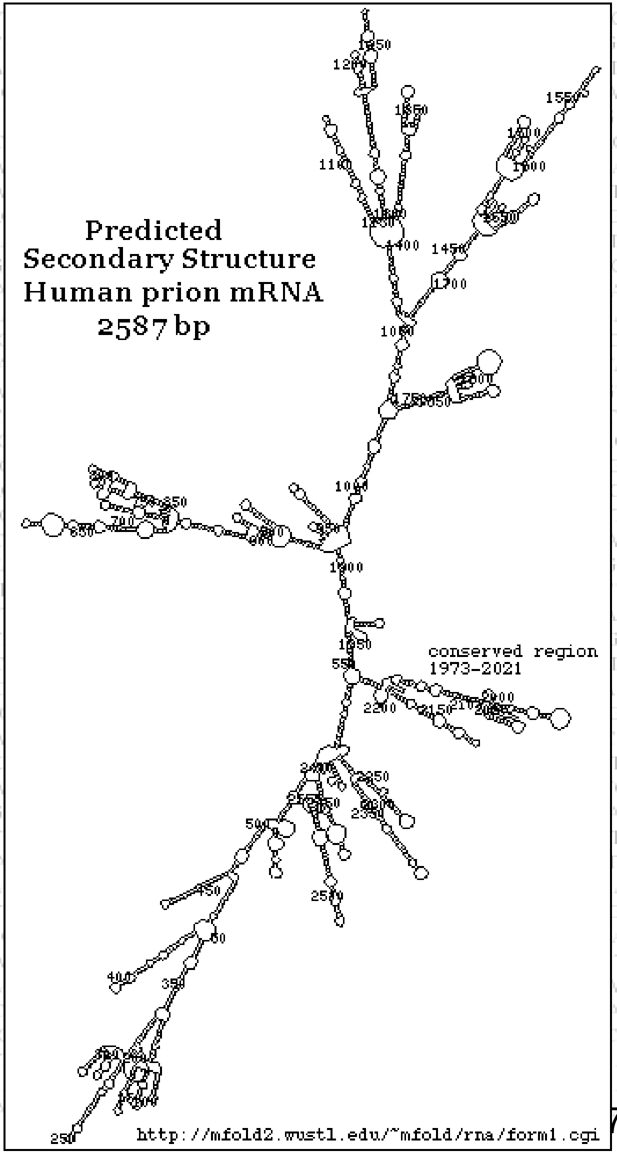


Tema 11. Traducción

Iniciación

When 40S subunits scan the leader region, they melt secondary structure hairpins with stabilities above -30 kcal.

Hairpins of greater stability impede or prevent migration.



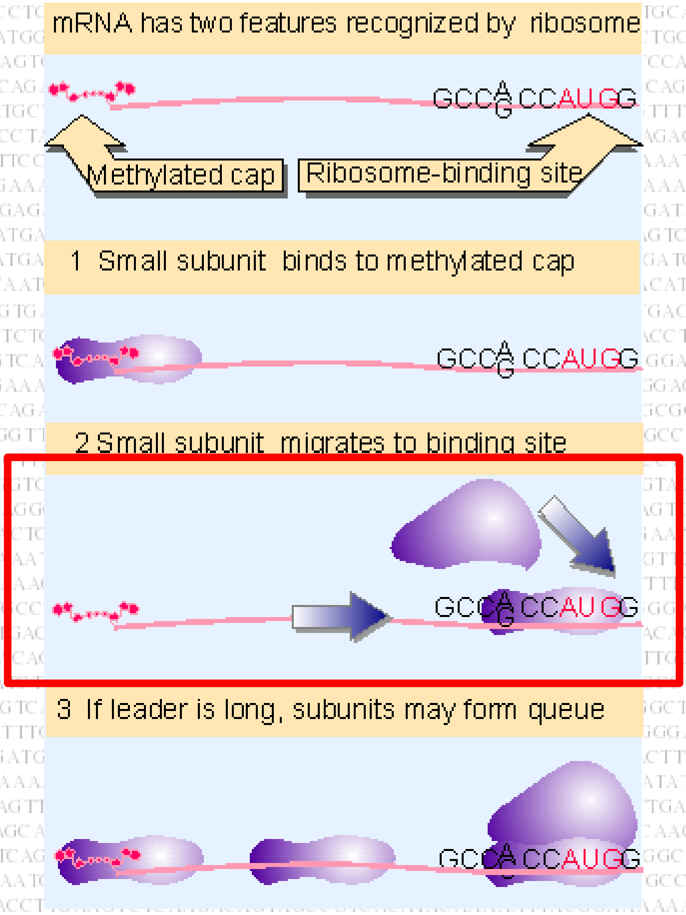
Iniciación

Migration stops when the 40S subunit encounters the AUG initiation codon.

Usually, although not always, the first AUG triplet sequence will be the initiation codon.

The AUG triplet by itself is not sufficient to halt migration; it is recognized efficiently as an initiation codon only when it is in the right context.

The optimal context consists of the sequence GCC$^{A}_{G}$CCAUGG "Kozak Consensus Sequence"

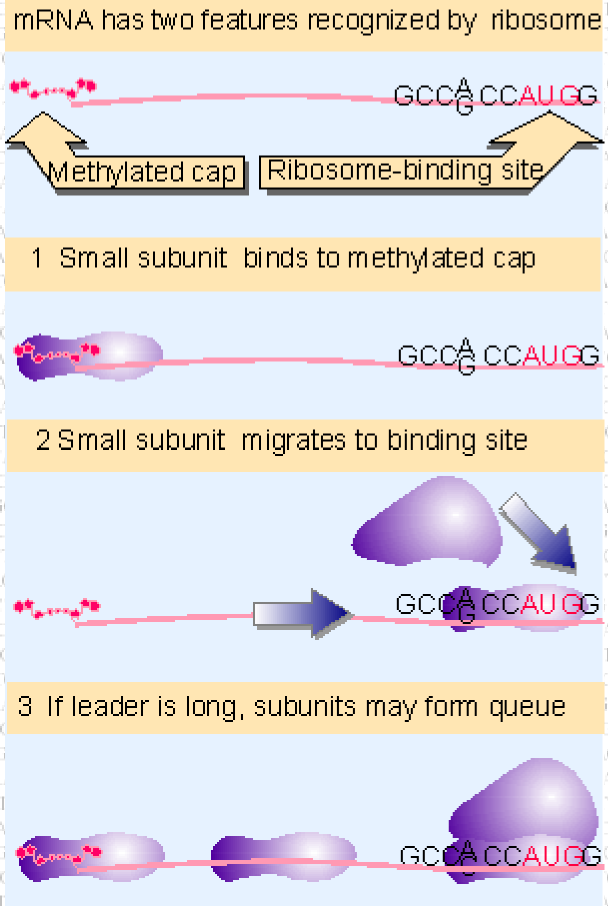


Iniciación

“Kozak Consensus Sequence”

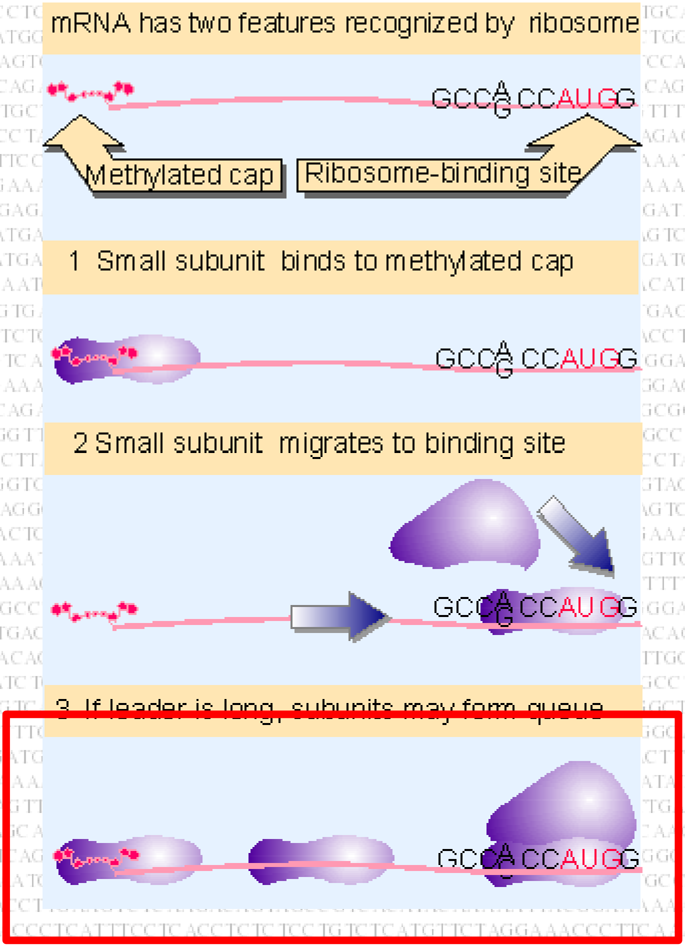
GCCAGCCAUGG

The purine (A or G) 3 bases before the AUG codon, and the G immediately following it, are the most important, and influence efficiency of translation by 10X ; the other bases have much smaller effects.



Iniciación

When the leader sequence is long, further 40S subunits can recognize the 5’ end before the first has left the initiation site, creating a queue of subunits proceeding along the leader to the initiation site.

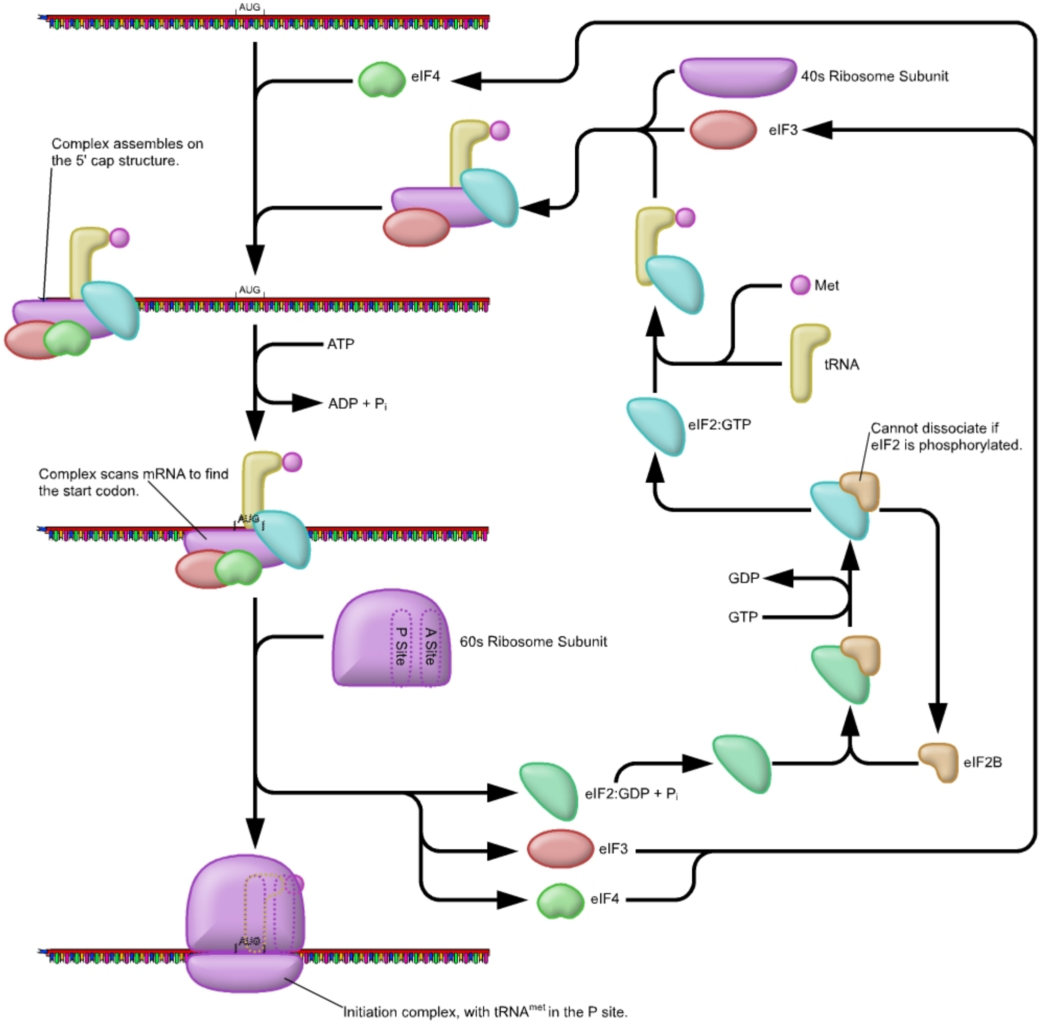


Iniciación

The process of initiation in eukaryotes is analogous to that in *E. coli*.

Eukaryotic cells have more initiation factors than bacteria

The factors are named similarly to those in bacteria, given the prefix "e" to indicate their eukaryotic origin.



Iniciación

We have dealt with the process of initiation as though the ribosome-binding site is always freely available.

However, its availability may be impeded by secondary structure.

