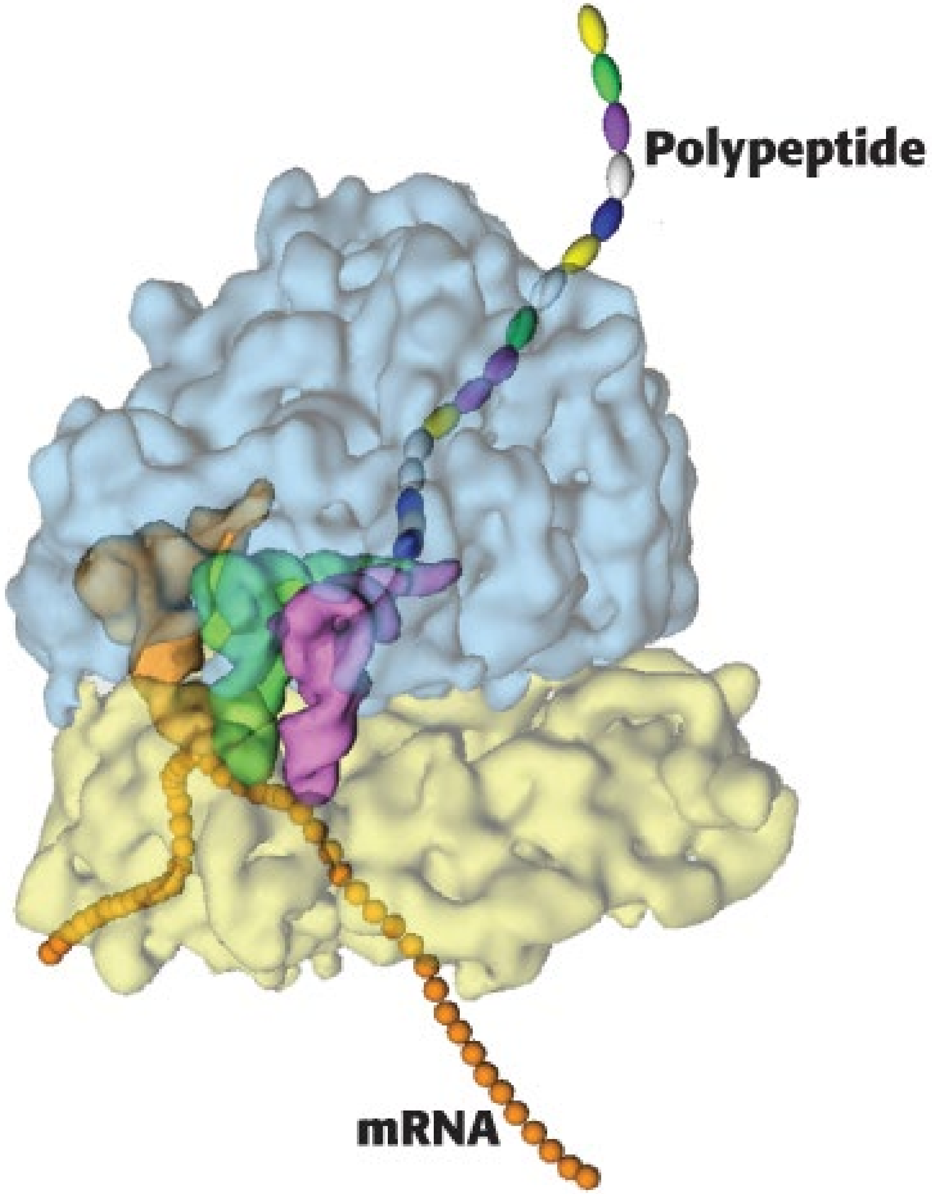

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Protein Biosynthesis
(Translation)

Chapter 8 and Chapter 20

Ana Luis
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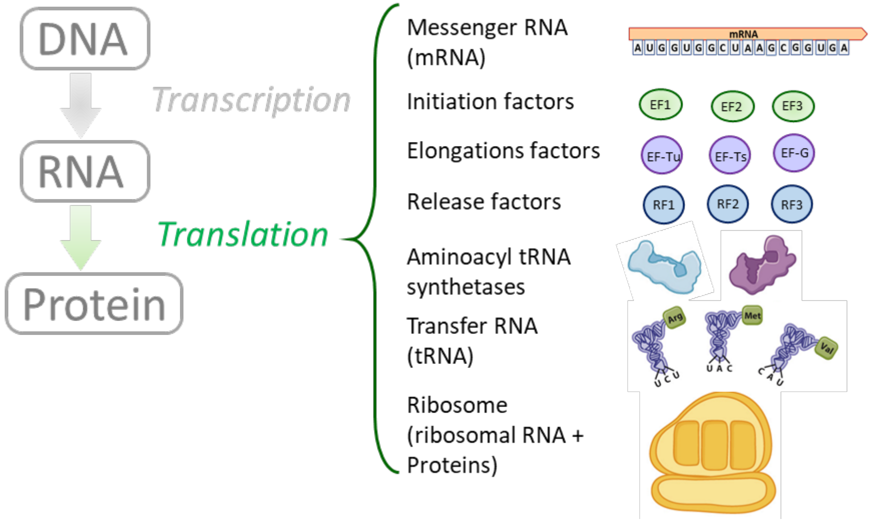
2025/04/28

Learning goals

- Explain how nucleic acid information is translated into an amino acid sequence and define the role of aminoacyl tRNA synthetases in this process
  - Protein biosynthesis requires the translation of nucleotide sequences into amino acid sequences
  - Describe features of the genetic code
  - tRNA structure and function
  - Aminoacyl-tRNA synthetases establish the genetic code

- Define the role of ribosomes in protein synthesis
  - Structure and function of ribosomes
  - Mechanism of protein synthesis (initiation, elongation, translocation and termination)

- Describe how certain chemicals can inhibit protein synthesis
  - Antibiotics and toxins inhibit protein synthesis



DNA

Transcription

RNA

Translation

Protein

Messenger RNA (mRNA)

Initiation factors

Elongations factors

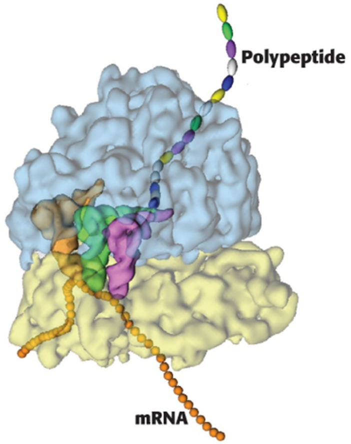
Release factors

Aminoacyl tRNA synthetases

Transfer RNA (tRNA)

Ribosome (ribosomal RNA + Proteins)

Why is important to understand the translation mechanisms?



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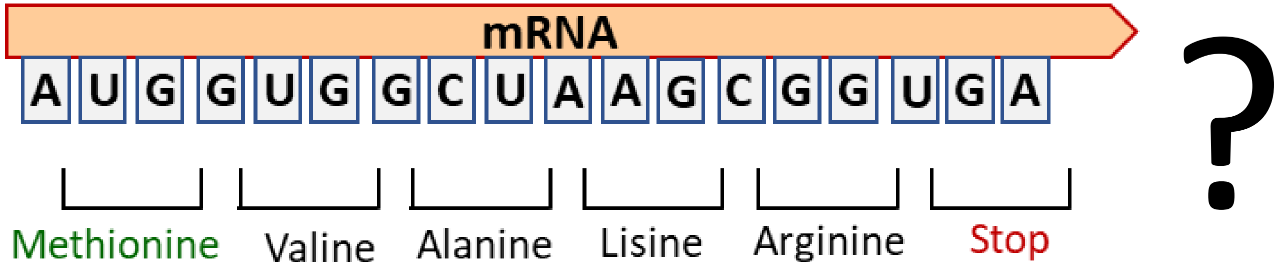


TRANSLATION

Inhibition of translation of specific mRNAs can lead to **diseases**

Fragile X mental retardation syndrome:
absence of the set of protein isoforms, derived from alternative splicing of the Fragile X mental retardation gene 1 (FMR1)

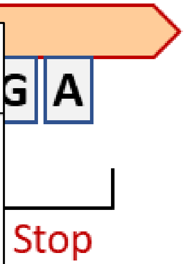
Bacterial toxins can block protein biosynthesis

Diphtheria toxin

Several **antibiotics** are inhibitors of protein biosynthesis

Streptomycin
Tetracycline
…..

Why is important to understand translation?



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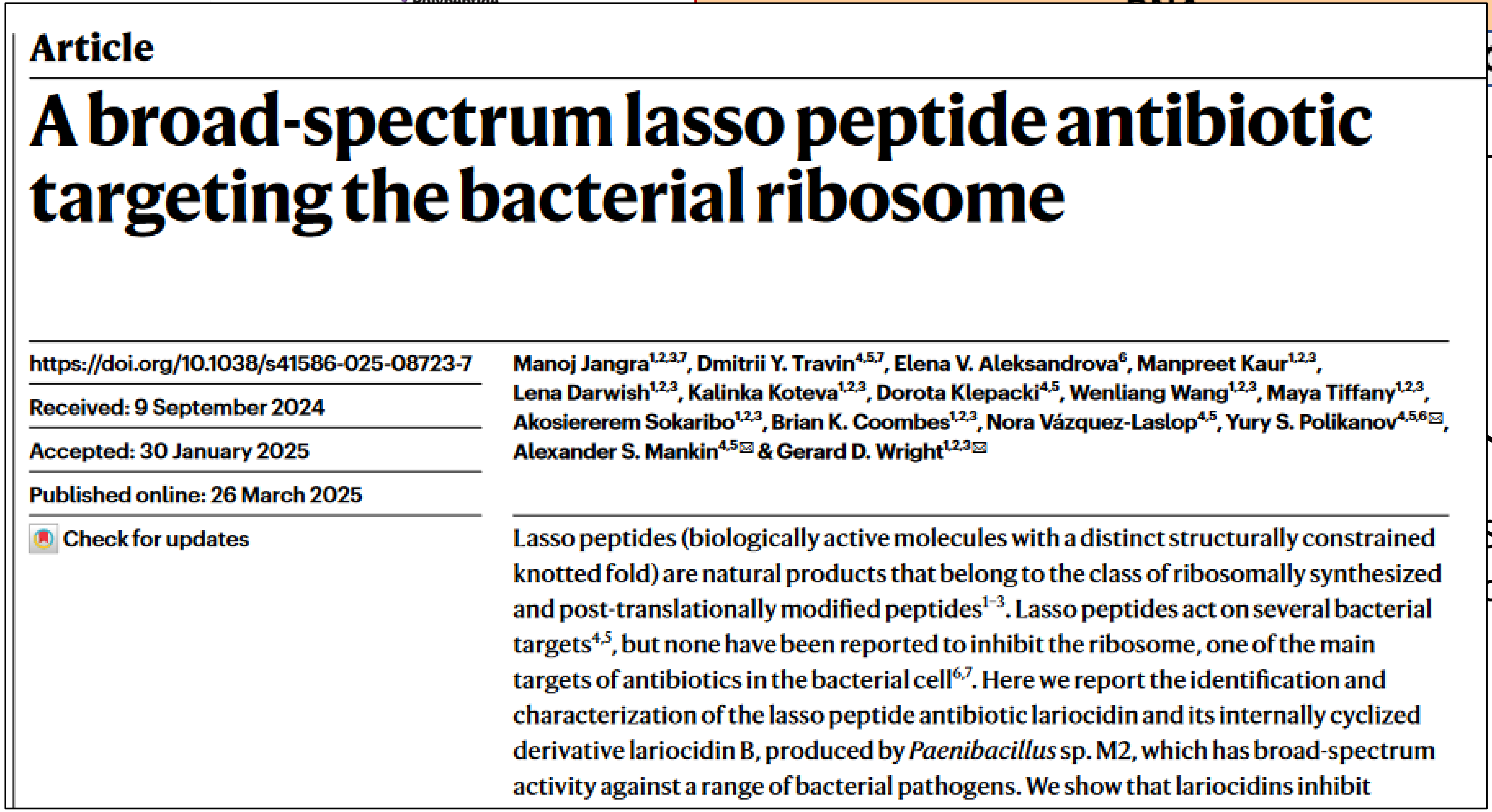


Article

A broad-spectrum lasso peptide antibiotic targeting the bacterial ribosome

https://doi.org/10.1038/s41586-025-08723-7
Received: 9 September 2024
Accepted: 30 January 2025

Manoj Jangra[1,2,3,7], Dmitrii Y. Travin[4,5,7], Elena V. Aleksandrova[6], Manpreet Kaur[1,2,3], Lena Darwish[1,2,3], Kalinka Koteva[1,2,3], Dorota Klepacki[4,5], Wenliang Wang[1,2,3], Maya Tiffany[1,2,3], Akosiererem Sokaribo[1,2,3], Brian K. Coombes[1,2,3], Nora Vázquez-Laslop[4,5], Yury S. Polikanov[4,5,6] ✉, Alexander S. Mankin[4,5] ✉ & Gerard D. Wright[1,2,3] ✉

Lasso peptides (biologically active molecules with a distinct structurally constrained knotted fold) are natural products that belong to the class of ribosomally synthesized and post-translationally modified peptides[1–3]. Lasso peptides act on several bacterial targets[4,5], but none have been reported to inhibit the ribosome, one of the main targets of antibiotics in the bacterial cell[6,7]. Here we report the identification and characterization of the lasso peptide antibiotic lariocidin and its internally cyclized derivative lariocidin B, produced by *Paenibacillus* sp. M2, which has broad-spectrum activity against a range of bacterial pathogens. We show that lariocidins inhibit

Several **antibiotics** are inhibitors of protein biosynthesis

Streptomycin
Tetracycline
…..

