# *La traduzione*

**Introduction**

**Key-components during translation**

- tRNAs
- Aminoacil-tRNA sintetasi
- Ribosoma

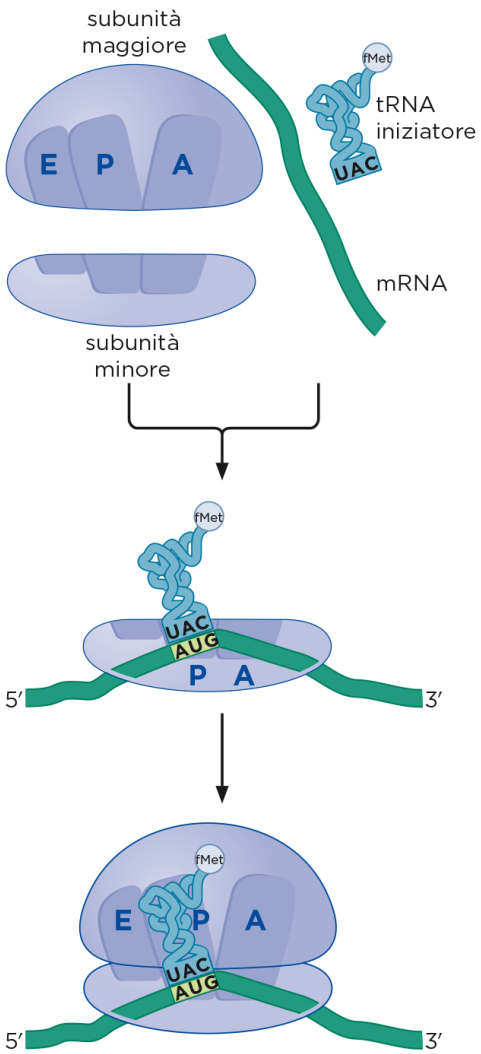
**The process of translation**

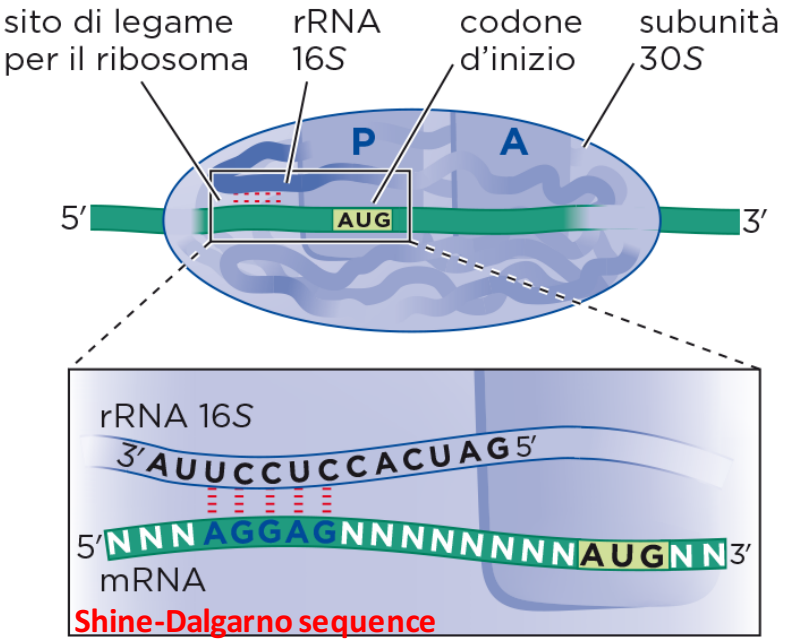
- Iniziazione
- Allungamento
- Terminazione

# *Il codice genetico*

# La iniziazione della traduzione

## Procarioti



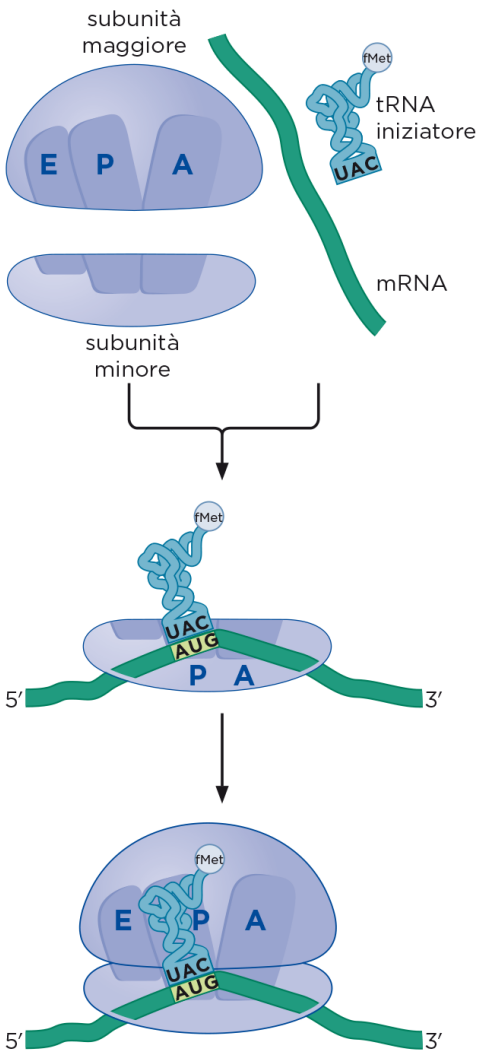

→Exact positioning of 30S subunit on start codon

→Loading of P-site with initiator tRNA

→ Recruitment of 50S subunit

# La iniziazione della traduzione

## Procarioti




### The initiator tRNA/tRNA iniziatore

→First aminoacid in procaryotic proteins is N-formylmethionine

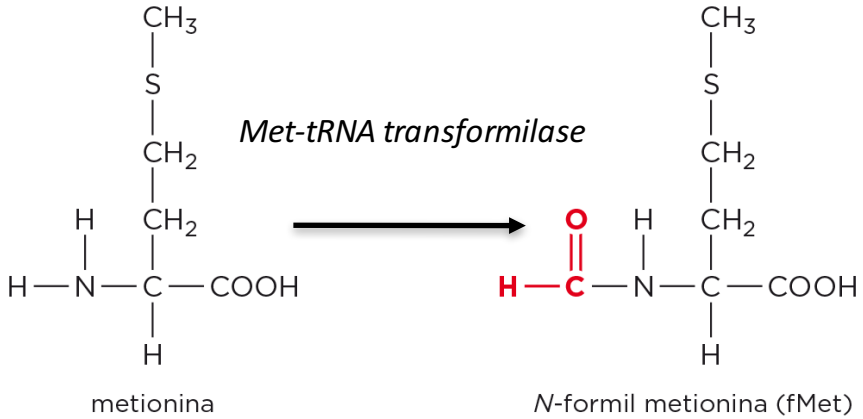
→ the initiator RNA is specific for AUG, GUG start codons ($tRNA_i^{fMet}$)

→tRNAi is loaded with Methionin: Met-$tRNA_i^{fMet}$

→Methionine tRNA transformylase adds formyl group (gruppo formile) to methinin fMet-$tRNA_i^{fMet}$

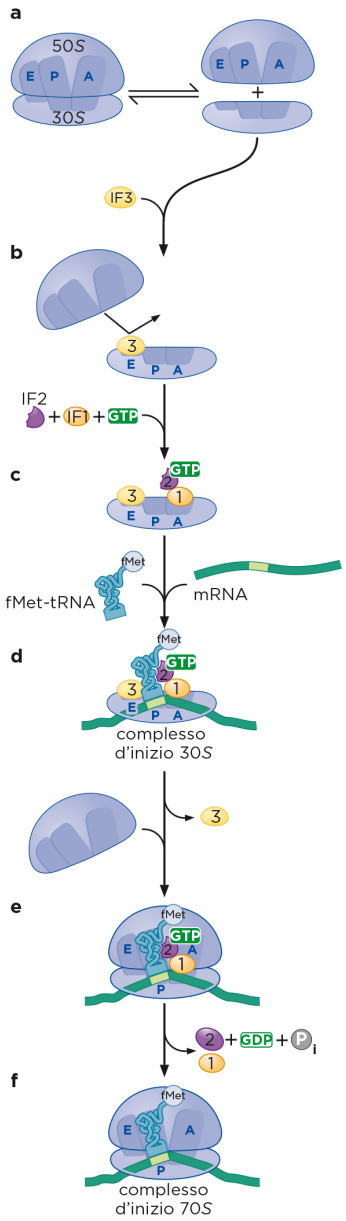
→ fMet-$tRNA_i^{fMet}$ is incorporated into the P-site of the 30S ribosomal subunit

(after translation finished, deformylases remove formyl group (gruppo formile), also 1-2 AA)



# La iniziazione della traduzione

## Procarioti




**Exact positioning of 30S subunit on start codon, assembly of initiation complex**

3 initiation factors (IFs) that mediate the exact loading of the initiator tRNA to the P-site of the 30S ribosomal subunit

→Loading of IF3 to the E-site blocks 30S portion of the E-site and prevents premature full-assembly of the ribosome

→IF1 blocks 30S portion of the A-site

→GTPase IF2 binds do IF1 and contacts fMet-tRNA$_i^{fMet}$

→mRNA contacts 30S minor subunit, fMet-tRNA$_i^{fMet}$ pairs with start codon =30S initiation complex

→70S initiation complex forms: structural change – exit of IF3

→ stimulation of GTPase activity of IF2 (GTP→GDP), affinity of IF2 to 30S reduced, IF2 and IF1 leave 30S

→ P-site loaded with loaded initiator tRNA, A-site ready to be loaded; E-site free.

# *La iniziazione della traduzione*

## *Eucarioti*

→minor subunit associated with loaded initiator tRNA and eukaryotic initiation factors of translation (eIFs)

→Recruitment of this complex to mRNA via the 5'Cap

→minor subunit/initiator tRNA scans on mRNA until FIRST start condon "AUG"