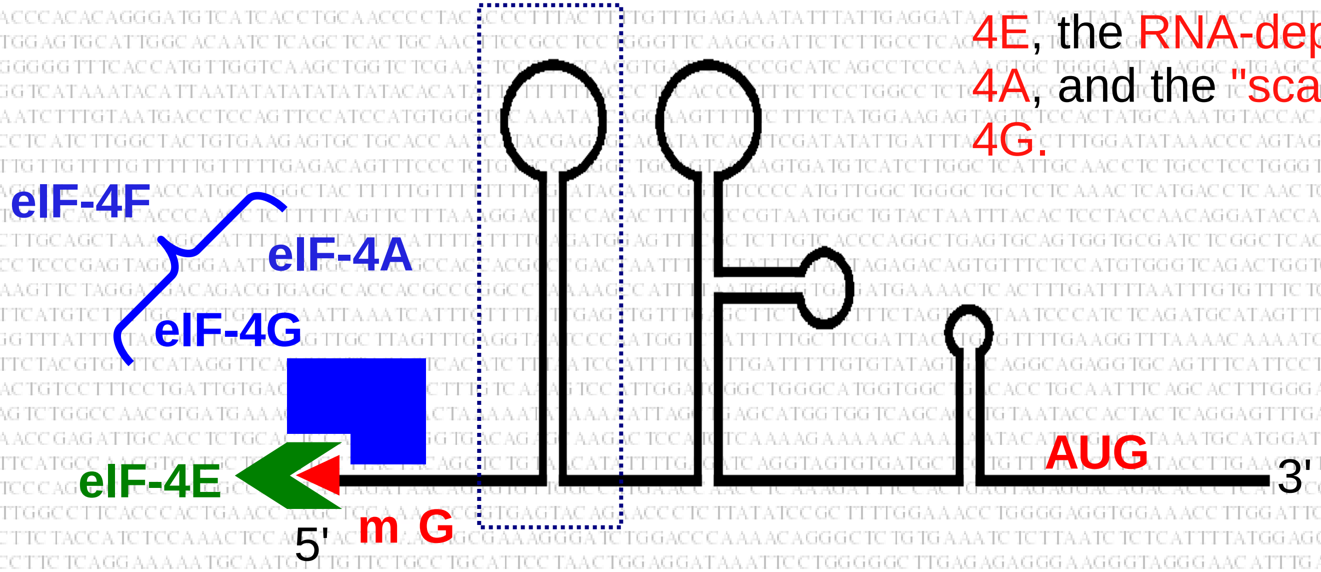
The recognition of mRNA requires several additional factors; an important part of their function is to remove any secondary structure in the mRNA.

Iniciación

The factor eIF4F is a protein complex that regulates key events in recruiting ribosomes to mRNA.

It is not clear whether it preassembles as a complex before binding to mRNA or whether the individual subunits are added individually.

It includes the cap-binding subunit eIF-4E, the RNA-dependent ATPase eIF-4A, and the "scaffolding" subunit eIF-4G.

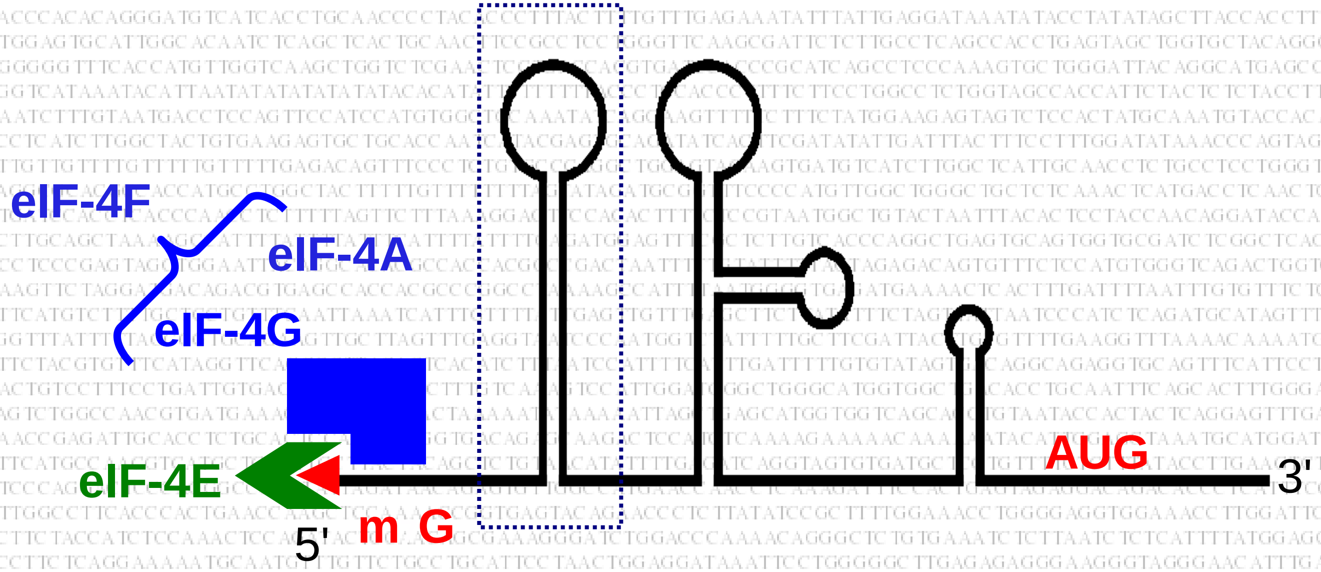


Iniciación

1.- eIF-4E Recognizes the 5' Cap on mRNA.

2.- eIF-4G Recognizes eIF-4E bound to the 5' Cap on mRNA.

3.- eIF-4A Binds to eIF-4G and helps unwind immediate secondary structure (first 15 bases).



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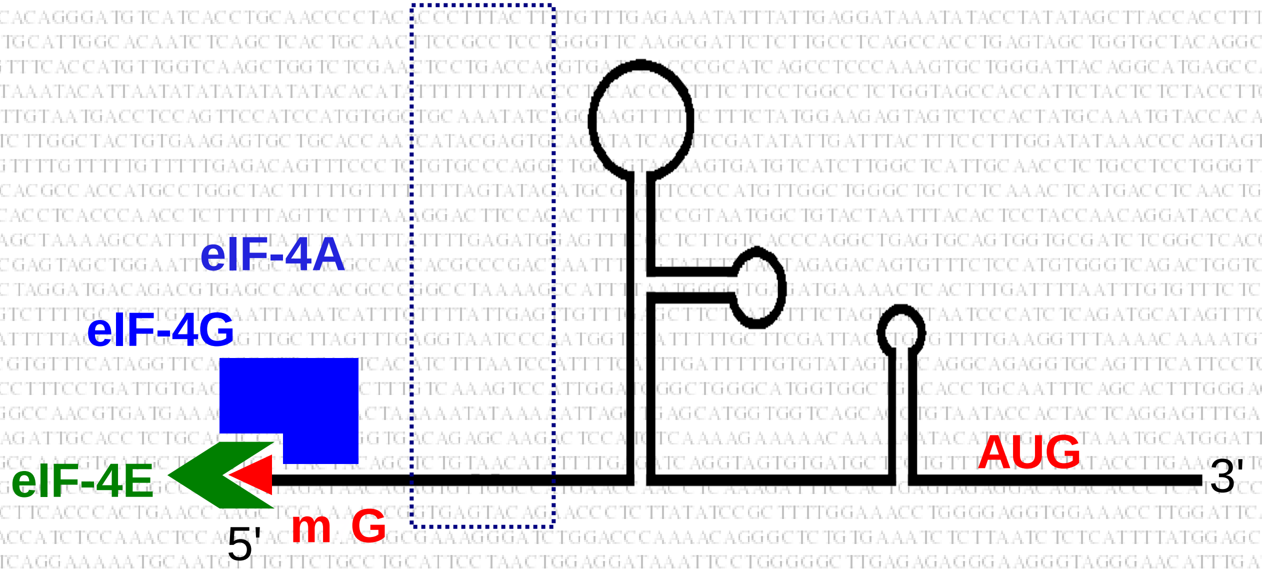
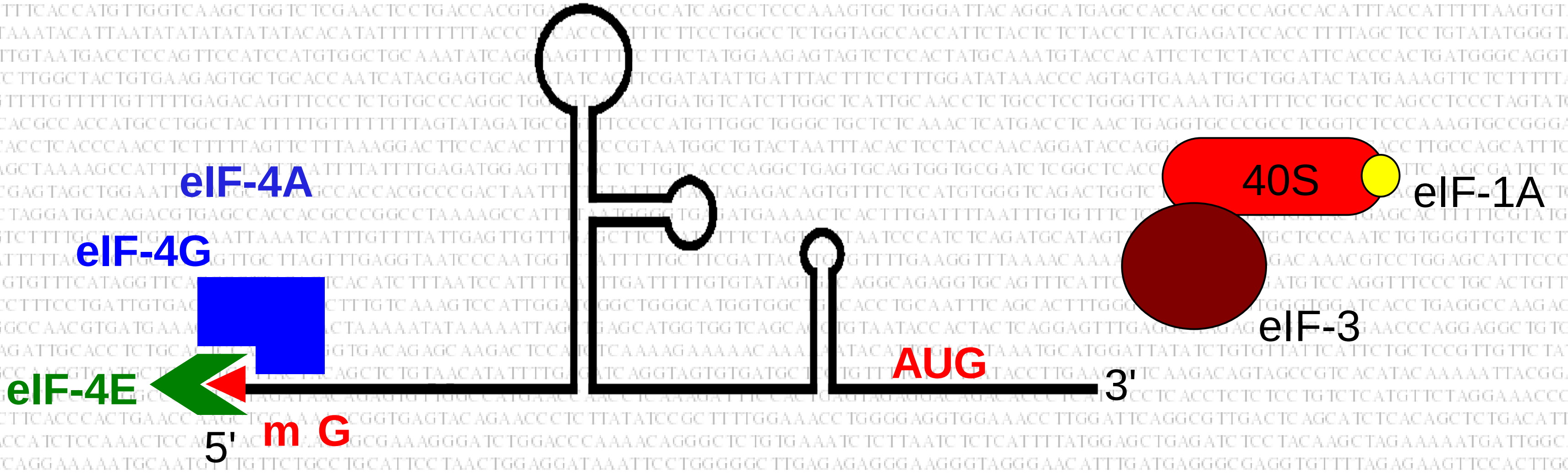


Iniciación

Meanwhile, eIF-1A & eIF-3 stabilize small subunits.

eIF-3 is a very large complex, with 8-10 subunits.

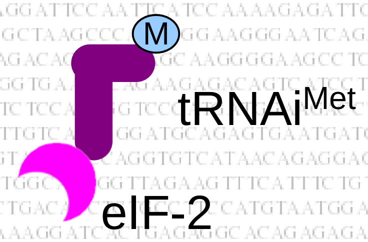


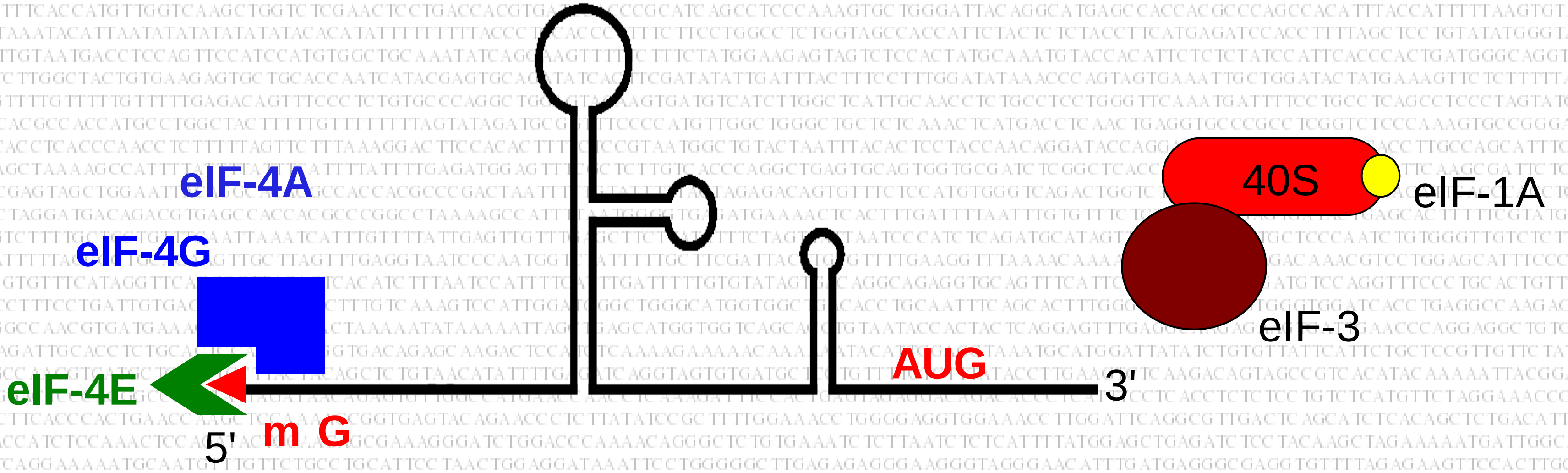
Iniciación

Meanwhile, eIF-1A & eIF-3 stabilize small subunits.

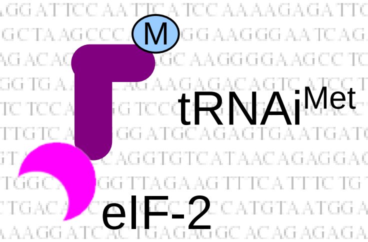
eIF-3 is a very large complex, with 8-10 subunits.

tRNAi^{Met} is bound by eIF-2 (ternary complex)





Iniciación

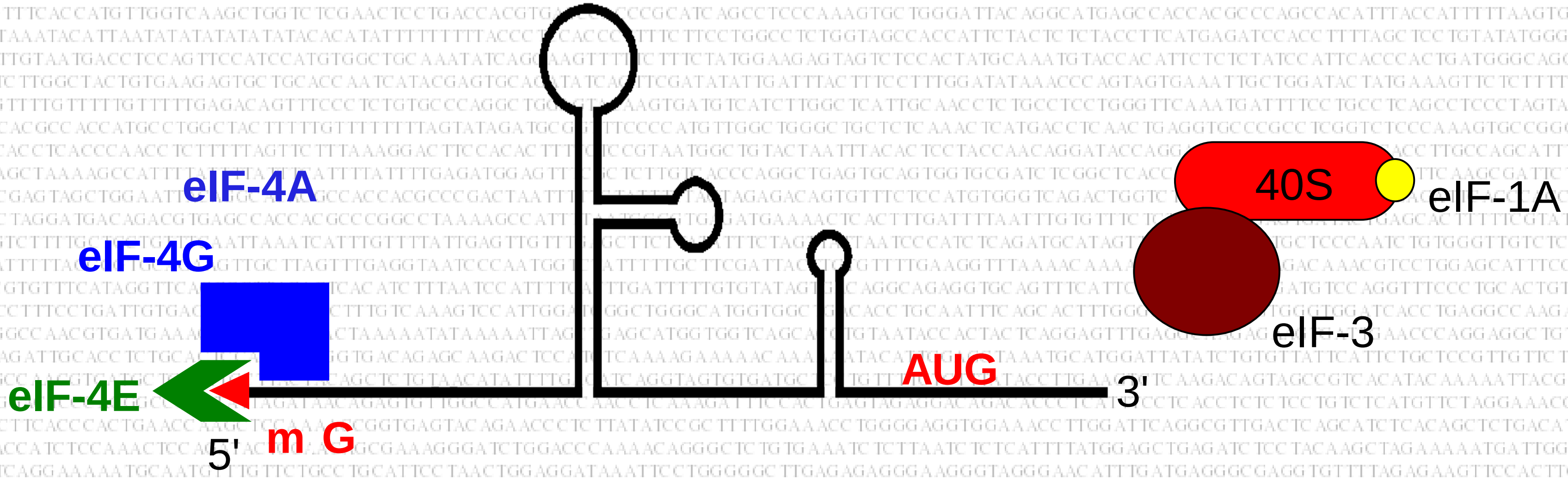


Ternary complex contains Met-tRNA$_i$, eIF2, and GTP.