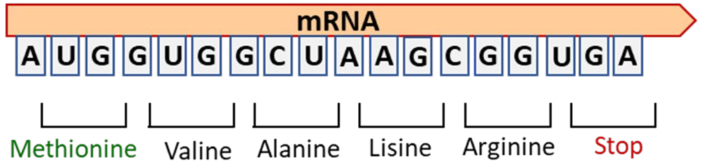
Translation

= the process of protein biosynthesis

Nucleic acid

Adenine **(A)**
Cytosine **(C)**
Guanine **(G)**
Uracil **(U)**

Translation



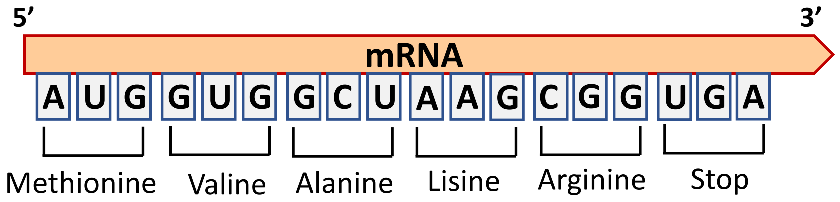
Amino acids

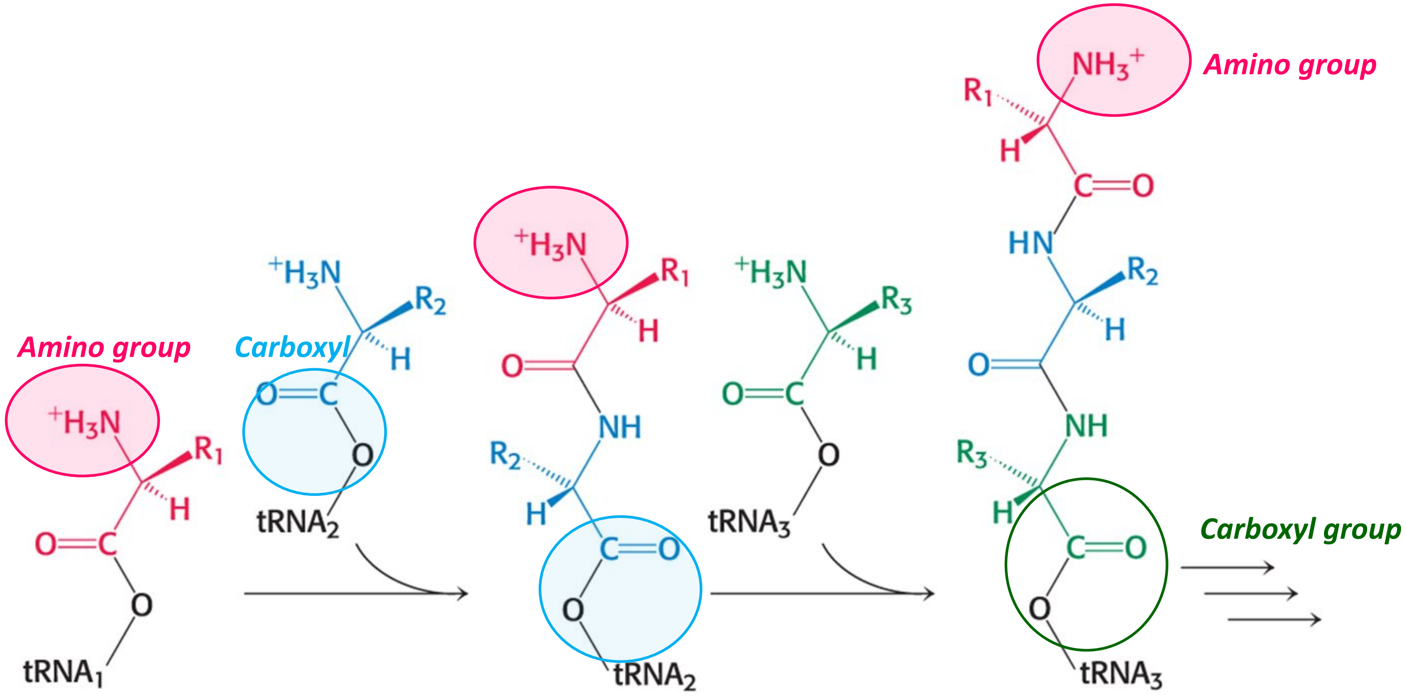
(A) Alanine
(R) Arginine
(N) Asparagine
(D) Aspartic acid
(C) Cysteine
(E) Glutamic acid
(Q) Glutamine
(G) Glycine
(H) Histidine
(I) Isoleucine
(L) Leucine
(K) Lysine
(M) Methionine
(F) Phenylalanine
(P) Proline
(S) Serine
(T) Threonine
(W) Tryptophan
(Y) Tyrosine
(V) Valine

Translation = the process of protein biosynthesis

The basics of protein biosynthesis are the same across all kingdoms of life:

- mRNA is decoded in the 5′-to-3′ direction one codon at a time
- the protein is synthesized in the amino-to-carboxyl direction





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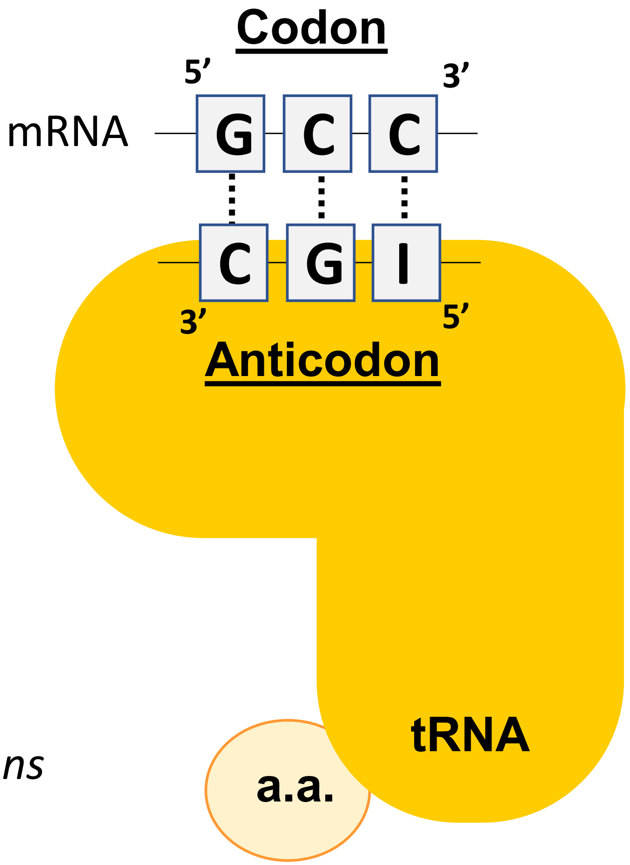
Translation = the process of protein biosynthesis

The basics of protein biosynthesis are the same across all kingdoms of life:

- **codon** - three coding bases on the mRNA template
- **transfer RNA (tRNA)** - function as adaptor molecules between a codon and an amino acid (a.a.)
- **anticodon** - portion of the tRNA that base pairs with the codon

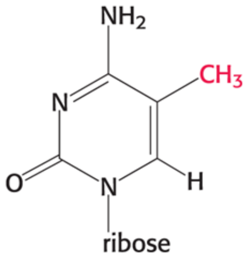
The correct protein biosynthesis requires an accurate recognition of codons by anticodons



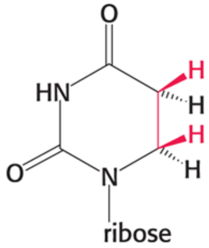
General characteristics of Transfer RNA (tRNA) molecules

- Each tRNA is a **single chain** containing between 73 and 93 **nucleotides**.

- tRNAs contain 7 to 15 **unusual bases**
  - methylated or demethylated derivatives of A, U, C, and G



5-Methylcytidine (mC)

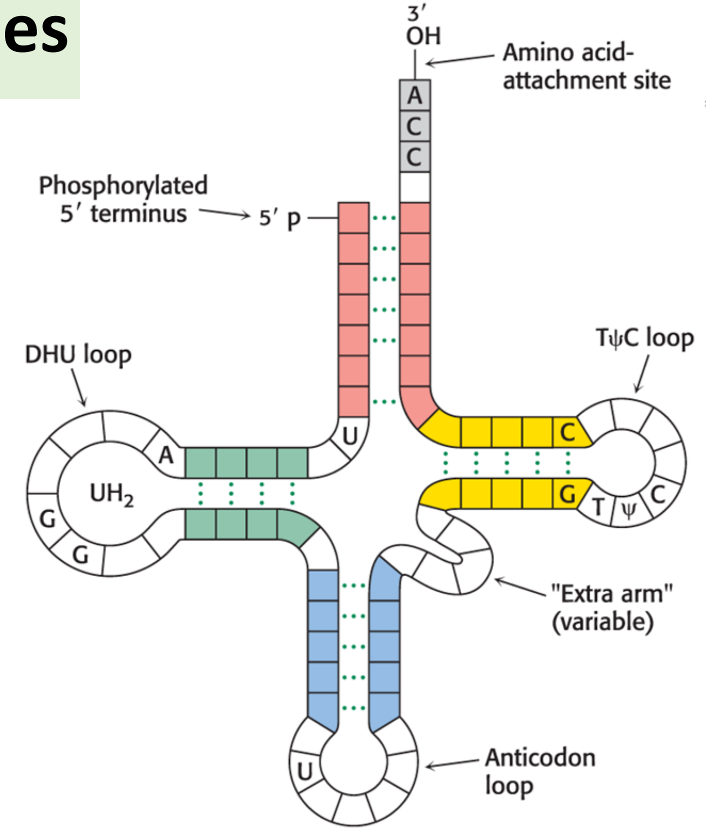


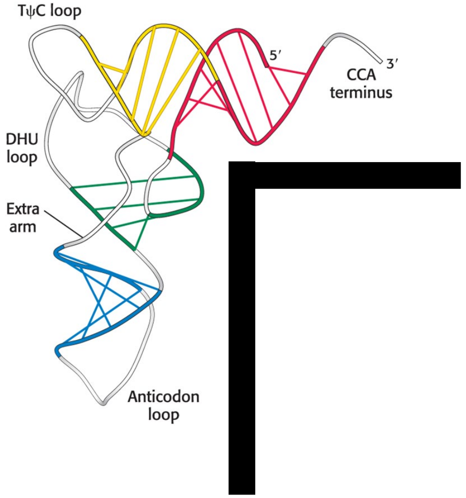
Dihydrouridine (UH_2)

- The **secondary structure** resembles a **cloverleaf**
  (~50% of the nucleotides are base-paired)

Five groups of bases are not base-paired, but participate in hydrogen-bonding interactions:
  - 3′ CCA terminal region (*acceptor stem*)
  - TψC loop
  - “extra arm”
  - anticodon loop
  - DHU loop

- The **three-dimensional structure is L-shaped**





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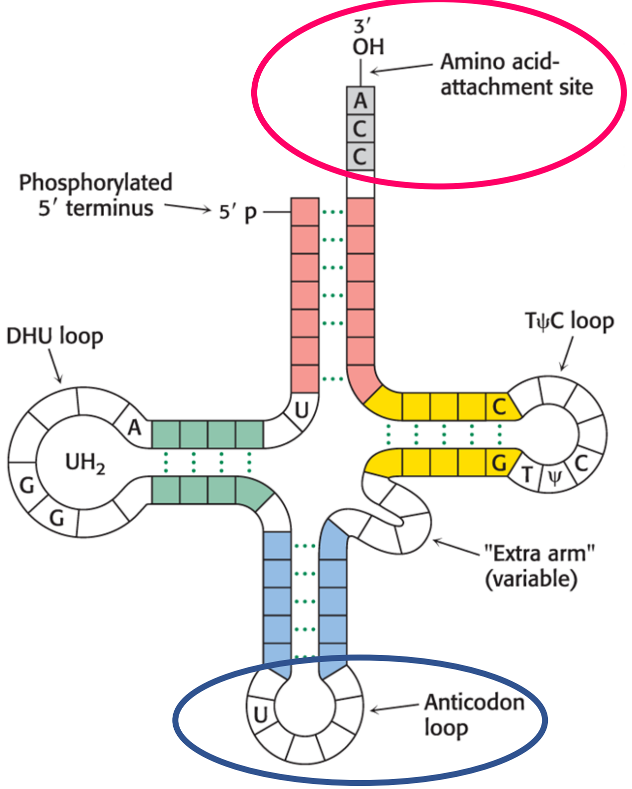
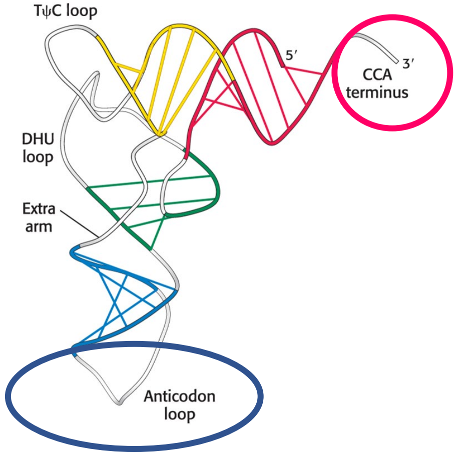
General characteristics of tRNA molecules

- The **anticodon loop** is near the center of the sequence

- **At the 3’ end, an activated amino acid is attached to** a hydroxyl group of **adenosine in the CCA region of the acceptor stem**
  - the CCA region has the ability to change its conformation during protein synthesis





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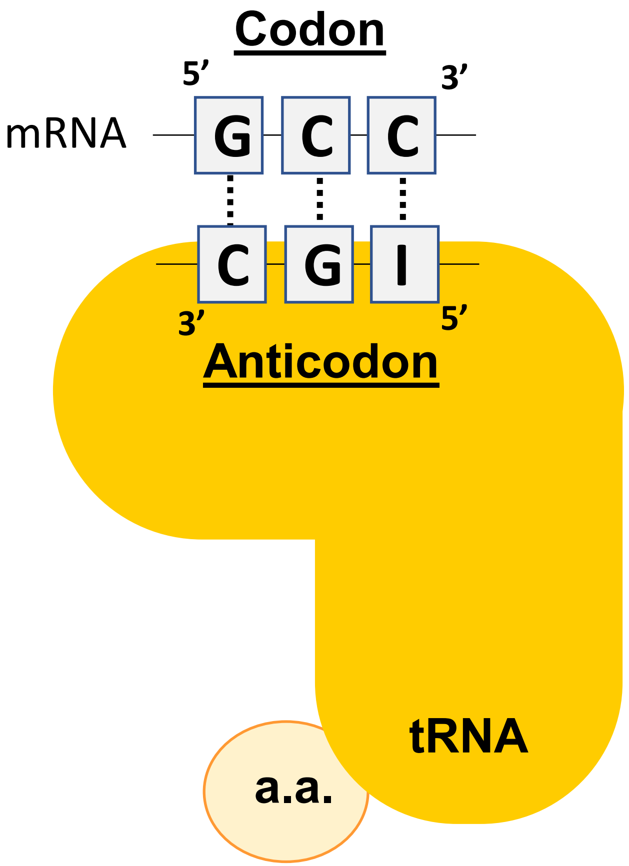
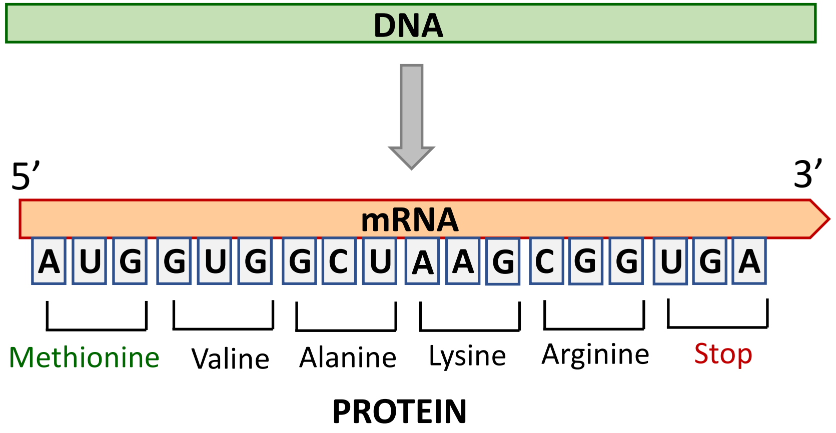
Genetic code