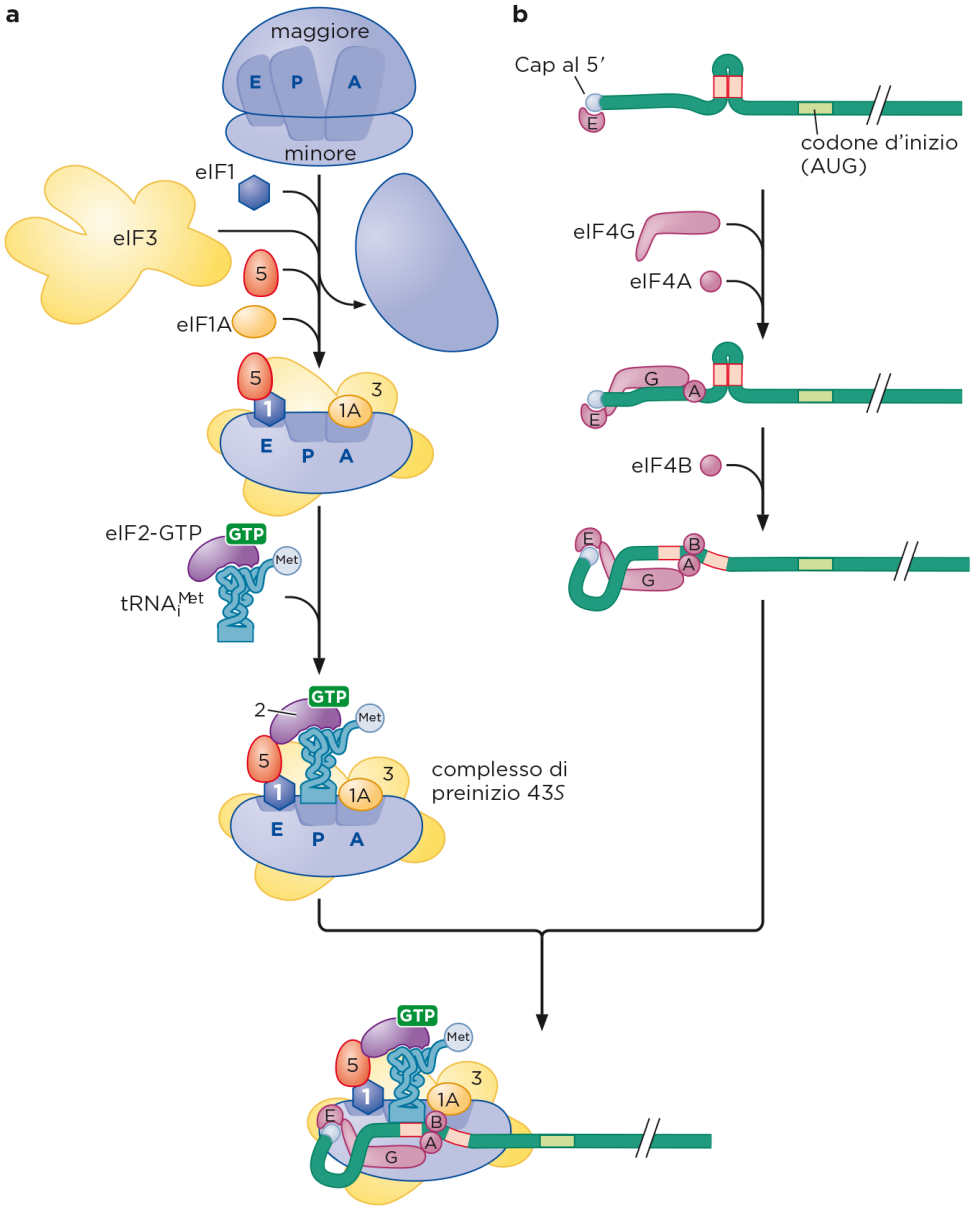
→Major subunit loaded to form initiator complex

**3 steps in eukaryotic initiation of translation**

1. Ternary complex, TC (complesso ternario)
2. 43S pre-initiation complex, 43S PIC (complesso di preinizio 43S)
3. 48S pre-initiation complex 48S PIC (complesso di preinizio 48S)

# La iniziazione della traduzione

## Eucarioti




## A. 40S ribosomal subunit

### 1. Ternary complex (complesso ternario)

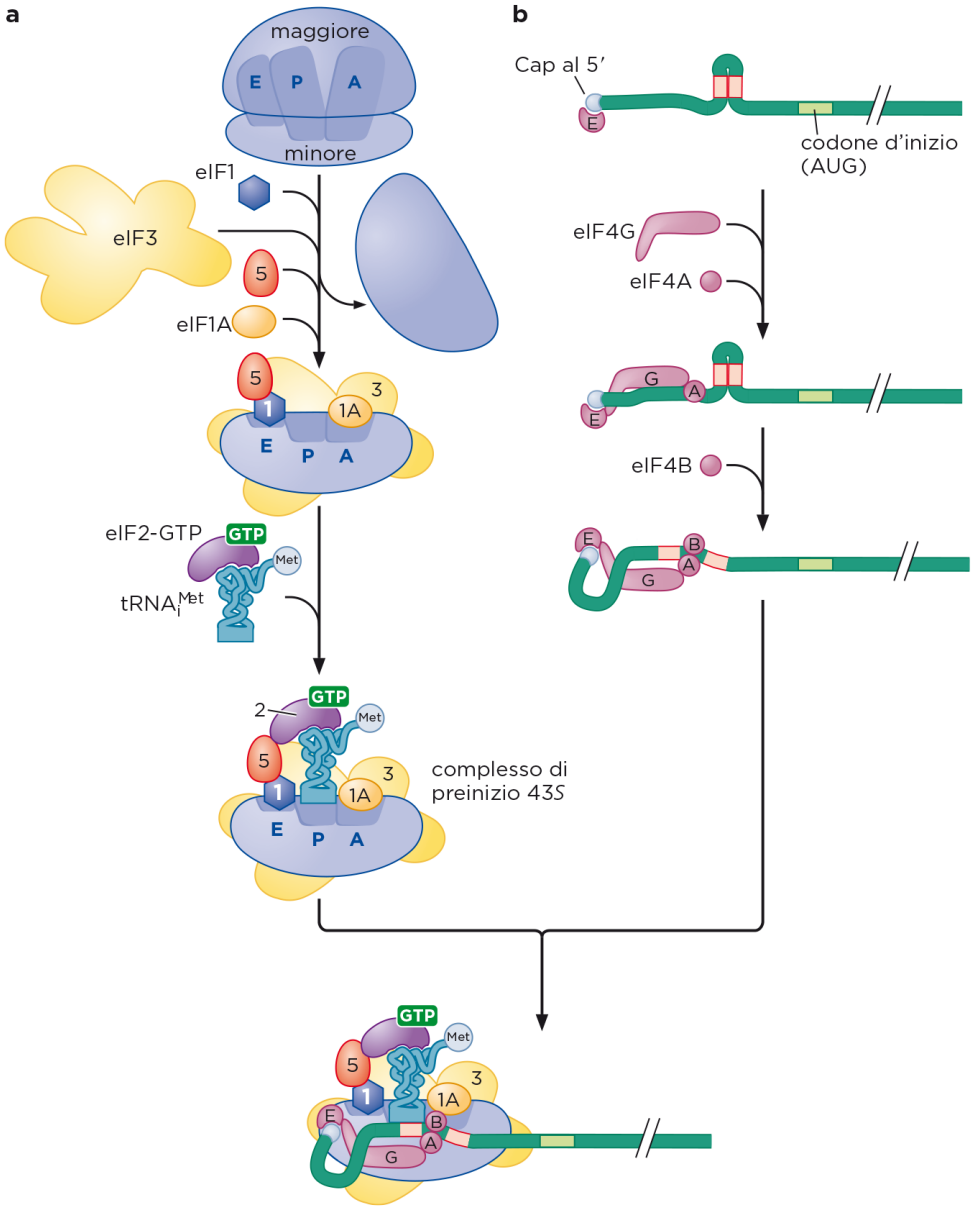
→eIF1, eIF5 bind E-site; eIF1A binds A-site: blocking of aberrant loading of initiator tRNA to A-site and premature assembly of 80S ribosome

→eIF2 GTPase binds intitator tRNA

= Met-tRNA$_i^{Met}$

# *La iniziazione della traduzione*

## *Eucarioti*




### A. 40S ribosomal subunit

1. **Ternary complex (complesso ternario)**

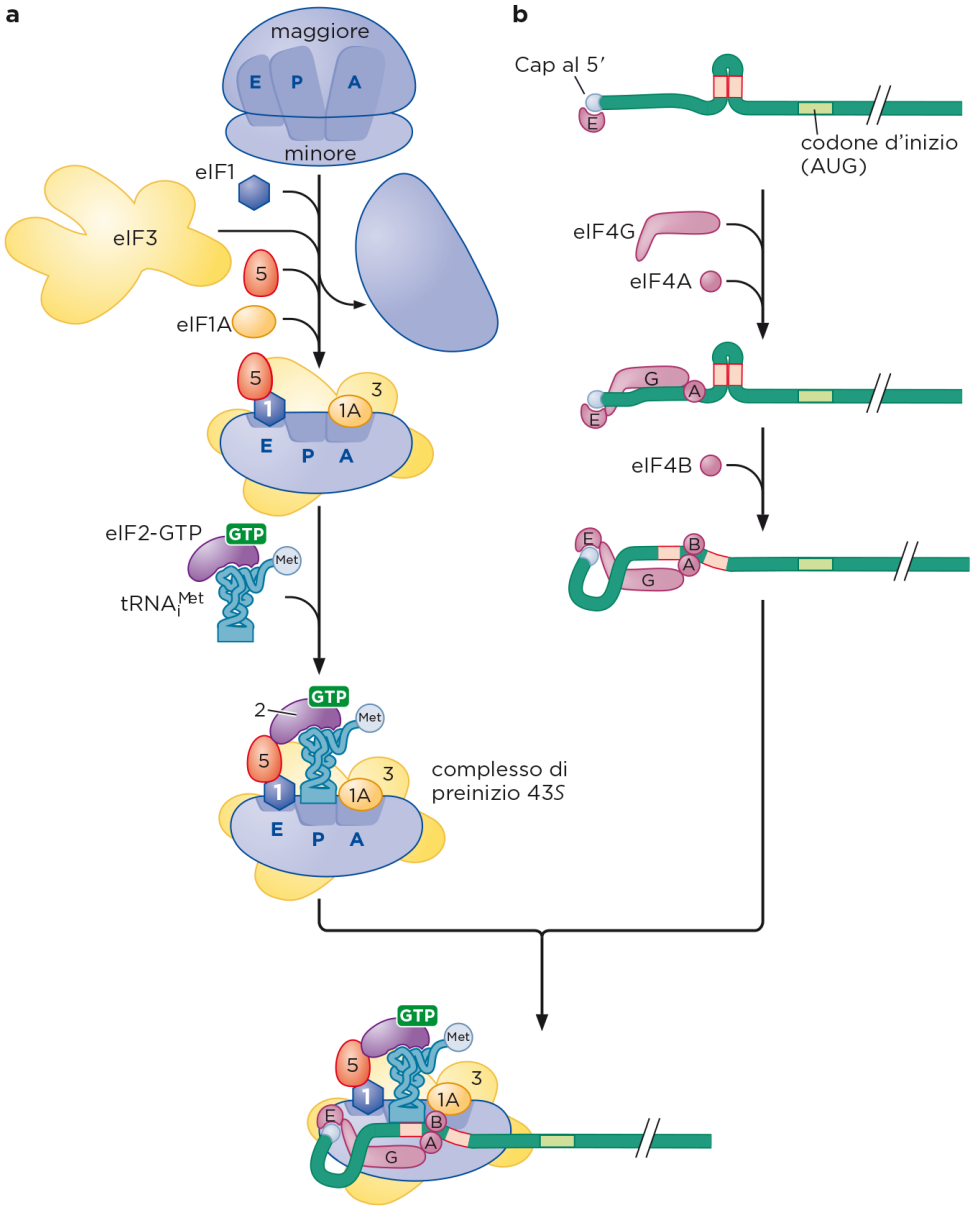
2. **43C pre-initiation complex**

→eIF2 binds to initiator tRNA and fits initiator tRNA into the P-site

eIF3 is large and associated with minor subunit, interacts with other eiFs and supports assembly of 43S PIC

