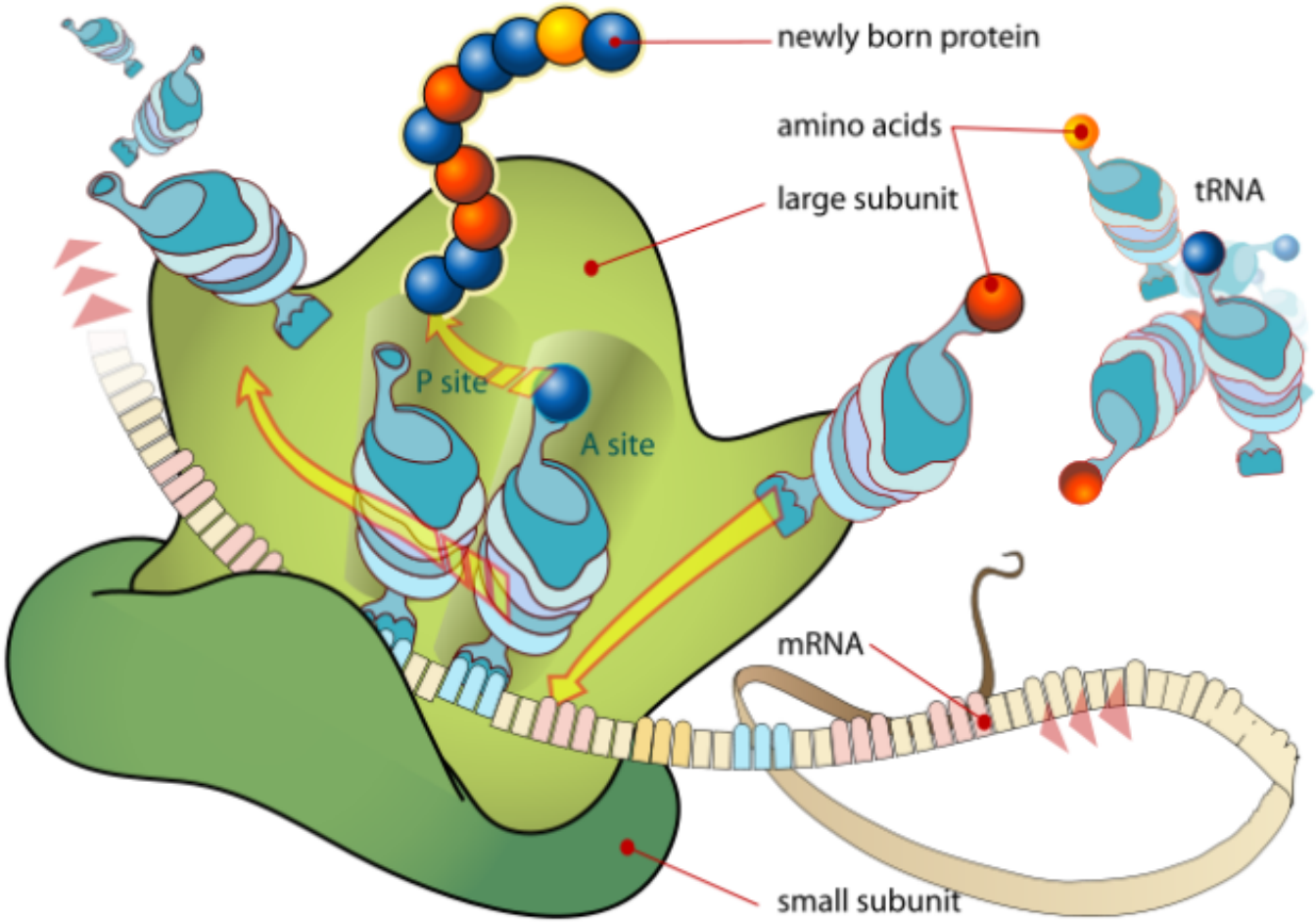
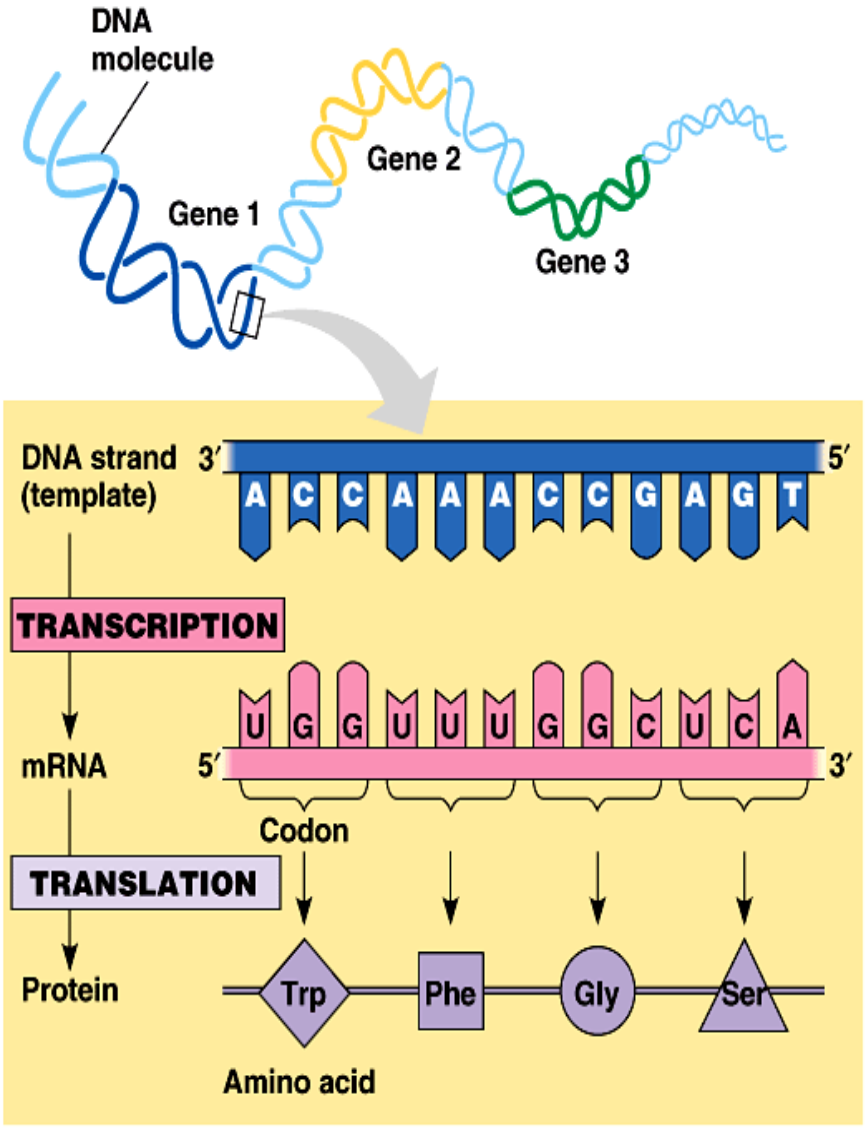
Translation



Watson et al. MOLECULAR BIOLOGY OF THE GENE (BIOLOGIA MOLECOLARE DEL GENE. Zanichelli)

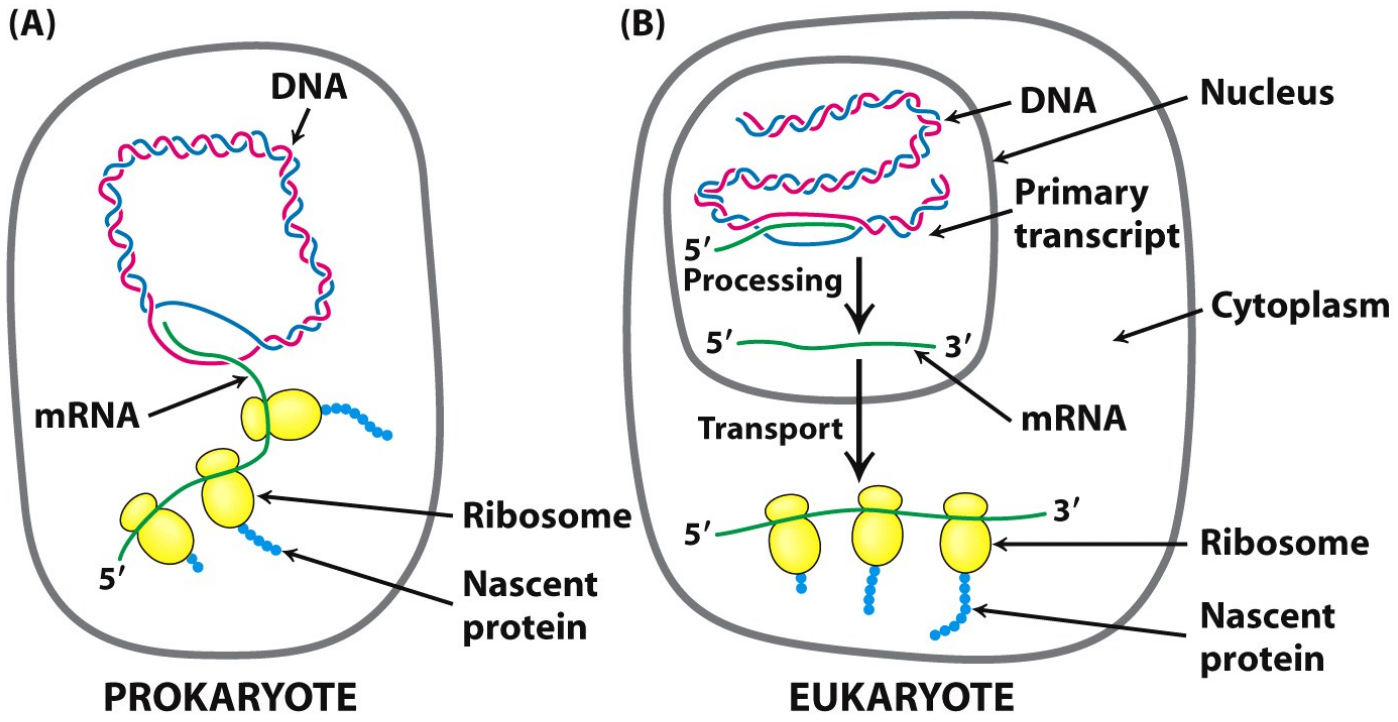
Alessandro Fatica (alessandro.fatica@uniroma1.it)

Flow of information through the cell



Francis Crick 1956

Translation: generates the linear sequences of amino acids in proteins from the genetic information contained within the order of nucleotides in messenger RNA (mRNA)



20 aa/sec

2-5 aa/sec

The machinery responsible for translating is composed of four primary components

1. messenger RNA (mRNA)
2. transfer RNA (tRNA)
3. amminacyl-tRNA synthetases
4. ribosome

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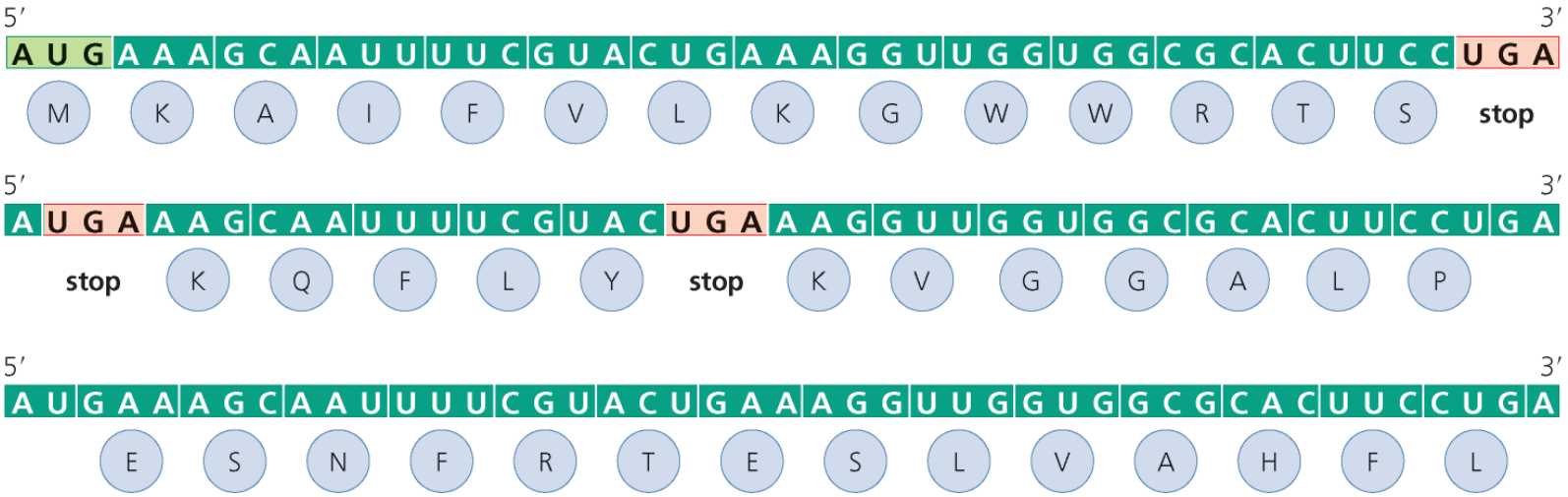
1- messenger RNA (mRNA)

- The information for protein synthesis is in the form of three-nucleotide codons, which each specify one amino acid.
- The protein coding-region(s) of each mRNA is composed of contiguous, non-overlapping strings of codons called **O**pen **R**eading **F**rame (ORF).
- The first and last codons of an ORF are known as the **start** and the **stop codons**



	START	STOP
Proc.	AUG, GUG, UUG	UAG, UGA, AGA
Euc.	AUG	UAG, UGA, AGA

Messenger RNA (mRNA)

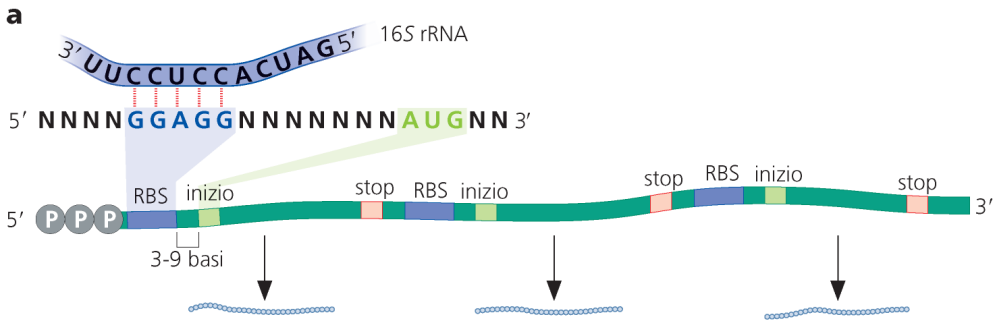
•Eukaryotic mRNAs contain a single ORF (**monocistronic**). In contrast, prokaryotic mRNAs frequently contain two or more ORFs (**polycistronic**).

•Many prokaryotic ORFs contain the ribosome binding site (**RBS** or **Shine-Dalgarno sequence**), which facilitate binding by a ribosome.

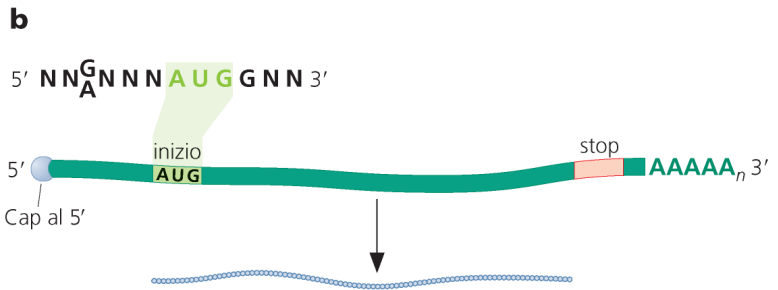
•Eukaryotic mRNAs recruit ribosome using a specific chemical modification called the **5'-cap**, which is located at the extreme 5' end of the message. Then, the ribosome moves (**scanning**) until it encounters a start codon (AUG).

•In eukaryotic mRNAs, translation is stimulated by the presence of a poly-A tail at the extreme 3'-end of mRNA.

Prokaryotic mRNA



Eukaryotic mRNA



The machinery responsible for translating is composed of four primary components

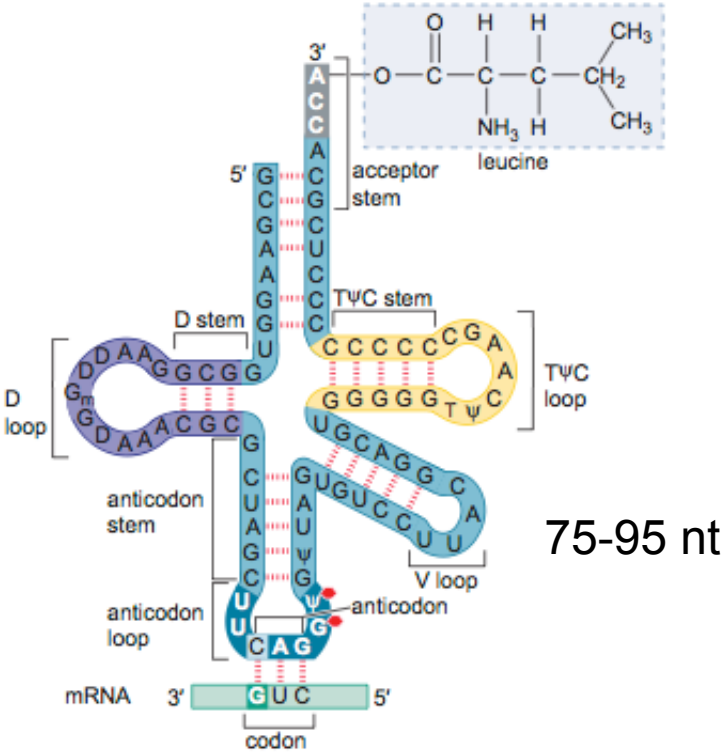
1. messenger RNA (mRNA)
2. transfer RNA (tRNA)
3. amminacyl-tRNA synthetases
4. ribosome

2- transfer RNA (tRNA)

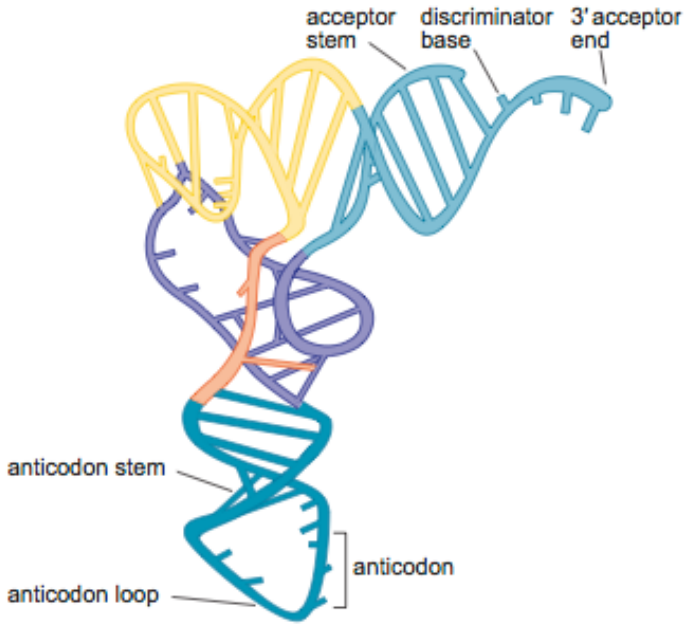
•tRNAs act as adaptors between codons and the amino acids (aa) they specify.