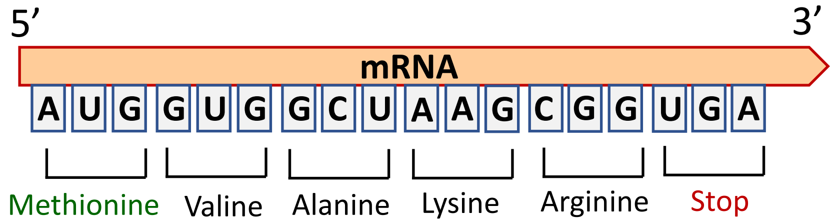


- Genetic code: the relation between the **sequence of bases in DNA** and the **sequence of amino acids in proteins**

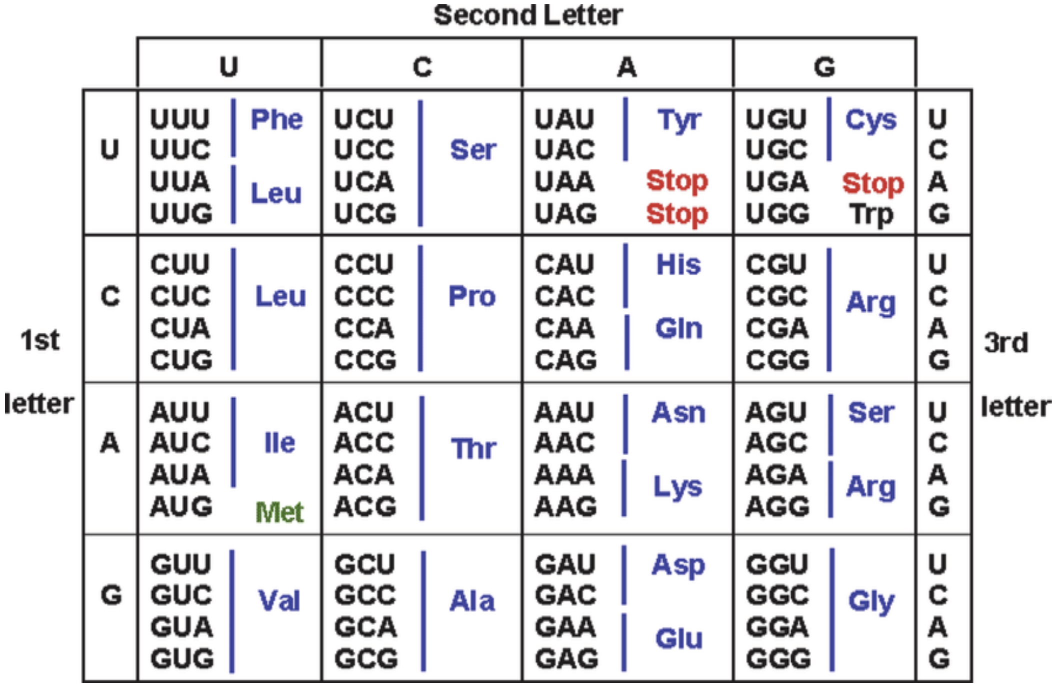


Amino acids are encoded by groups of three bases starting from a fixed point

- <u>Features of the Genetic code</u>:
  - three nucleotides (codon) encode an amino acid
  - has directionality
  - nonoverlapping
  - has no punctuation
  - is degenerate (most amino acids are encoded by more than one codon)



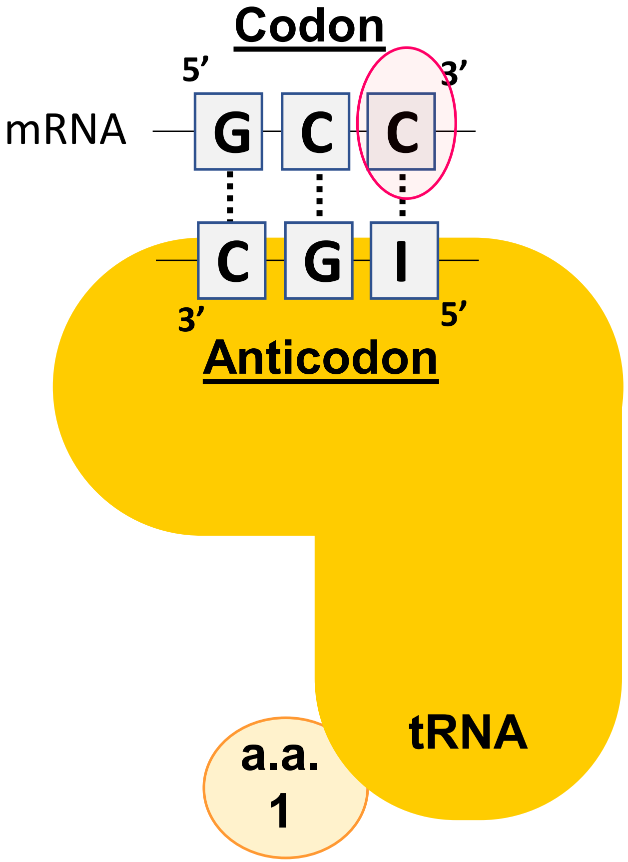
- 61 codons encode specify amino acids (20 in total)
- 3 codons are stop codons that designate termination of translation.

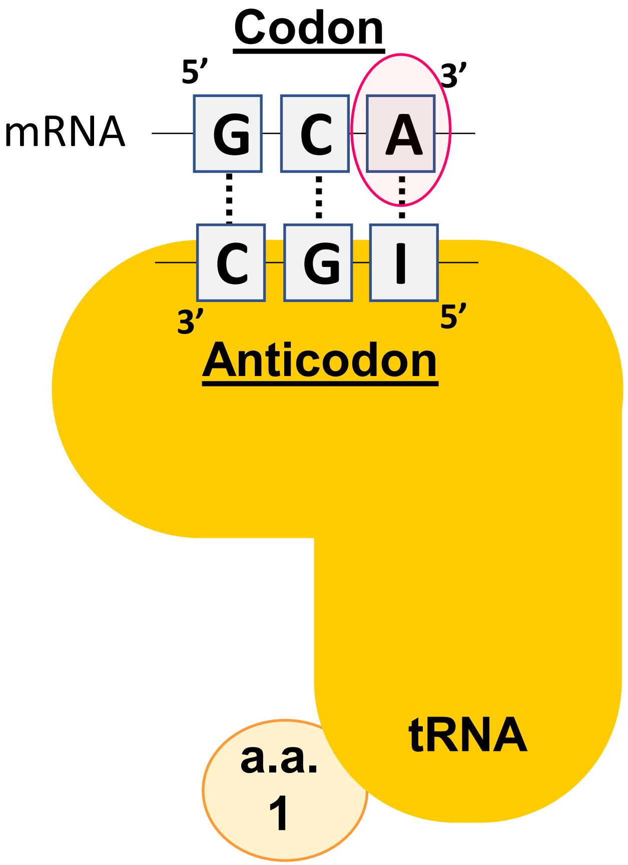


	Second Letter								
1st letter	U		C		A		G		3rd letter
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	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

’Wobble’ effect in base-pairing

’Wobble’ effect: Some tRNA molecules can recognize more than one codon

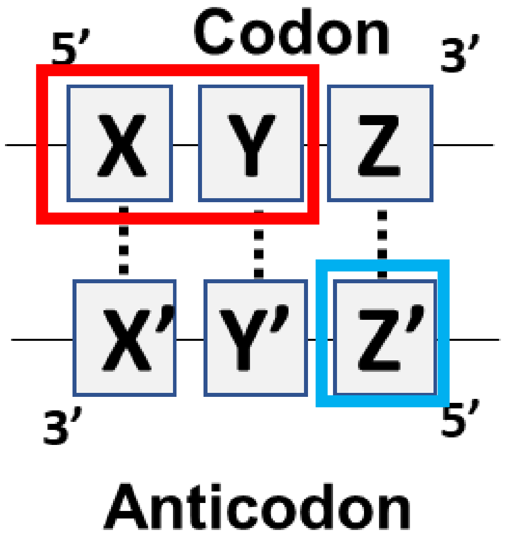




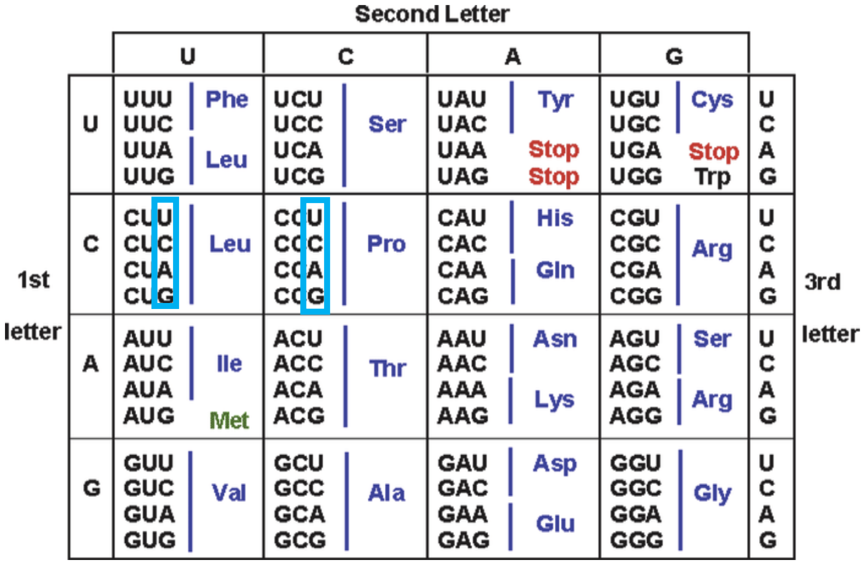
’Wobble’ effect in base-pairing

’Wobble’ effect: Some tRNA molecules can recognize more than one codon

Anticodons base-pair with codons



- The redundancy, or degeneracy, of the genetic code indicates that recognition of the third base of a codon is sometimes less discriminating than the other two



Second Letter

1st letter	U		C		A		G		3rd letter
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	Met	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

🟥 Codons that differ in either of their first two bases (from 5’) must be recognized by different tRNAs.

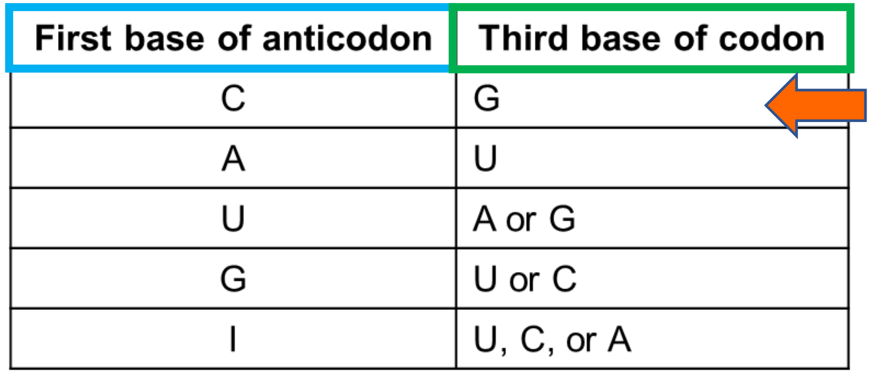
🟦 The **first base of the anticodon (5’) determines the degree of wobble**

“wobble” = steric freedom in the pairing of the first base of the anticodon with the third base of the codon

’Wobble’ effect in base-pairing

“wobble hypothesis ”: established hypothesis that predicts the binding of anticodons to codons

TABLE 30.2 Allowed pairings at the third base of the codon according to the wobble hypothesis



First base of anticodon	Third base of codon
C	G
A	U
U	A or G
G	U or C
I	U, C, or A

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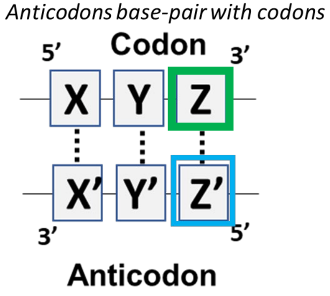

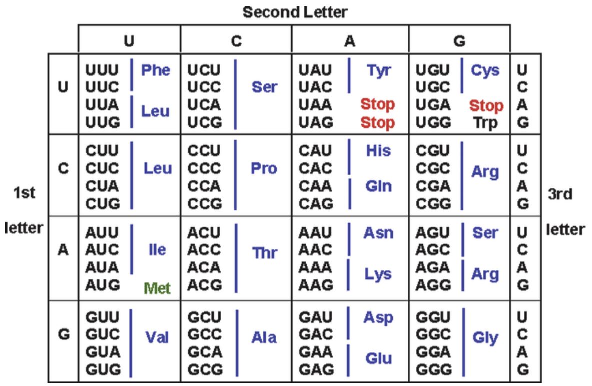

Second Letter

1st letter	U		C		A		G		3rd letter
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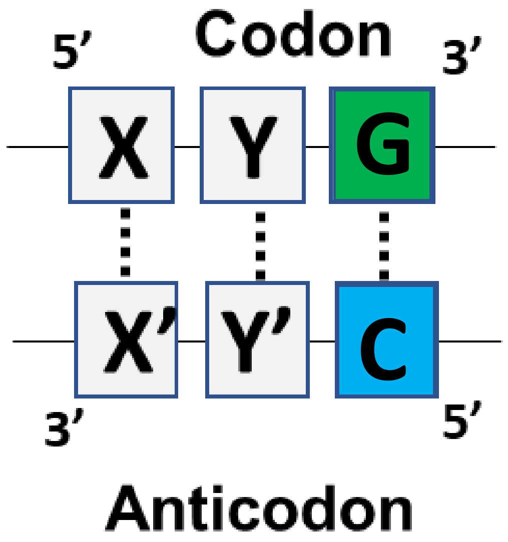
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		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