# *La iniziazione della traduzione*

## *Eucarioti*




### A. 40S ribosomal subunit

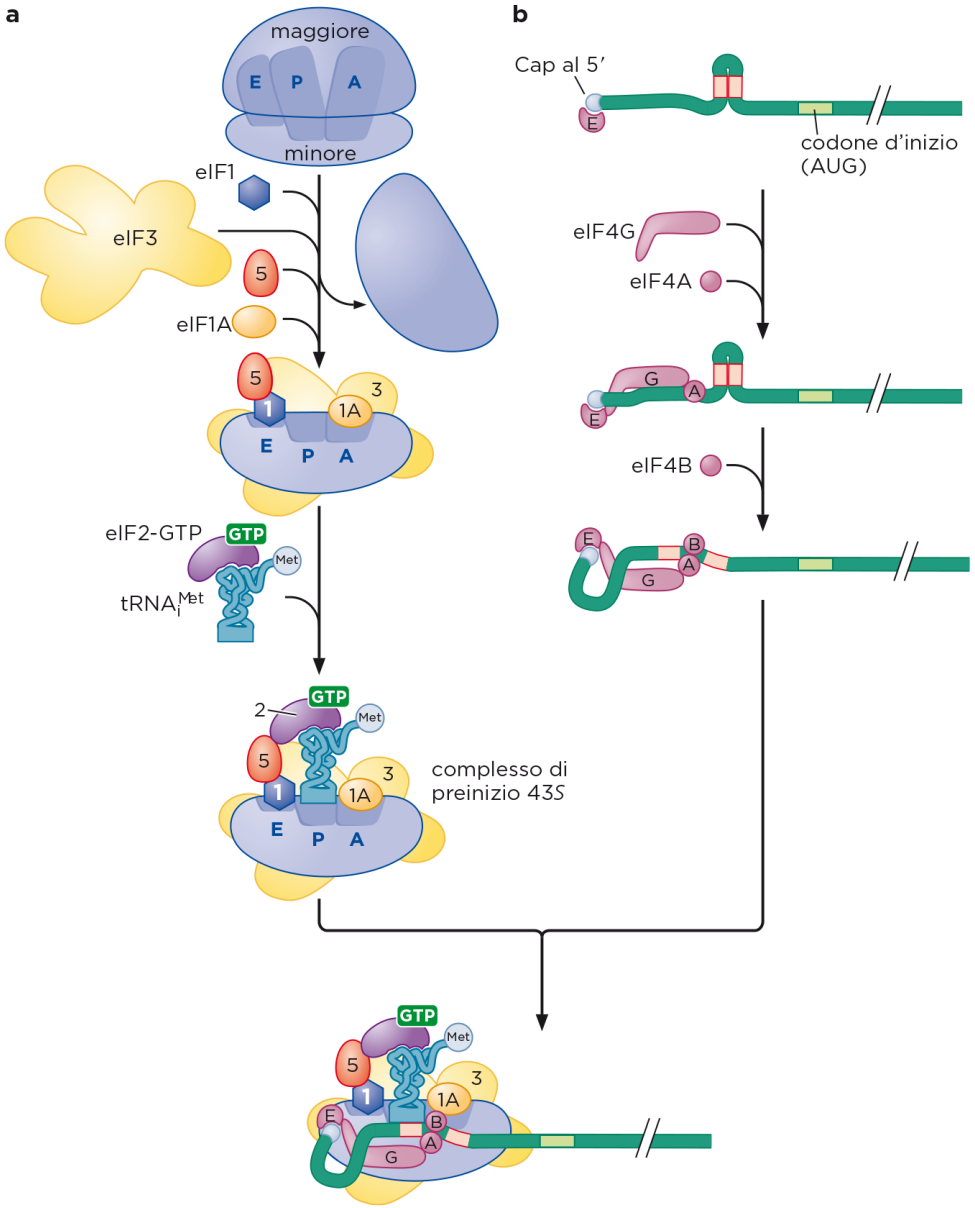
1. **Ternary complex (complesso ternario)**
2. **43C preinitiation complex**

### B. mRNA + eIF4 initiation factors

1. 5'Cap bound by eIF4E
2. eIF4G bind eIF4E and mRNA
3. eIF4A binds eIF4G and mRNA
4. eIF4G-E interaction is major regulator of initiation; diverse factors can compete with binding to eIF4E → regulates initiation
5. eIF4B recruited that activates the helicase activity of eIF4A.
   eIF4A helicase opens secondary structures upstream of start codon

# La iniziazione della traduzione

## Eucarioti




**A. 40S ribosomal subunit**

1. **Ternary complex (complesso ternario)**
2. **43C pre-initiation complex**

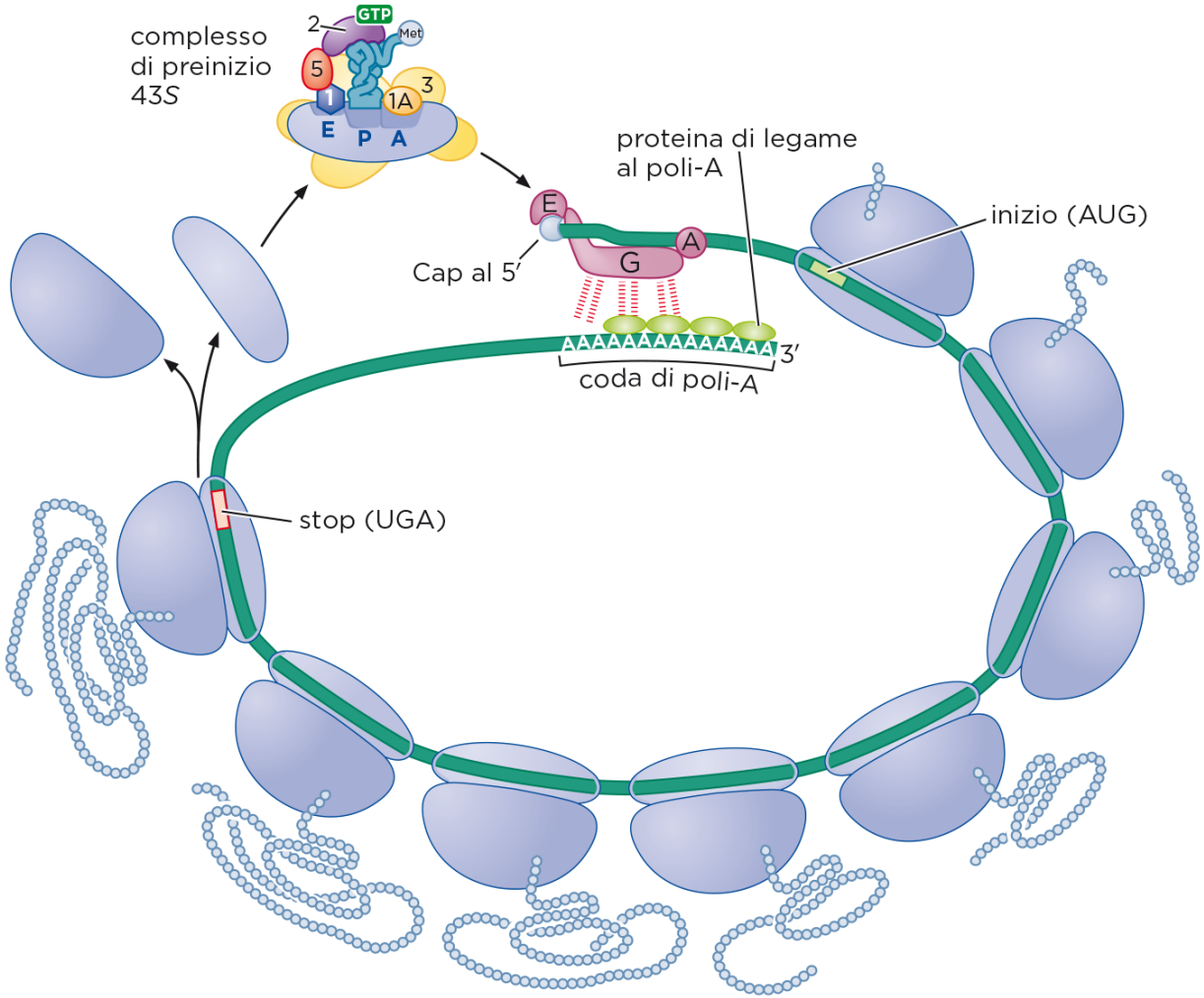
**B. mRNA + eIF4 initiation factors**

**C. 48S pre-initiation complex**

mRNA with eIF4s interacts with
43C pre-initiation complex
to form the 48S pre-initiation complex

# *Interaction between intitation factors and Poly-A tail increases effciency of initiation of translation*

## *Eucarioti*



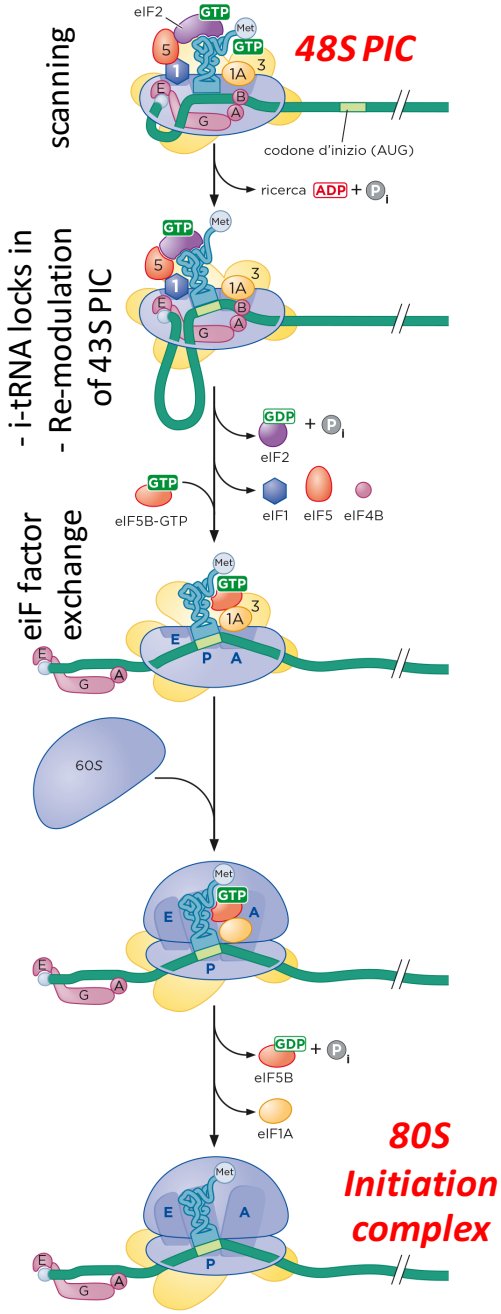

→Interaction of eIF4G with poly-A binding proteins

→circle formation

--> **Quality check of mRNA**

**→More efficient recycling of ribosomes after one cycle of translation**

# *Identification of the AUG start codon by the 48S PIC*




→ eIF4A/B helicase activity moves 30S subunit 5'→3' to find AUG (ATP→ADP+Pi)

→ Initiator tRNA (Met-tRNA$_i^{Met}$) locks in at FIRST AUG

Series of events:

1. eIF1 leaves 43S PIC
2. Structural change of eIF5
3. This mediates GTP hydrolysis by eIF2; eIF2 no longer able to interact with initiator tRNA and leaves complex together with eIF5

→ Now initiator tRNA (Met-tRNA$_i^{Met}$) can be bound be eIF5B-GTP; this stimulates the assembly of the 60S with the 40S subunit