•There are many types of tRNA molecules, but each <u>is attached to a specific aa</u> and each recognizes a particular codon, or codons, in the mRNA.

•The hydrolysis of the high-energy acyl linkage between the aa and tRNA helps drive the formation of the peptide bonds that links aa to each others in proteins.



75-95 nt



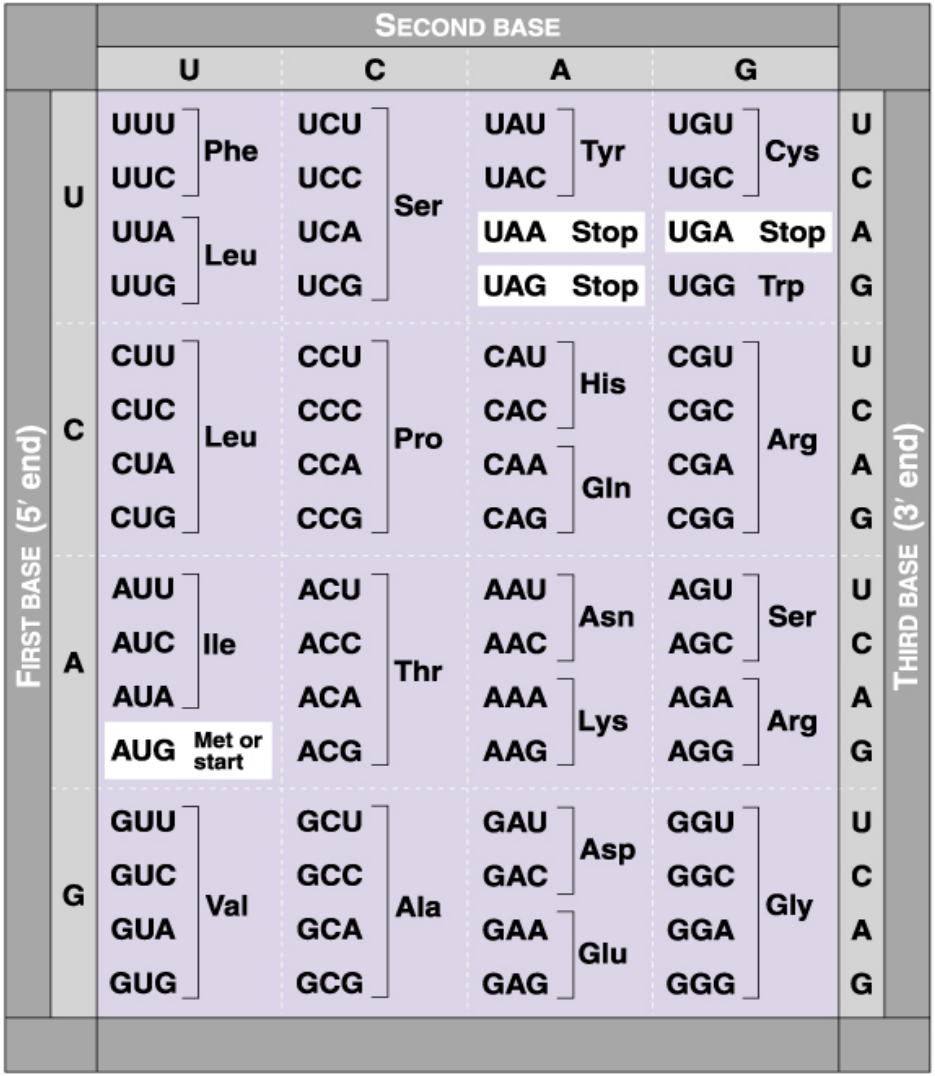
The Genetic Code

- Proteins are composed by the combination of **20 amino acids**
- DNA and RNA are composed by the combination of **4 nucleotides**
- How can 4 bases-nucleic acids code for 20 aa-proteins?

  - 1 base code, 4^1 = 1 aa, it can code for 4 aa.
  - 2 bases cpde, 4^2= 1 aa, it can code for 16 aa.
  - 3 bases (Codon) code, 4^3= 1 aa, it can code for 64 aa
  - The genetic code is redundant: **each amino acid is encoded by more than one codon**

The genetic code is universal, any changes would be lethal, and is one of the most valid evidence for the origin of life from a single organism.

The Genetic Code



The machinery responsible for translating is composed of four primary components

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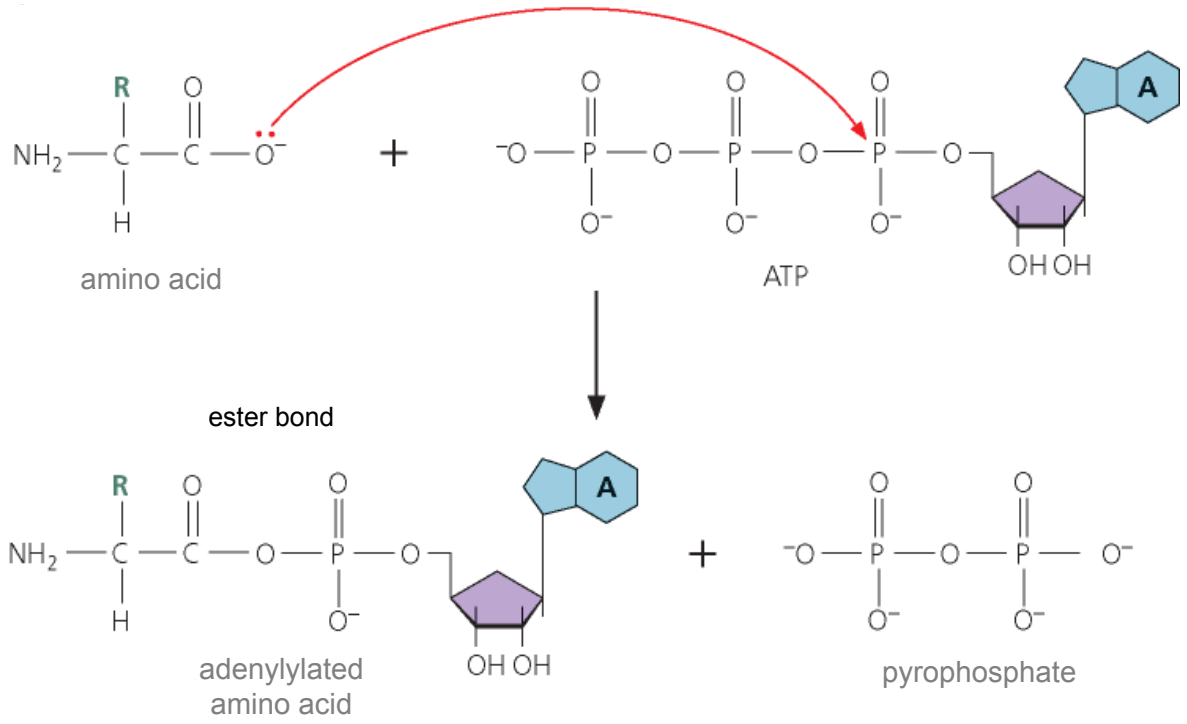
3- Amminacyl-tRNA synthetases

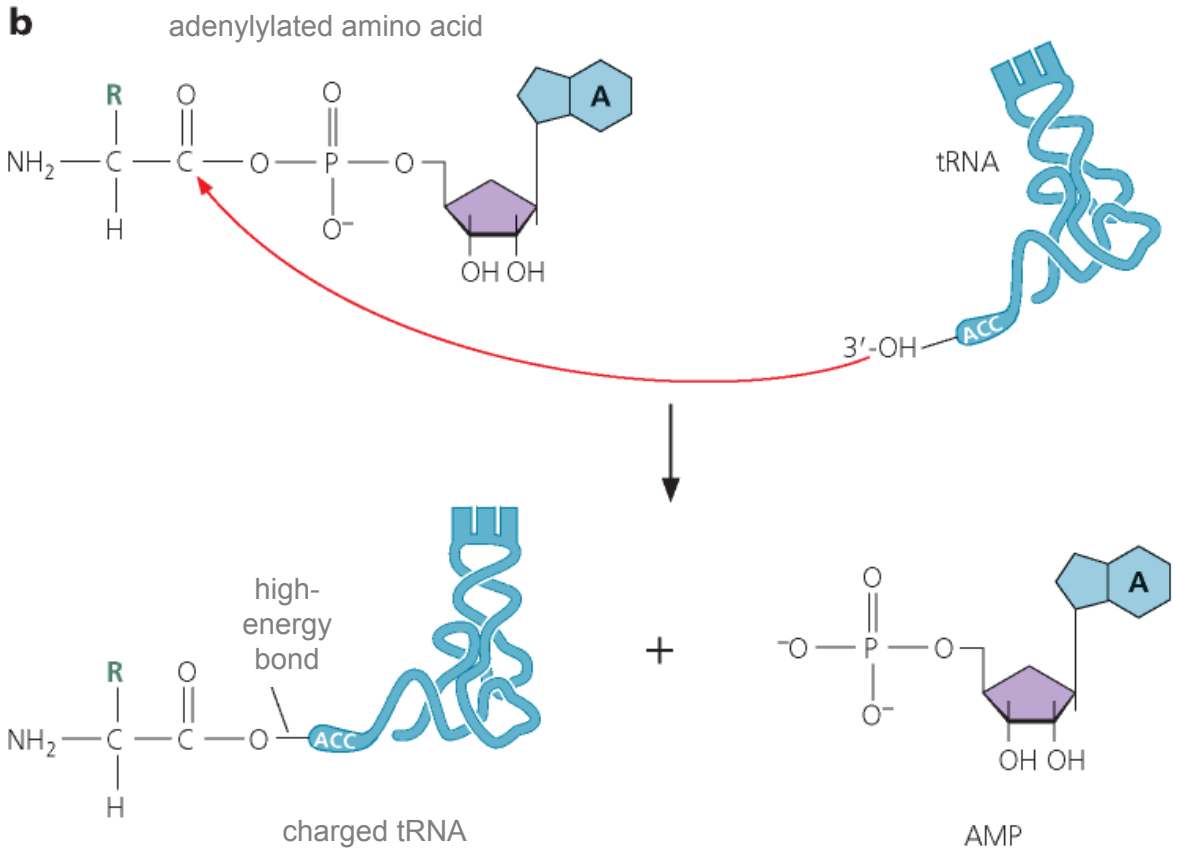
All amminacyl-tRNA synthetases attach an amino acid to a tRNA in 2 enzymatic steps;

1- Adenylylation: in which the aa reacts with ATP to became adenylylated with the concomitant release of pyrophosphate



3- Amminacyl-tRNA synthetases

2- tRNA charging: in which the adenylylated amino acid, which remains bond to the synthetases, reacts with tRNA.

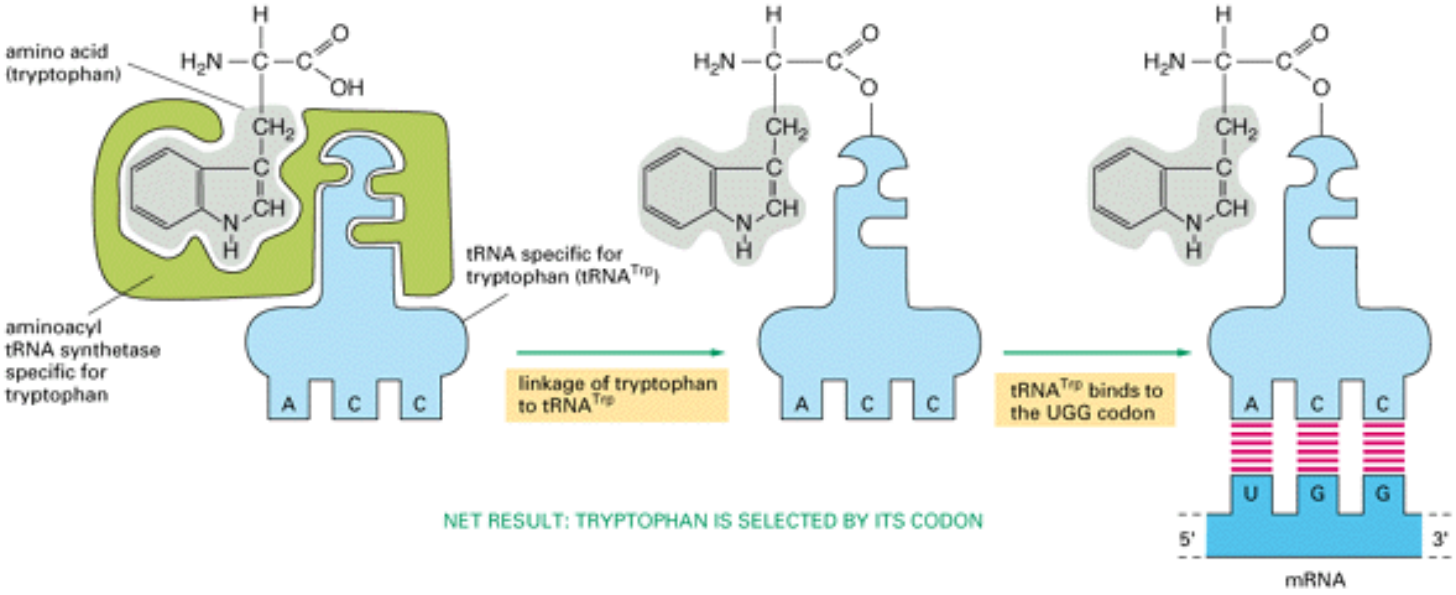


3- Amminacyl-tRNA synthetases

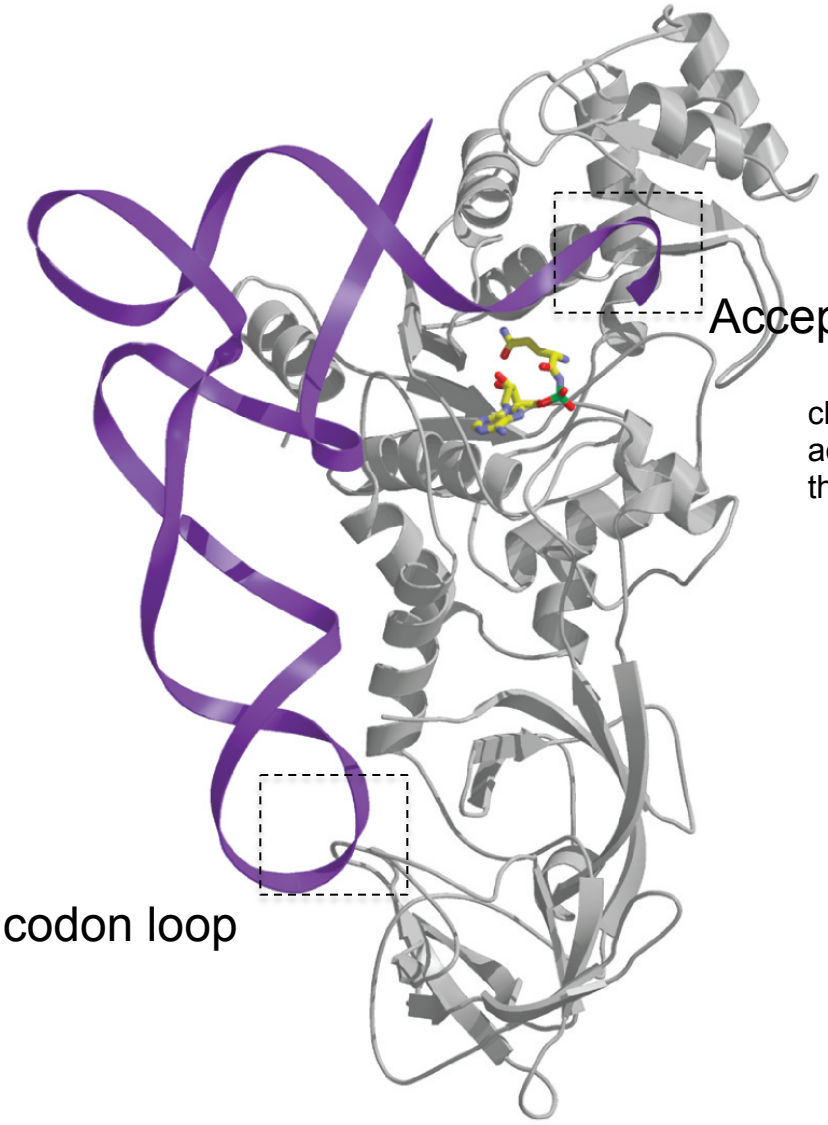
•Most organisms have 20 different aminoacyl-tRNA synthetases, one for each amino acid, and are highly specific.

•They must recognize the correct set of tRNA for a particular aa, and they must charge all of these isoaccepting tRNAs with the correct aa. Bot processes must be carried out with high fidelity.

•The **acceptor stem** is an especially important determinant for the specificity of tRNA synthetases recognition. The **anticodon loop** frequently contributes to discrimination as well.