Anticodons base-pair with codons

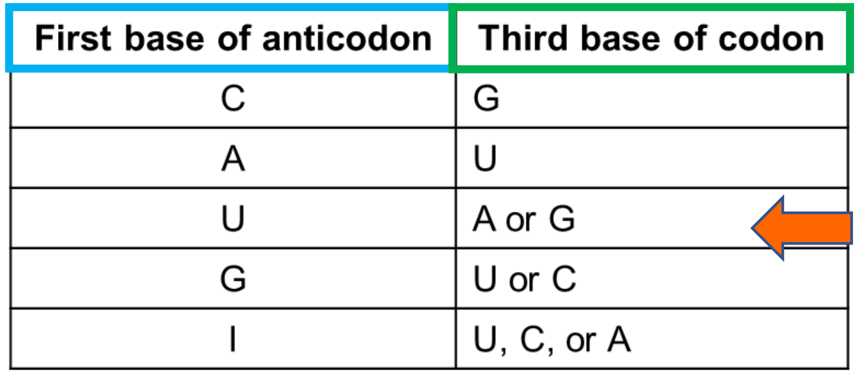


C

’Wobble’ effect in base-pairing

“wobble hypothesis ”: established hypothesis that predicts the binding of anticodons to codons

TABLE 30.2 Allowed pairings at the third base of the codon according to the wobble hypothesis



First base of anticodon	Third base of codon
C	G
A	U
U	A or G
G	U or C
I	U, C, or A

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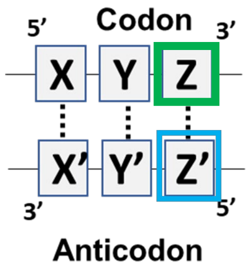
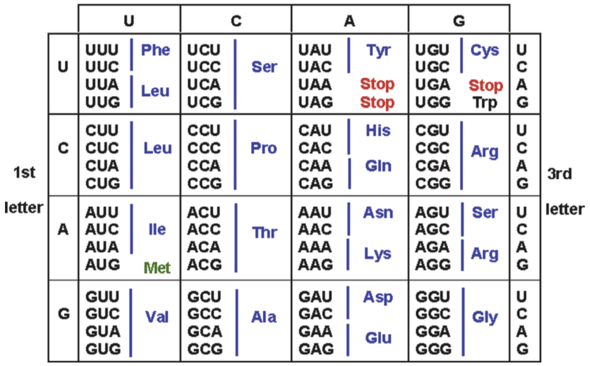
Anticodons base-pair with codons



Second Letter



1st letter	U		C		A		G		3rd letter
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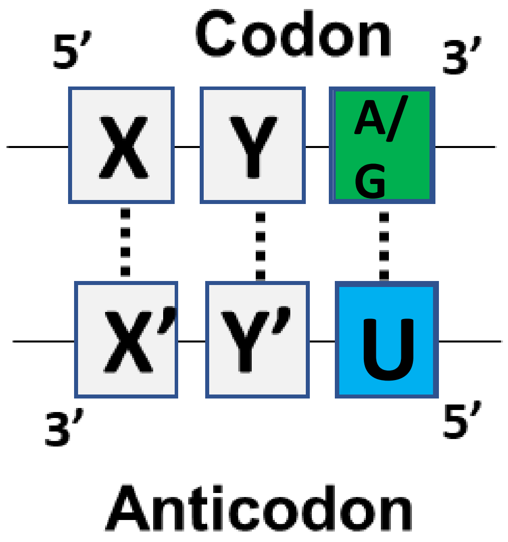
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		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

Anticodons base-pair with codons



C

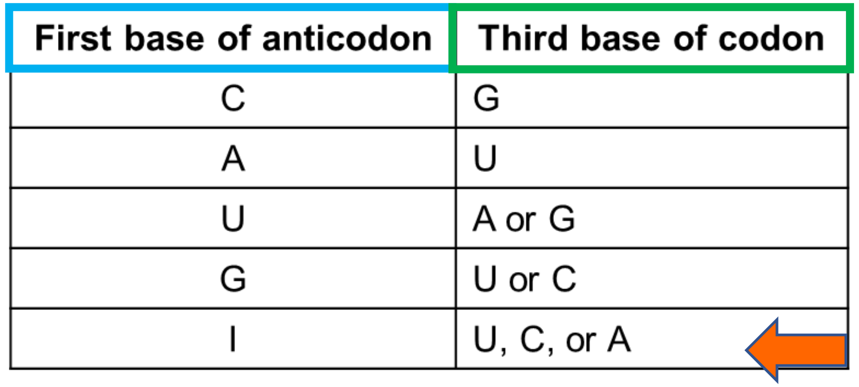
’Wobble’ effect in base-pairing

“wobble hypothesis ”: established hypothesis that predicts the binding of anticodons to codons

<u>Example:</u> If first base of the anticodon is inosine, the anticodon can recognize three different codons

The purine base inosine pairs with cytidine, uridine or adenosine

TABLE 30.2 Allowed pairings at the third base of the codon according to the wobble hypothesis



First base of anticodon	Third base of codon
C	G
A	U
U	A or G
G	U or C
I	U, C, or A

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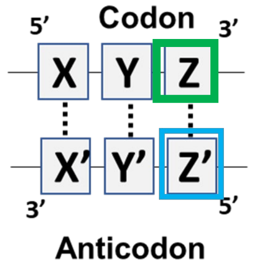
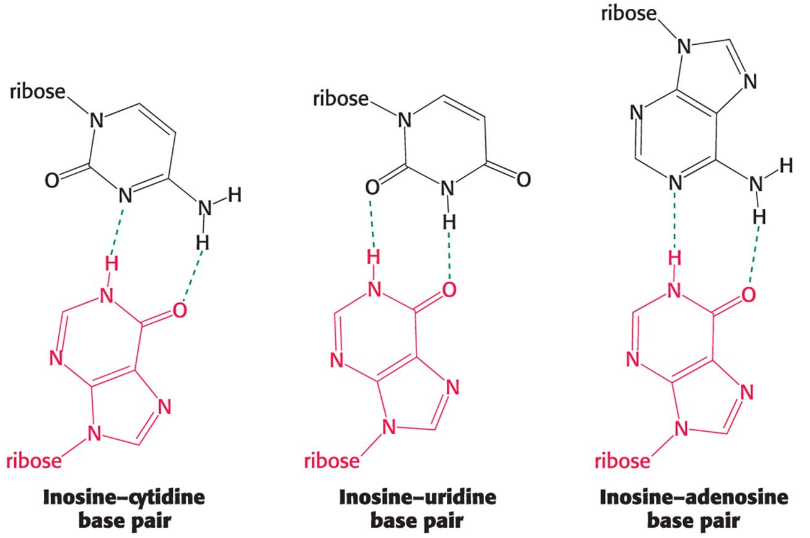
Anticodons base-pair with codons





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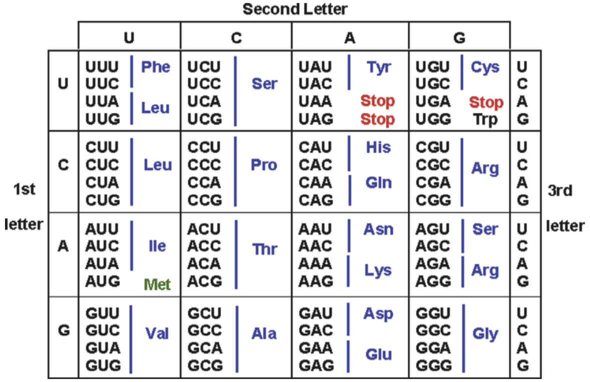
Second Letter

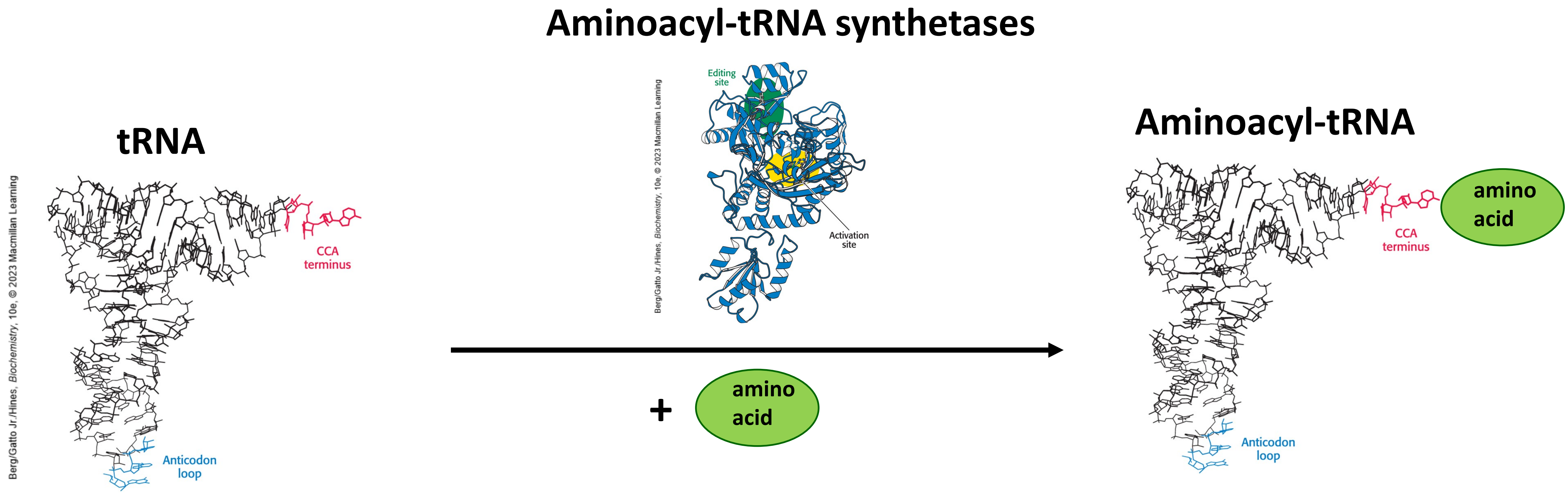


1st letter	U		C		A		G		3rd letter
U	UUU UUC UUA UUG	Phe Leu	UCU UCC UCA UCG	Ser	UAU UAC UAA UAG	Tyr Stop Stop	UGU UGC UGA UGG	Cys Stop Trp	U C A G
C	CUU CUC CUA CUG	Leu	CCU CCC CCA CCG	Pro	CAU CAC CAA CAG	His Gln	CGU CGC CGA CGG	Arg	U C A G
A	AUU AUC AUA AUG	Ile Met	ACU ACC ACA ACG	Thr	AAU AAC AAA AAG	Asn Lys	AGU AGC AGA AGG	Ser Arg	U C A G
G	GUU GUC GUA GUG	Val	GCU GCC GCA GCG	Ala	GAU GAC GAA GAG	Asp Glu	GGU GGC GGA GGG	Gly	U C A G

Ionosine is formed by the deamination of adenosine

- has a heterocyclic nitrogen base that can form hydrogen bonds with adenine, cytosine and uracil

Aminoacyl-tRNA synthetases attach specific amino acids to tRNAs



Aminoacyl-tRNA synthetases

tRNA

Aminoacyl-tRNA

+

amino acid

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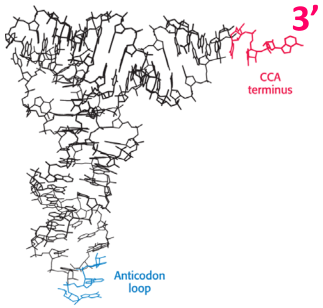
Amino acids required for protein biosynthesis must first be attached to specific tRNA molecules

- The attachment of a given amino acid to a particular tRNA establishes the genetic code

Ester linkages couple amino acids to tRNA



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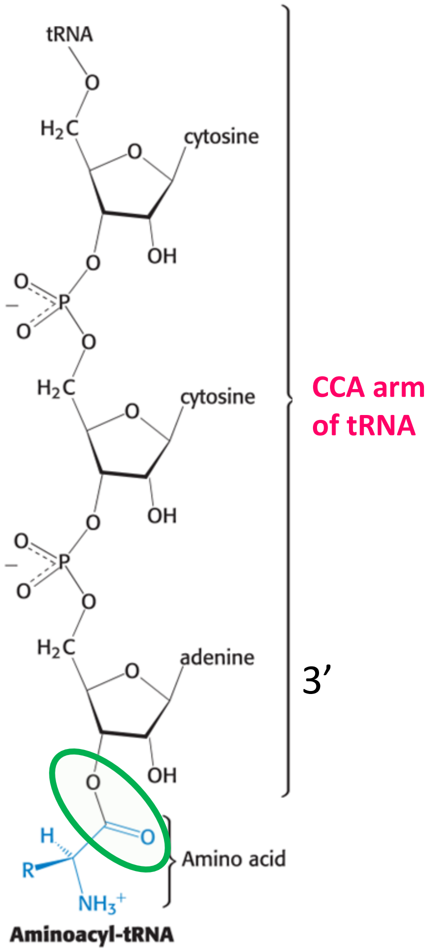


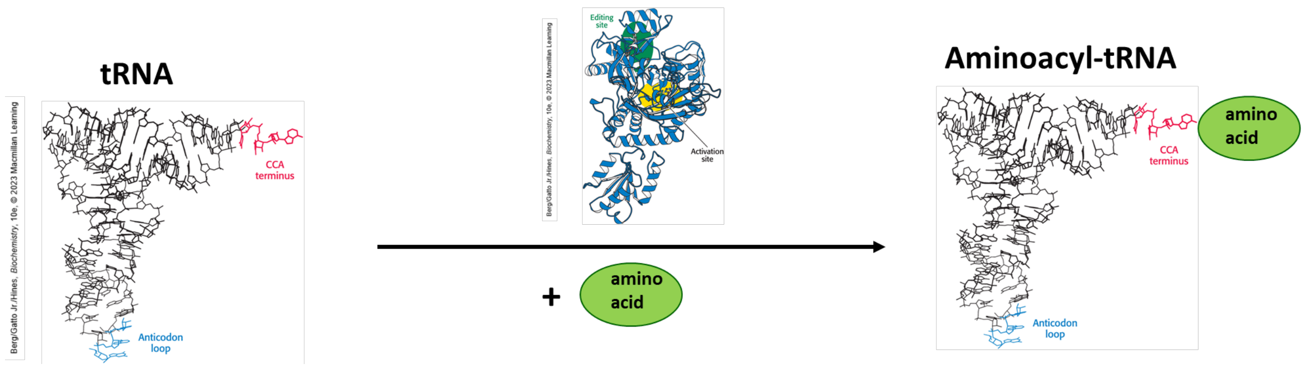
The process of attaching an amino acid to tRNA is called **aminosylation**

- Amino acids are bound to the 3’end of the tRNA via an ester bond between the carboxyl group on the amino acid and either the 2’ or 3’ hydroxyl group of the terminal adenosine of the tRNA

Aminoacyl-tRNA: Amino acid bound to tRNA

Each aminoacyl-tRNA synthetase is specific for a given amino acid

How aminoacyl-tRNA synthetases evolve to differentiate between different amino acids?