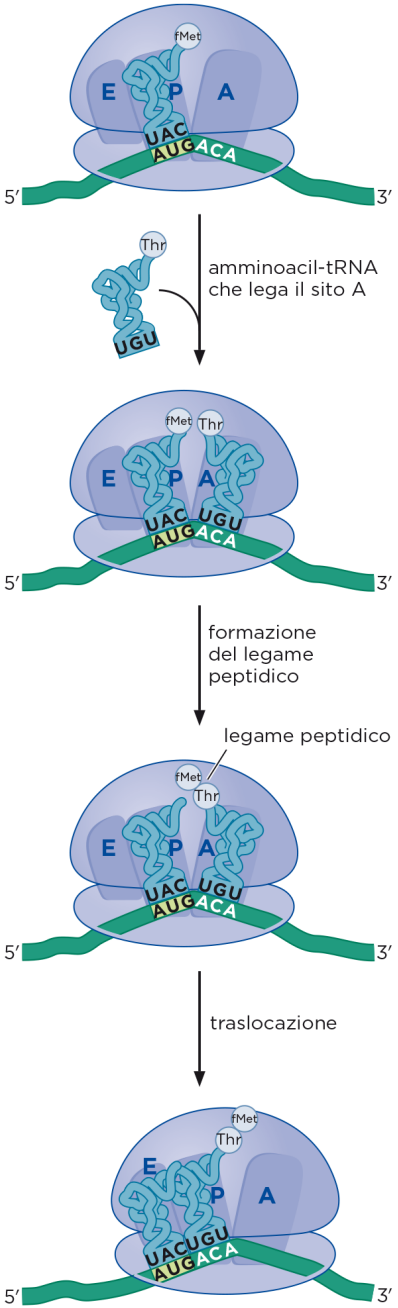
→ GTP hydrolysis by eIF5B, eIF5B-GDP and eiF4A leave complex

→ Initiator tRNA (Met-tRNA$_i^{Met}$) located in P-site; A-site and E-site are accessible = ***80S Initiation complex***

**READY FOR ELONGATION**

***Iniziazione; Eucarioti***

# Allungamento della traduzione - Elongation




***Translational elongation is HIGHLY CONSERVED between eukaryotes and prokaryotes***

*(slides are from **prokaryotes** unless otherwise indicated)*

*1. initiation complex*

*2. Correct aminoacyl-tRNA is loaded into A-site*

**Controlled by elongation factors (EFs)**

*3. Peptidyl-transferase reaction*

*4. Translocation of ribosome*

*5. A-site again free*

# *Allungamento della traduzione - Elongation*

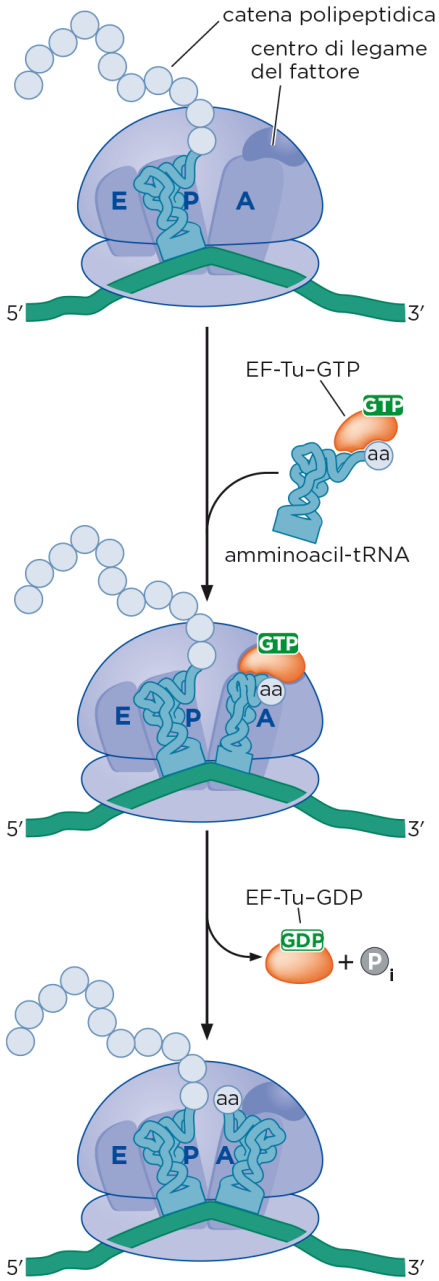
***Translational elongation is HIGHLY CONSERVED between eukaryotes and prokaryotes***

## 5.5 SINTESI PROTEICA

| Fattori proteici | | |
|---|---|---|
| **Procarioti** | **Eucarioti** | **Funzione** |
| Fattori di inizio | | |
| IF1 | | |
| IF2 | eIF2 | Formazione del complesso di inizio |
| IF3 | eIF3 | |
| | eIF4C, CBP | Legame del cappuccio 7-MG |
| | eIF5 | Dissociazione di eIF2, eIF3 e eIF4c |
| | eIF6 | Dissociazione dei ribosomi inattivi |
| Fattore di allungamento | | |
| EF-Tu | eEFLα | Inserimento dell'amminoacidil-tRNA |
| EF-Ts | eEF1βγ | Rigenerazione del fattore precedente |
| EF-G | eEF2 | Fattore di traslocazione |
| Fattore di rilascio | | |
| RF1 | eRF | |
| RF2 | | Rilascio della catena polipeptidica |
| RF3 | | |

Tabella 5.4
Elenco dei fattori proteici di inizio, allungamento e rilascio della catena polipeptidica di procarioti ed eucarioti.

# *EF-Tu assembles aminoacyl-tRNA into the A-site*




***EF-Tu is a GTP binding protein** that interacts with the major Ribosome subunit and charged tRNA to control the loading of aminoacyl-tRNA to the A-site and to protects from a premature peptidyl-transferase reaction*

→ EF-Tu-GTP binds to 3'-terminus of aminoacyl-tRNA

→ aminoacyl-tRNA - EF-Tu-GTP complex enters A-site of major subunit

→Only when the aminoacyl-tRNA anti-codon pairs with the mRNA codon (minor ribosome subunit) AND EF-Tu fits perfectly into a binding site (EF-Tu binding site) of the major subunit EF-Tu-GTP complex, GTPase activity is activated

→GTP-hydrolysis by EF-Tu GTPase activity results in the release of aminoacyl-tRNA; EF-Tu leaves ribosome to be recycled.

**NEXT STEP: PEPTIDYL-TRANSFERASE REACTION**

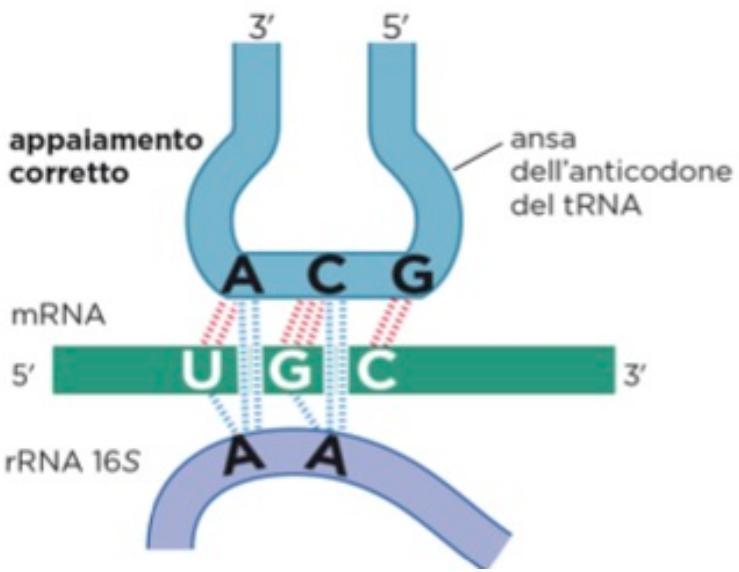
***Allungamento; Eucarioti***

# *Mechanisms that ensure correct reading of mRNA codons*

## *1. 2 adenine of 16S rRNA stabilize codon-anticodon interaction*

**ACCURATEZZA**

Correct codon-anticodon pairing



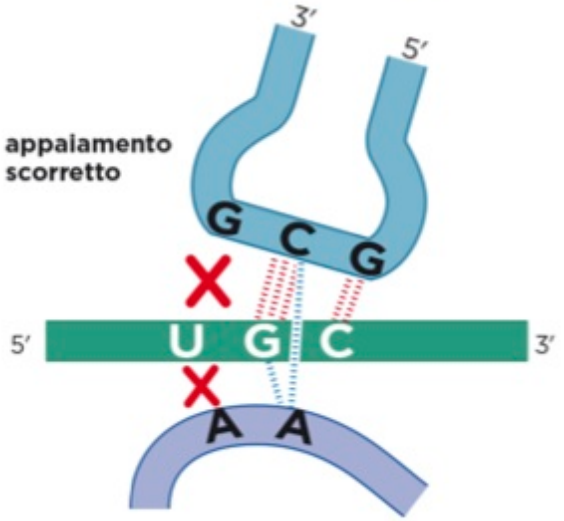

→2 adenine in 16S rRNA (minor subunit)
can form hydrogen bonds with the
minor groove of the paired codon-anticodon region

→Stabilisation of aminoacyl-tRNA – EF-Tu-GTP complex on codon

→EF-Tu-GTP interacts with its ribosomal binding site and activates GTPase activity

→ Peptidyltransferase reaction

In-Correct codon-anticodon pairing




→ missmatch between codon and anticodon

→2 adenine in 16S rRNA (minor subunit)
CANNOT form hydrogen bonds with the
minor groove of the paired codon-anticodon regic

→EF-Tu-GTP CANNOT activate GTPase activity

→aminoacyl-tRNA – EF-Tu-GTP complex leaves A-site

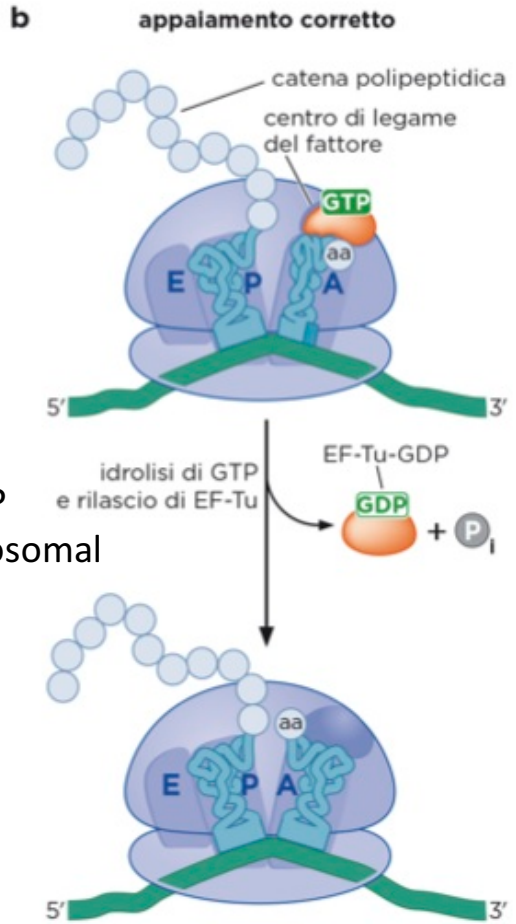
→New attempt of correct loading of aminoacyl-tRNA