3- Amminacyl-tRNA synthetases

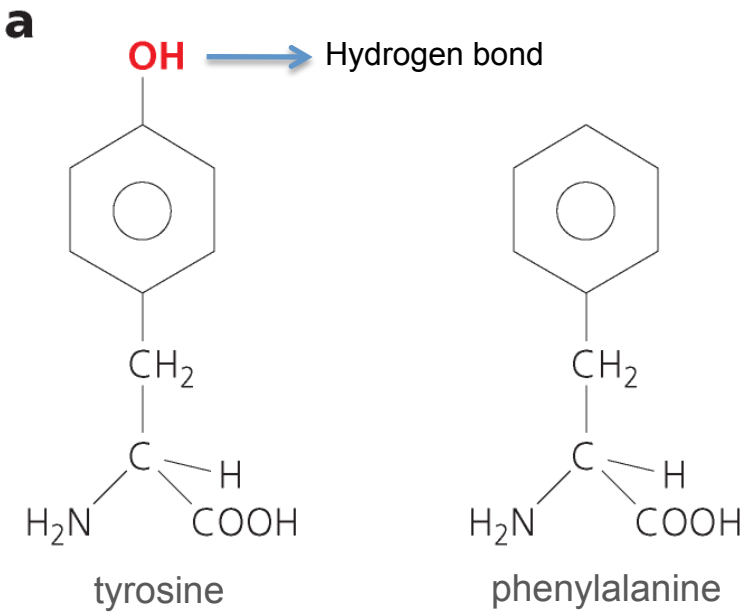


changing a single case pair in the acceptor stem is sufficient to convert the recognition specificity

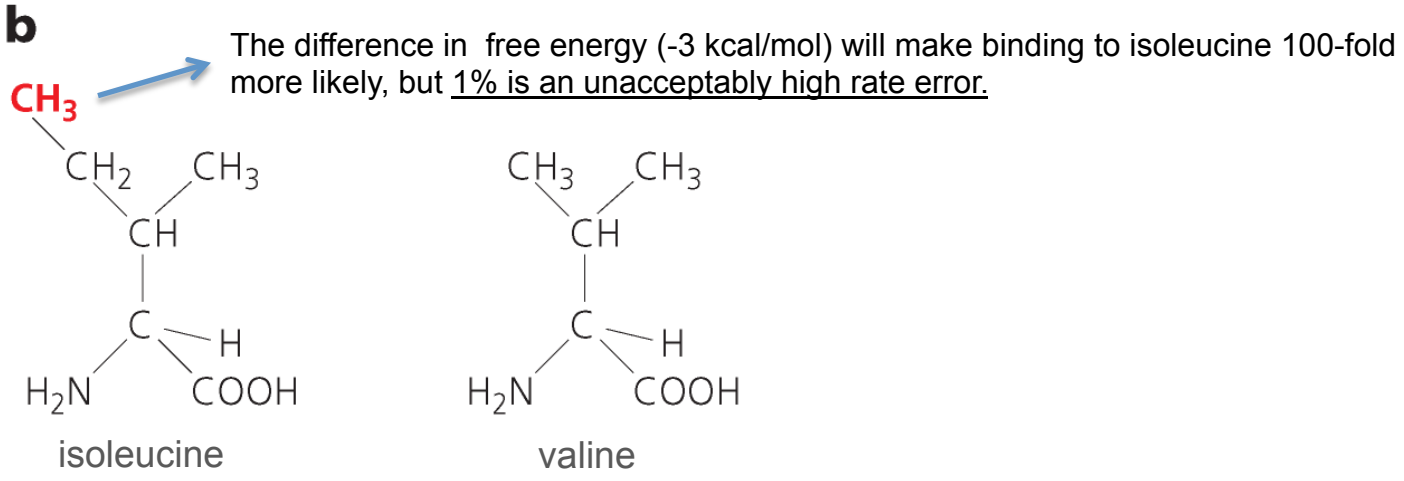
3- Amminacyl-tRNA synthetases

Distinguishing features of similar amino acids:

Easy

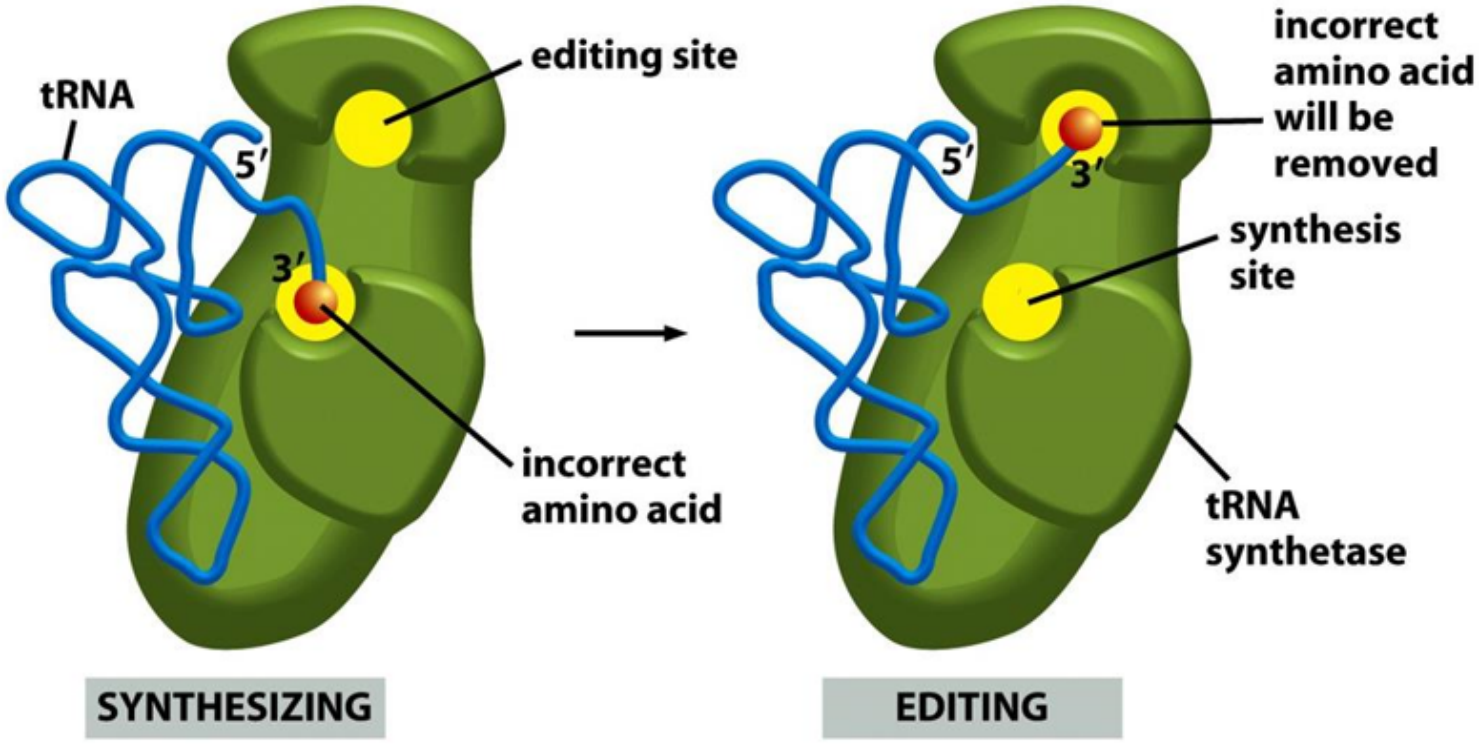


Difficult

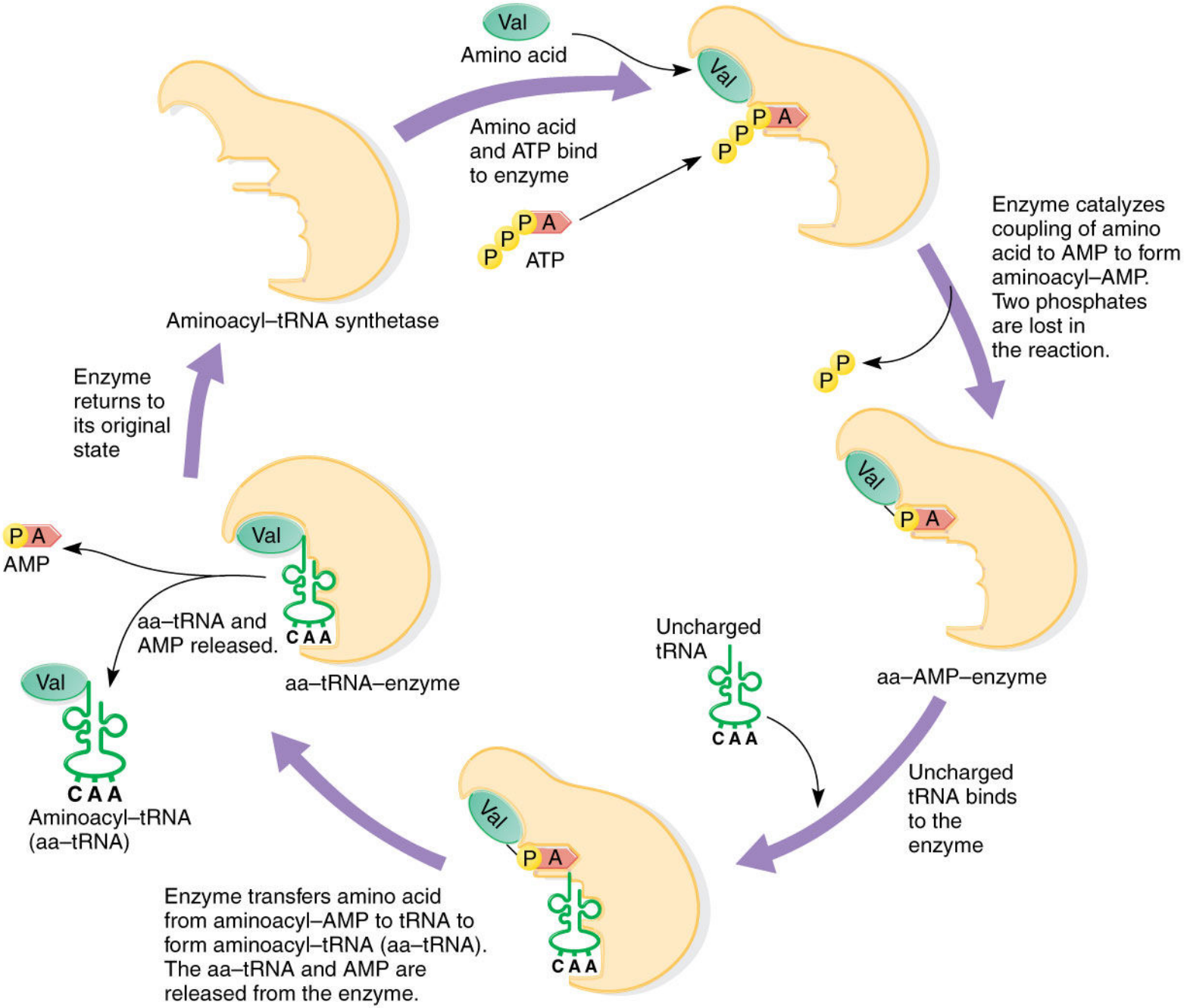


3- Amminacyl-tRNA synthetases

•It use an **editing pocket** to increase the fidelity of tRNA charging (<u>error rate < 0,1%</u>) that allows it to proofread the product of the adenylylation reaction.



<u>The ribosome is unable to discriminate between correctly and incorrectly charged tRNAs</u>

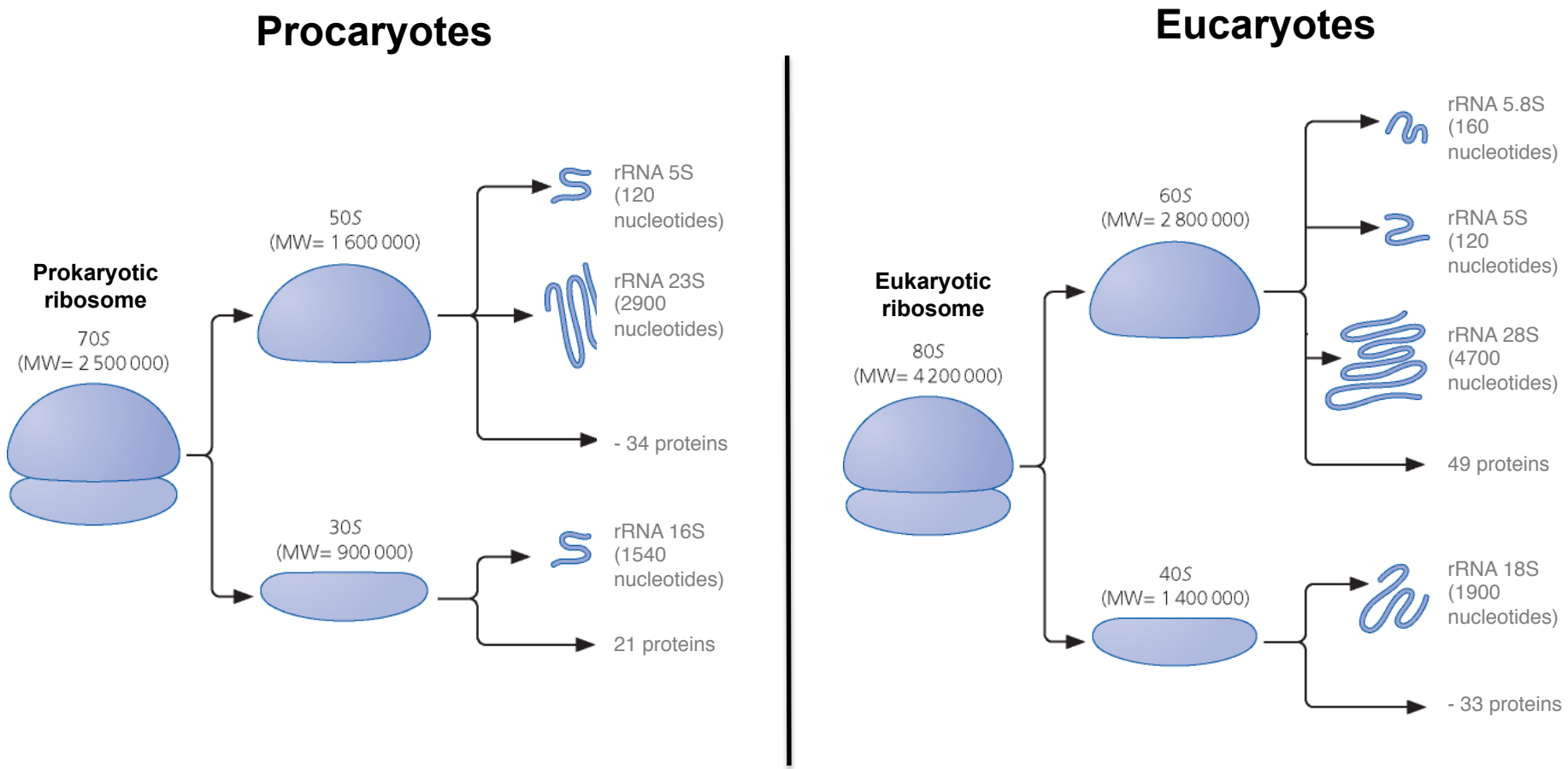


The machinery responsible for translating is composed of four primary components

1. messenger RNA (mRNA)
2. transfer RNA (tRNA)
3. amminacyl-tRNA synthetases
4. ribosome

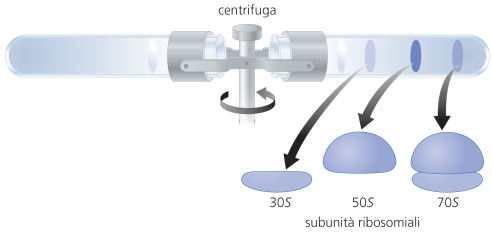
4- Ribosome

The ribosome is the macromolecular machine that directs the synthesis of proteins and is composed of a large and a small subunit. By convention ribosomal subunits are named according to the velocity of their sedimentation when subjected to a centrifugal force (S = Svedberg unit).



Ribosomal RNAs (rRNAs) are 2/3 of the mass of the ribosome

Svedberg Unit



The sedimentation coefficient **s** of a particle is used to characterize its behaviour in sedimentation processes, notably centrifugation. It is defined as the ratio of a particle's sedimentation velocity to the acceleration that is applied to it. The sedimentation speed υ_t (in ms^{-1}) is also known as the terminal velocity. It is constant because the force applied to a particle by gravity or by a centrifuge (measuring typically in multiples of tens of thousands of gravities in an ultracentrifuge) is cancelled by the **viscous resistance** of the medium through which the particle is moving. The applied acceleration **a** (in ms^{-2}) is the centrifugal acceleration ωr^2, ω is the angular velocity of the rotor and **r** is the distance of a particle to the rotor axis (radius).
The viscous resistance is given by the **Stokes' law: $6\pi\eta r_0 v$** where **η** is the viscosity of the medium, $\mathbf{r_0}$ is the radius of the particle and **v** is the velocity of the particle.

$$s = \frac{v_t}{a} \qquad v_t = \frac{mr\omega^2}{6\pi\eta r_0} \longrightarrow s = \frac{v_t}{r\omega^2} = \frac{m}{6\pi\eta r_0}$$

For most of the biological particles sedimentation coefficients are very small values and, by convention, their unit value is:

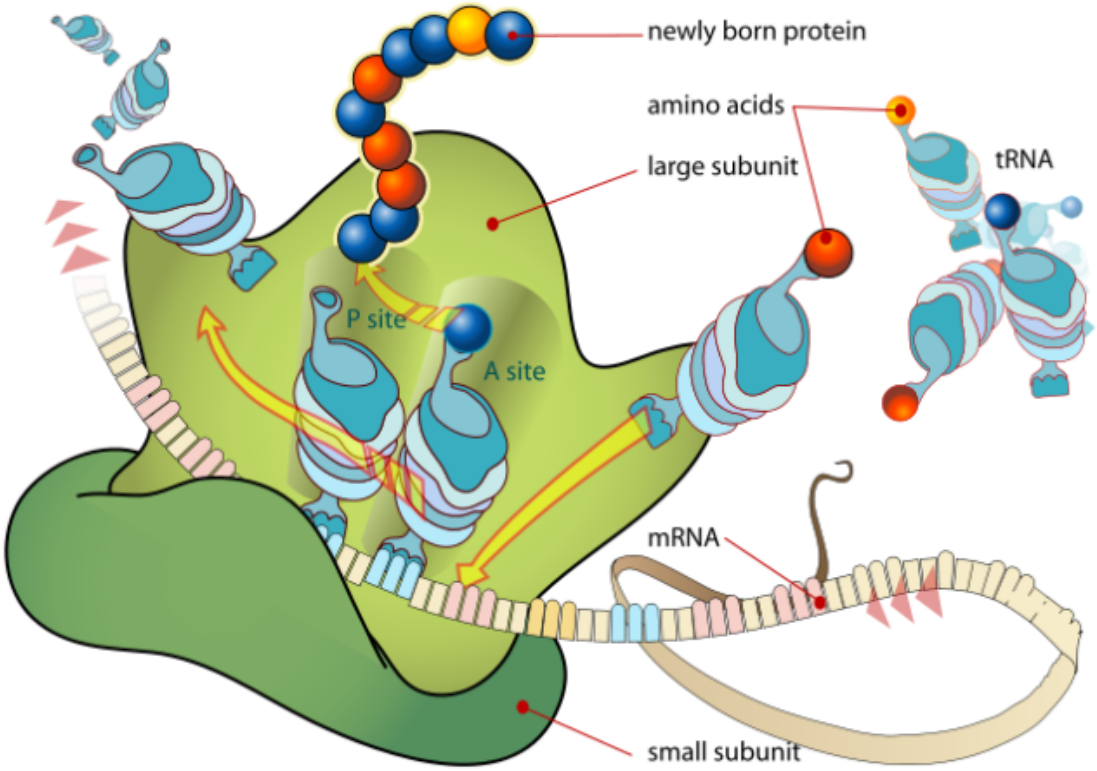
10^{-13} s = ***Svedberg unit (S)***

For example, a molecule of rRNA having a sedimentation coefficient equal to $5x10^{-13}$ s has a value of 5S