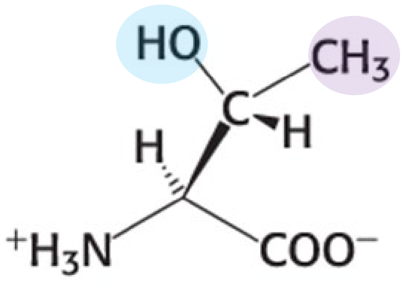
tRNA

Aminoacyl-tRNA synthetases

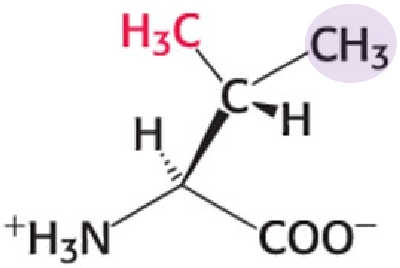
\+ **amino acid**

Aminoacyl-tRNA **amino acid**

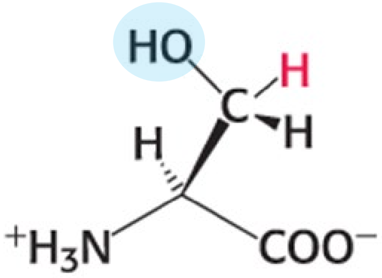
A closer look at the amino acid threonine, valine and serine



Threonine



Valine

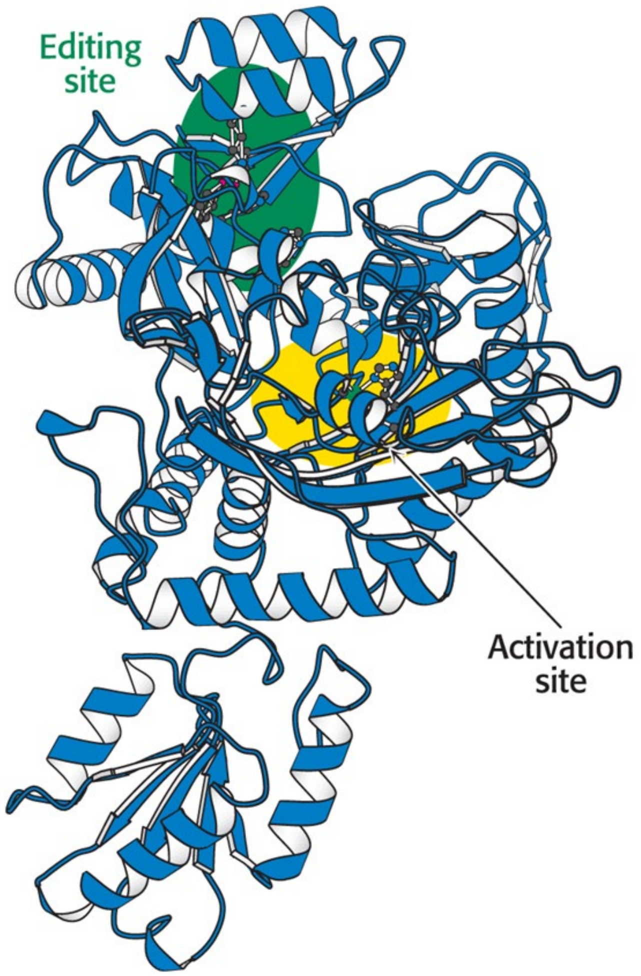


Serine

Each aminoacyl-tRNA synthetase is specific for a given amino acid

How aminoacyl-tRNA synthetases evolve to differentiate Threonine, Valine and Serine?

Threonyl-tRNA Synthetase contains an **activation site**



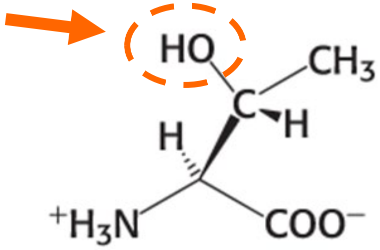
Activation site

responsible for activating threonine by binding it to adenosine triphospate (ATP) and further transfer of these amino acid to the tRNA molecule

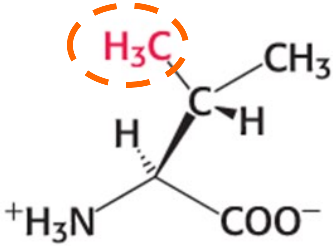
- *Aminoacyl-tRNA synthetases have highly discriminating amino acid activation sites*

Each aminoacyl-tRNA synthetase is specific for a given amino acid

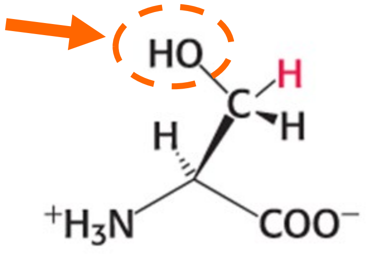
How aminoacyl-tRNA synthetases evolve to differentiate Threonine, Valine and Serine?



Threonine (Thr)



Valine (Val)



Serine (Ser)

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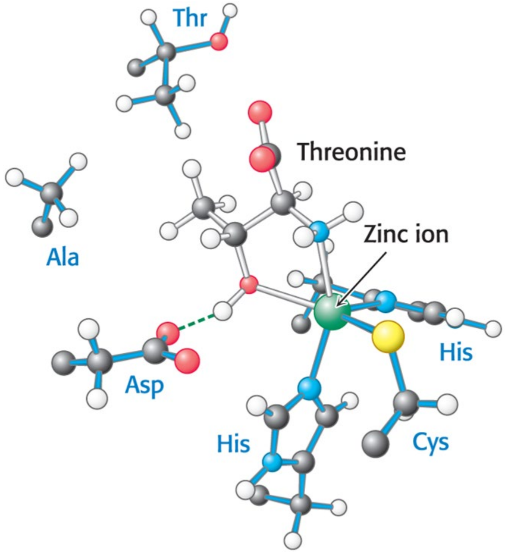
- To avoid coupling to the incorrect amino acid, threonyl-tRNA synthetase ($tRNA^{Thr}$) contains a **zinc ion at the active site that *binds* to the amino and hydroxyl groups of threonine**

- **Valine** is similar in overall structure to threonine but **lacks the hydroxyl group**, so it does not bind to $tRNA^{Thr}$

- **Serine** is occasionally linked to $tRNA^{Thr}$ because of the presence of **the hydroxyl group**



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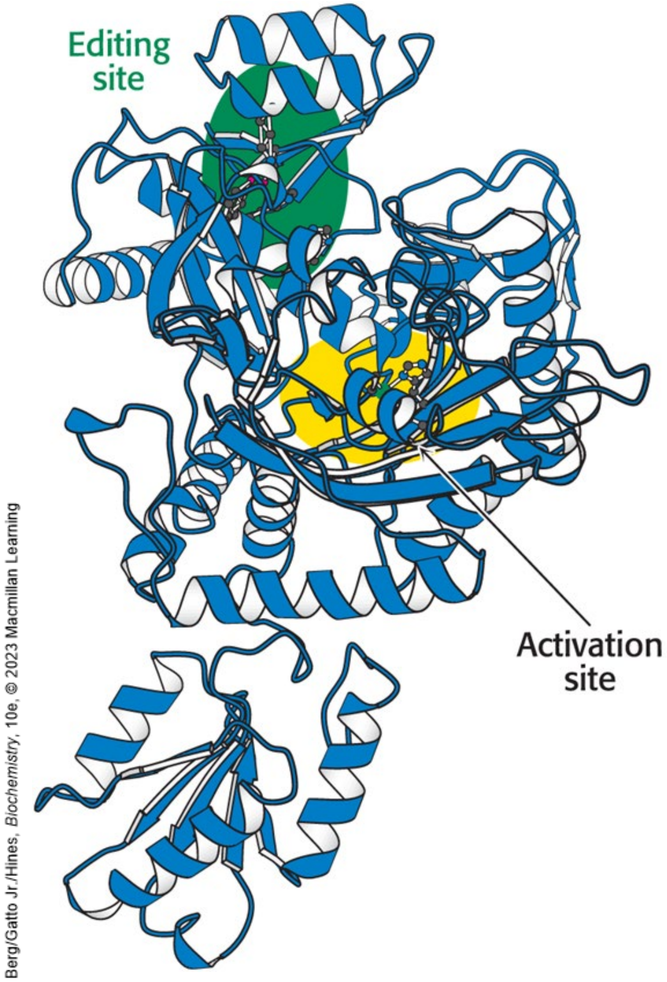
Each aminoacyl-tRNA synthetase is specific for a given amino acid

How aminoacyl-tRNA synthetases evolve to differentiate Threonine, Valine and Serine?

Threonyl-tRNA Synthetase contains an **activation site** and an **editing site**



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Editing site:
acts as a profreader and removes any incorrect bound amino acid from the tRNA molecule

Activation site
responsible for activating threonine by binding it to adenosine triphospate (ATP) and further transfer of these amino acid to the tRNA molecule

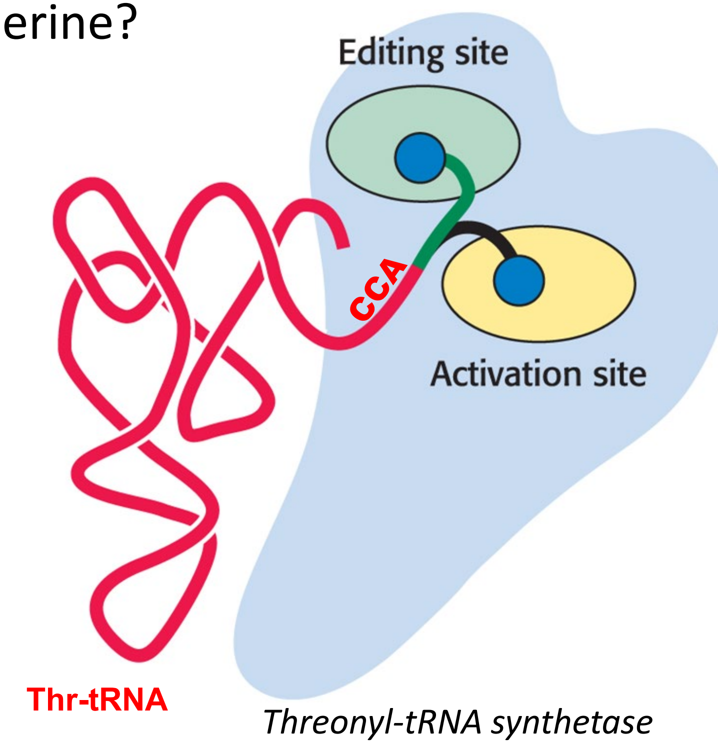
Each aminoacyl-tRNA synthetase is specific for a given amino acid

How aminoacyl-tRNA synthetases evolve to differentiate Threonine, Valine and Serine?

- Threonyl-tRNA synthetase has an **editing site that hydrolyzes Serine if this is linked to threonine-tRNA**

  - The aminoacylated CCA arm of the tRNA is flexible and can swing out of the activation site and into the editing site to remove Ser

  - Because Thr contains an extra methyl group, it is sterically excluded from the editing site



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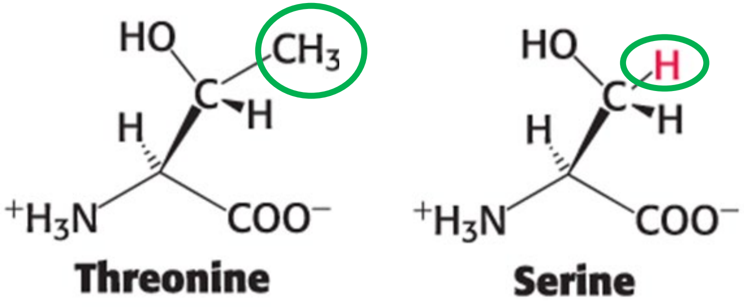
- Most aminoacyl-tRNA synthetases contain editing sites and activation sites to ensure very high fidelity.