The complex is formed in two stages.

1.- GTP binds to eIF-2 which increases the factor’s affinity for Met-tRNA$_i$

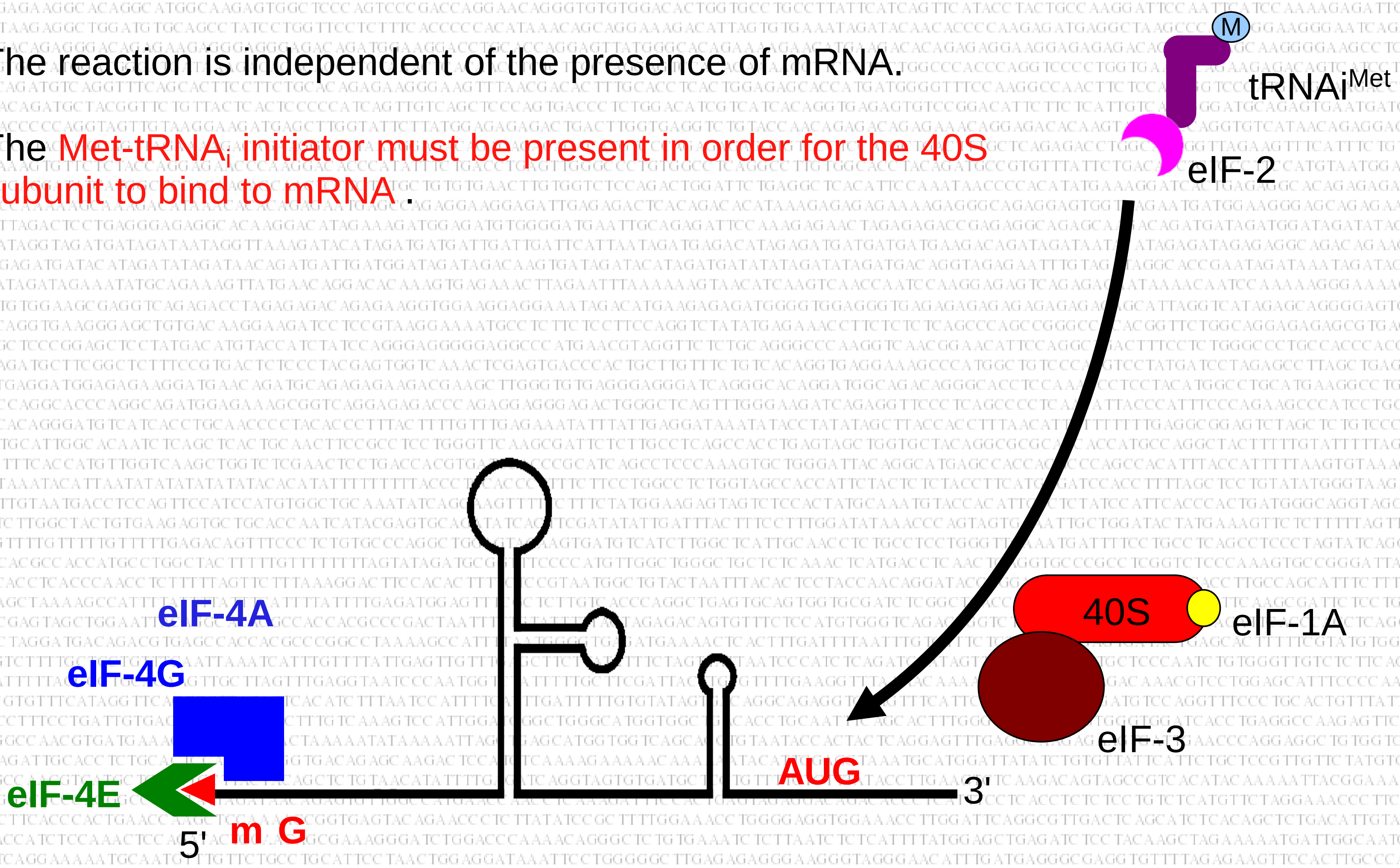
2.- Met-tRNA$_i$ is then bound.



Iniciación

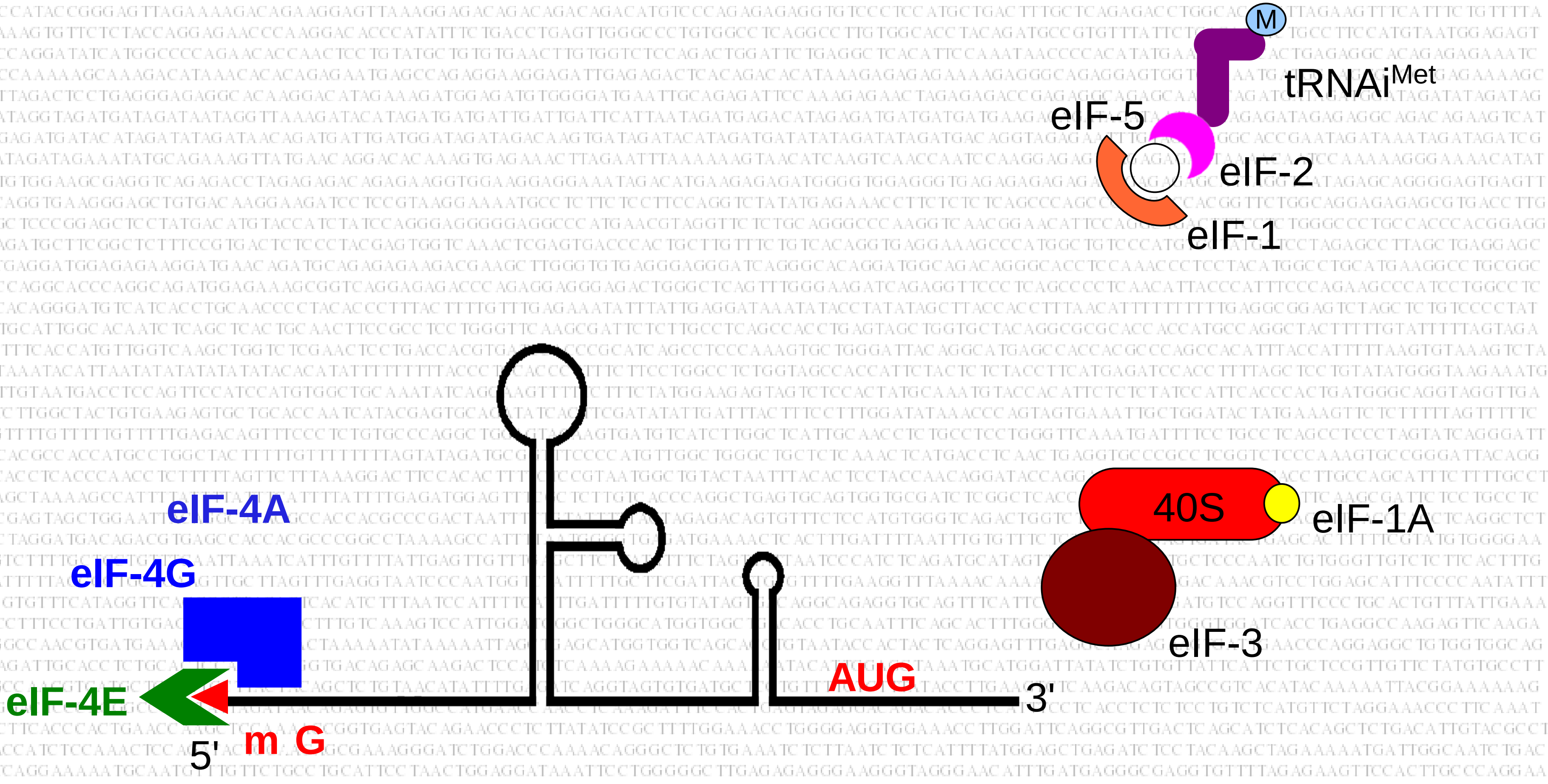
The reaction is independent of the presence of mRNA.

The Met-tRNA$_i$ initiator must be present in order for the 40S subunit to bind to mRNA .



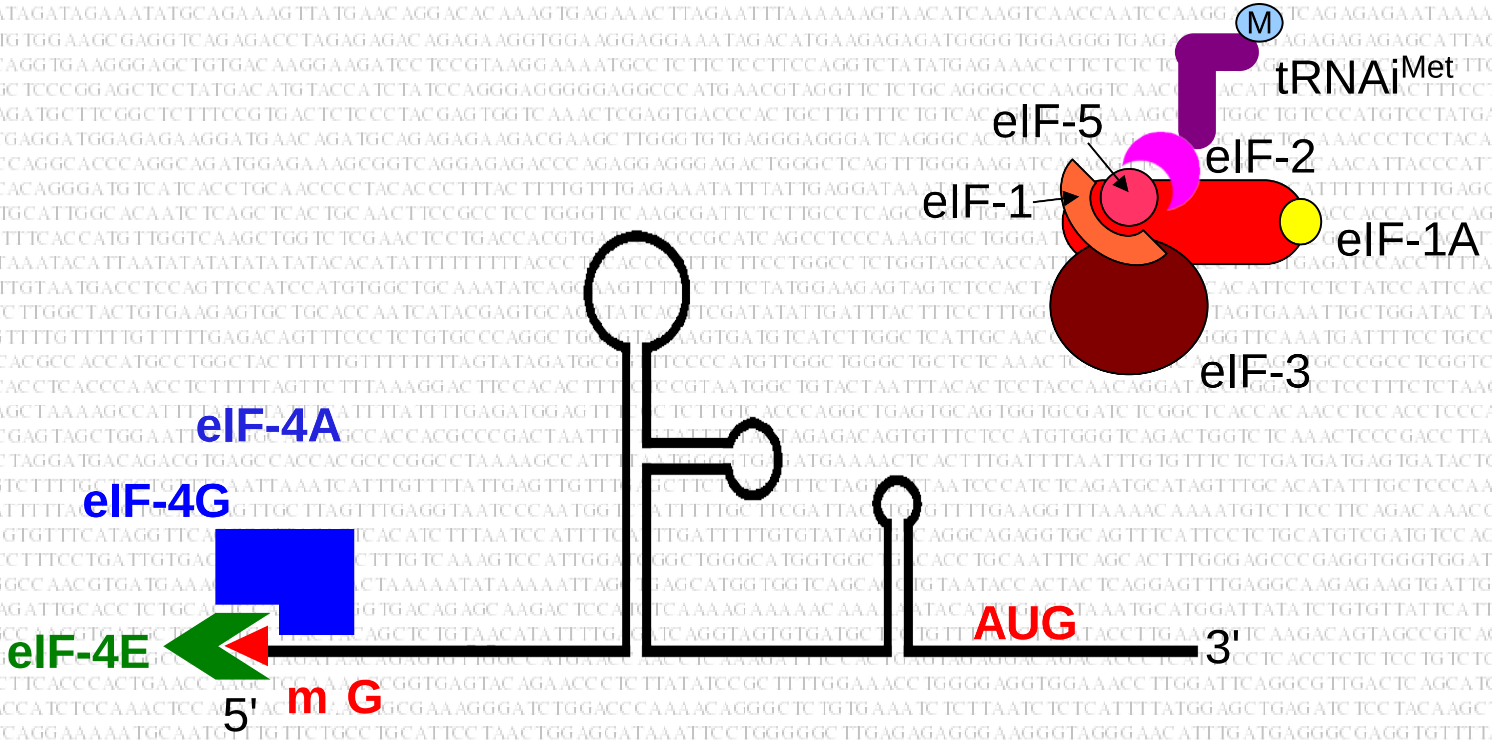
Iniciación

Ternary complex is stabilized with eIF-1 & -5



Iniciación

Ternary complex + eIF-5 eIF-1 are loaded unto small subunit.