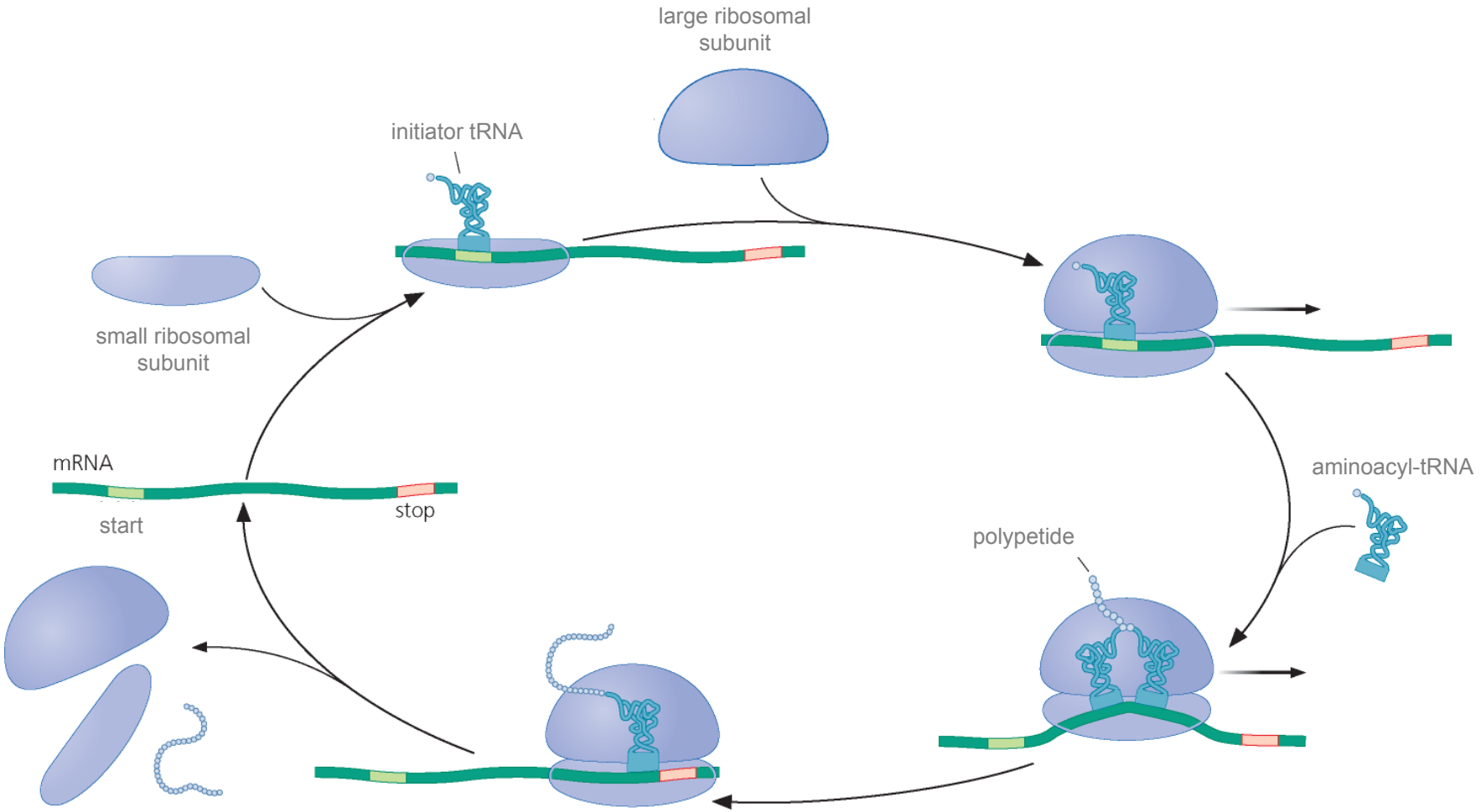
Bigger particles sediment faster and have higher sedimentation coefficients. Sedimentation coefficients are, however, not additive.

The Ribosome machine

1. It has to translate code written with an alphabet of 4 bases in a second written by 20 amino acids
2. It has to synthesize protein (peptidyl transferase reaction)
3. It has to guarantee the moving on the mRNA and tRNA molecules exchange

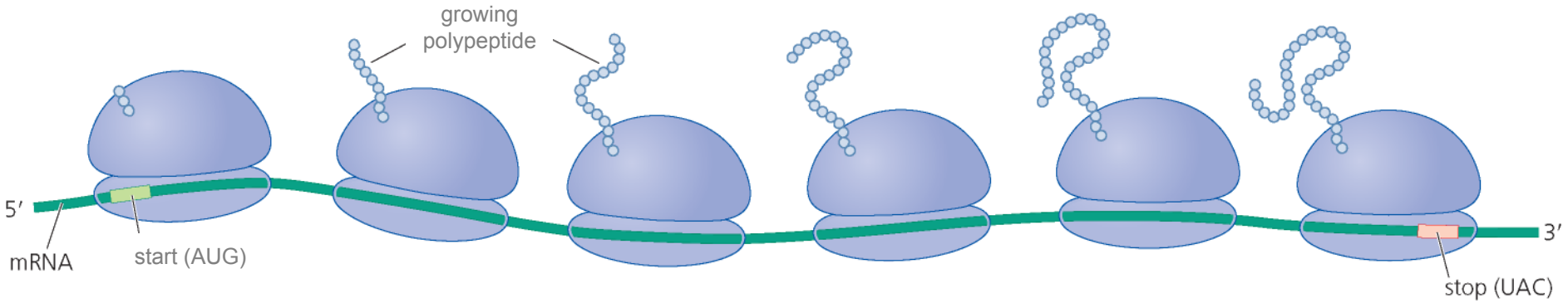


The ribosome cycle



Many machines running on the same RNA

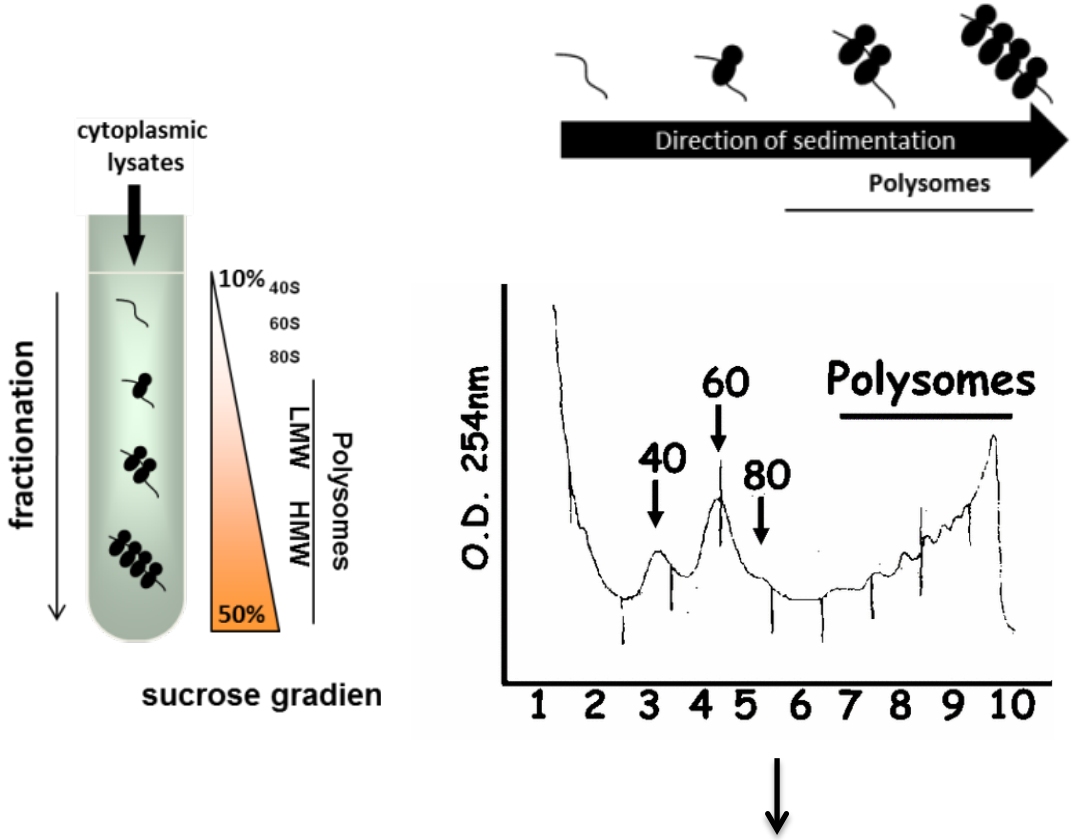
Although a ribosome can synthetize only one polypeptide at a time, each mRNA can be translated simultaneously by multiple ribosomes. An mRNA bearing multiple ribosomes is known as **polyribosome** or **polysome**.



A single ribosome is in contact with about 30 nt of mRNA, but its large size only allows a density of one ribosome every 80 nt.

Polysome profile

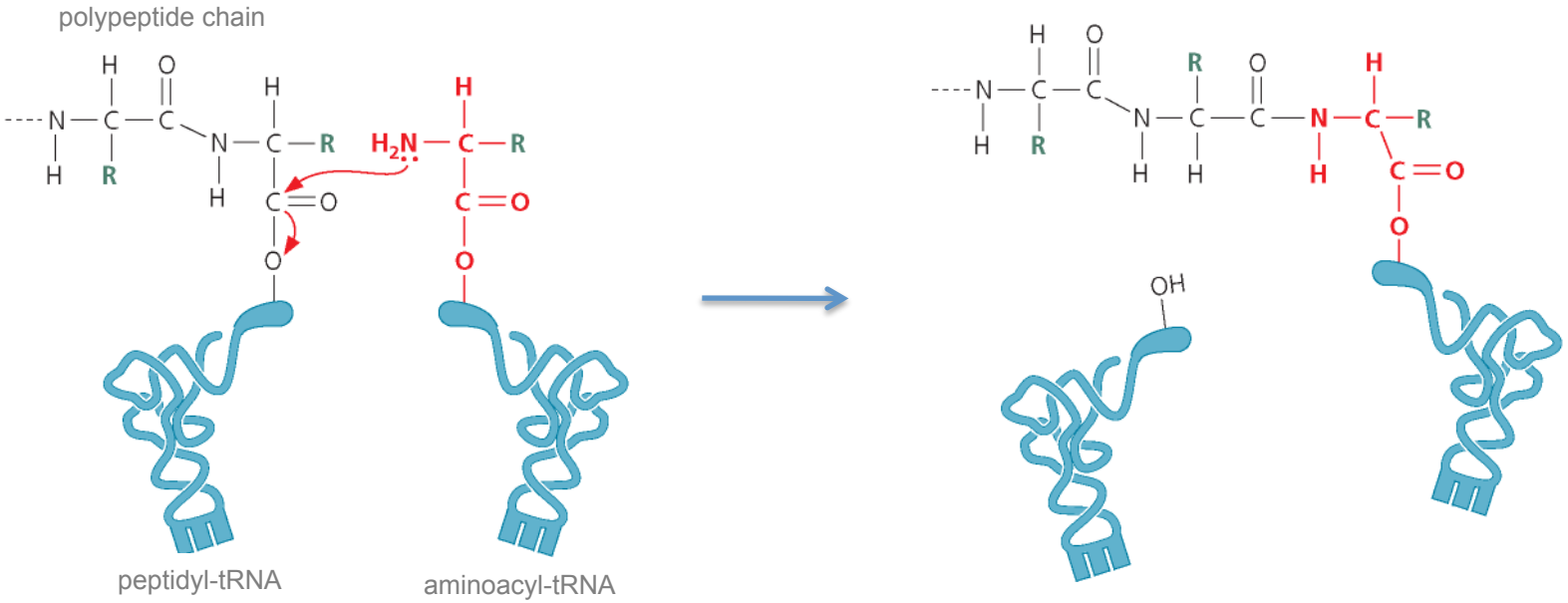
Polysome profiles obtained by ultracentrifugation of cell lysates in sucrose gradients (10% - 50%) allow the identification of the mRNA in active translation (associated with polysomes).



The individual fractions can be analyzed by Northern, qRT-PCR, microarray or RNA-seq

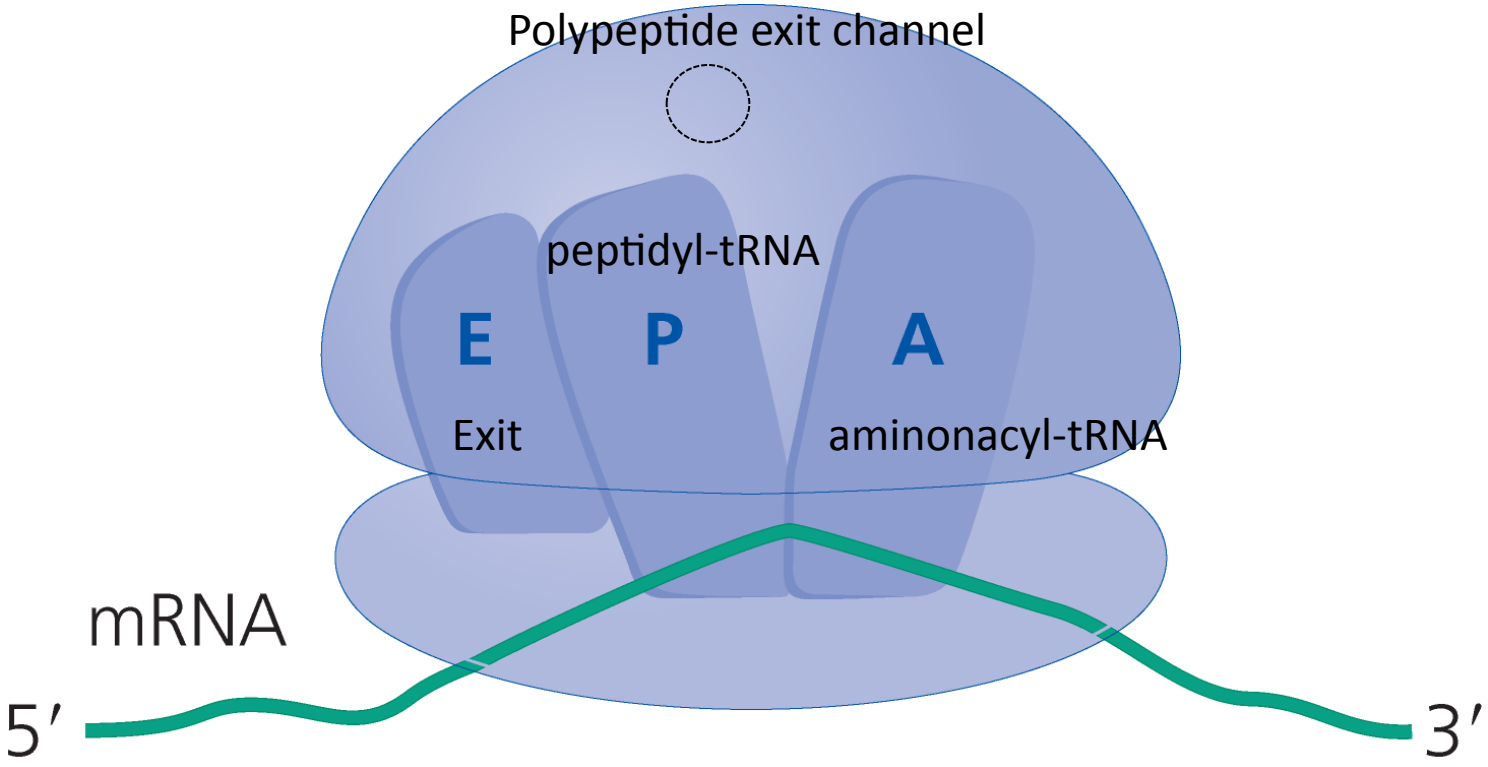
The peptidyl transferase reaction

The peptidyl transferase reaction occurs inside the ribosome and produces the polypeptide chain

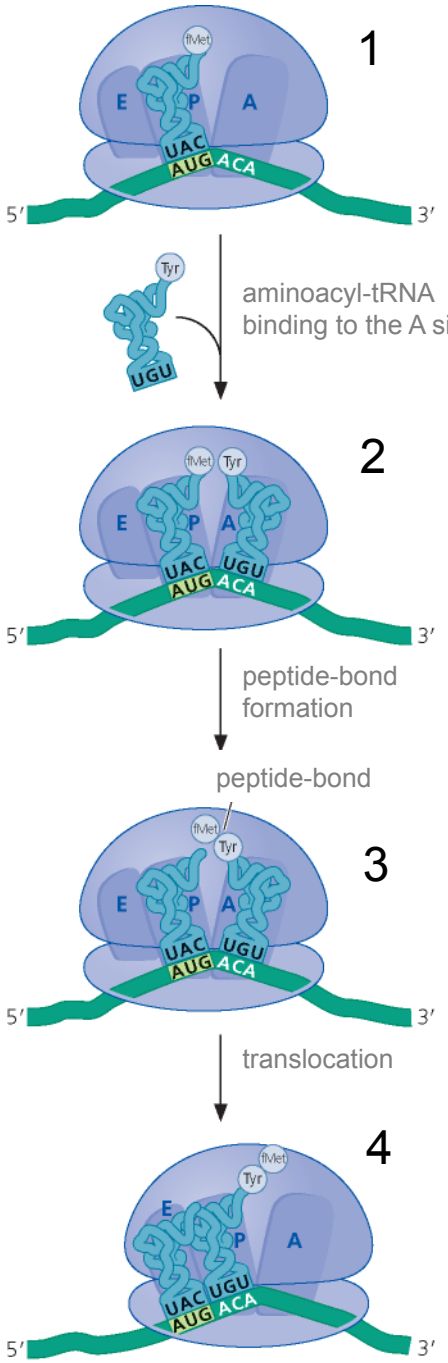


The energy for peptide bond formation originates from the breaking of the bond that link the polypeptide chain to the tRNA. High-energy bond that formed during the reaction catalyzed by the tRNA synthetase by using one ATP molecule.