Proofreading by Aminoacyl-tRNA Synthetases Increases the Fidelity of Protein Biosynthesis



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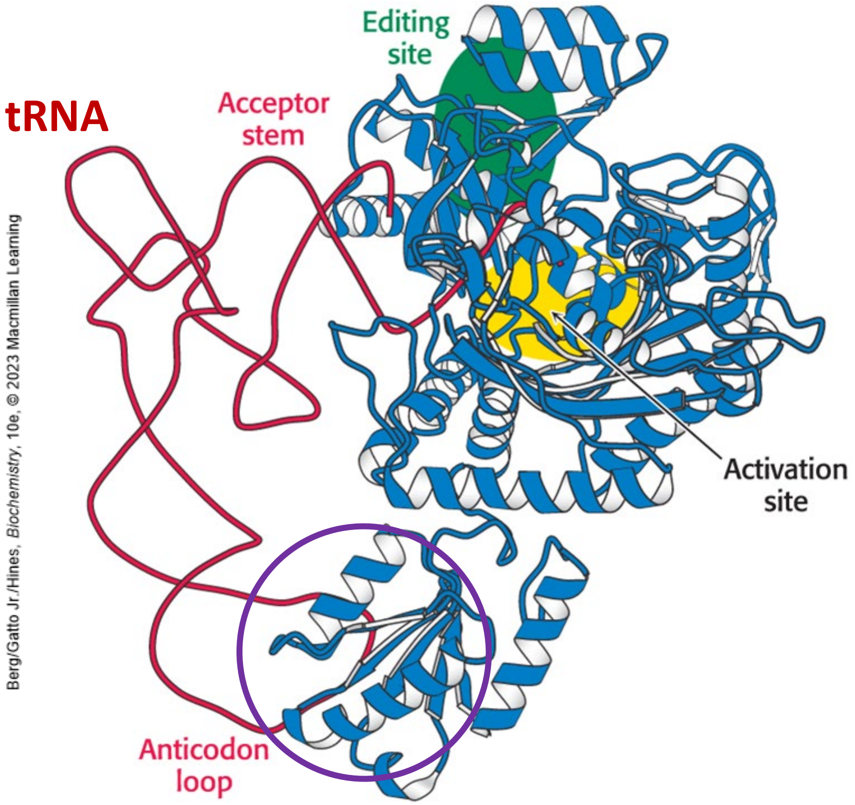
Aminoacyl-tRNA synthetases interaction with tRNA

Aminoacyl-tRNA synthetases assign a particular amino acid to a specific tRNA - the true translators of the genetic code

Threonine-tRNA synthetase



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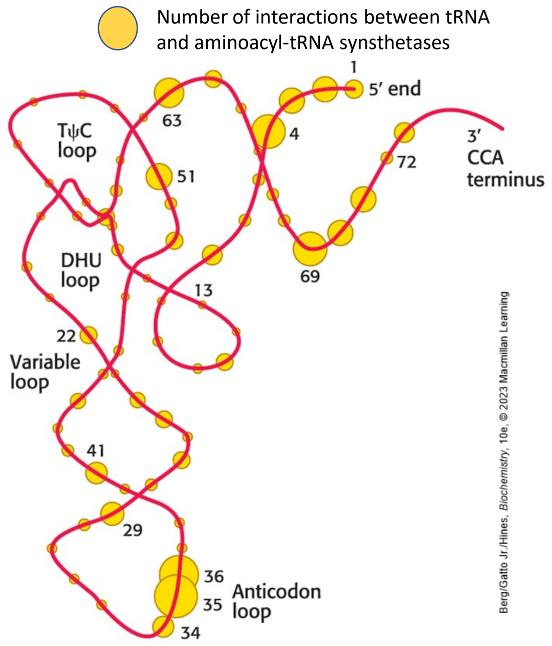
- Threonine-tRNA synthetases **bind** to both the **acceptor stem** and the **anticodon loop** of the tRNA

- Some synthetases **recognize their tRNA** partners primarily on the basis of their **anticodons**

- Synthetases may also **recognize other aspects of tRNA structure** that vary among different tRNAs

  - many of the recognition sites **are loops rich in unusual bases**



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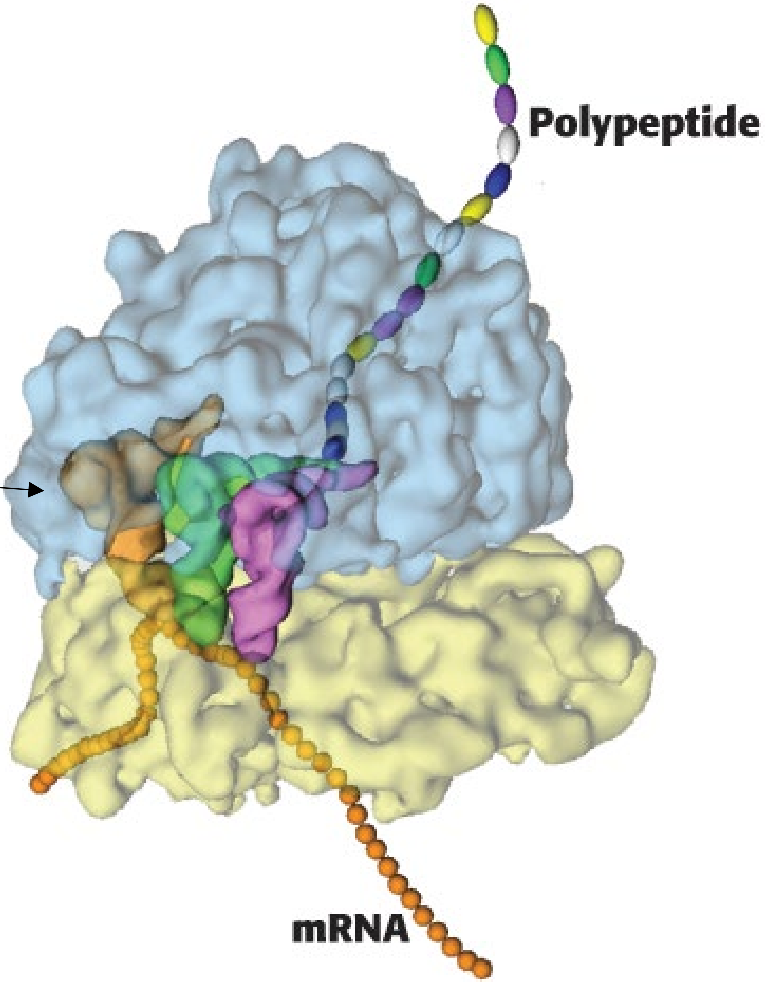
The ribosome is the site of protein synthesis

Ribosomes coordinate the interplay of aminoacyl-tRNAs, mRNA, and proteins



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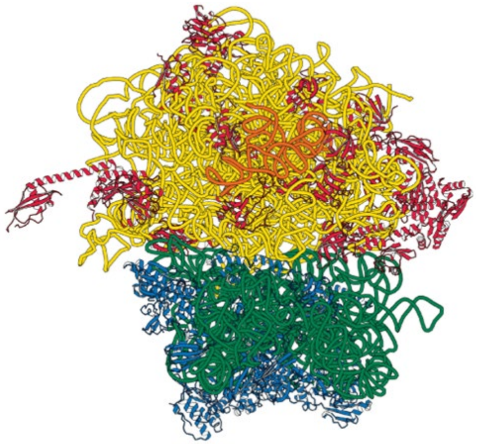
The ribosome is the site of protein synthesis

Ribosomes coordinate the interplay of aminoacyl-tRNAs, mRNA, and proteins

70S ribosome



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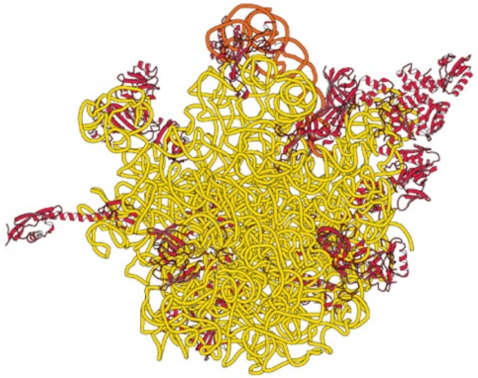
=

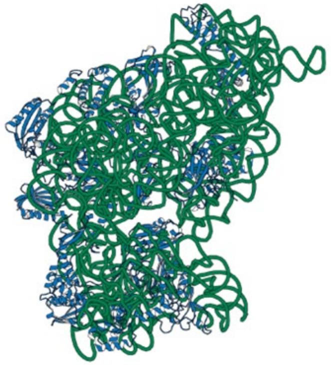
50S subunit



34 proteins (L1 to L34)
23S rRNA
5S rRNA

+

30S subunit



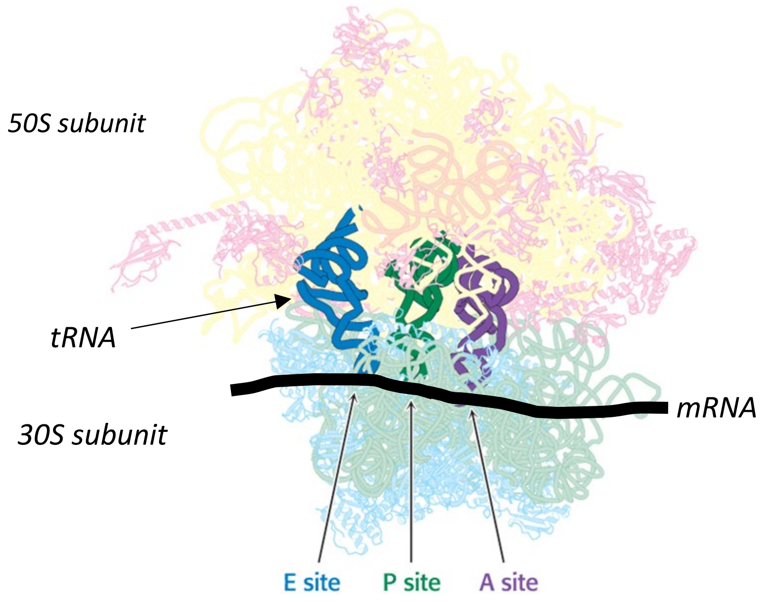
21 proteins (S1 to S21)
16S rRNA

The *E. coli* ribosome has a sedimentation coefficient of 70S and is composed of two subunits:

- a large (50S) subunit
- smaller (30S) subunit.

- Two-thirds of the mass of ribosomes is RNA
- **Ribosomal RNA (rRNA) is the actual catalyst for protein synthesis**, with the ribosomal proteins making only a minor contribution

The ribosome has three binding sites for transfer RNAs



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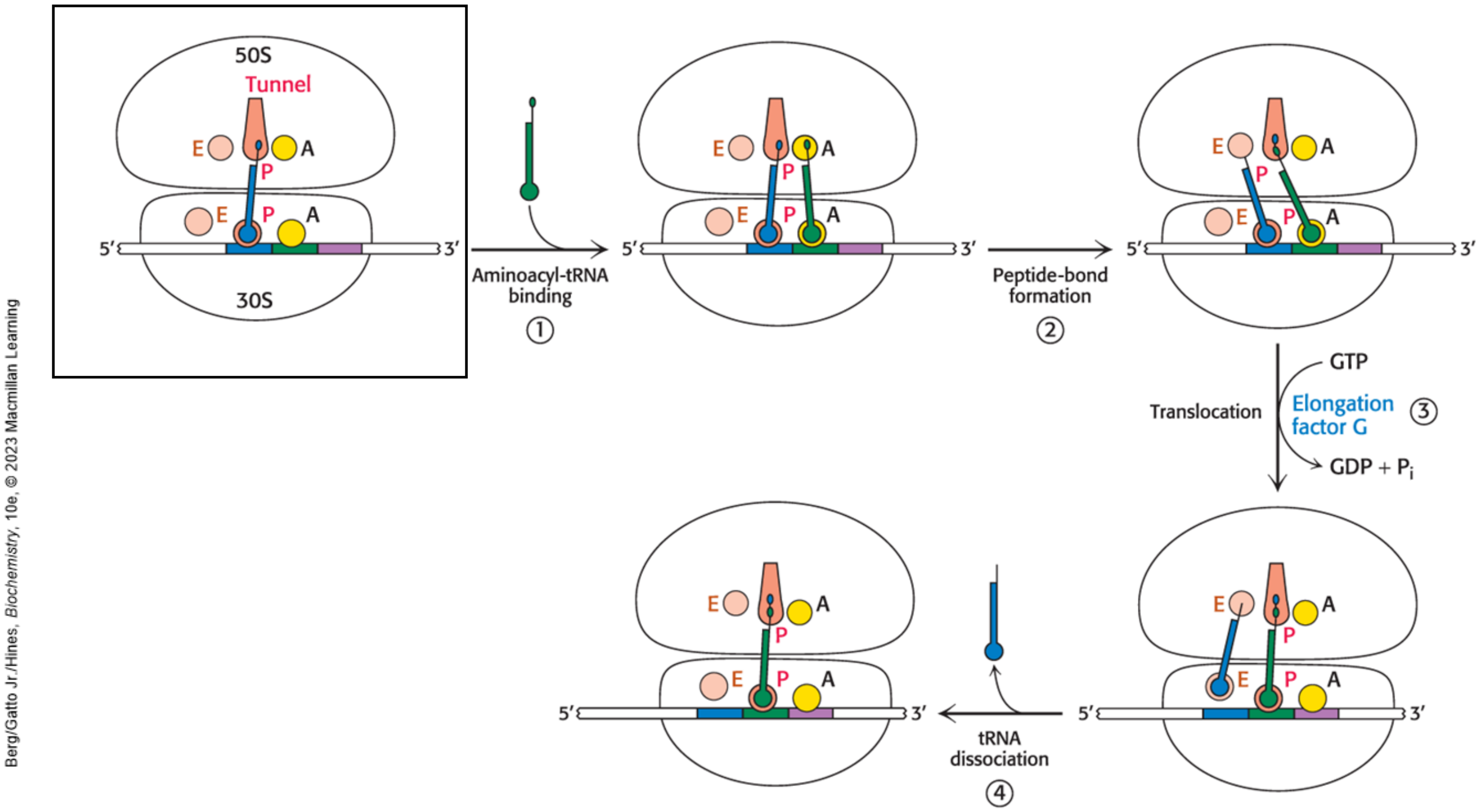
- The **mRNA fragment** is **bound** within the **30S** subunit
- Each **tRNA** is in **contact** with the **30S** and **50S** subunits
- Three **tRNA-binding sites** in ribosomes allow the formation of peptide bonds
  - the aminoacyl **(A) site**
  - the peptidyl **(P) site**
  - the exit **(E) site**

Overview of mechanism of protein biosynthesis

Initiation, Elongation, Translocation and Termination

Initiation



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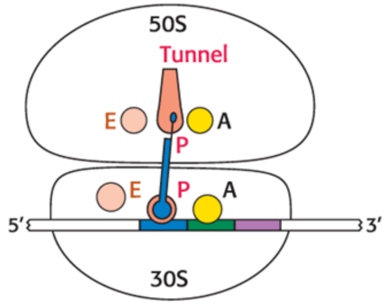
Initiation of translation

- In bacteria, many mRNA molecules encode two or more polypeptide chains.
- All **mRNA molecules contain signals that define the beginning and end of each encoded polypeptide chain.**