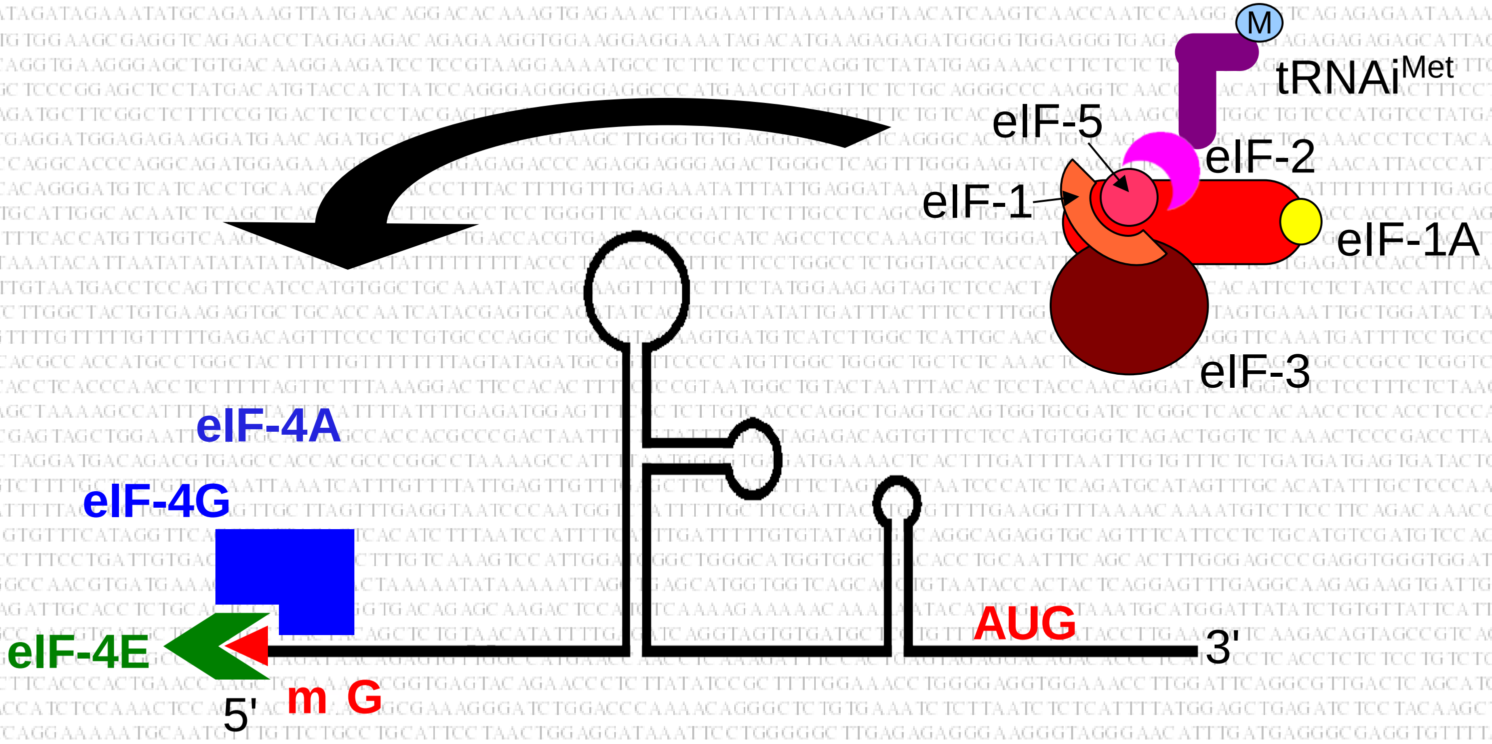


Iniciación

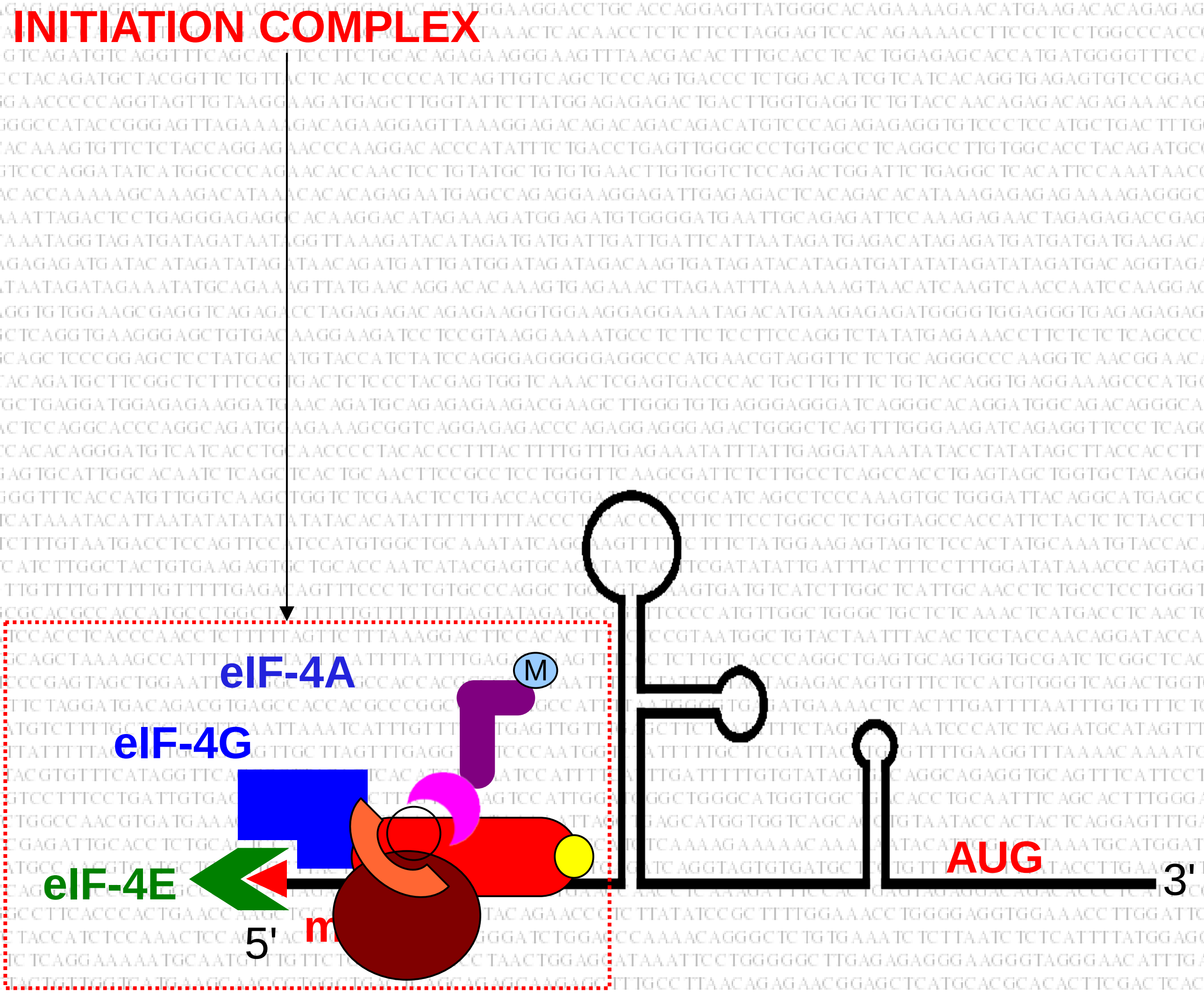
Small subunit is loaded unto eIF-4G/eIF-4A complex

eIF-4G binds to eIF-3 associated with the small ribosomal subunit.

This provides the means by which the 40S ribosomal subunit binds to eIF-4F.

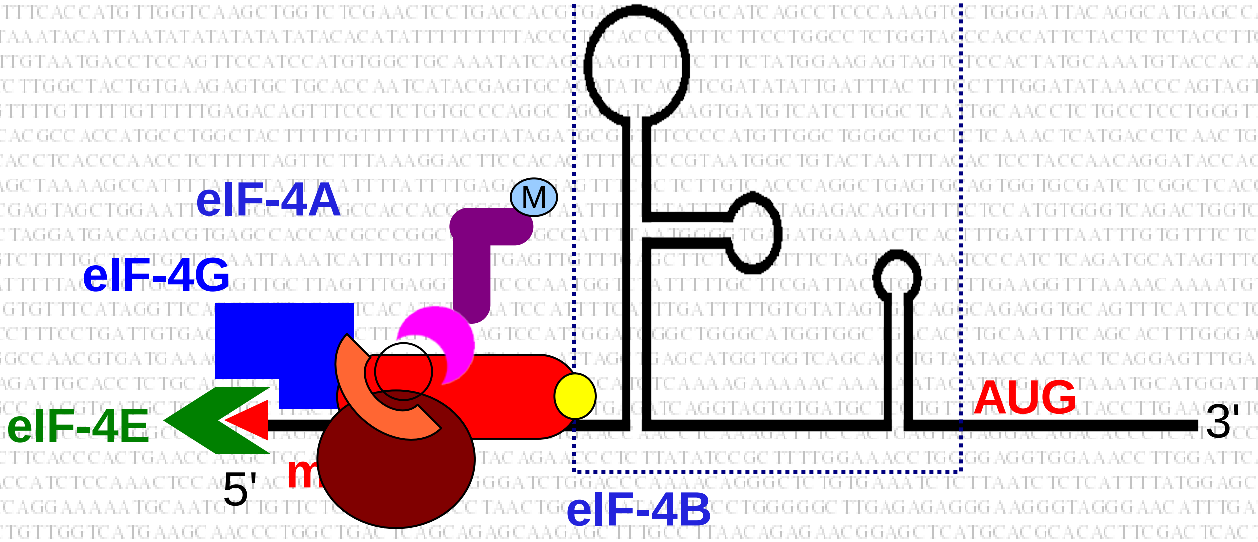


Iniciación



Iniciación

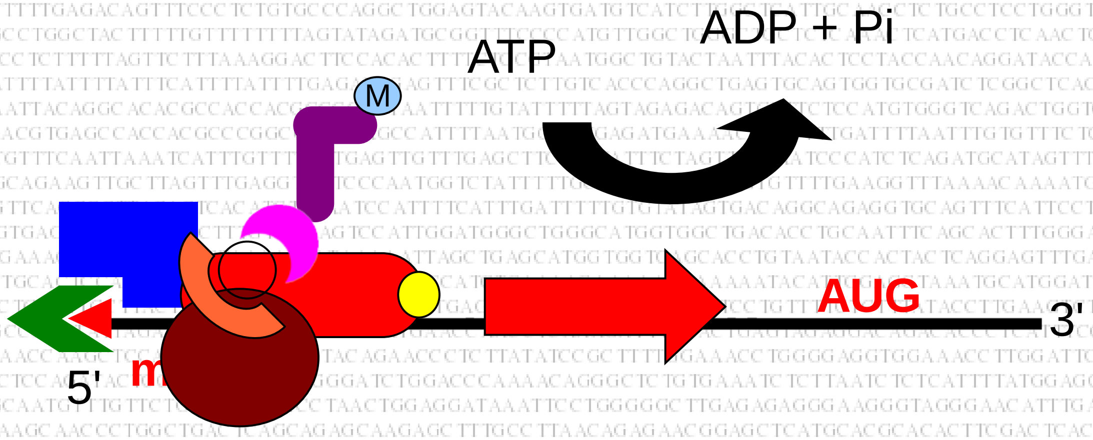
eIF-4B is recruited to the initiation complex to denature further secondary structures.



Iniciación

Once all secondary structures have been melted the initiation complex “scans” for a start codon.

This process requires energy in the form of ATP



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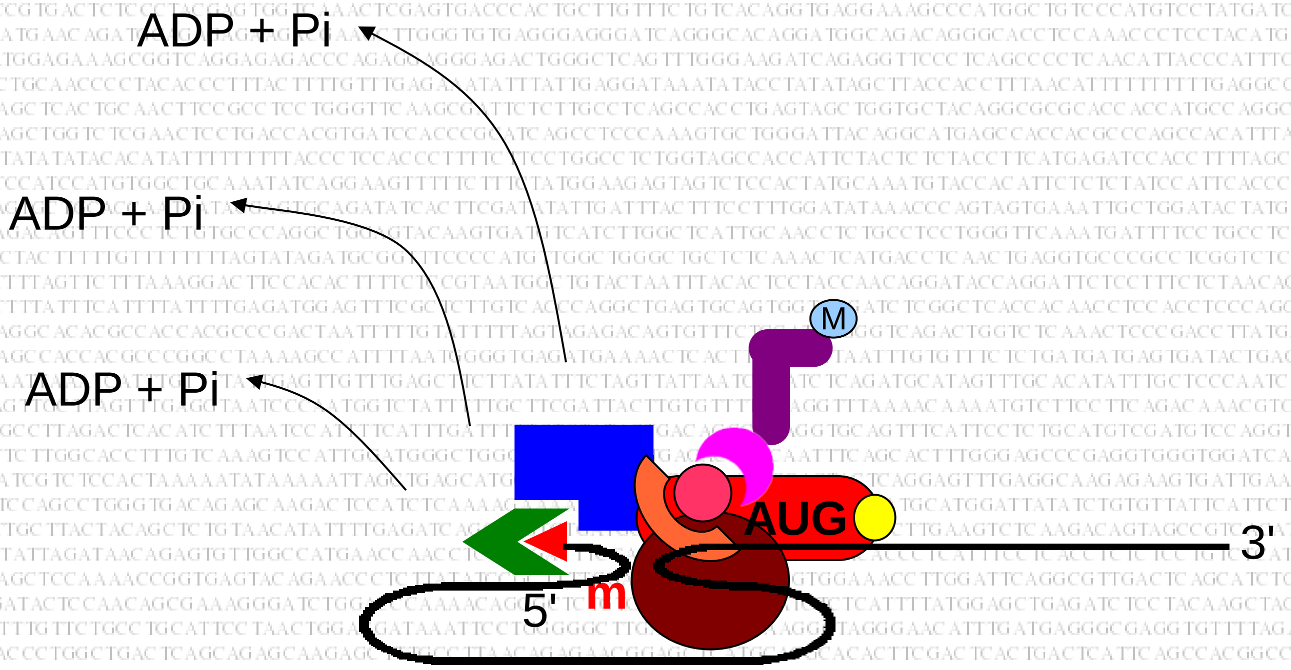
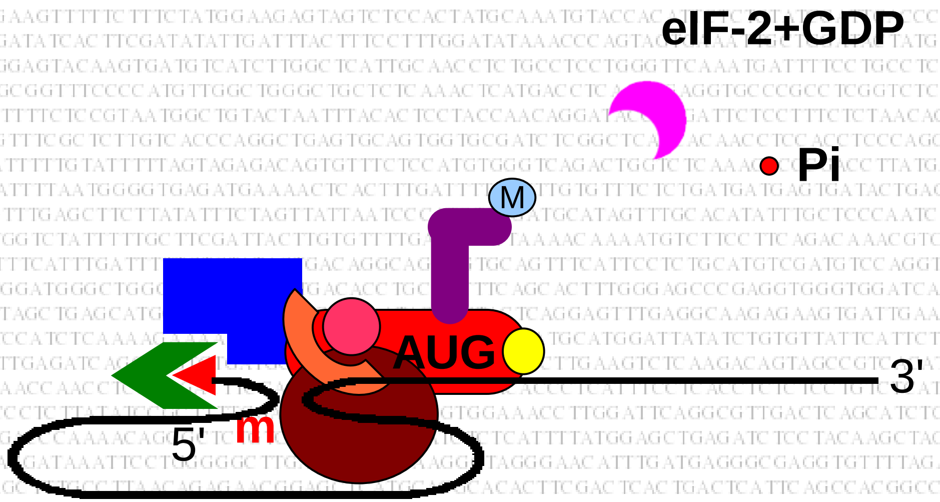


Iniciación

Initiation in eukaryotes almost always uses AUG as the start codon.