The ribosome has three tRNA binding sites and one exit channel for the peptide

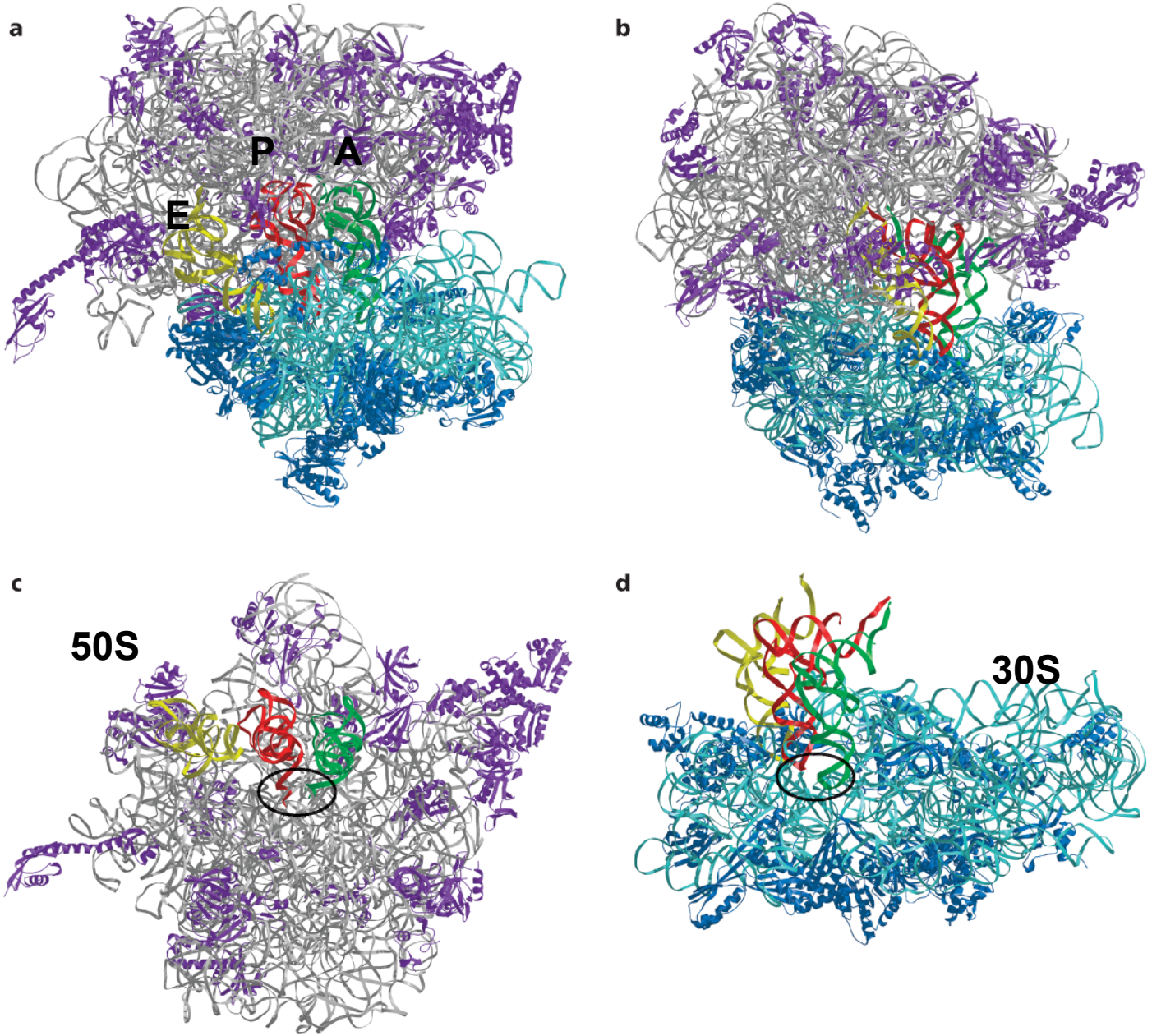


An overview of translation



1. The charged initiator-tRNA is loaded in the P site
2. The correct aminoacyl-tRNA is loaded in the A site
3. A peptide bond is formed
4. The resulting peptidyl-tRNA is **translocated** in the P site

1998-2000



Crystallographic structures (ribosomal proteins are absent from the peptidyl-transferase site), and subsequent biochemical analysis determined that the catalytic component of the ribosome is the rRNA.

The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath *"for studies of the structure and function of the ribosome"*.

Photo: U. Montan

Venkatraman Ramakrishnan

Prize share: 1/3

Photo: U. Montan

Thomas A. Steitz

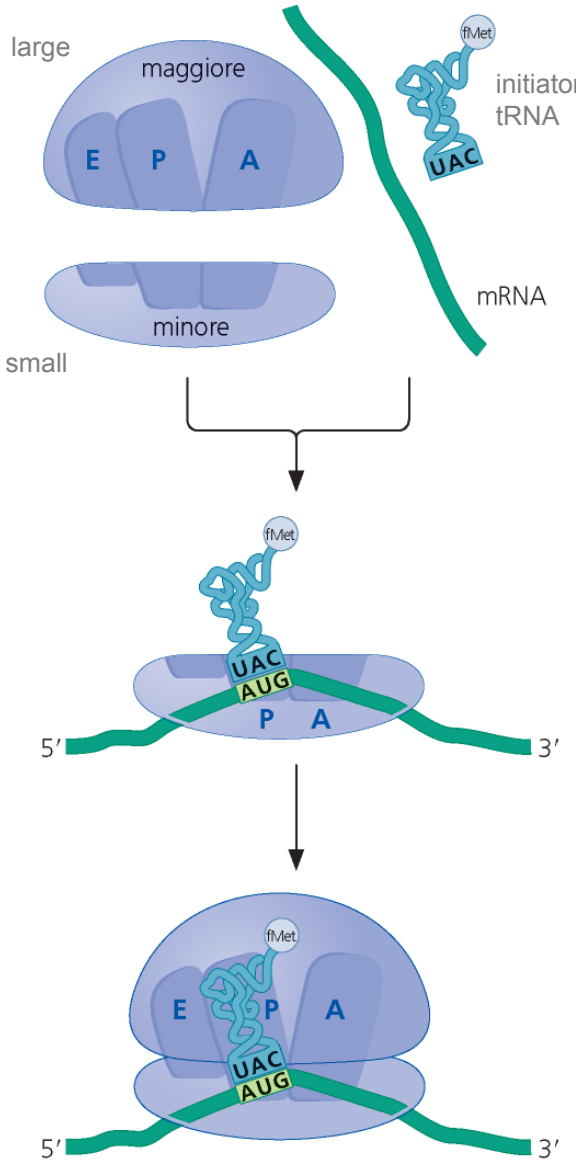
Prize share: 1/3

Photo: U. Montan

Ada E. Yonath

Prize share: 1/3

Initiation of translation



For translation to be successfully initiated, three events must occur:

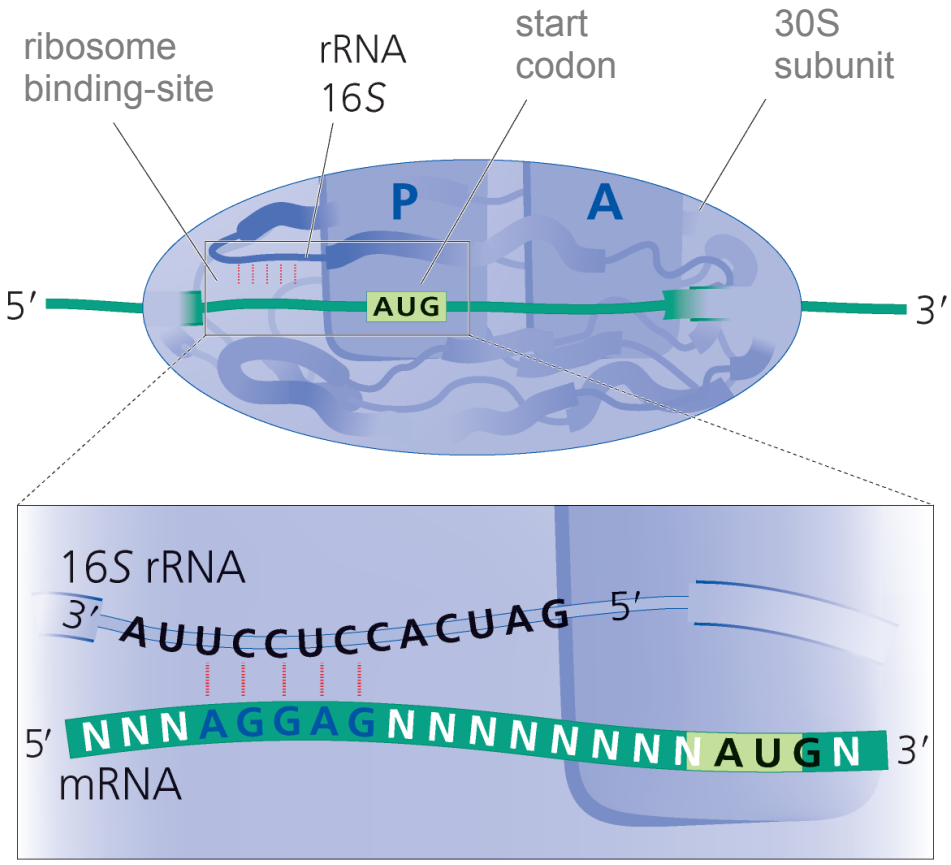
1- The ribosome must be recruited to the mRNA.

2- A charged tRNA must be placed into the P-site.

3- the ribosome must be precisely positioned over the start codon.

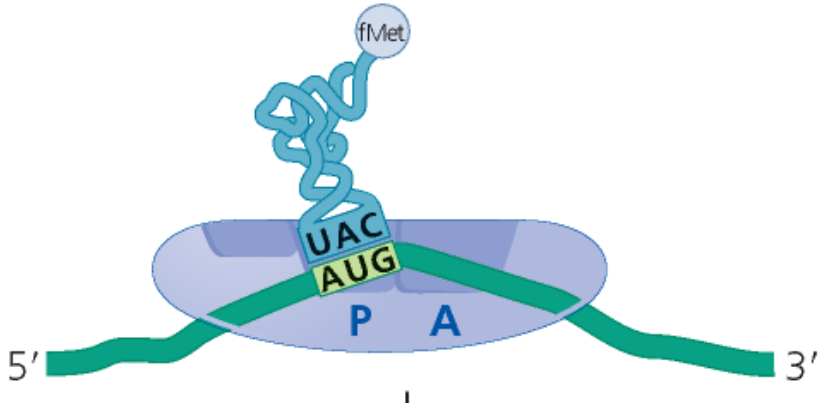
Translation initiation in prokaryotes

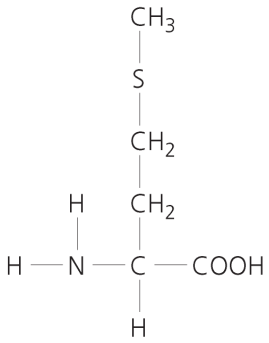
In prokaryotes, the association of the small subunit with the mRNA is mediated by base-pairing interactions between the ribosome binding site (**RBS** or **Shine-Dalgarno sequence**) and the 16S rRNA.



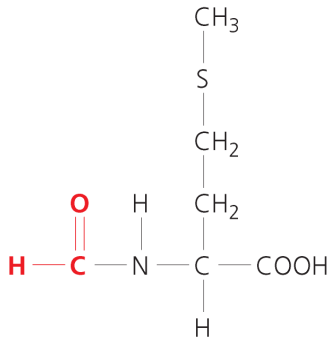
Translation initiation in prokaryotes

During initiation a charged tRNA enters the P site. This event requires a special tRNA known as the **initiator tRNA**, which base-pairs with the start codon (AUG or GUG). Initiator-tRNA is always charged with a modified form of methionine, the ***N*-formyl methionine,** and is referred to as **fMet-tRNA$_i^{fMet}$** . However, not all proteins start with fMet as an enzyme known as **deformylase** remove the formil group after the synthesis of the polypeptide.



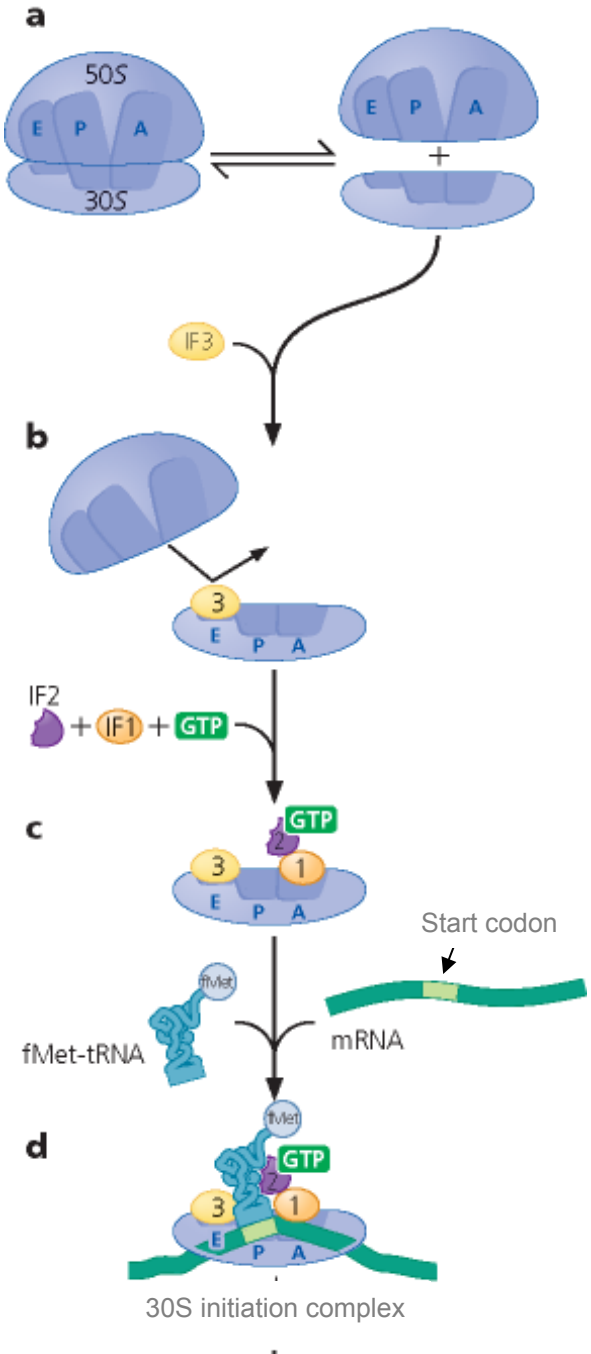


methionine



N-formil-methionine (fMEt)

Prokaryotic translation initiation factors

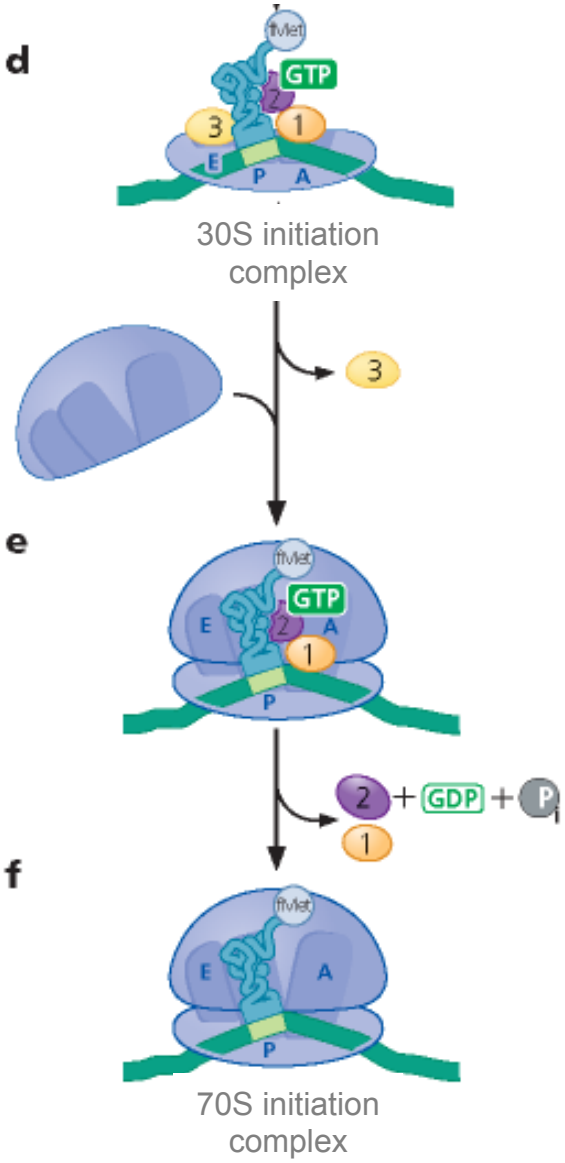


IF-1, IF-2, IF-3

- **IF1** prevents tRNAs from binding to the portion of the 30S that will became part of the A site.
- **IF2** is GTPase (a protein that binds and hydrolizes GTP) that interacts with IF1, fMet-$tRNA_i^{fMet}$ and the small subunit. IF2 facilitates the association of the initiator tRNA to the 30S.
- **IF3** binds the 30S and blocks it from reassociating with the 50S. It is critical for a new cycle of translation.

With all three IFs bound, the small subunit is prepared to bind to the mRNA and the initiator-tRNA to form the **30S initiation complex**.

Translation initiation in prokaryotes

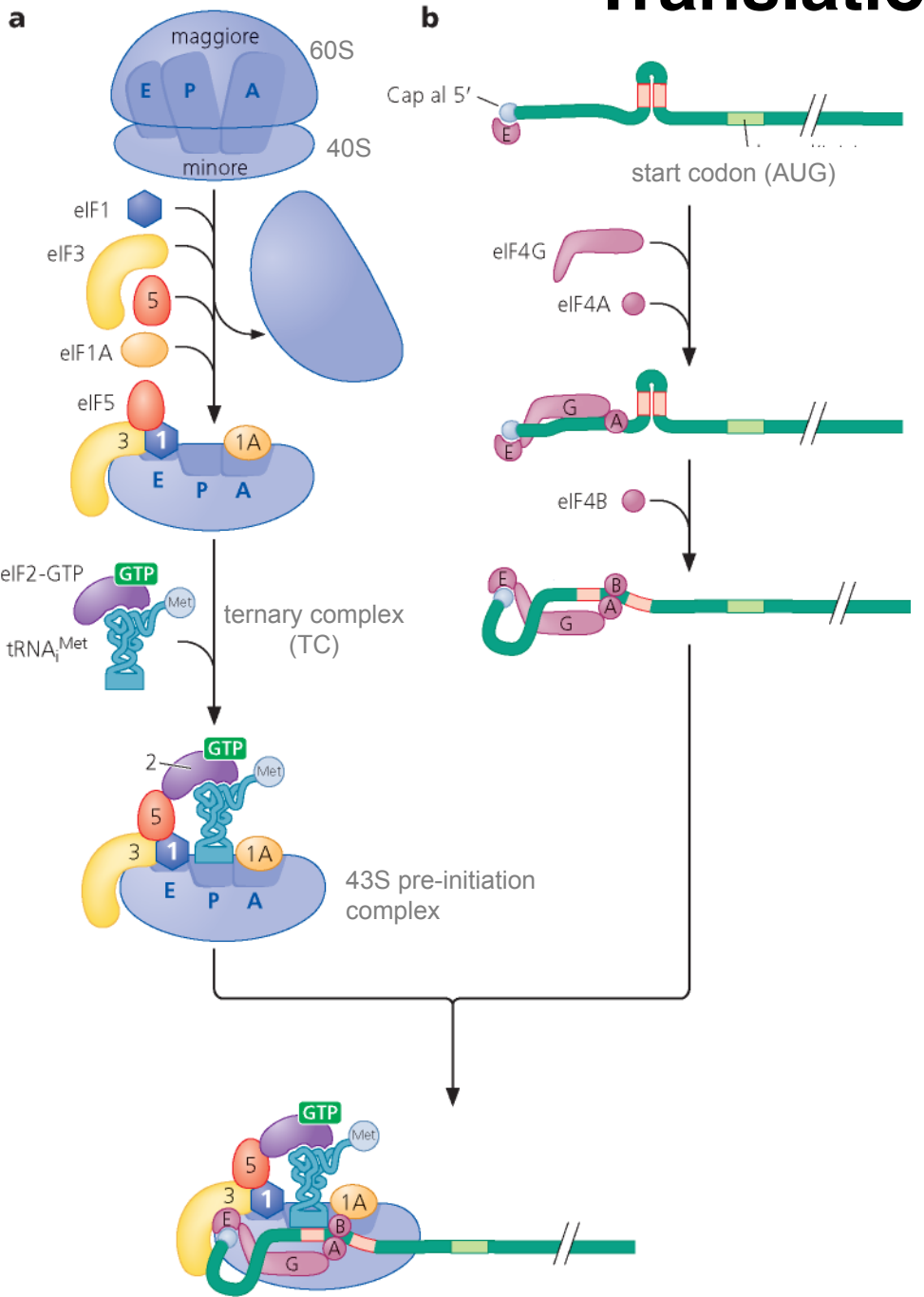


- When the start codon and the fMet-tRNA$_i^{fMet}$ base-pair, the 30S initiation complex undergoes a change in conformation that results in the release of IF3 and the binding of the 50S.