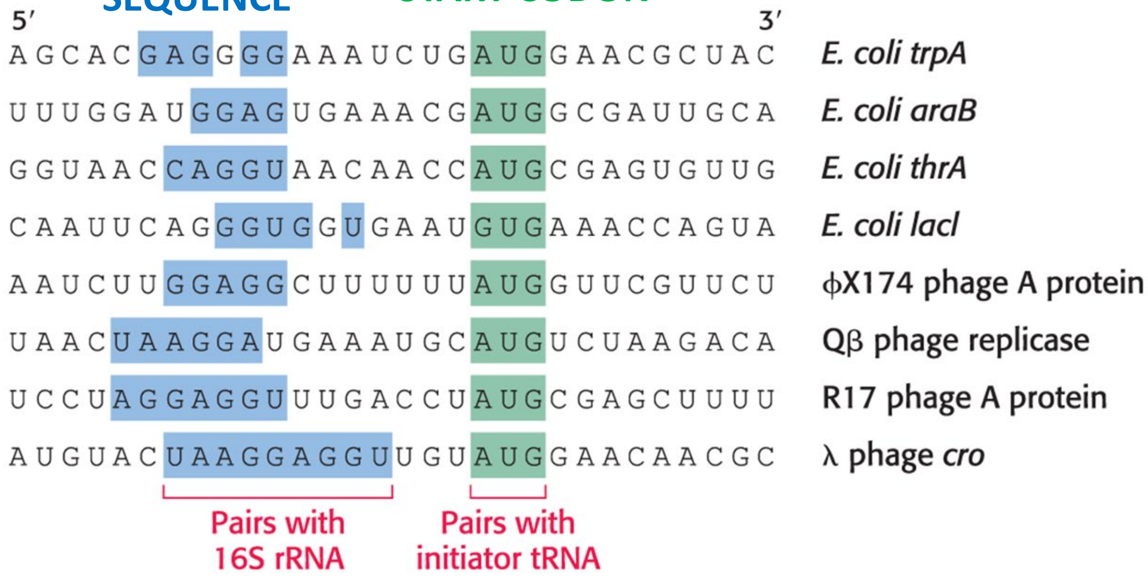
- Each **initiator region** usually displays:
    - an **start codon**: AUG codon (Met) or, less frequently, GUG (Val) or, rarely, UUG (Leu)
    - a **purine-rich sequence** centered ~10 nucleotides on the 5′ side of the initiator codon

- **Shine–Dalgarno sequence** = (in bacteria) the purine-rich region that binds to the rRNA to position the initiator codon of the mRNA in the P site of the ribosome

Examples of mRNA initiator regions

PURINE-RICH SEQUENCE

START CODON



5′ → 3′	
AGCACGAGGGGAAAUCUGAUGGAACGCUAC	*E. coli trpA*
UUUGGAUGGAGUGAAACGAUGGCGAUUGCA	*E. coli araB*
GGUAACCAGGUAACAACCAUGCGAGUGUUG	*E. coli thrA*
CAAUUCAGGGUGGUGAAUGUGAAACCAGUA	*E. coli lacI*
AAUCUUGGAGGCUUUUUUAUGGUUCGUUCU	ϕX174 phage A protein
UAACUAAGGAUGAAAUGCAUGUCUAAGACA	Qβ phage replicase
UCCUAGGAGGUUUGACCUAUGCGAGCUUUU	R17 phage A protein
AUGUACUAAGGAGGUUGUAUGGAACAACGC	λ phage *cro*

Pairs with 16S rRNA

Pairs with initiator tRNA

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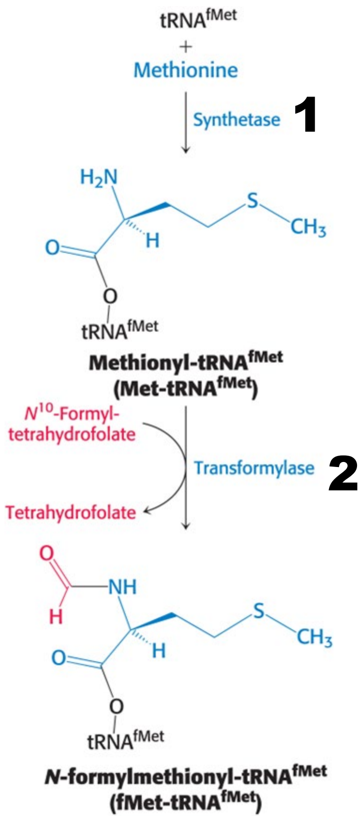
Bacterial protein synthesis is initiated by *N*-formylmethionyl-transfer RNA

Initiator tRNA ($tRNA^{fMet}$) - distinct tRNA that brings ***N*-formylmethionine to the ribosome** to initiate protein biosynthesis (Note: $tRNA^{Met}$, not $tRNA^{fMet}$, inserts Met in internal positions)



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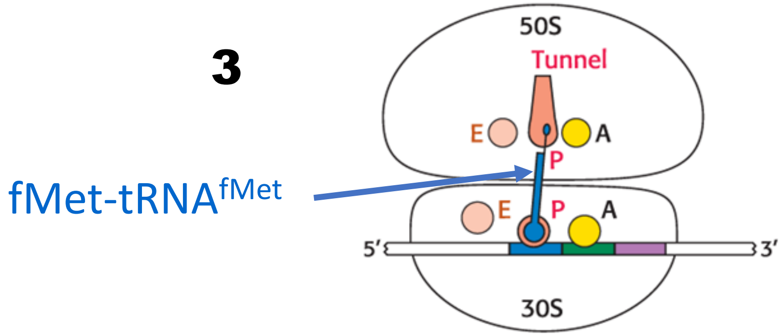
1) Met is linked to $tRNA^{fMet}$ by the aminoacyl-tRNA synthetase

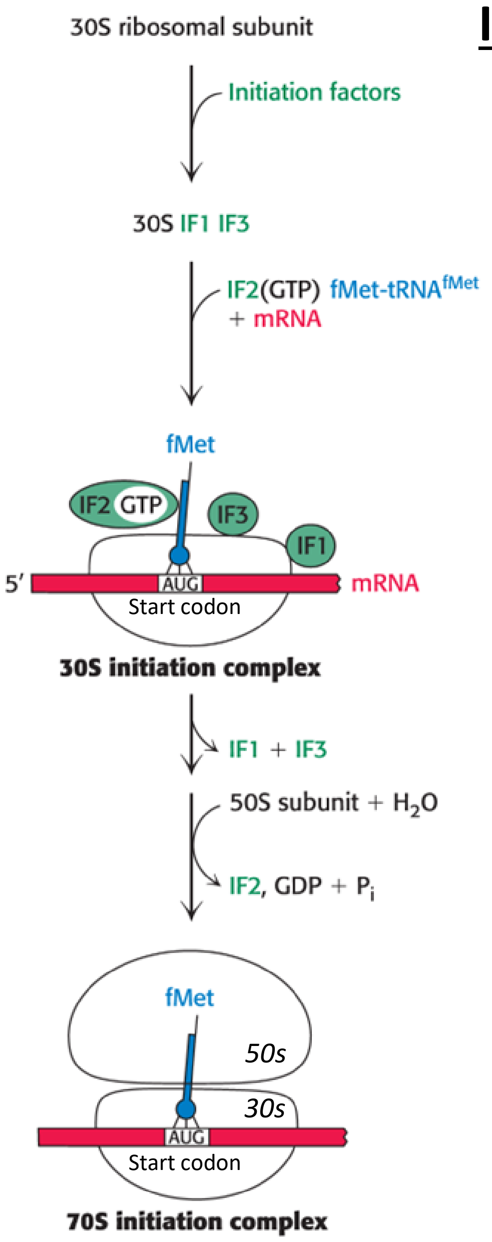
2) Amino group of the Met attached to $tRNA^{fMet}$ is formylated

3) *N*-formylmethionyl-tRNA is placed in the P site of the ribosome



Met (methionine)

Bacterial protein synthesis is initiated by *N*-formylmethionyl-tRNA (fMet-tRNA^{fMet})



30S ribosomal subunit

Initiation factors

30S IF1 IF3

IF2(GTP) fMet-tRNA^{fMet}
+ mRNA

30S initiation complex

IF1 + IF3

50S subunit + H_2O

IF2, GDP + P_i

70S initiation complex

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Initiation factors (IF1, IF2, IF3) assist in the assembly of the protein-synthesizing machinery

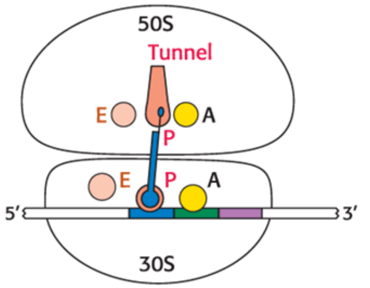
1) **IF1 and IF3** bind the 30S subunit to prevent premature binding to the 50S subunit

2) **IF2(GTP) initiator- fMet-tRNA^{fMet}** complex binds with mRNA and the 30S subunit to form the 30S initiation complex

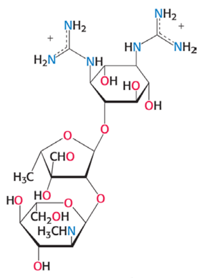
3) Structural changes lead to release of IF1 and IF3

4) IF2 stimulates the association of the 50S subunit to the complex and GTP hydrolysis releases IF2, resulting in the 70S initiation complex

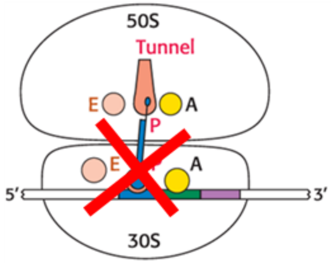
70S initiation complex formation is the rate-limiting step in protein biosynthesis



Antibiotic Streptomycin Binds to 30S ribosomal subunit and interferes with the binding of fMet-tRNA^{fMet} *(Specific to bacteria)*

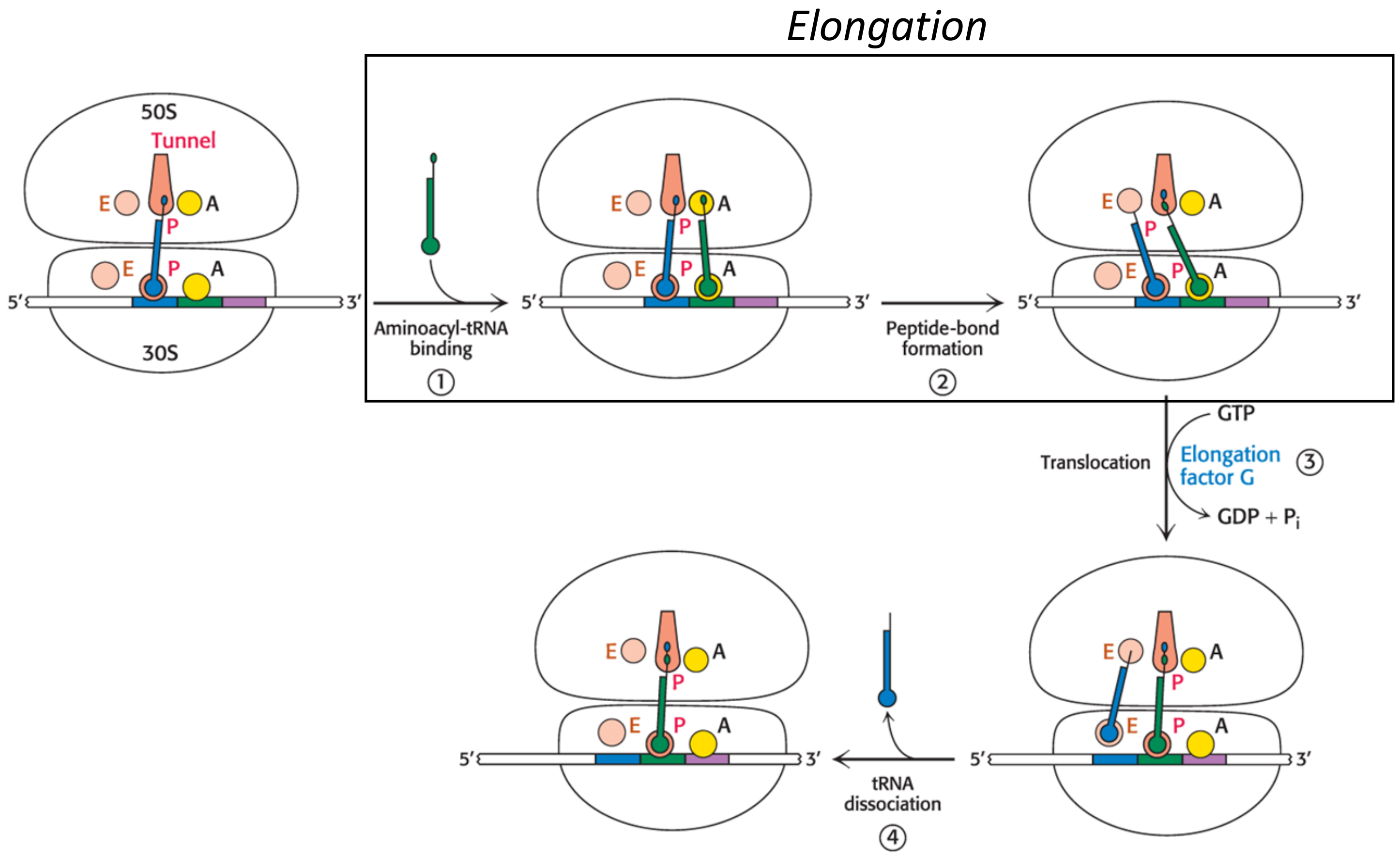


Streptomycin



Overview of mechanism of protein biosynthesis

Initiation, Elongation, Translocation and Termination



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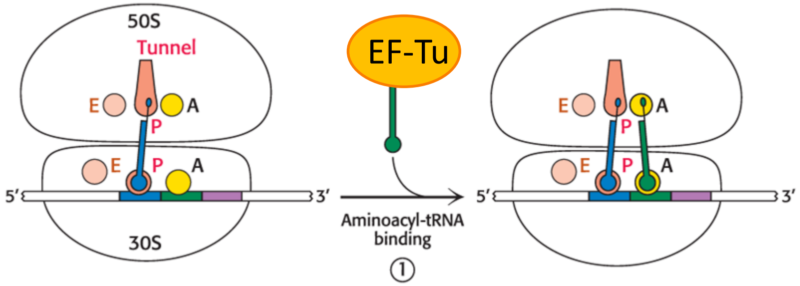
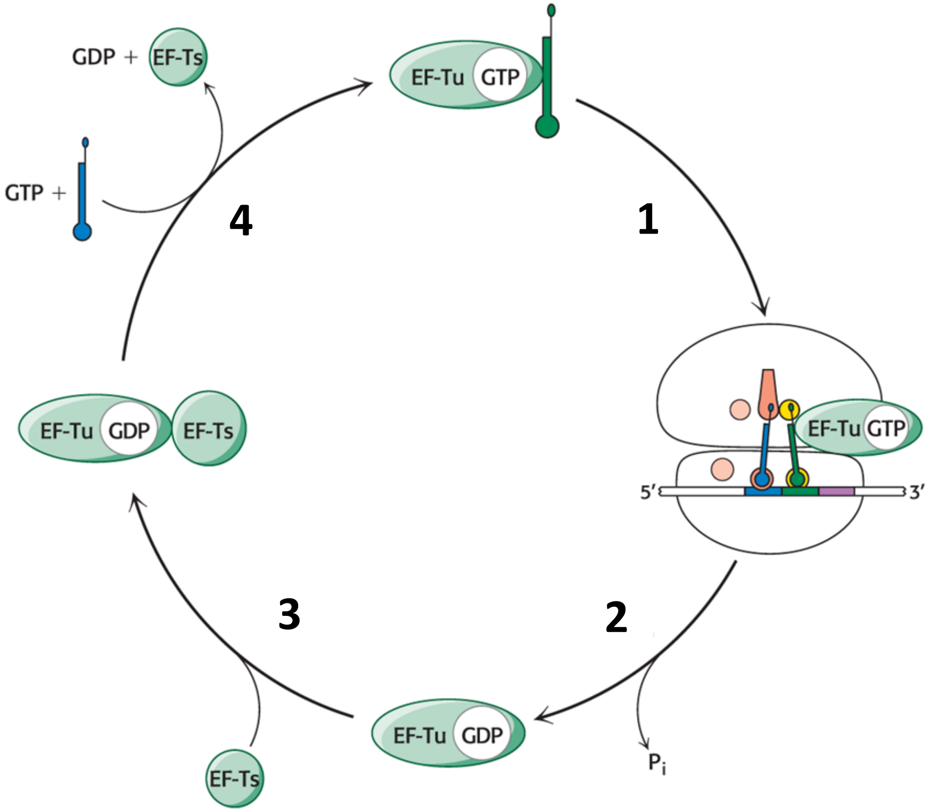
Elongation factors deliver aminoacyl-tRNAs to the ribosome

In the 70S initiation complex, fMet-tRNAfMet occupies the P site and the A site is vacant





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1. EF-Tu-GTP binds aminoacyl-tRNA and delivers it to the A site on the ribosome.