# *Mechanisms that ensure correct reading of mRNA codons*

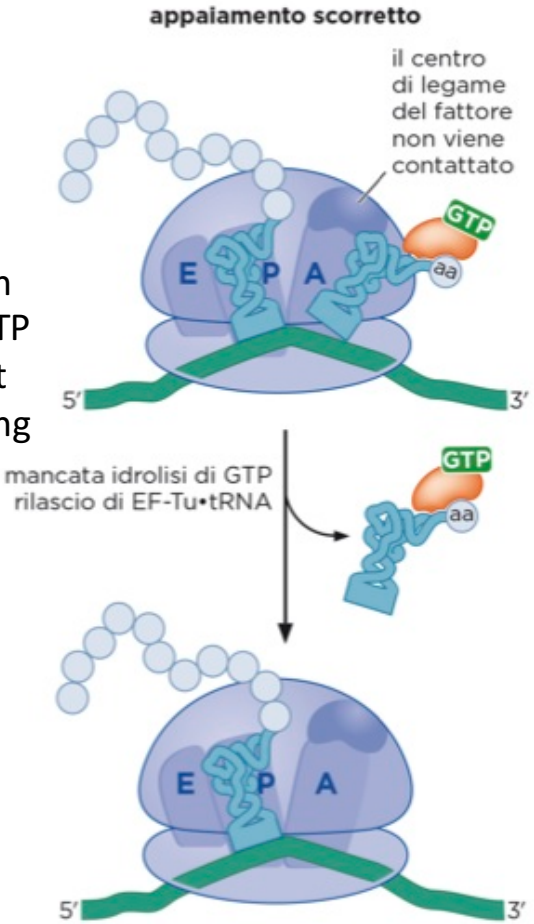
## *2. Interaction of EF-Tu-GTP with itsribosomal binding site (EF-Tu binding site)*

**ACCURATEZZA**

Perfect codon-anticodon Pairing: EF-Tu-GTP Interacts with ribosomal binding site




Inperfect codon-anticodon Pairing: EF-Tu-GTP CANNOT interact with EF-Tu binding site



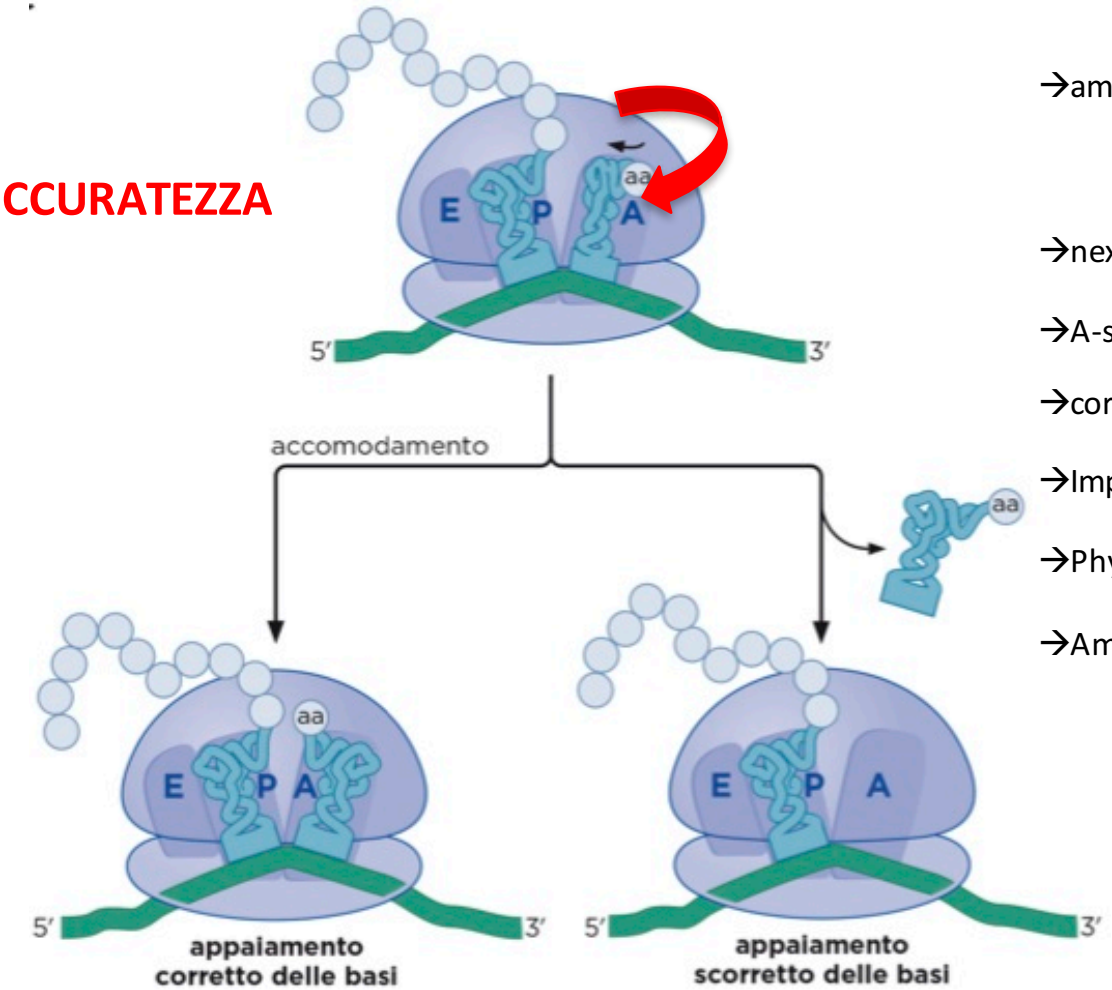

**NEGATIVE SELECTION**

→DE-stabilisation of aminoacyl-tRNA – EF-Tu-GTP complex on codon

# *Mechanisms that ensure correct reading of mRNA codons*

## *3. Accomodation of amminoacyl-tRNA in A-site*

**ACCURATEZZA**




→amminoacyl-tRNA is loaded to A-site
with aminoacid pointing outwards. This prevents unwanted peptidyltransferase reactions

→next, 3'terminus of aminiacyl-tRNA will be twisted to pull

→A-site aminoacid close to nascent peptide chain

→correct codon-anticodon pairing resist physical force

→Imperfect correct codon-anticodon pairing

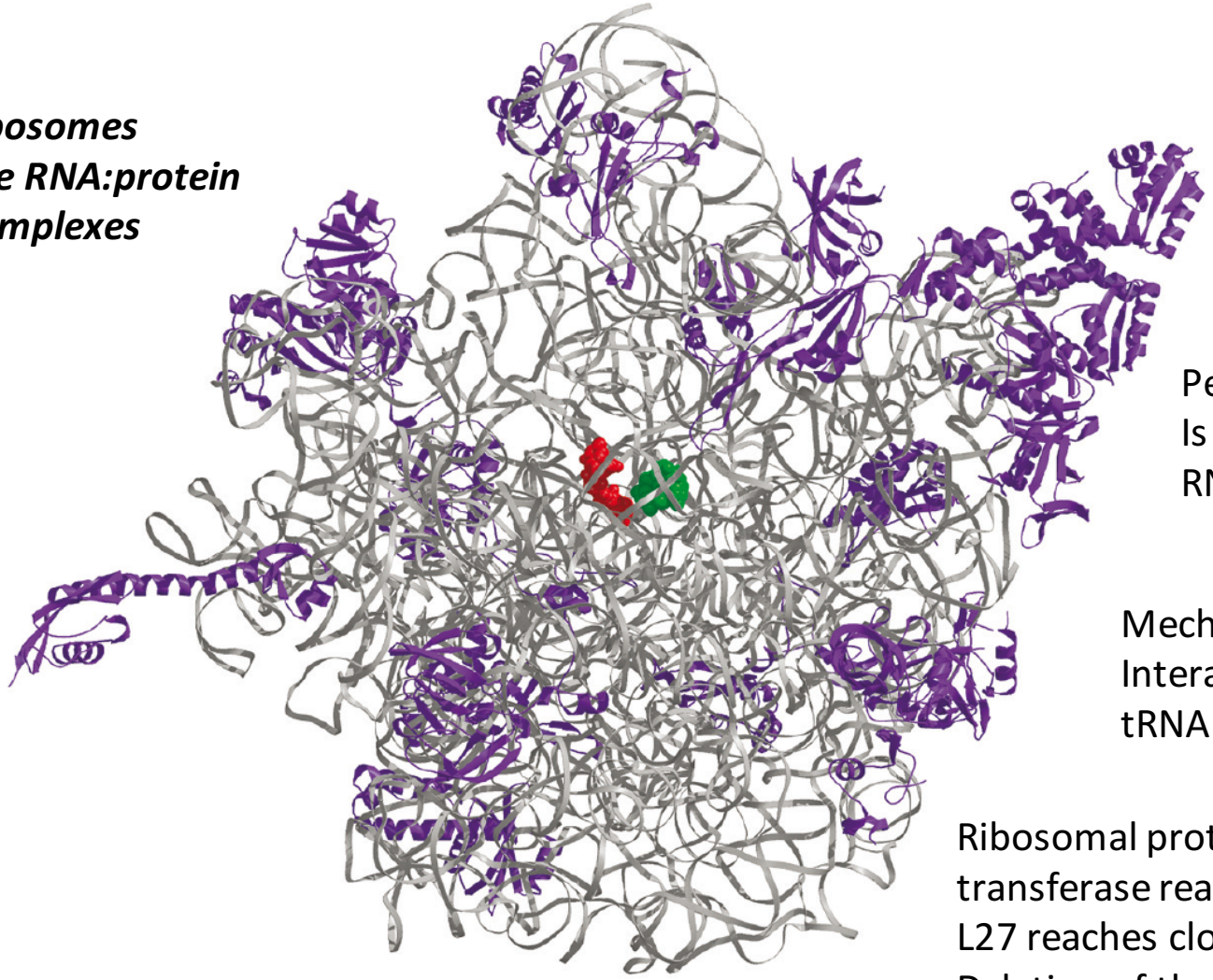
→Physical force seperates codon from anitcodon

→Aminoacyl-tRNA leaves ribosome

**NEGATIVE SELECTION**

# *The peptidyl-transferase reaction and translocation*

***ribosomes are RNA:protein complexes***



Major ribosome subunit

P-site

A-site

Peptide synthesis
Is catalyzed by ribosomal
RNA (23S rRNA, major subunit)

Mechanism involves
Interaction between rRNA and
tRNA but is unclear

Ribosomal protein L27 supports peptidyl-transferase reaction.
L27 reaches close to active center;
Deletion of these parts slow down
Synthesis, but do not abolish peptidyl-
Transferase activity