- The 50S stimulates the GTPase activity of IF2-GTP, causing it to hydrolyze GTP. The resulting IF2-GDP has reduced affinity for the ribosome and the initiator tRNA leading to the release of IF2 and IF1 from the ribosome.

The net results of initiation is the formation of an intact 70S ribosome assembled at the start site with a fMet-tRNA$_i^{fMet}$ in the P site and an empty A site (**70S initiation complex**)

Translation initiation in eukaryotes



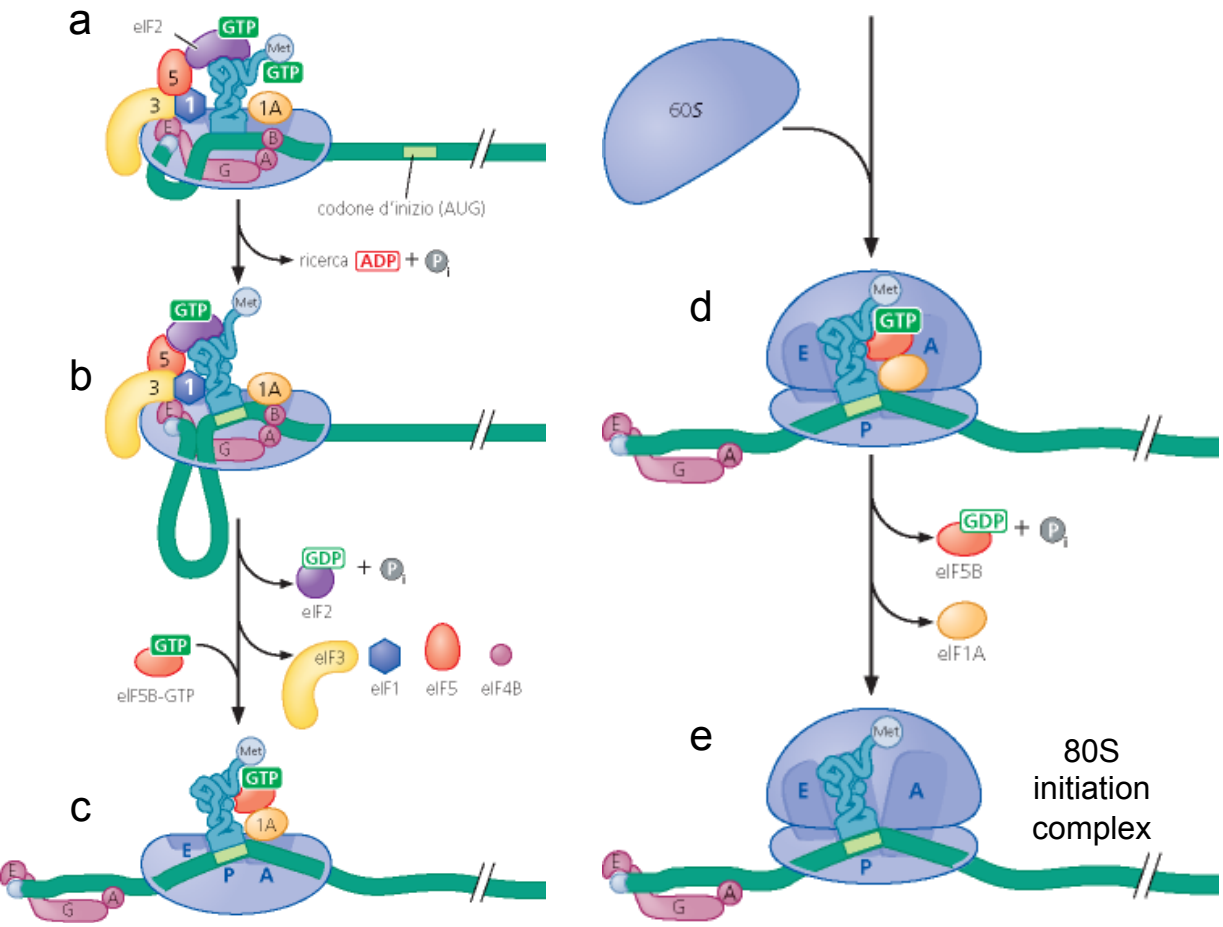
The small subunit is already associated with the initiator tRNA (**Met-tRNA$_i^{Met}$**) when is recruited to the capped 5'-end of the mRNA. It then scans along the mRNA in a 5'->3' direction until it reaches the first start codon AUG.

eIF3 and **eIF1A** correspond to IF3 e IF1.

eIF2 e **eIF5B** correspond to IF2. eIF5B-GTP recruits the complex formed by eIF2-GTP and the Met-tRNA$_i^{Met}$ **(ternary complex-TC)** on the 40S to form **the 43S pre-initiation complex.**

Recognition of mRNAs by the 43S is mediated by **eIF4F** (**4E**, **4G** e **4A**), which binds the 5'-cap, and **eIF4B**, which activates the helicase activity of 4A.

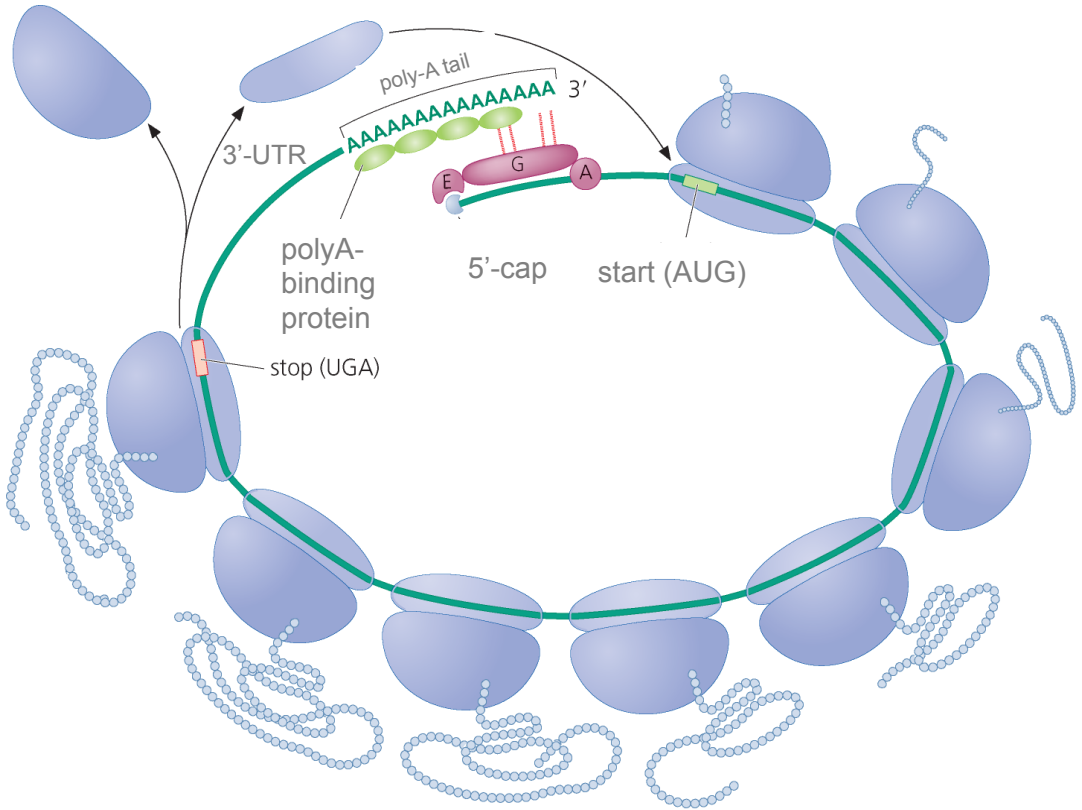
Translation initiation in eukaryotes



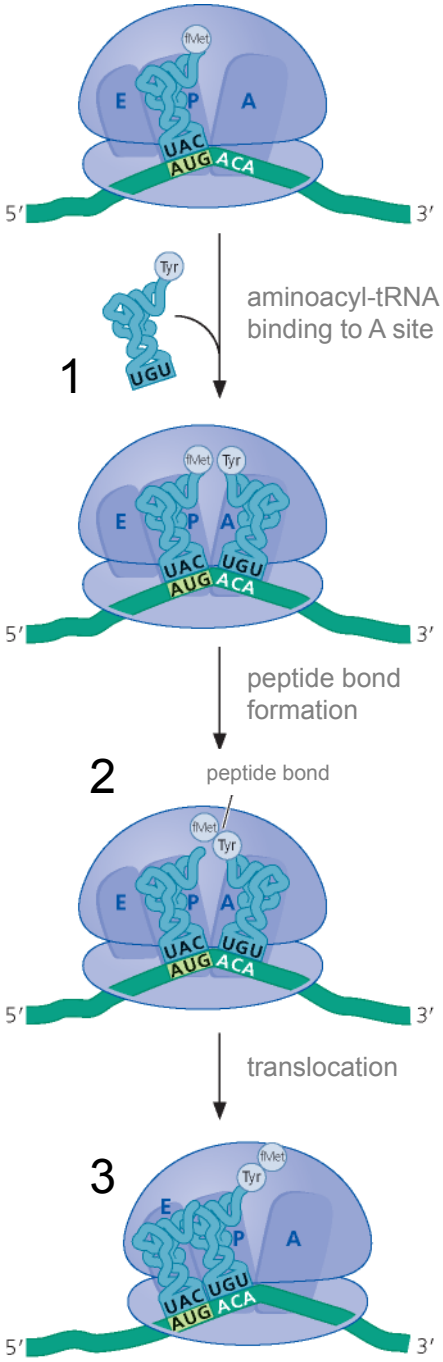
Once assembled at the 5'-end of the mRNA, the small subunit with IFs scans the mRNA for the first start codon. Correct base-pairing triggers the release of eIF2 and eIF3, which allows the binding of the 60S. This leads to the release of the remaining IFs by stimulating GTP hydrolysis. As a result, the initiator tRNA is placed in the P site of the resulting **80S initiation complex**.

Translation initiation factors hold eukaryotic mRNAs in circles

The polyA binding protein (**PABP**), which coats the poly-A tail of mRNAs, interacts with eIF4F inducing a circular configuration. As a result, once a ribosome finishes translating the newly released ribosome is ideally positioned to re-initiate translation of the same mRNA . Furthermore, many factors that regulates translation of specific mRNAs act by binding the 3'-Untranslated Region (3'-UTR) and inhibiting eIF4F activity.



Translation elongation

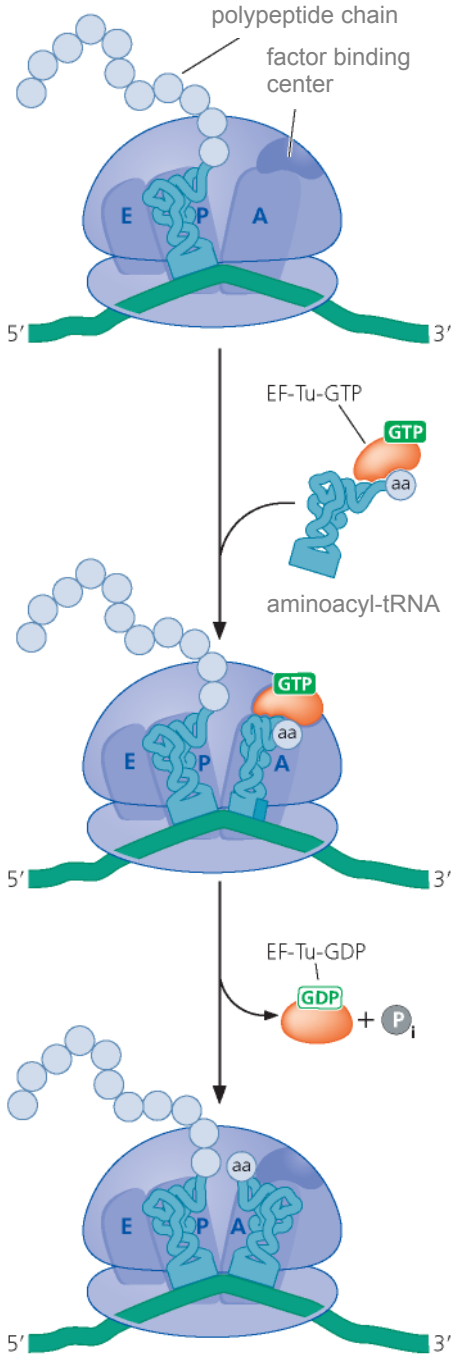


There are three key events that must occur for elongation of the peptide chain:

1. The correct aminoacyl-tRNA (aa-tRNA) is loaded into the A site.
2. A peptide bond is formed between the aa-tRNA in the A site and the peptide chain that is attached to the peptidyl-tRNA in the P site. As a result, the growing polypetide is trasfered from the tRNA in the P site to the aa of the tRNA in the A site.
3. The resulting polypeptidil-tRNA in the A site and its associated codon must be translocated to the P-site.

These events are controlled by the **elongation factors (EFs)**

Elongation Factors

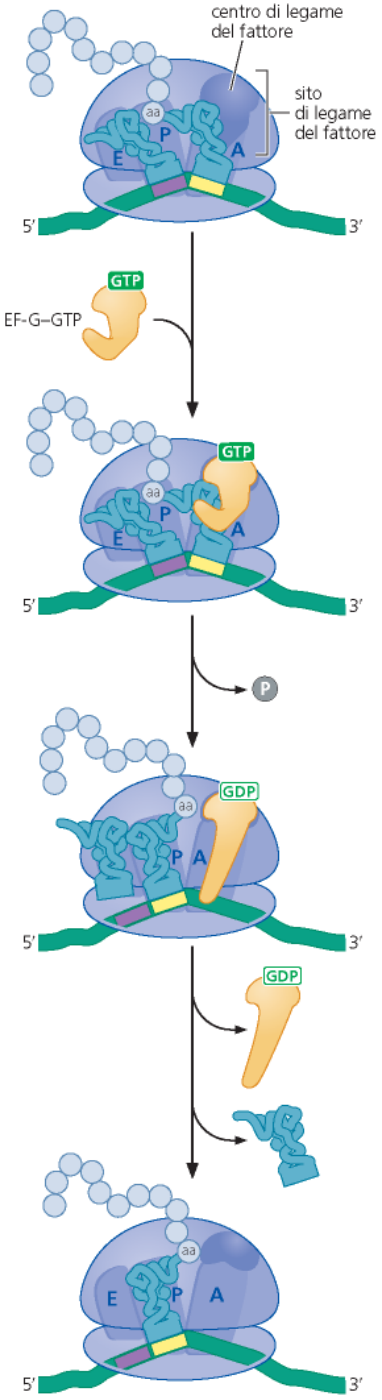


- Recruit the aa-tRNAs to the ribosome
- Guarantee a correct base-pairing between tRNA and mRNA codons
- Favor the translocation (moving of tRNAs from P to E and from A to P)

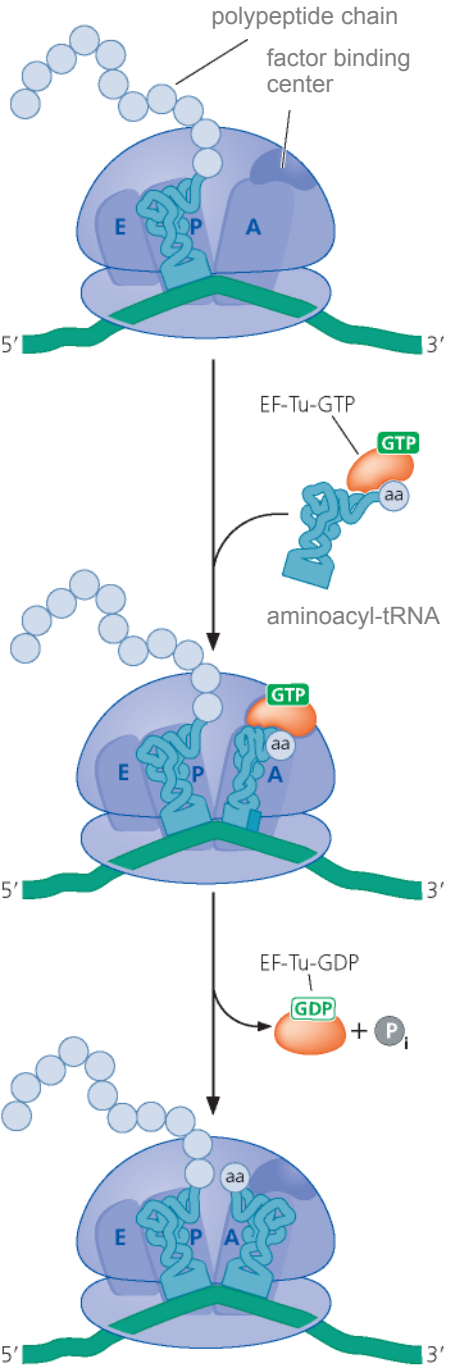
Prokariotes:
EF-Tu
EF-G
EF-Ts

Eukaryotes:
eEF1 (from α to γ)
eEF2

<u>Elongation is conserved between eukaryotes and prokaryotes</u>



Elongation factors: EF-Tu



When is associated to GTP, EF-Tu binds to charged tRNAs, masking the coupled aa. Thus preventing its incorporation in the peptide chain. When EF-Tu hydrolyzes its bound GTP, any associated aa-tRNA is released.