Start codon recognition by ternary complex displaces eIF-2 by hydrolizing GTP to produce eIF-2-GDP + Pi.

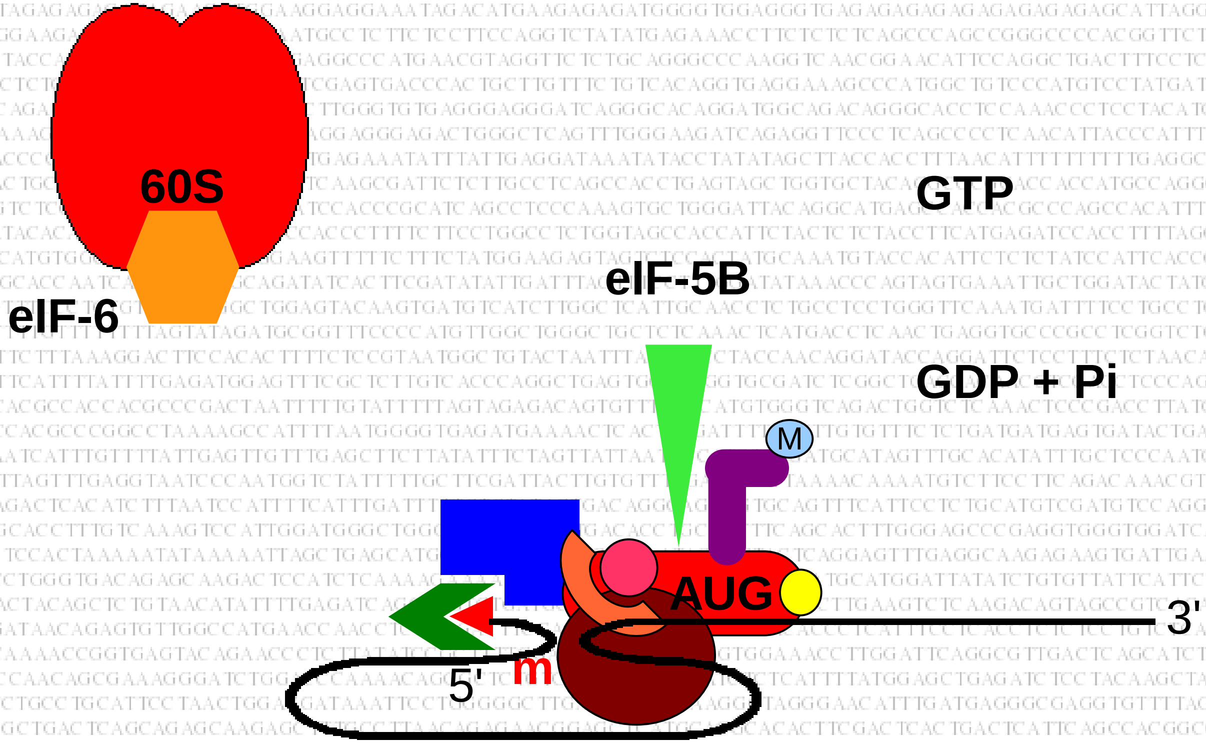
eIF-2 removal necessary for 60S to join 40S.



Iniciación

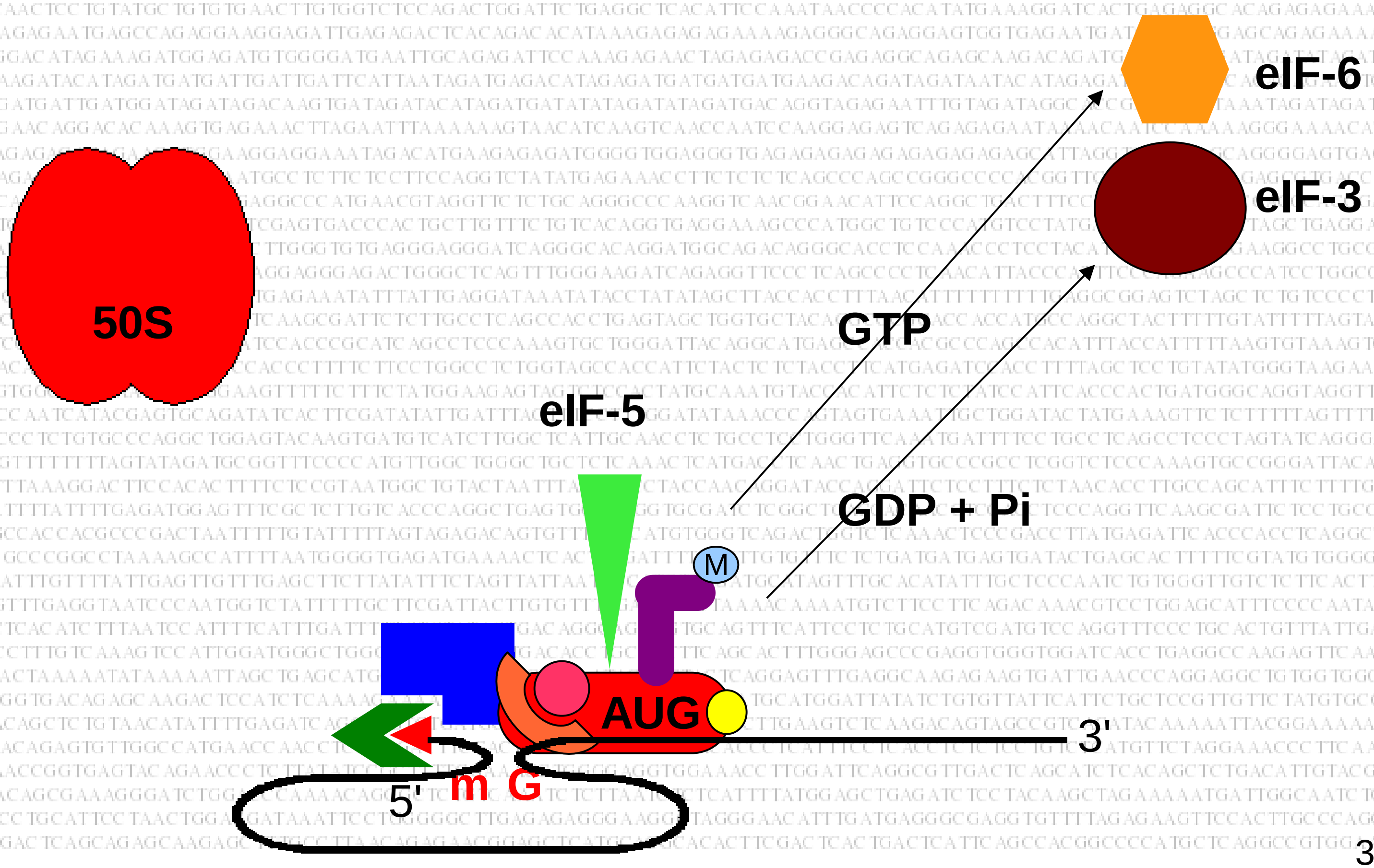
eIF-6 is required to maintain large subunits in their dissociated state.

eIF-2, eIF-3 & eIF-6 are released when the large subunit joins the initiation complex (60S will not load otherwise).



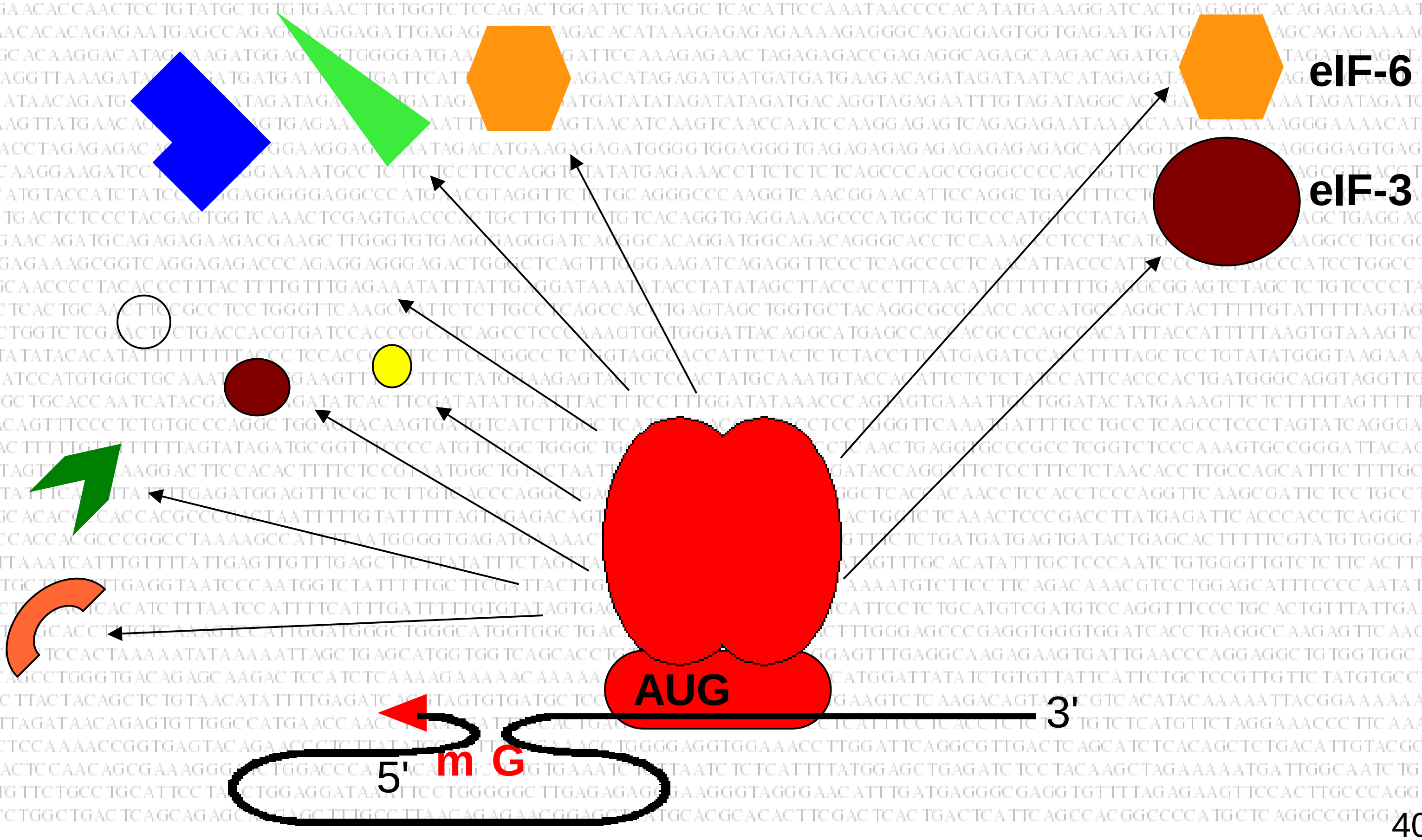
Iniciación

This is mediated by eIF-5, which is a GTPase.



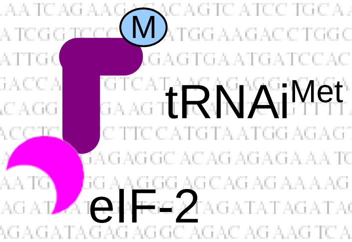
Iniciación

All of the remaining factors are released when the complete 80S ribosome is formed.



tRNA iniciador

The initiator tRNA is a distinct species = $\text{tRNA}_{\text{iMet}}$



		At initiation	At elongation
tRNA species (moiety) employed	Prokaryotes	$\text{tRNA}_f^{\text{Met}}$	$\text{tRNA}_m^{\text{Met}}$
	Eukaryotes	$\text{tRNA}_i^{\text{Met}}$	

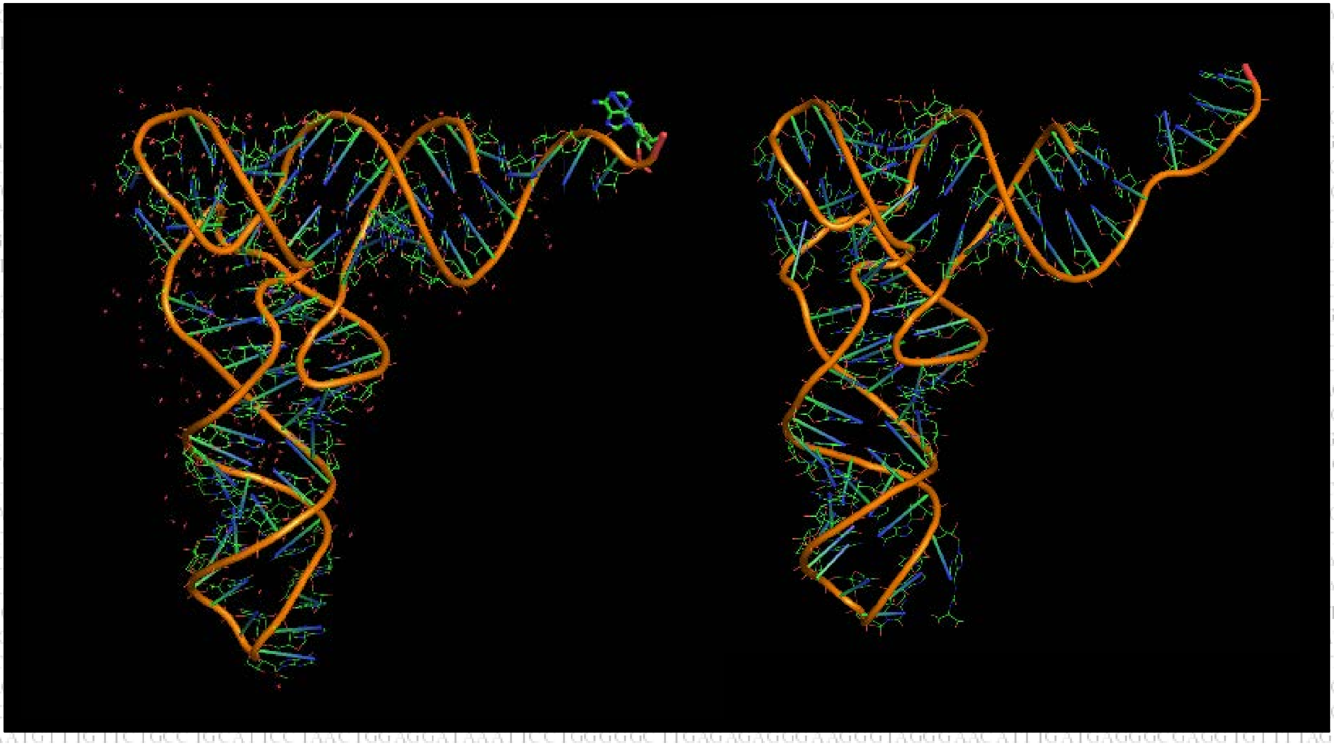
Methionine not formylated.