# *The peptidyl-transferase reaction and translocation*

## A. "Major subunit action"

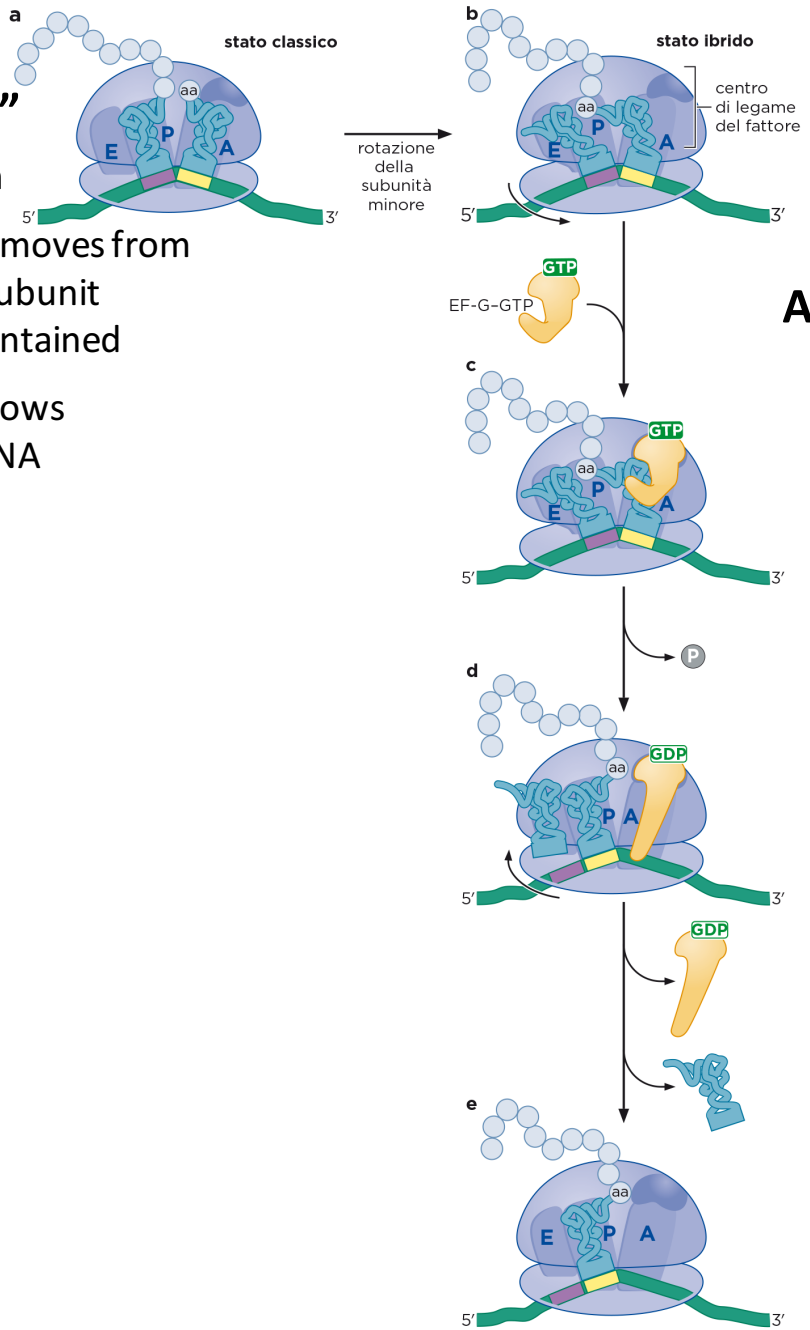
→ peptidyl-transferase reaction

→3' end of new peptidyl-tRNA moves from A-site to P-site in major subunit

→Codon-anticodon pairing maintained

→Minor subunit rotates and allows new interaction tRNA-rRNA

**= hybrid state**




## A. "EF-G action"

→EF-G-GTP binds to EF-G binding site after peptidyl-transferase reaction

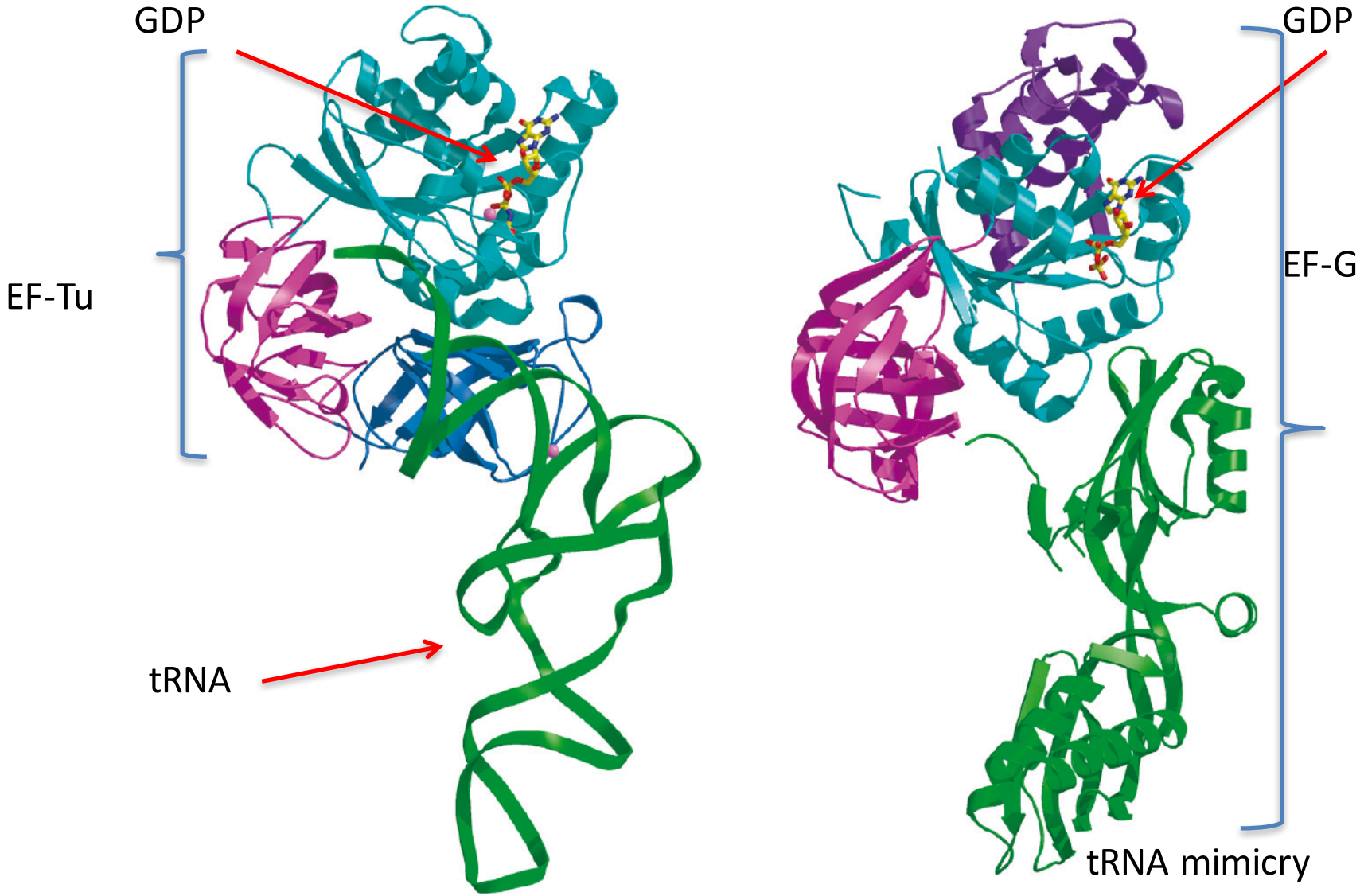
→GTP hydolysis, structural change EF-G-GDP occupies A-site

→"gate" between A and P site opens and permits passage of A-site Peptidyl-tRNA to P-site
P-site tRNA shifted to E-site

→New codon becomes available in A-site (ribosome moves forward)

→Minor subunit shifts back into original position

# *EF-G represents a EF-Tu-tRNA molecular mimicry*




"mimetism molecolare"

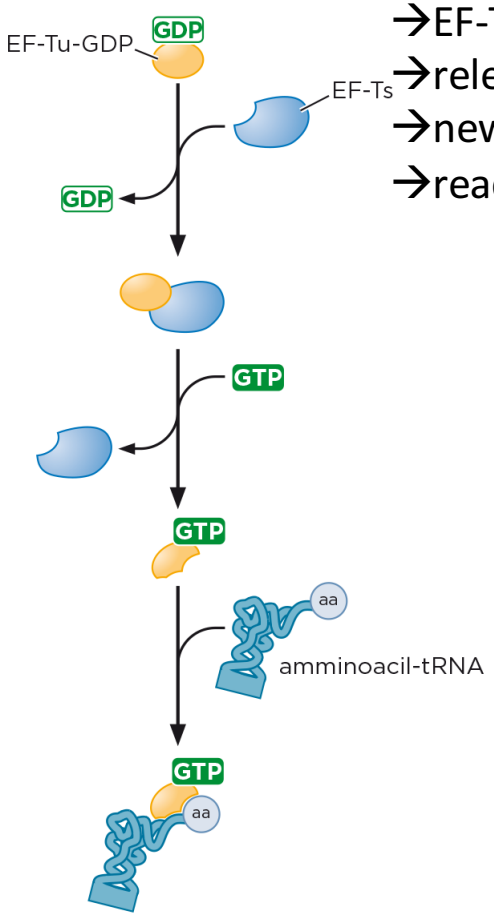
# *Recycling of EF-Tu and EF-G*

→For each peptidyl-transferase reaction 1 EF-G-GTP and 1 EF-Tu-GTP are hydrolyzed
→ Recycling by exchanging GDP for GTP

## EF-G

→ GTP hydrolyzed to GDP+Pi
→ GDP has low affinity for EF-G
→ new GTP replaces GDP
→ EF-G-GTP ready for next elongation step

## EF-Tu




→EF-Ts binds EF-Tu-GDP
→release of GDP
→new GTP added to EF-Tu
→ready for next elongation

# *ENERGY CONSUMPTION DURING TRANSLATION*

Amminoacyl-tRNA synthetase: 1 ATP for adding aminoacid to tRNA 3’ end

EF-Tu: 1 GTP for fitting correct aminoacyl-tRNA into A-site (proofreading function)

EF-G: 1 GTP translocation of ribosome (A-P-E site passage)

**Peptide+1 AA: 1 ATP, 2 GTPs**

# TERMINATION OF TRANSLATION

**STOP CODONS:**

UAG: codone Ambra (scoperto da Harris Bernstein
UAA: codone Ocra
UGA: codone Opale

**→No tRNA-aminoacyl loading in A-site**
**→Polypeptide chain must be cleaved from peptidyl-aminoacyl tRNA located in P-site**