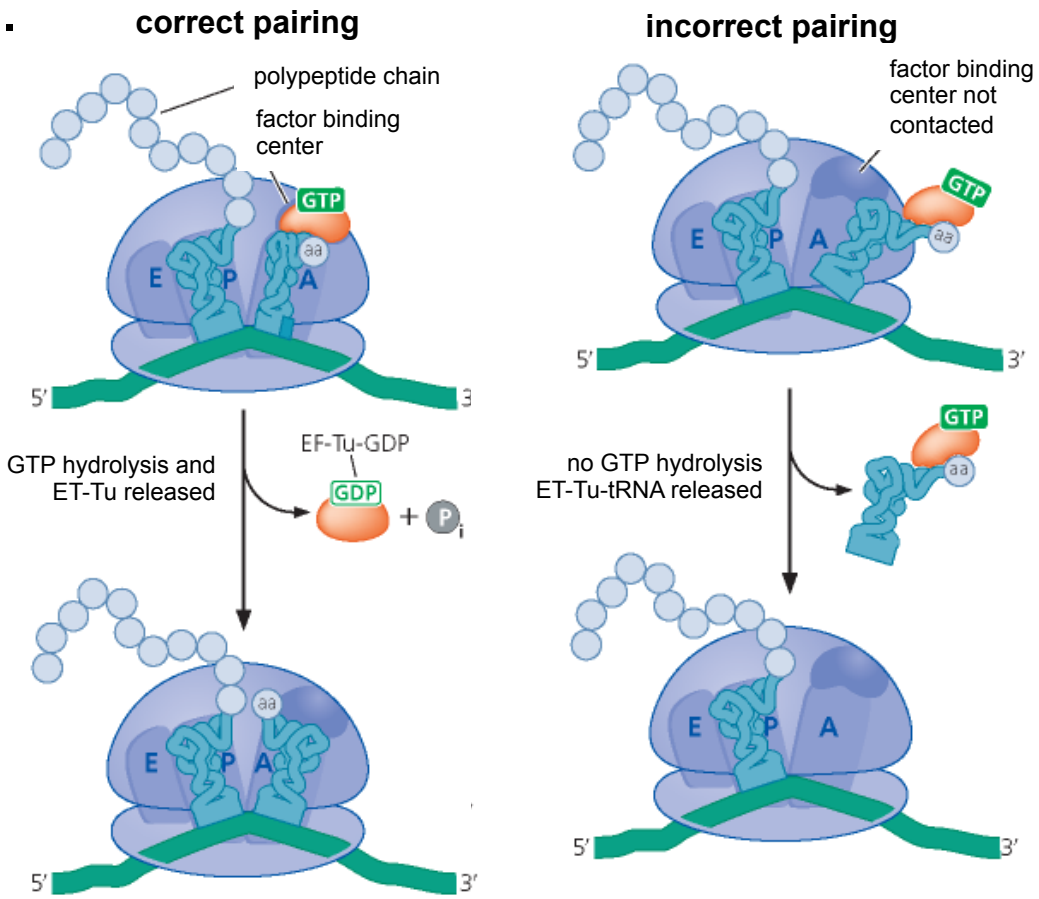
The trigger that activates the EF-Tu GTPase is the **factor binding center** of the large subunit (the same domain that activates IF2).

<u>EF-Tu is critical to the specificity of translation</u>

The ribosome use multiple mechanisms to select against incorrect aminoacy-tRNAs

The error rate in translation is between 10^{-3} to 10^{-4}. At least two mechanisms contribute to this:

1.

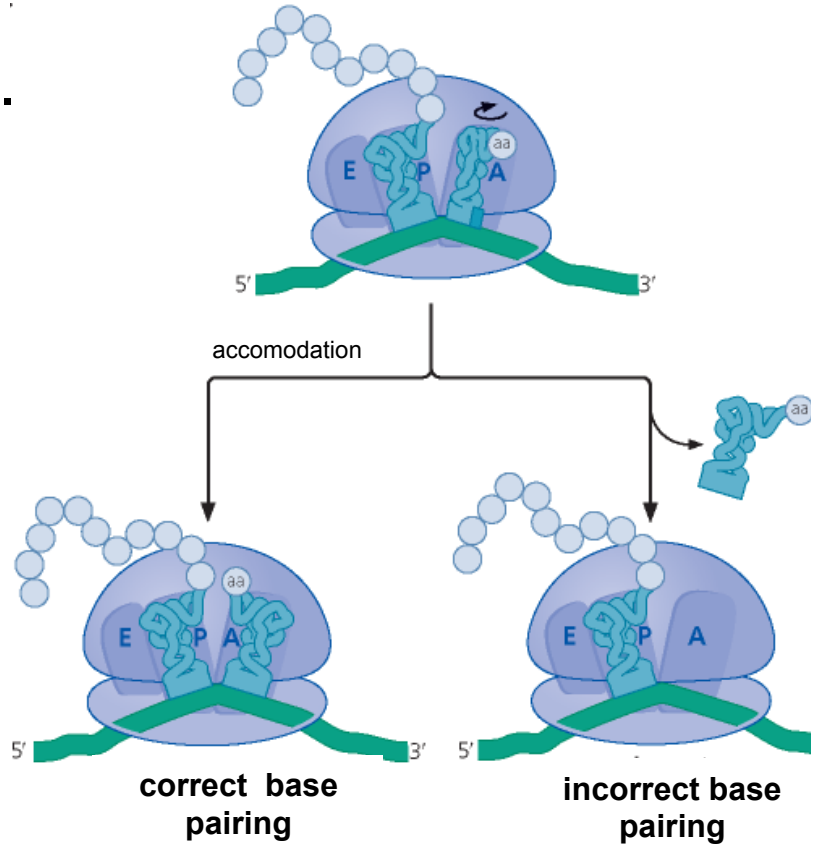


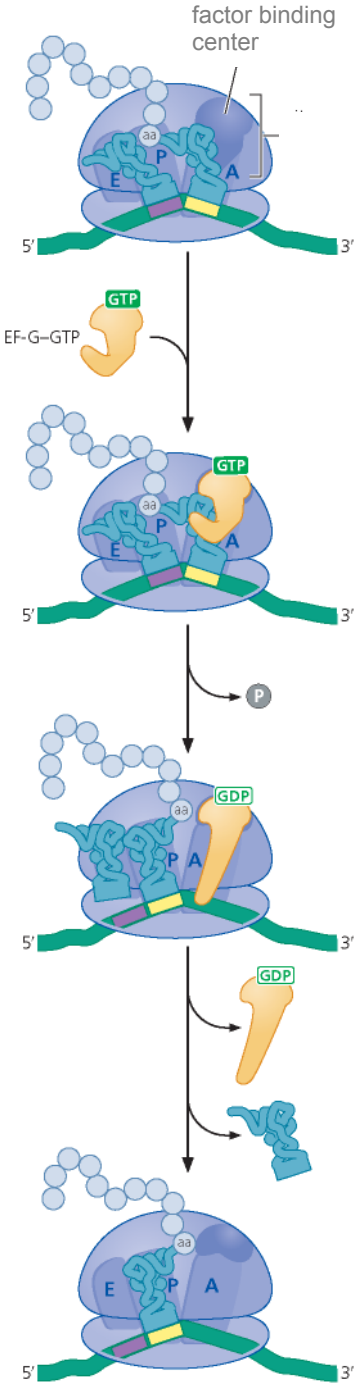
EF-Tu only interacts with the factor binding center after the tRNA is loaded into the A site and a correct codon-anticodon match is made.

The ribosome use multiple mechanisms to select against incorrect aminoacy-tRNAs

When the charged tRNA is introduced into the A site, its 3'-end is distant from the site of peptide bond formation. To participate in the peptidyl transferase reaction, the tRNA must rotate in a process called **accomodation**. Incorrectly paired tRNAs dissociate from the ribosome during accomodation.

2.





Elongation factors: EF-G

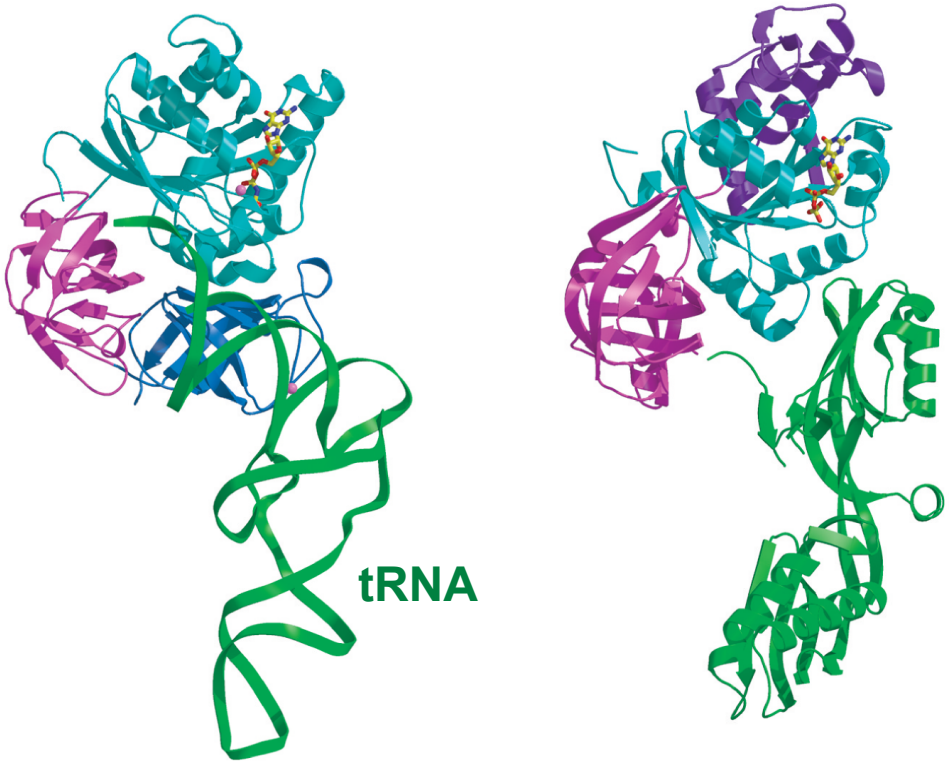
EF-G controls the **translocation** process: movement of the P-site tRNA to the E site; movement of the A-site tRNA to the P-site and mRNA movement by three nucleotides to expose the next codon. The initial steps of translocation are coupled to the peptidyl transferase reaction.

EF-G recognizes the peptidyl-tRNA in the A site only when associated to GTP. Then EF-G binds the ribosome, it contacts the factor-binding center, which stimulates GTP hydrolysis. This changes the conformation of EF-G-GDP, allowing it to reach the small subunit and trigger translocation of the A-site tRNA into the P site, the P-site tRNA into the E site and the movement of the mRNA by one codon.

When translocation is complete, the resulting structure has reduced affinity for EF-G-GDP, allowing its release from the ribosome.

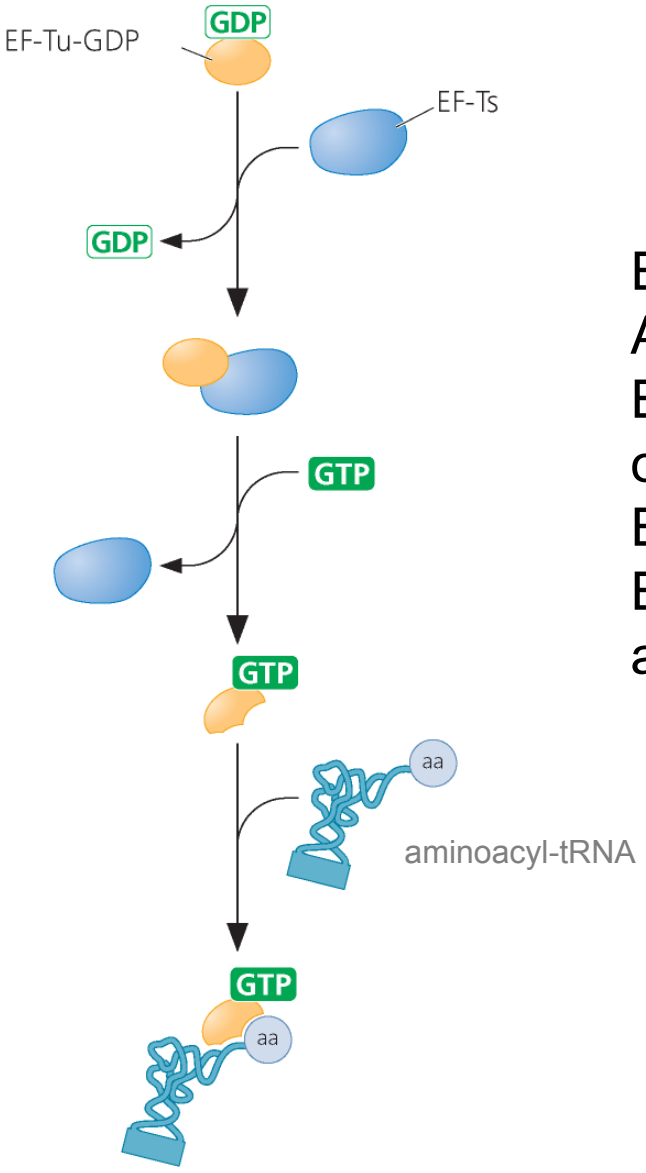
Elongation factors

EF-Tu-GTP-tRNA and EF-G-GDP have a similar structure. This is an example of “molecular mimicry” in which a protein takes on the appearance of a tRNA to facilitate association with the same binding site.



EF-Tu-GDP-Phe-tRNA **EF-G-GDP**

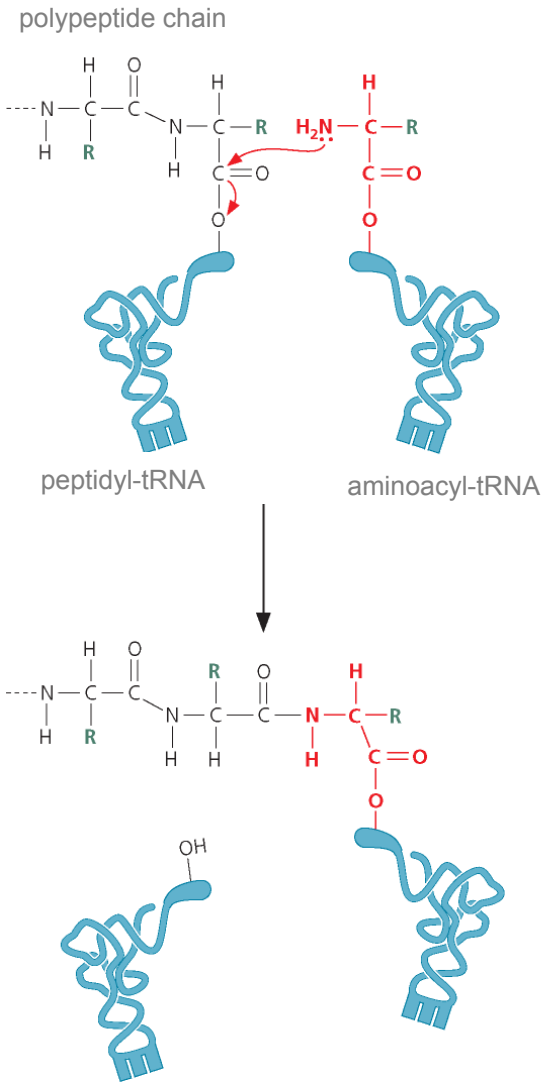
Elongation factors: EF-Ts



EF-Ts acts as a **GTP exchange factor** for EF-Tu. After EF-Tu-GDP is released from the ribosome, EF-Ts binds to EF-Tu, causing the displacement of GDP. Next, GTP binds to the resulting EF-Tu-EF-Ts complex, causing its dissociation into free EF-Ts and EF-Tu-GTP. Finally, EF-Tu-GTP binds a molecule of charged tRNA.

Cost per round of peptide bond formation

- This reaction is catalysed by the rRNA of the large subunit: the ribosome is a ribozyme.



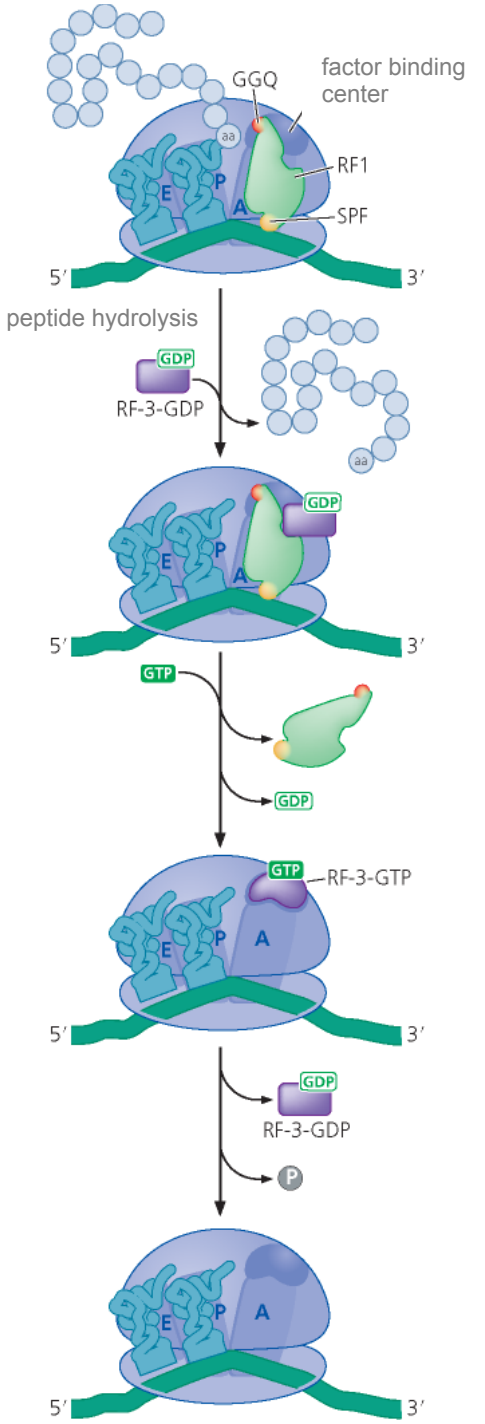
Energy cost:

1 ATP is consumed by the aminoacyl-tRNA synthetase in creating the high energy acyl bond that links the amino acid to the tRNA. The breakage of this bind drives the petydil transferase reaction.