Difference between the initiating and elongating Met-tRNAs lies solely in the tRNA moiety, with Met-tRNA_i used for initiation and Met-tRNA_m used for elongation.

tRNA iniciador

Two unique features of the initiator $tRNA_i^{Met}$ in yeast:

1.- it has an unusual tertiary structure

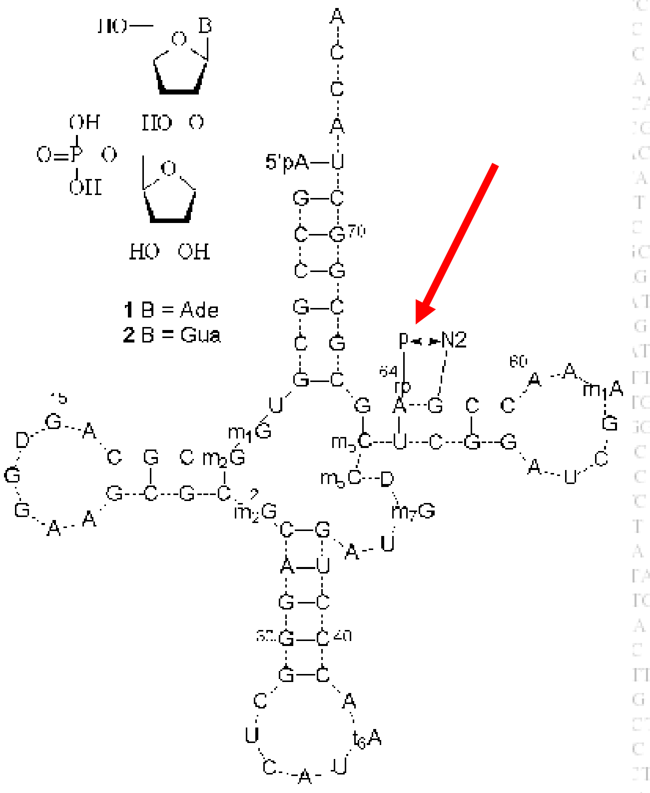


tRNA iniciador

Two unique features of the initiator tRNA$_i^{Met}$ in yeast:

2.- it is modified by phosphorylation
of the 2' ribose position on base 64
(if this modification is prevented, the initiator can be used in elongation).

So the principle of a distinction between initiator and elongator Met-tRNAs is maintained in eukaryotes, but its structural basis is different from that in bacteria.



Iniciación

The presence of the 3' poly-A tail stimulates the formation of an initiation complex at the 5' end.

The poly(A)-binding protein (**PAB1P** in yeast) is required for this effect.

Pab1p binds to **eIF-4G**, which in turn is bound to **eIF4E**.

This implies that the mRNA must (transiently) have a circular organization, with both the 5' and 3' ends held in this complex.

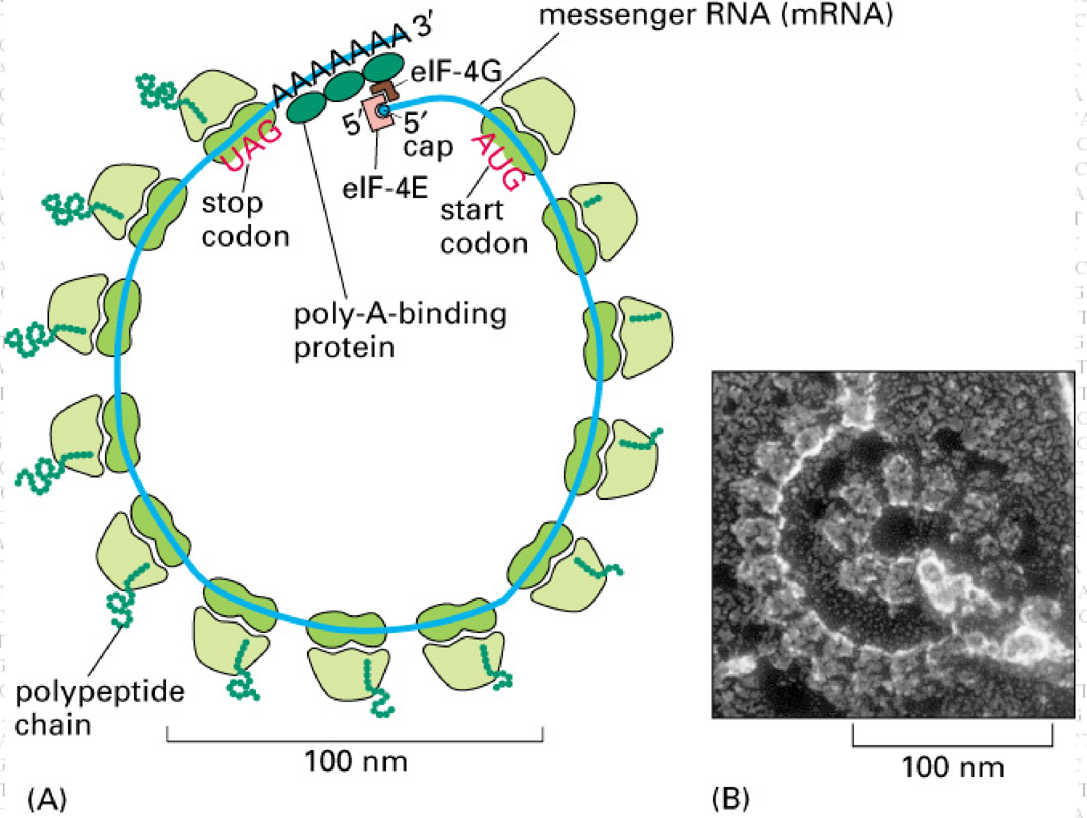


Figure 6–75. Molecular Biology of the Cell, 4th Edition.

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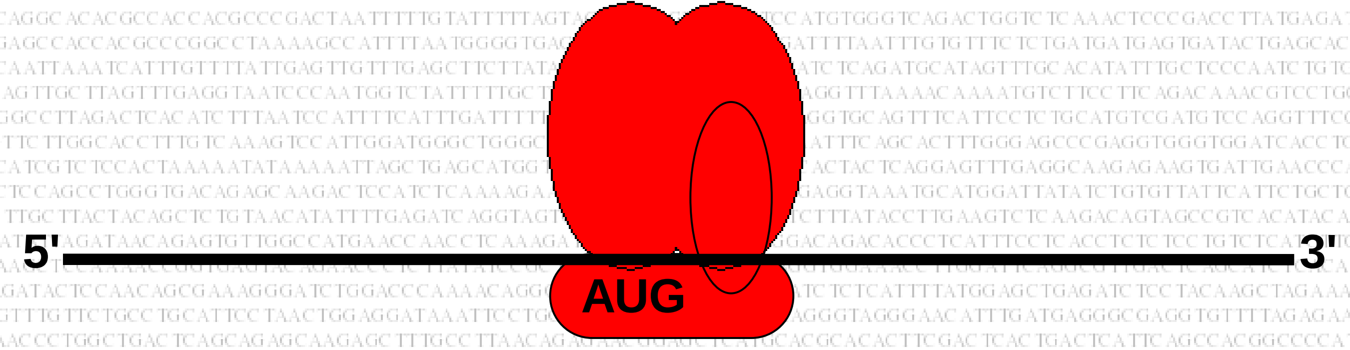
Elongación

Once the complete ribosome is formed at the initiation codon, the stage is set for a cycle in which aminoacyl-tRNA enters the **A site** of a ribosome whose **P site** is occupied by $tRNA_i^{Met}$.

Any aminoacyl-tRNA except the initiator can enter the A site.

Its entry is mediated by an elongation factor (EF-Tu in bacteria eEF-1 or eEF-T in eukaryotes).

The process is similar in eukaryotes & prokaryotes.

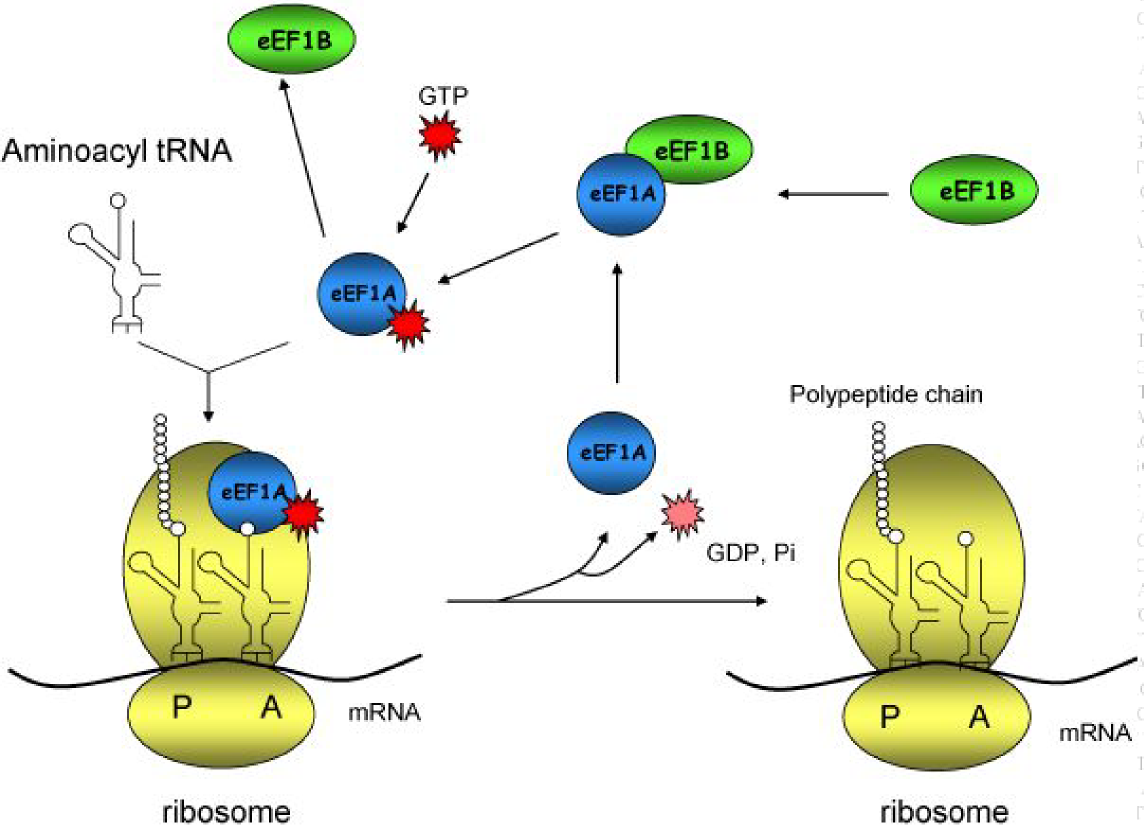


Elongación

In eukaryotes, **eEF-1A** is responsible for bringing aminoacyl-tRNA to the ribosome, in a reaction that involves cleavage of GTP.

It is homologous to its prokaryotic counterpart (**EF-Tu**).

It is regenerated by **eEF-1B**, an **EF-Ts** homologue.



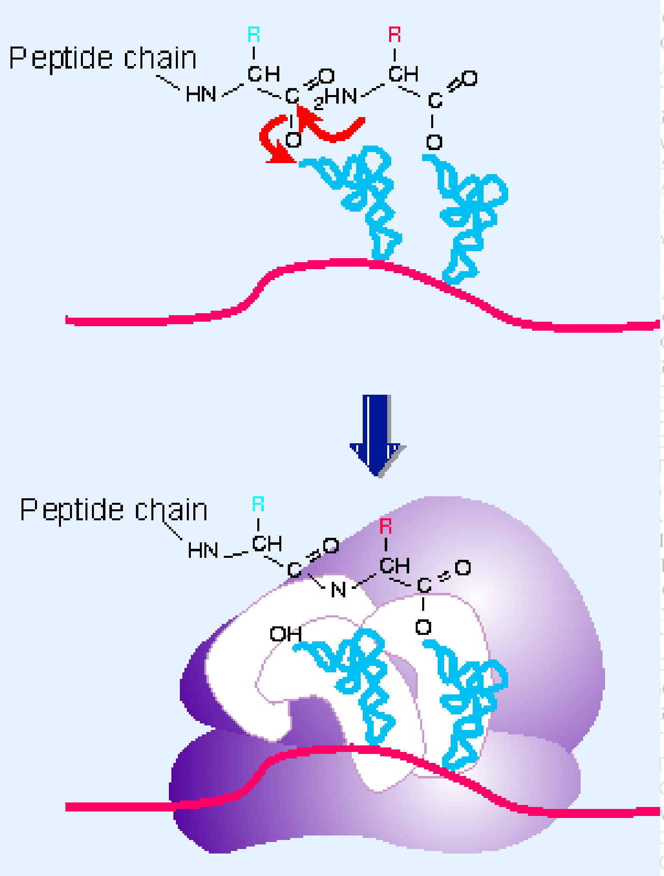
Elongación

The peptidyl transferase reaction involves elongating the polypeptide chain by transferring the polypeptide of the P-site-tRNA to the A-site-tRNA.

Peptidyl transferase is a function of the large (50S or 60S) ribosomal subunit.

The transferase is part of a ribosomal site close to the upper ends of both tRNAs.

Both rRNA and 50S subunit proteins are necessary for this activity.