**TERMINATION IS A 2 STEP PROCESS CONTROLLED BY RELEASE FACTORS (RFs)**

**Prokaryotes:**
Class 1 RFs: RF1: recognizes UAG
RF2: recognizes UGA
RF1+RF2 recognize UAA

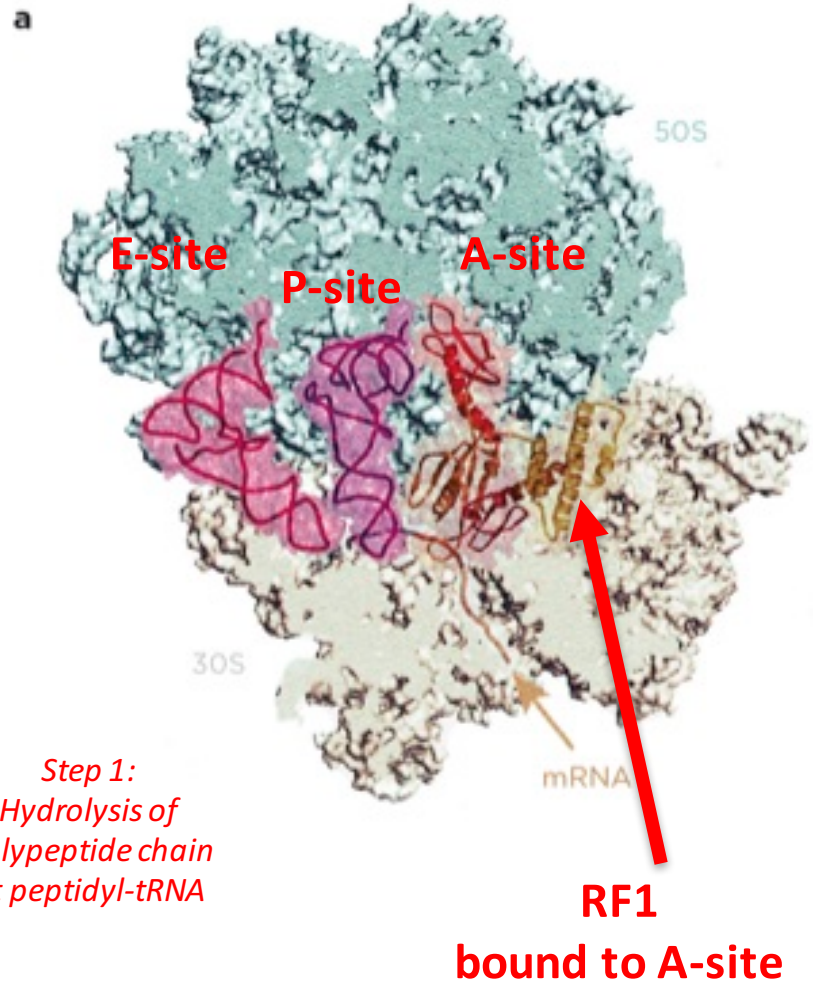
**Eukaryotes:** only one class I factor: eRF1 recognizes UAG, UAA, UGA

*Step 1: Hydrolysis of Polypeptide chain at peptidyl-tRNA*

**Prokaryotes:** one class II RF: RF3

**Eukaryotes:** one class II RF: eRF3

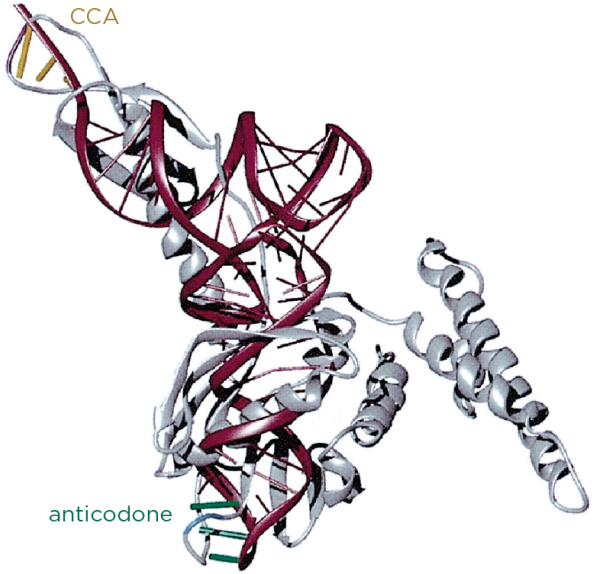
*Step 2: Release of class 1 RFs from ribosome*



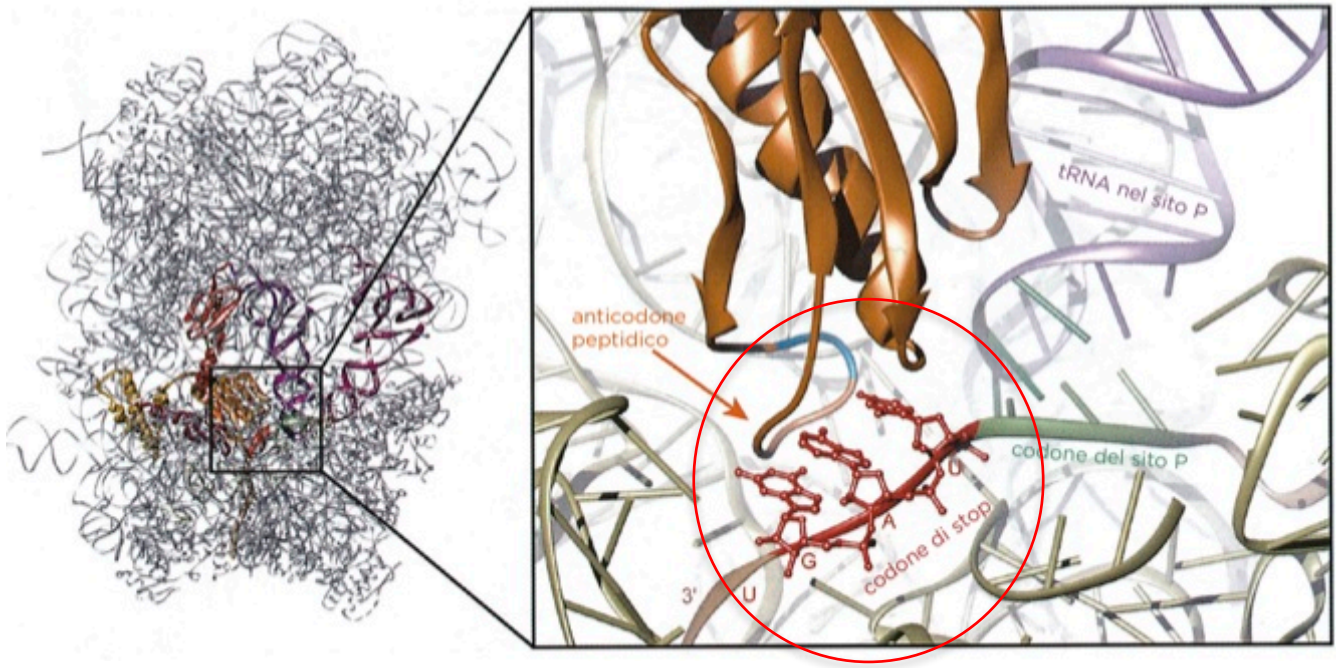
# *TERMINATION OF TRANSLATION BY RFs*

Class 1 RFs contain **PEPTIDE ANTICODONs** (anticodon peptidico)

→3 aminoacids recognize STOP codons in mRNA template
→ exchanging peptide anticodons between RF1 (UAG) and RF2 (UGA) exchanges stop codon specificity!!




*RF1 and RF2*
***structure is similar to tRNAs***



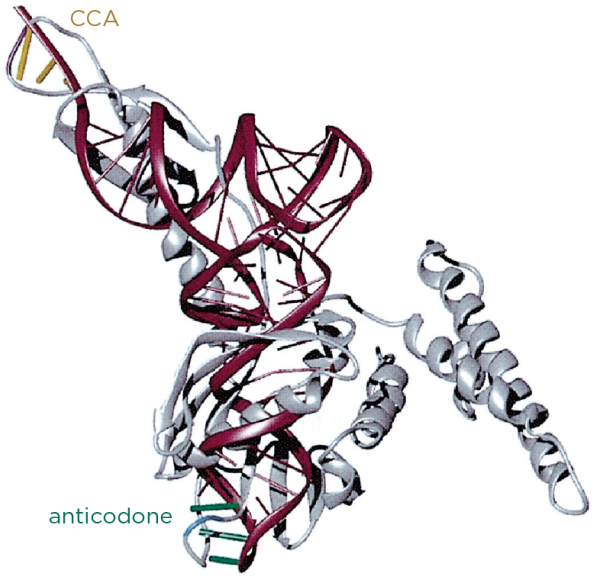

Peptide anticodon
interacts with stop codon
in A-site
(other protein motifs support interaction of RF1/RF2 with ribosome)

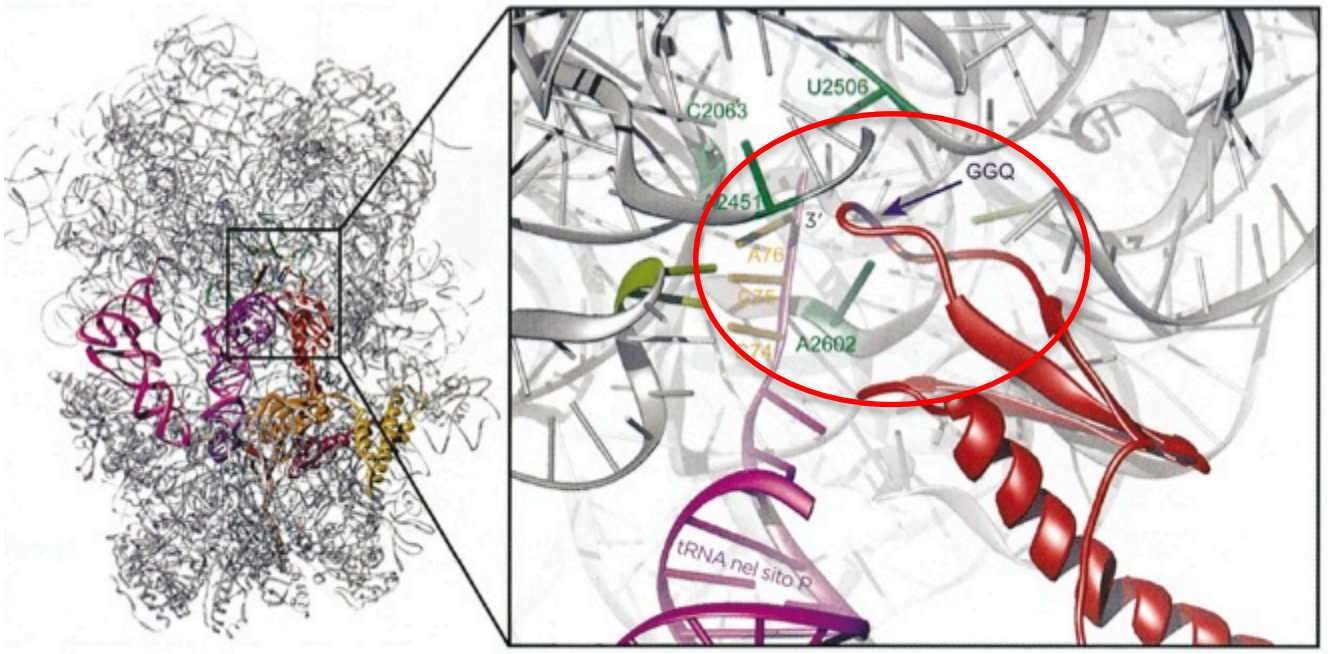
# TERMINATION OF TRANSLATION BY RFs

RF1 an RF2 contain a GGQ (Gycin-Glycin-Glutamin) motif that is located close to the peptidyl-transferase center of the major ribosomal subunit