1 GTP is consumed by EF-Tu in delivering a charged tRNA to the A site.

1 GTP is consumed by EF-G in the translocation process

Termination of translation: release factors



•Stop codons are recognized by **release factors (RFs)** that activate the hydrolysis of the polypeptide from the peptidyl-tRNA (Class I RFs)

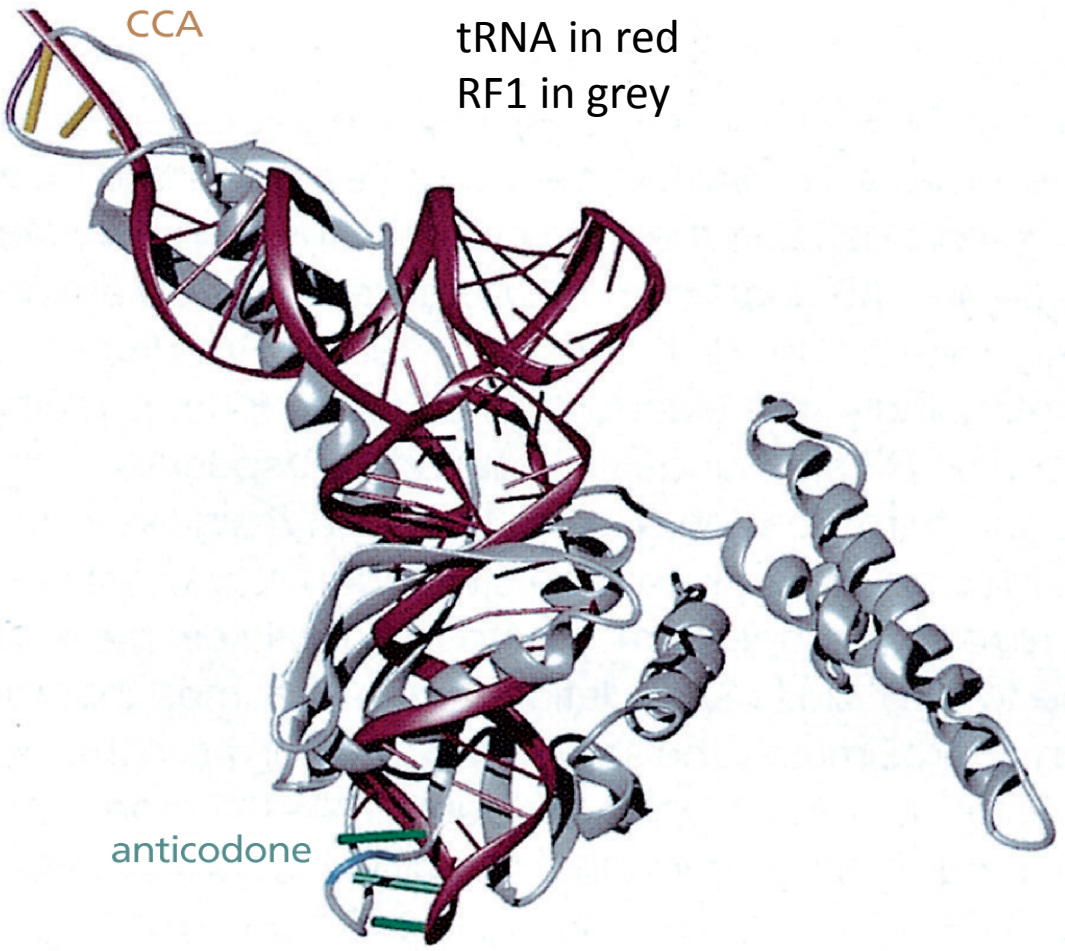
Class I release factors:

Prokaryotes:	Eukaryotes:
UAG – RF1	UAG – eRF1
UGA – RF2	UGA – eRF1
UAA – RF1, RF2	UAA – eRF1

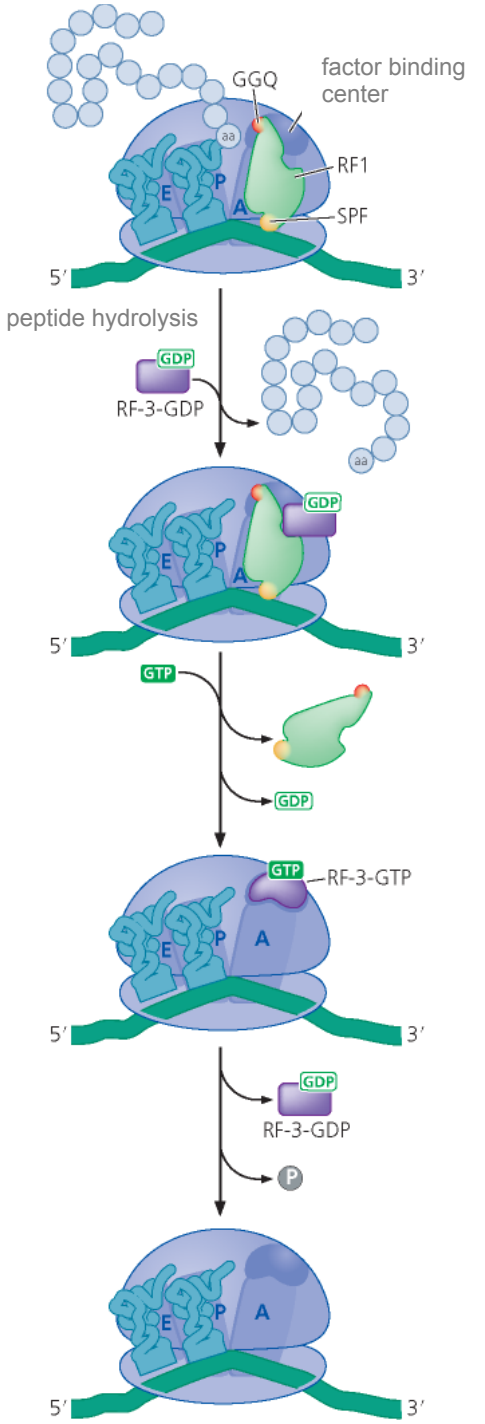
Classe II release factors (stimulate the release of Class I RFs):

Prokaryotes:	Eukaryotes:
RF3	eRF3

To interact with the A site of ribosome, Class I RFs have a structure similar to tRNA



Termination of translation



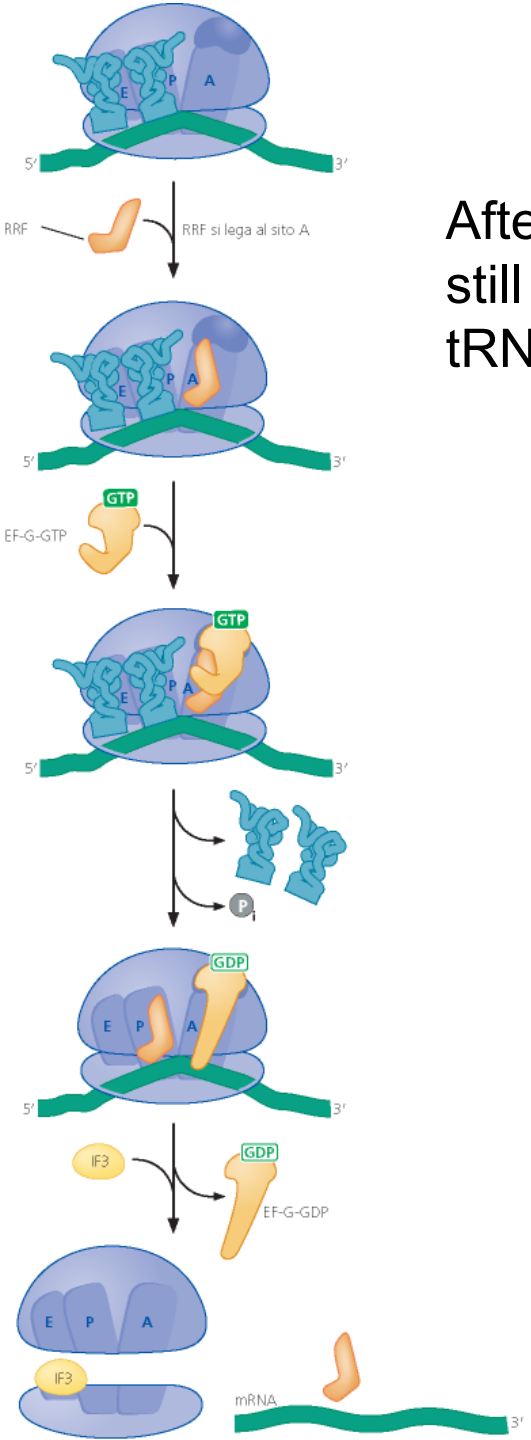
A Class I RF binds the A and triggers the hydrolysis of the peptidyl-tRNA linkage. Then, it must be removed from the ribosome. A Class II RF is required for this.

RF3 (o eRF3), unlike other factors involved in translation, has a higher affinity for GDP than GTP. The binding of RF3-GDP to the ribosome depend on the presence of RF1. After RF1 stimulates polypeptide release, a change in conformation stimulates RF3 to exchange its bound GDP for a GTP.

This change allows RF3 to associate with the factor binding center. This interaction stimulates the hydrolysis of GTP.

In the absence of RF1, RF3-GDP has a low affinity for the ribosome and is released.

Ribosome recycling factors



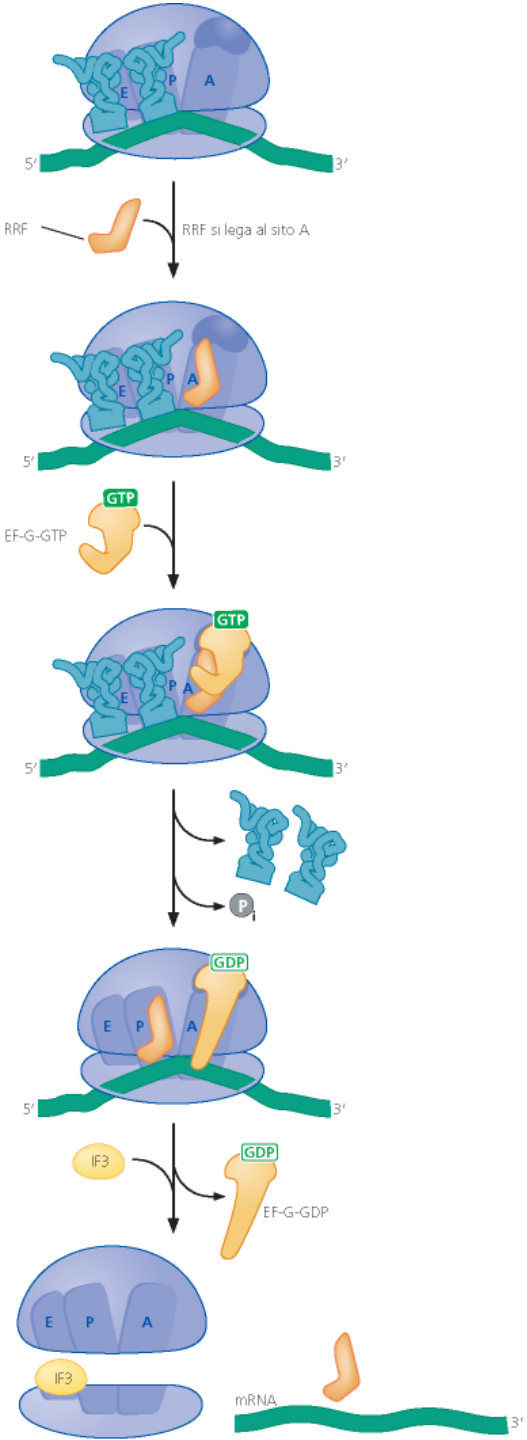
After the release of the polypeptide chain, the ribosome is still bound to the mRNA and left with two deacylated tRNAs (in the P and E sites).

Recycling factors are required for:

- removing tRNAs e mRNA from the ribosome,
- dissociate the ribosome into its large and small subunits.

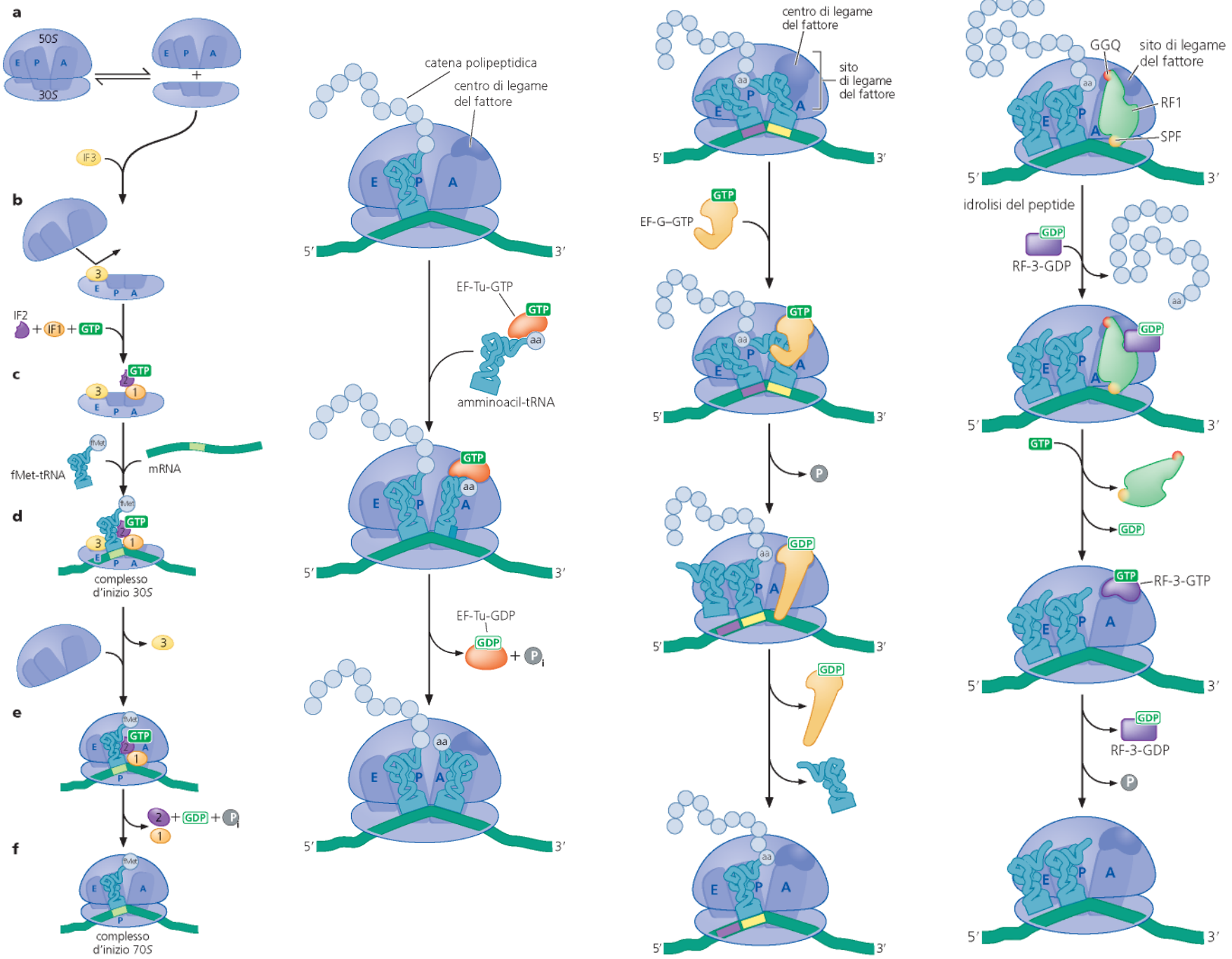
<u>Prokaryotes:</u>
RRF cooperates with EF-G and IF3 for ribosome recycling

Ribosome recycling



RRF binds to the empty A site (it mimics a tRNA) and recruits **EF-G-GFP** stimulating the release of the uncharged tRNAs bound to the P and E sites, in events that mimic EF-G function during elongation. Once the tRNAs are removed, EF-G and RRF are released from te ribosome along with mRNA.

IF3 participates in mRNA release and is required to separate the two ribosomal subunits fro each others. The small subunit bound to IF3 can now participate in a new round of translation.


a
50S
30S
E P A
IF3
b
IF2
IF1
GTP
c
fMet-tRNA
mRNA
d
complesso d'inizio 30S
e
f
complesso d'inizio 70S
GDP
catena polipeptidica
centro di legame del fattore
EF-Tu-GTP
amminoacil-tRNA
EF-Tu-GDP
5′
3′
sito di legame del fattore
EF-G–GTP
idrolisi del peptide
RF-3-GDP
GGQ
RF1
SPF
RF-3-GTP

EPILOGUE

Initiation, elongation, and termination of translation is mediated by an ordered series of interdependent factor binding an release events. This ordered nature of translation ensures that no one step occurs before the previous step is complete.

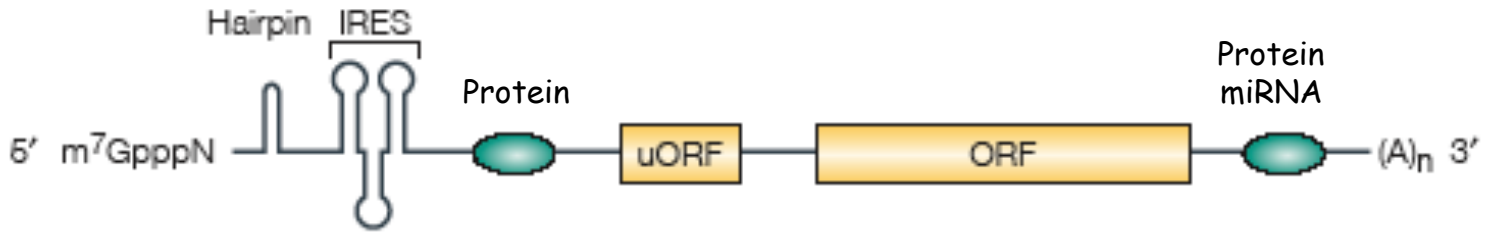
If any step cannot be completed, then the entire process stop.

It is this Achilles heel that antibiotics exploit when they target the translation process.

Antibiotics targets and consequences

Antibiotic/Toxin	Target Cells	Molecular Target	Consequence
Tetracycline	Prokaryotic cells	A-site of 30*S* subunit	Inhibits aminoacyl-tRNA binding to A-site
Hygromycin B	Prokaryotic and eukaryotic cells	Near A-site of 30*S* subunit	Prevents translocation of A-site tRNA to P-site
Paromycin	Prokaryotic cells	Adjacent to A-site codon–anticodon interaction site in 30*S* subunit	Increases error rate during translation by decreasing selectivity of codon–anticodon pairing
Chloramphenicol	Prokaryotic cells	Peptidyl transferase center of 50*S* subunit	Blocks correct positioning of A-site aminoacyl-tRNA for peptidyl transfer reaction
Puromycin	Prokaryotic and eukaryotic cells	Peptidyl transferase center of large ribosomal subunit	Chain terminator; mimics 3′ end of aminoacyl-tRNA in A-site and acts as acceptor for nascent polypeptide chain
Erythromycin	Prokaryotic cells	Peptide exit tunnel of 50*S* subunit	Blocks exit of growing polypeptide chain from the ribosome; arrests translation
Fusidic acid	Prokaryotic cells	EF-G	Prevents release of EF-G–GDP from the ribosome
Thiostrepton	Prokaryotic cells	Factor-binding center of 50*S* subunit	Interferes with the association of IF2 and EF-G with factor-binding center
Kirromycin		EF-Tu	Prevents conformational changes associated with GTP hydrolysis and therefore EF-Tu release
Ricin and α-sarcin (protein toxins)	Prokaryotic and eukaryotic cells	Chemically modifies RNA in factor-binding center of large ribosomal subunit	Prevents activation of translation factor GTPases
Diphtheria toxin	Eukaryotic cells	Chemically modifies EF-Tu	Inhibits EF-Tu function
Cycloheximide	Eukaryotic cells	Peptidyl transferase center of 60*S* subunit	Inhibits peptidyl transferase activity

Elements that influence translation of mRNA in eukaryotes



- **Cap structure** and **the polyA tails**: canonical motifs
- **Secondary structures** close to the 5 ‘- end block translation initiation
- **IRES**: ribosome entry site mediates cap-independant translation
- **Short ORF** reduced translation of the main ORF
- **Binding sites for trans-acting regulatory factors** (protein, miRNA…)