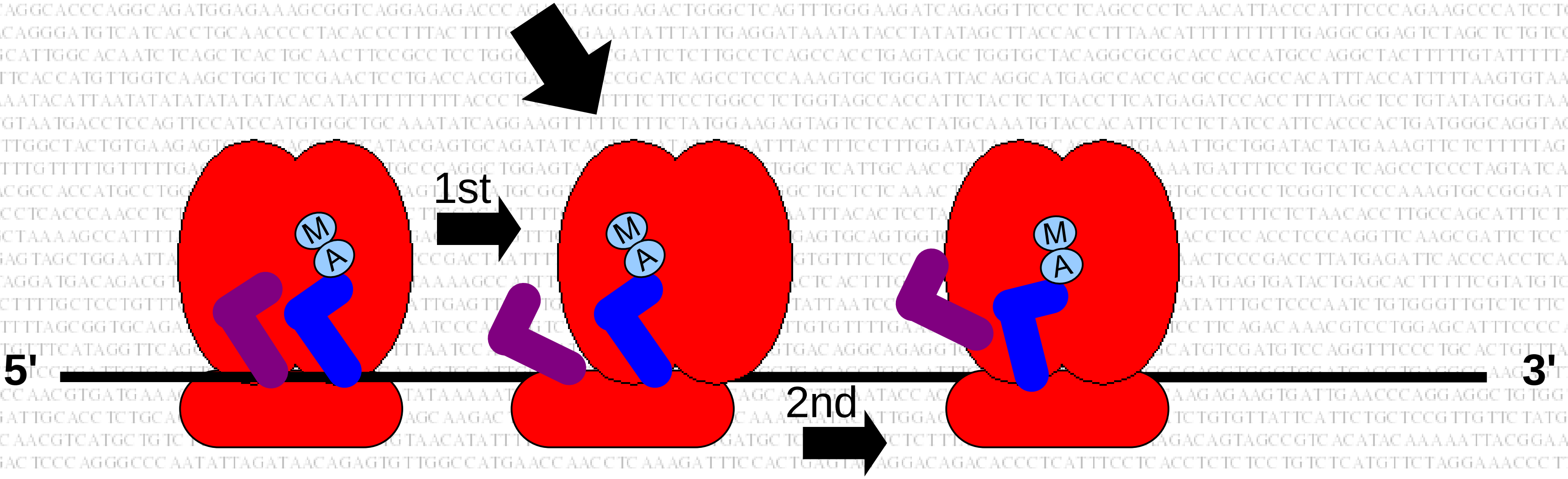


Elongación

After transpeptidation, the ribosome must move (translocate) one codon towards 3'.

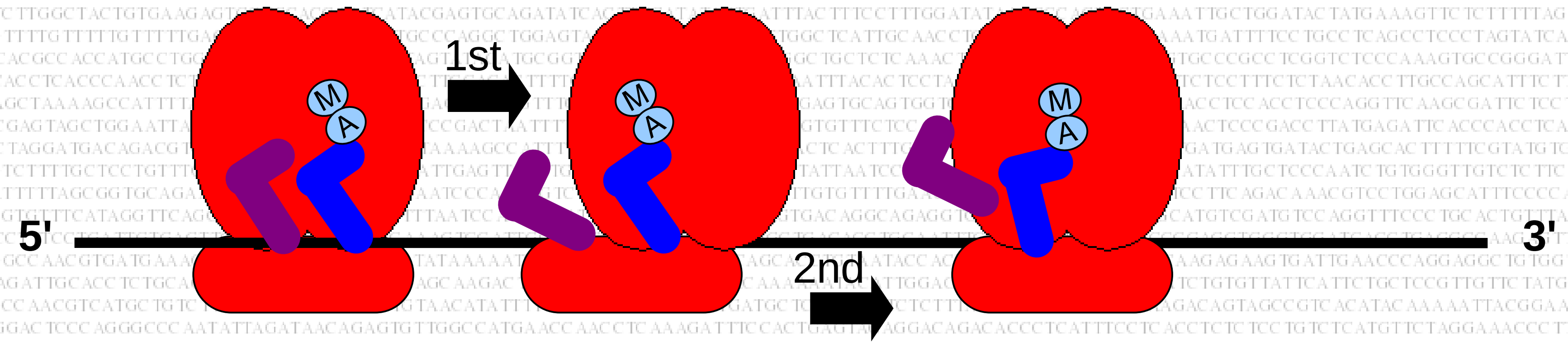
First the aminoacyl ends of the tRNAs (located in the 50S subunit) move into the new sites (while the anticodon ends remain bound to their codons in the 30S subunit).

At this stage, the tRNAs are effectively bound in **hybrid sites,** consisting of the 50S E/ 30S P and the 50S P/ 30S A sites.



Elongación

Then movement is extended to the 30S subunits, so that the anticodon-codon pairing region finds itself in the right site.

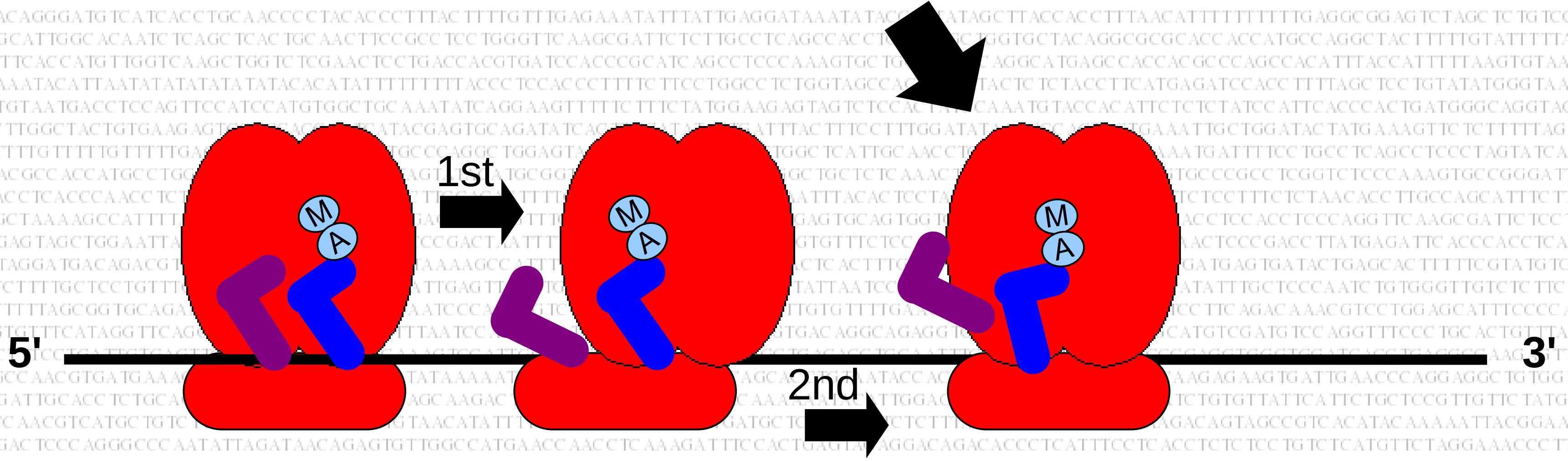


Elongación

Translocation expels the uncharged tRNA from the P site, so that the new peptidyl-tRNA can enter the A site.

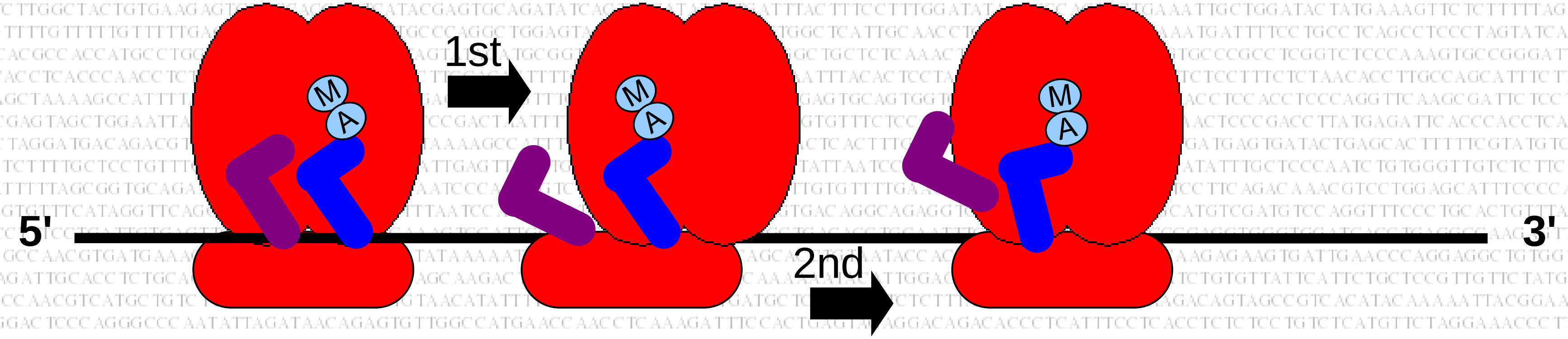
In bacteria the discharged tRNA leaves the ribosome via another site, the **E site**.

In eukaryotes it is **expelled directly** into the cytosol.



Elongación

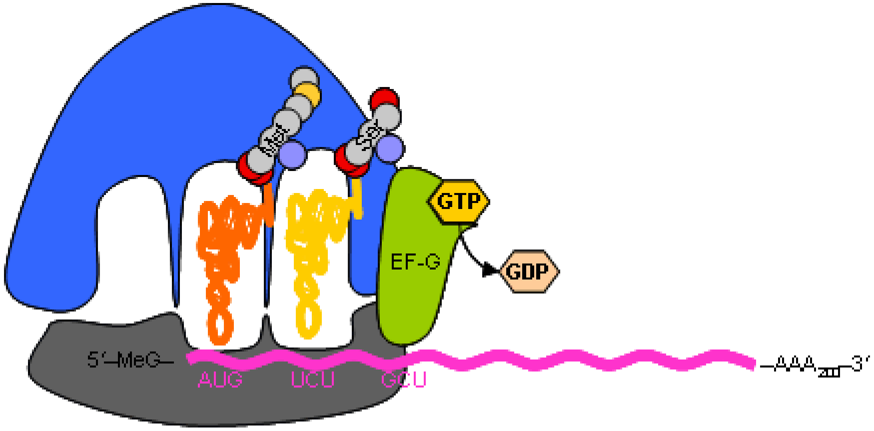
Another model hypothesizes a dissociative movement of subunits



Elongación en procariotas

Translocation requires GTP and another elongation factor, eEF-2.

This factor is a major constituent of the cell; it is present at a level of ~1 copy per ribosome (20,000 molecules per cell).



Ribosomes cannot bind eEF-1A (EF-Tu in prokaryotes) and eEF-2 (EF-G in prokaryotes) simultaneously, it's a two-step process!

Elongación en procariotas

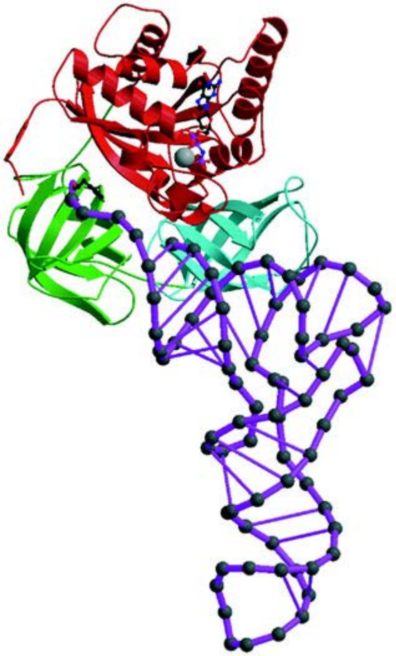
eEF-2 is a protein and not a special kind of tRNA.

Exhibit remarkable similarity to tRNA.

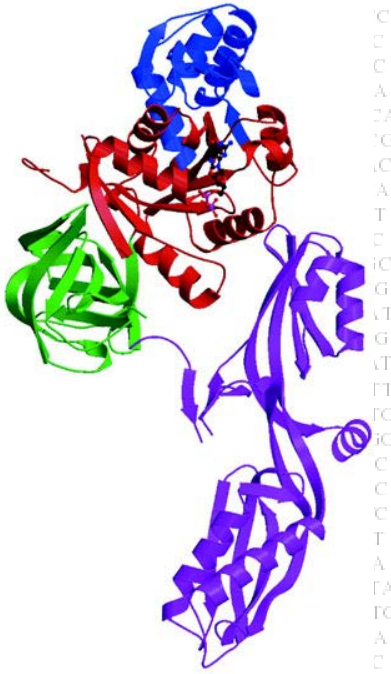
Convergent evolution !

eEF-2 mimics the overall structure of the aa complexed with tRNA in the ternary complex.

This creates the immediate assumption that they compete for the same binding site (presumably in the vicinity of the A site).



ternary complex of aminoacyl-tRNA:EF-Tu:GDP

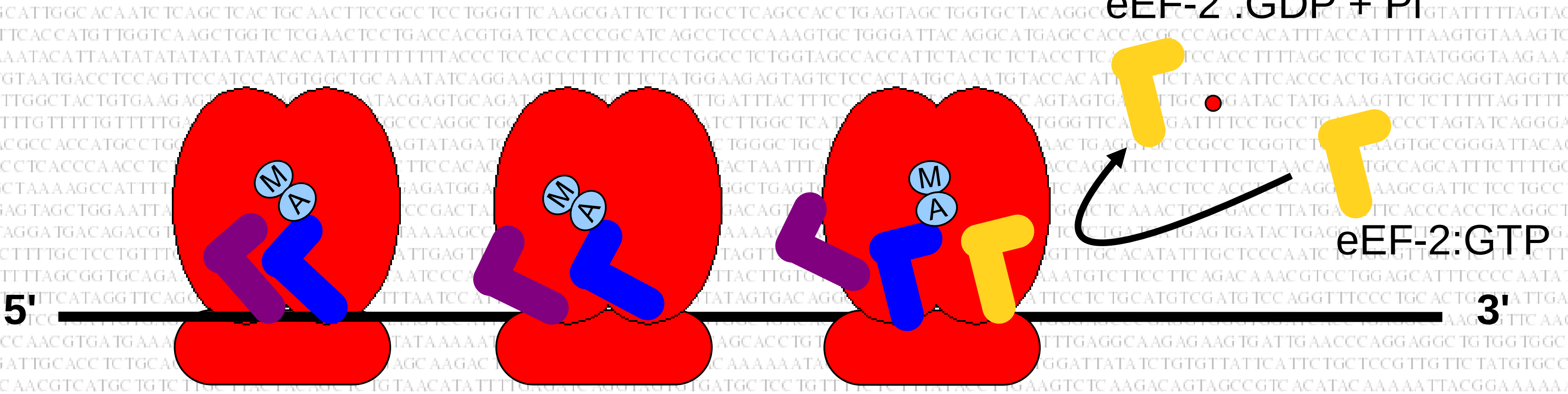


eEF-2.