GGQ (Gycin-Glycin-Glutamin) promotes hydrolysis of the nascent polypeptide chain

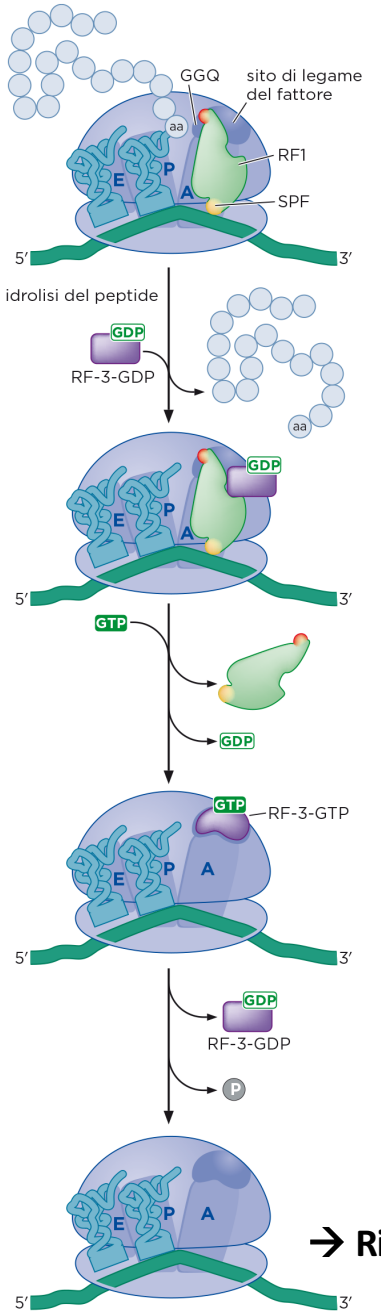
**→→ GGQ (Gycin-Glycin-Glutamin) motif and the peptide anticodon mimic central functions of a tRNA**




*RF1 and RF2*
***structure is similar***
***to tRNAs***
***→Anticodon peptide***
***→GQQ motif***

# TERMINATION OF TRANSLATION BY RFs




→ hydrolysis of polypeptide chain stimulated by class I RFs

→ RF3-GDP is loaded to the ribosome only when RF1 is present

→ release of polypeptide stimulated by class I RFs

→ This permits the exchange of GDP for GTP in RF3

→ Conformational change

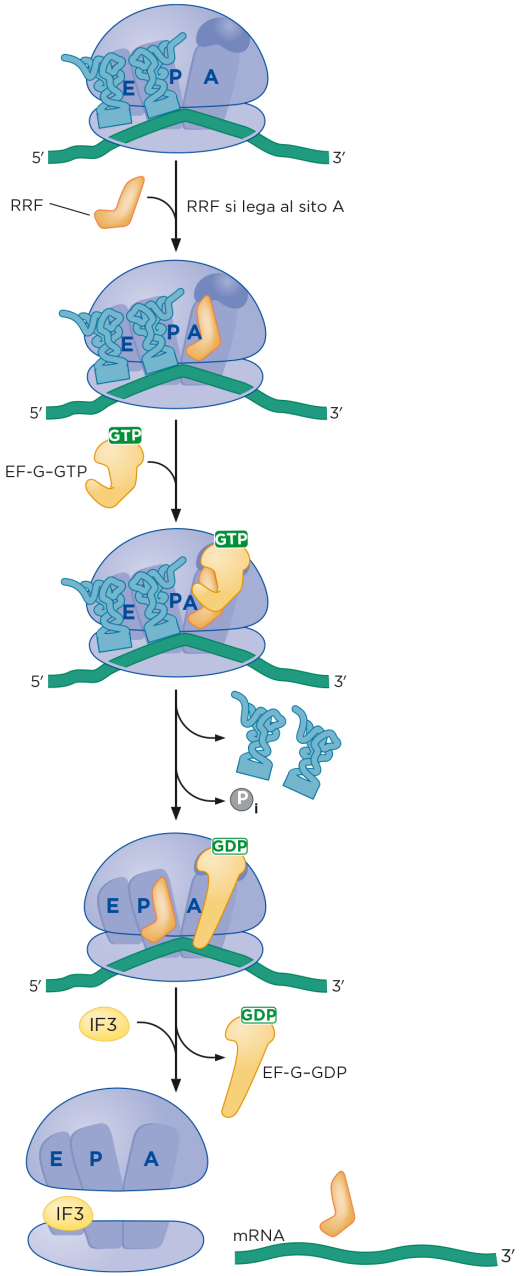
→ This allows to bind RF3-GTP to its binding site in the major subunit; RF1 expulsed

GTP hydolysis; RF3-GDP has low affinity for ribosome and dissociate

**TRANSLATION TERMINATED**

→ **Ribosome + E+P site tRNAs enters into recycling**

# *RIBOSOME RECYCLING – Riciclaggio del ribosoma*




## RIBOSOME RECYCLING FACTOR (RRF)

→ RRF mimics tRNA and enters A-site

→ RRF recruits EF-G-GTP into A-site

→GTP-hydrolysis, P and E site tRNAs will be removed and RFF enters P-site (mimicing the transfer of tRNA from A to P site)

→GTP-hydrolysis, P and E site tRNAs will be removed and RFF enters P-site (mimicing the transfer of tRNA from A to P site)

→EF-G-GDP leaves major subunit

→EF-G-GDP leaves major subunit and IF3 aims to bind the Minor ribosome subunit

→Disassembly of ribosome, mRNA

→IF3 is located again to E-site

→NEW RIBOSOME CYCLE CAN INITIATE

# THE RIBOZYME IS A PREFERRED TARGET FOR ANTIBIOTICS

→ Antibiotics are produced by bacteria and funghi

→ 40% of antibiotics target the ribosome, 1% of total antibiotics useful for medicine

→ Frequently bind components of the translation machinery

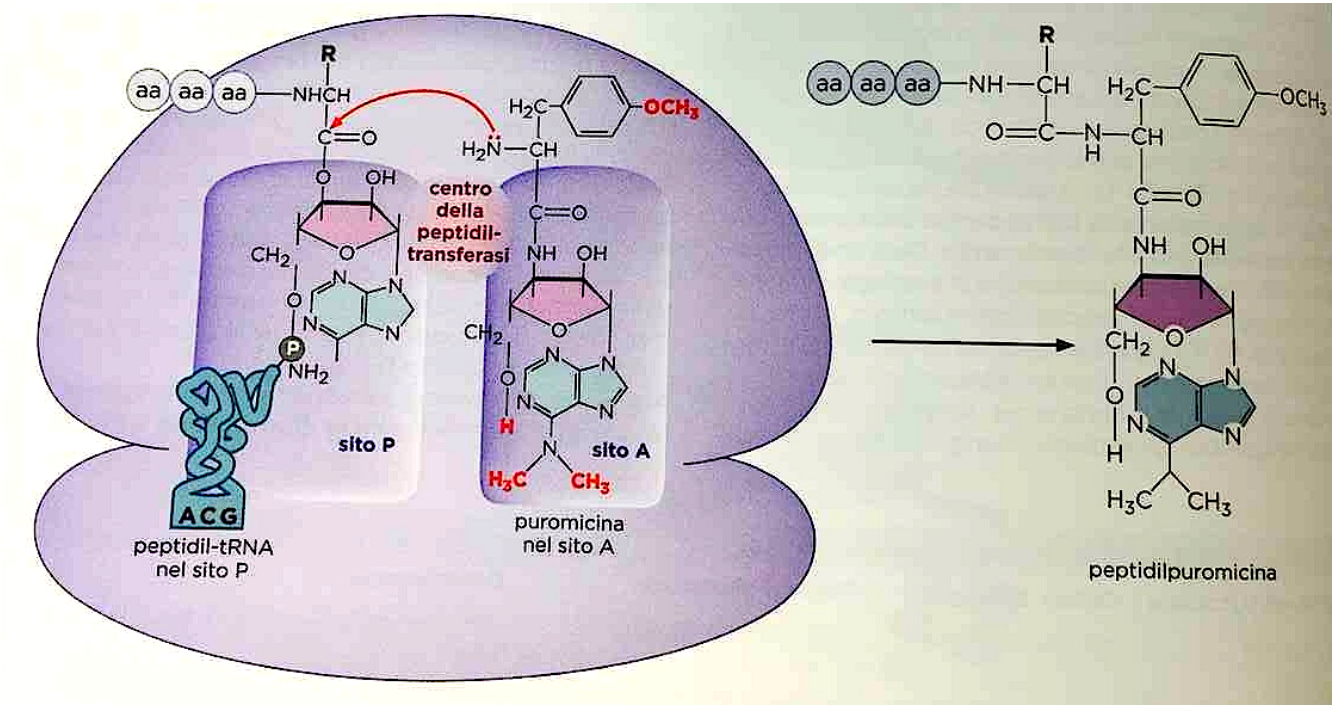
→ Antibiotics take advantage of the requirement for precise ribosome structure for translation (differences in structure can mediate antibiotics resistance!!)

→ Translation blocked – bacterium dies

Example: PUROMYCIN: very efficient anitbiotics
→ mimics an aminoacyl-tRNA in the ribosome A site
→ polypeptide chain will be transferred to puromycin
→polypeptide-puromycin released from ribosome

*The use of ribosome targeting antibiotics is a strong tool to Understand the ribosome function*

***PUROMYCIN***
***targets pro- and eukaryote ribosomes***



# THE RIBOZYME IS A PREFERRED TARGET FOR ANTIBIOTICS

| Antibiotici, tossine | Cellule bersaglio | Bersagli molecolari | Conseguenze |
|---|---|---|---|
| Tetraciclina | cellule procariotiche | sito A della subunità 30S | inibisce il legame dell'amminoacil-tRNA al sito A |
| Igromicina B | cellule procariotiche ed eucariotiche | prossimità del sito A della subunità 30S | impedisce la traslocazione del tRNA dal sito A al sito P |
| Paromicina | cellule procariotiche | adiacente al sito dell'interazione codone-anticodone nel sito A della subunità 30S | aumenta la frequenza di errori durante la traduzione, diminuendo la selettività dell'appaiamento codone-anticodone |
| Cloramfenicolo | cellule procariotiche | centro della peptidiltransferasi della subunità 50S | blocca il corretto posizionamento dell'amminoacil-tRNA nel sito A per la reazione di trasferimento della peptidiltransferasi |
| Puromicina | cellule procariotiche ed eucariotiche | centro della peptidiltransferasi della subunità ribosomiale maggiore | terminatore della catena; simula l'estremità 3' dell'amminoacil-tRNA del sito A e si comporta da accettore della catena polipeptidica nascente |
| Eritromicina | cellule procariotiche | canale d'uscita del peptide della subunità 50S | blocca l'uscita della catena polipeptidica nascente dal ribosoma; arresta la traduzione |
| Acido fusidico | cellule procariotiche | EF-G | impedisce il rilascio di EF-G-GDP dai ribosomi |
| Tiostreptone | cellule procariotiche | centro di legame del fattore della subunità 50S | interferisce con l'associazione di IF2 ed EF-G con il centro di legame del fattore |
| Chirromicina | | EF-Tu | impedisce i cambiamenti conformazionali associati all'idrolisi di GTP e di conseguenza il rilascio di EF-Tu |
| Ricino e α-sarcina (tossine proteiche) | cellule procariotiche ed eucariotiche | modificano chimicamente l'RNA nel centro di legame del fattore della subunità ribosomiale maggiore | impediscono l'attivazione delle GTPasi associate al fattore di traduzione |
| Tossina difterica | cellule eucariotiche | modifica chimicamente EF-Tu | inibisce la funzione di EF-Tu |
| Cicloesimmide | cellule eucariotiche | centro della peptidiltransferasi della subunità 60S | inibisce l'attività peptidiltransferasica |

→ (segu