Elongación en eucariotas

EF-G binds to the ribosome to sponsor translocation; and then is released following ribosome movement.

The hydrolysis of GTP causes a change in the structure of EF-G, which in turn forces a change in the ribosome structure.



Elongación y antibióticos

The steroid antibiotic fusidic acid "jams" the ribosome in its post-translocation state.

Fusidic acid stabilizes the ribosome:eEF-2:GDP complex, so that eEF-2 and GDP remain on the ribosome instead of being released.

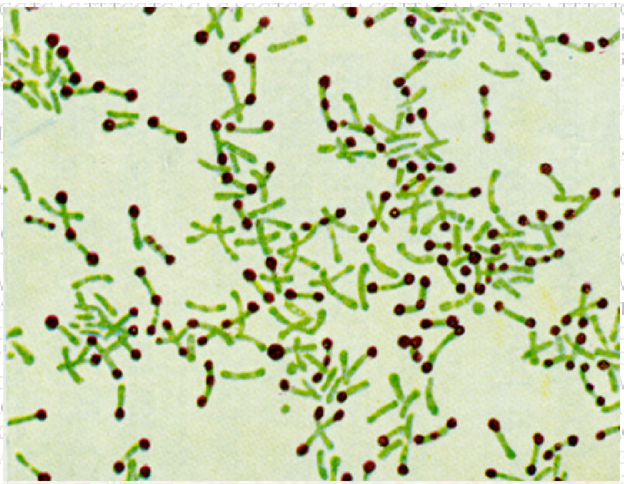
No further amino acids can be added to the chain.

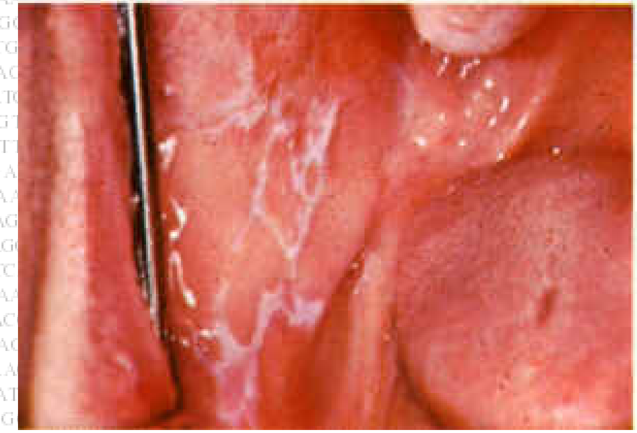
Use as antibiotic!

Contrarily,

eEF-2 is very susceptible to diphtheria toxin.

EF-G (the prokaryote version) is not.





Terminación

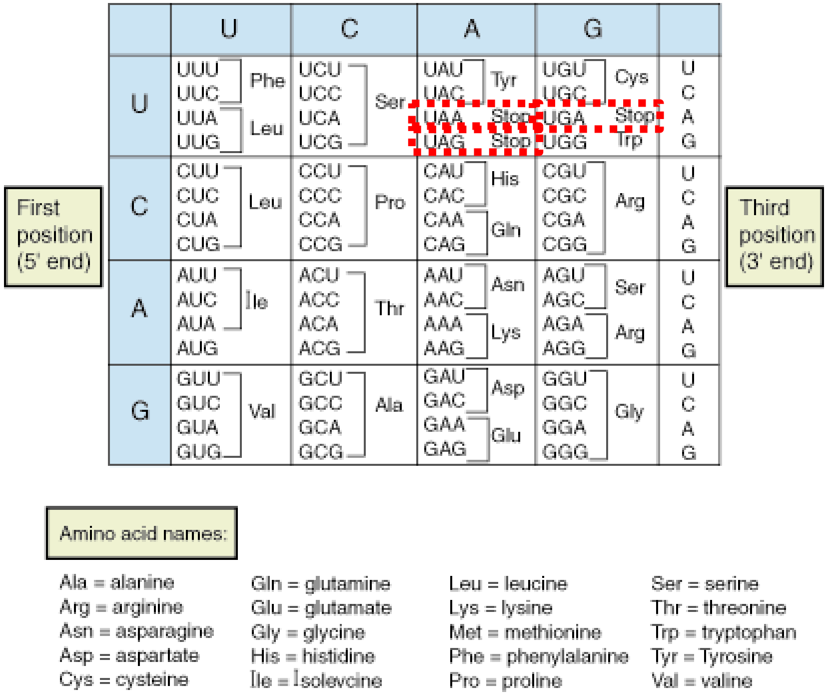
Only 61 triplets are assigned to amino acids.

The other three triplets are termination codons (or stop codons).

UAG = amber codon

UGA = opal codon

UAA = ochre codon



First position (5' end)	U		C		A		G		Third position (3' end)
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG		ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Amino acid names:

Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartate
Cys = cysteine
Gln = glutamine
Glu = glutamate
Gly = glycine
His = histidine
Ile = Isoleucine
Leu = leucine
Lys = lysine
Met = methionine
Phe = phenylalanine
Pro = proline
Ser = serine
Thr = threonine
Trp = tryptophan
Tyr = Tyrosine
Val = valine

In bacteria UAA is the most commonly used termination codon in eukaryotes its UAG.

There appear to be more errors reading UGA, which result in the continuation of protein synthesis until another termination codon is encountered.

Terminación

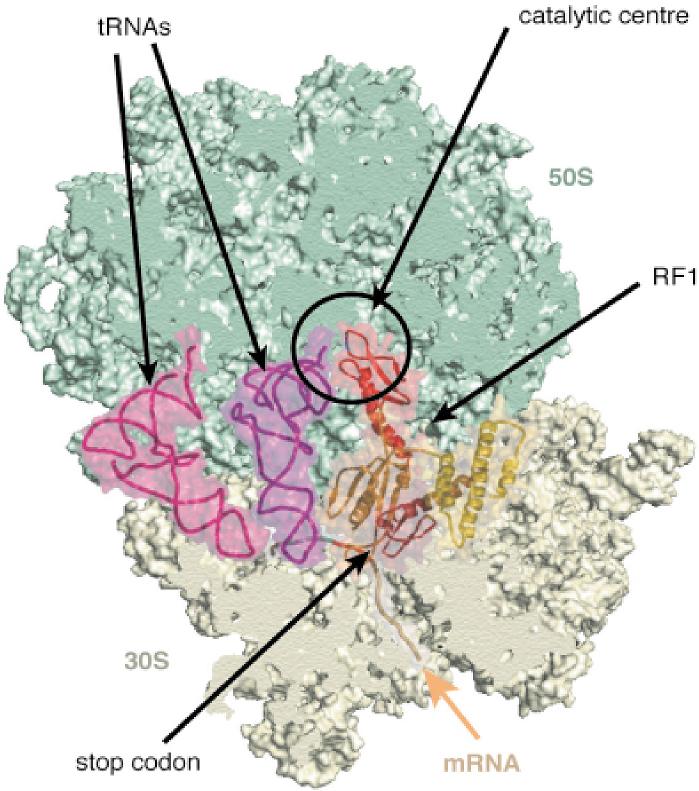
In *E. coli* two related proteins catalyze termination.

They are called release factors (RF), and are specific for different sequences.

RF-1 recognizes UAA and UAG.

RF-2 recognizes UGA and UAA.

The factors act at the ribosomal A site and require polypeptidyl-tRNA in the P site.



The release factors are present at much lower levels than initiation or elongation factors;~600 molecules of each per cell (1 RF per 10 ribosomes).

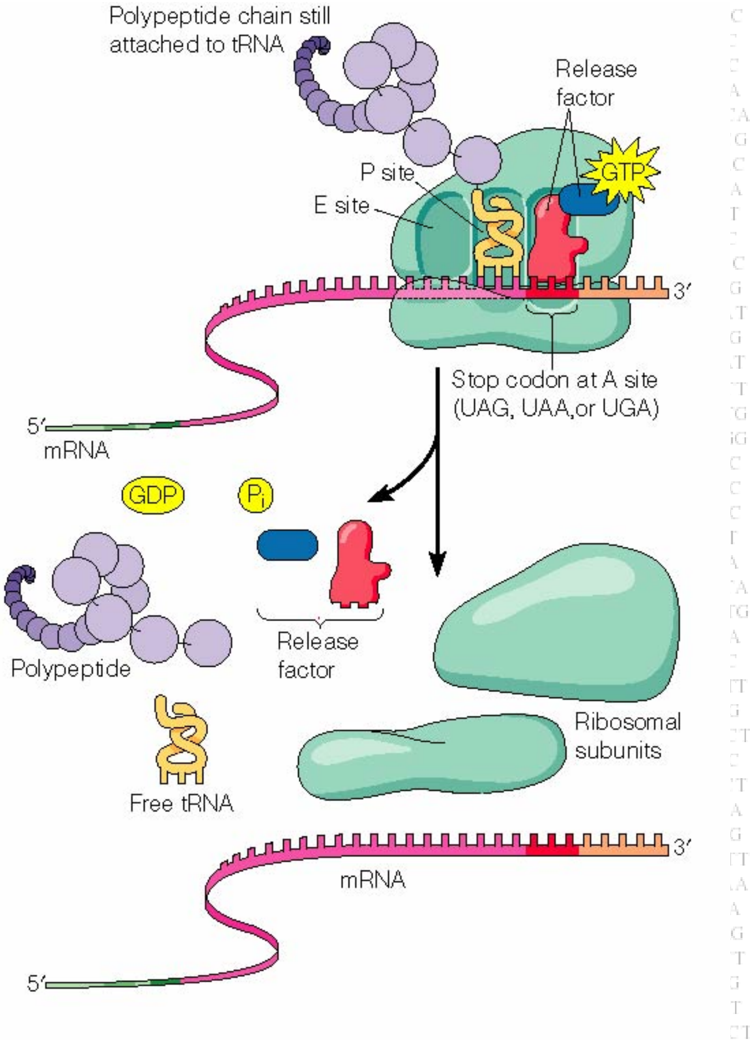
Terminación

RF1 and RF2 recognize the termination codons and activate the ribosome to hydrolyze the peptidyl tRNA.

Reaction analogous to the usual peptidyl transfer, except that the acceptor is H_2O instead of aminoacyl-tRNA.

RF1 or RF2 are released from the ribosome by RF-3, which is a GTP-binding protein related to EF-G.

RF3 resembles the GTP-binding domains of EF-Tu and EF-G, and RF1/2 resemble the C-terminal of EF-G, which mimics tRNA.



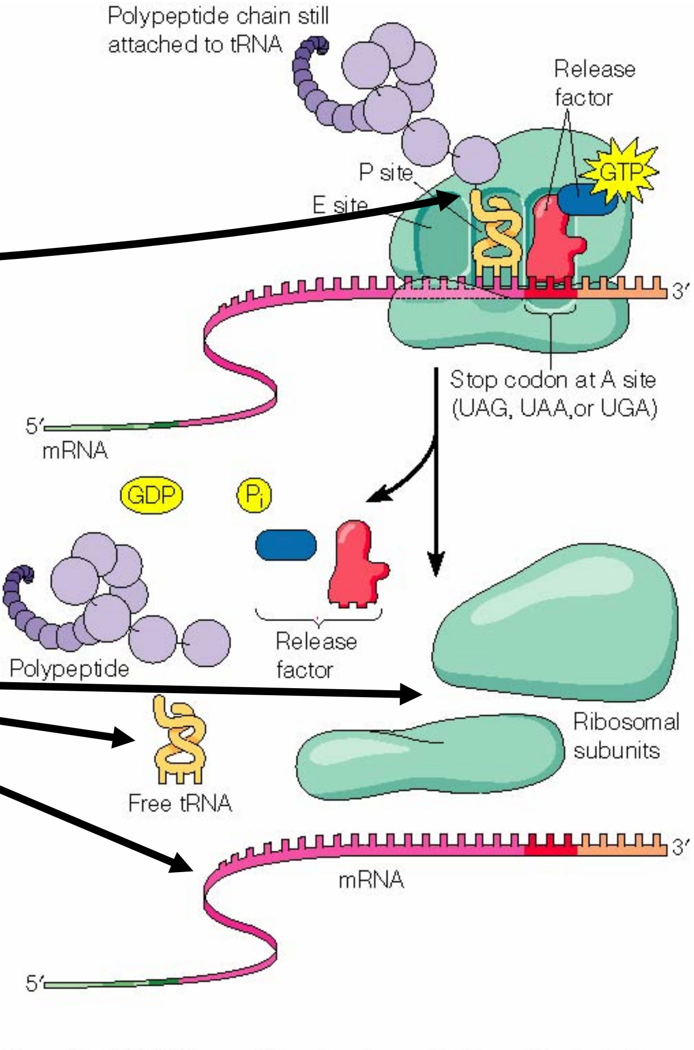
Terminación

Two stages are involved in ending translation.

The *termination reaction* itself involves release of the protein chain from the last tRNA.

The *post-termination reaction* involves release of the tRNA and mRNA, and dissociation of the ribosome into its subunits.

None of the termination codons is represented by a tRNA, they are recognized directly by protein factors!



PKR: dsRNA and stress sensor

Stress signals induced by viral infection arrest translation of both cellular and viral mRNAs.

In order to inhibit viral replication.

Host PKR activated by dsRNA, dimerizes, autophosphorylates and phosphorylates eIF2-α translation initiation factor.

eIF2 phosphorylation arrests translation.

Viruses have evolved different strategies to avoid complete arrest of translation.

1.- Directly modulating PKR activity

2.- Inhibiting phosphorylation of eIF2-α