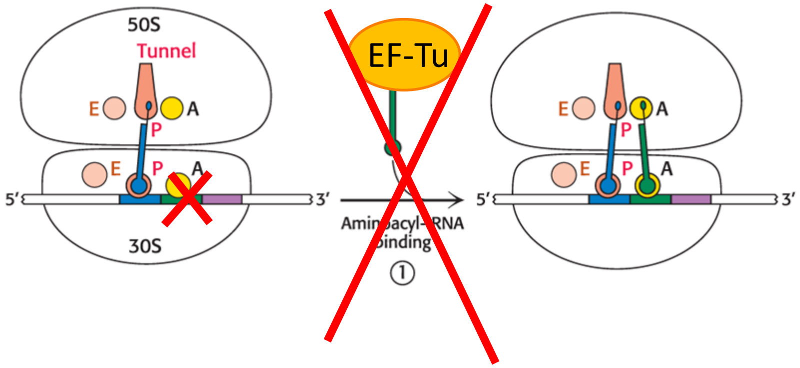
2. Correct codon recognition leads to structural changes that stimulate the GTPase activity of EF-Tu and EF-Tu-GDP departs from the A site

3. EF-Ts binds to EF-Tu-GDP

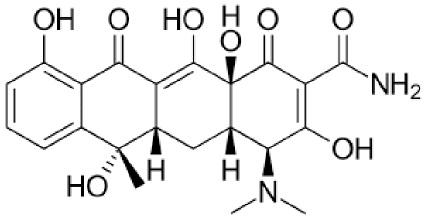
4. EF-Ts induces release of GDP. EF-Ts departs as another GTP and tRNA bind to form the EF-Tu-GTP complex that is ready for another delivery to the ribosome

Elongation factors deliver aminoacyl-tRNAs to the ribosome

In the 70S initiation complex, fMet-tRNAfMet occupies the P site and the A site is vacant

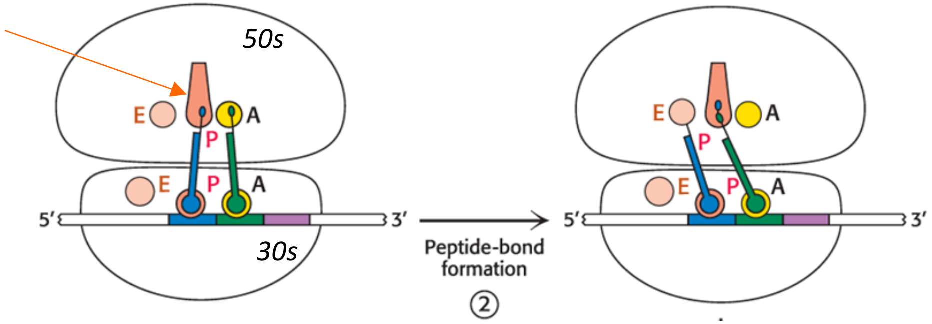


Antibiotic Tetracycline



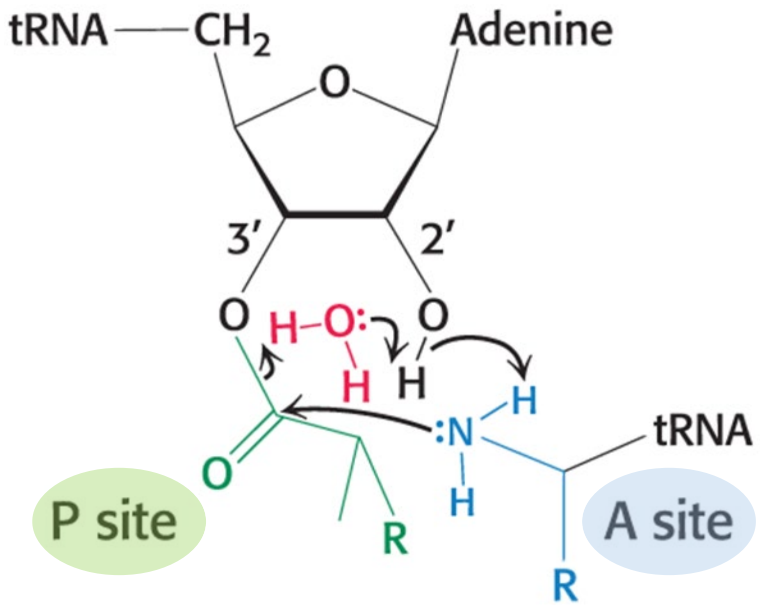
Binds to 30S ribosomal subunit and inhibits the binding of aminoacyl-tRNAs (bacteria)

Elongation – peptidyl transferase catalyzes peptide-bond formation



peptidyl transferase center = a site on the 50S subunit that catalyzes the thermodynamically spontaneous formation of the peptide bond

The ribosome enhances the rate of peptide bond synthesis by positioning and orienting the two substrates



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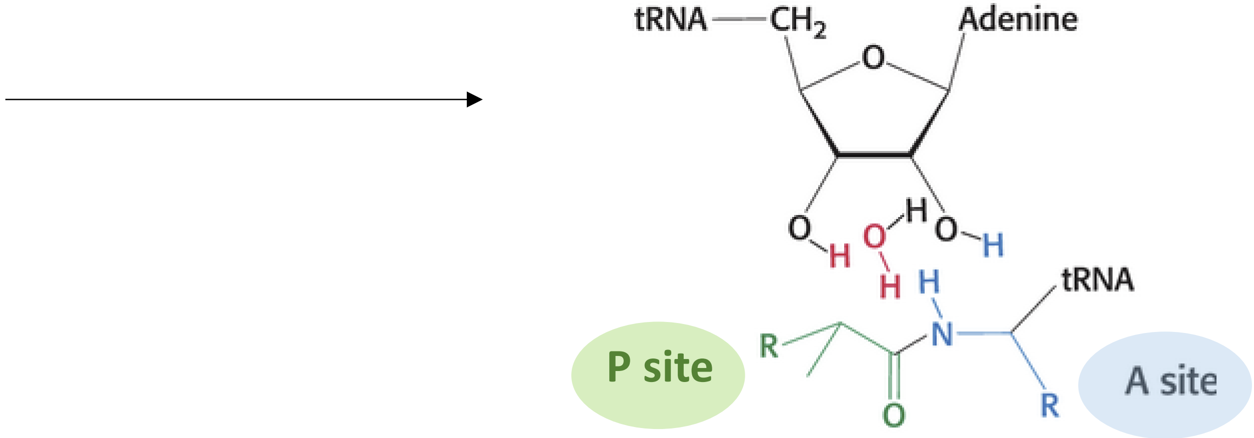
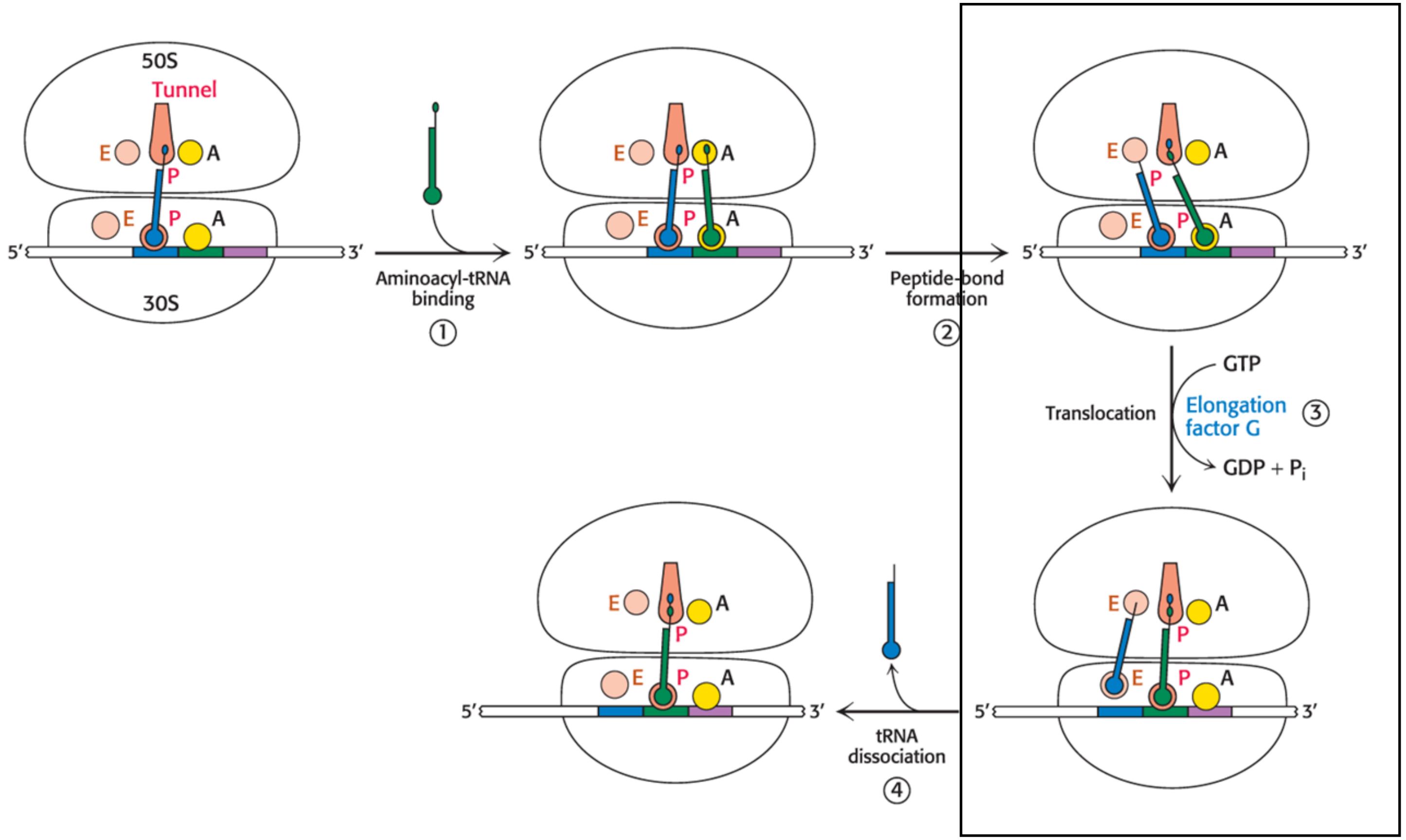
The amino group of the aminoacyl-tRNA in the A site attacks the carbonyl group of the ester linkage of the peptidyl-tRNA in the P site



Overview of mechanism of protein biosynthesis

Initiation, Elongation, Translocation and Termination



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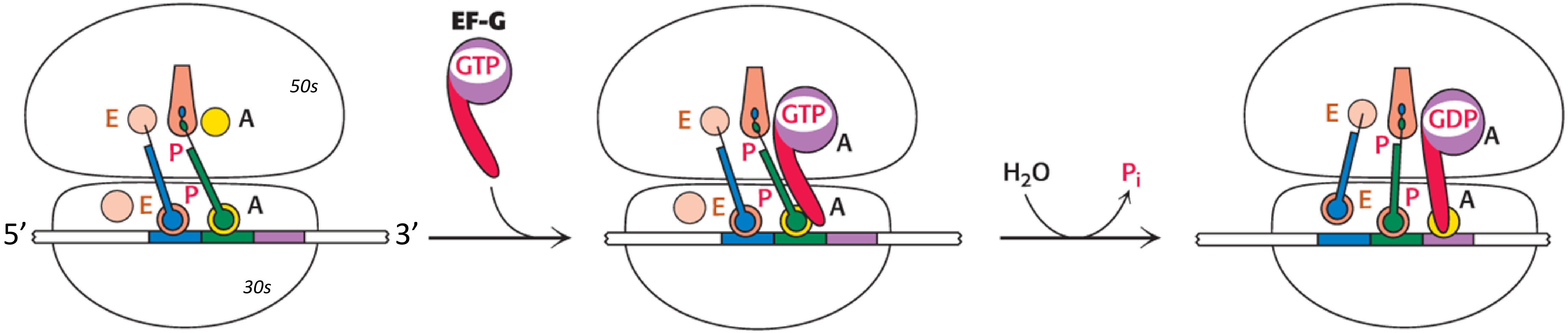
Translocation

Translocation repositions tRNAs and mRNA with respect to the ribosome

Upon peptide bond formation, the growing chain is in the P site of the 50S subunit while attached to the tRNA whose anticodon is in the A site on the 30S subunit



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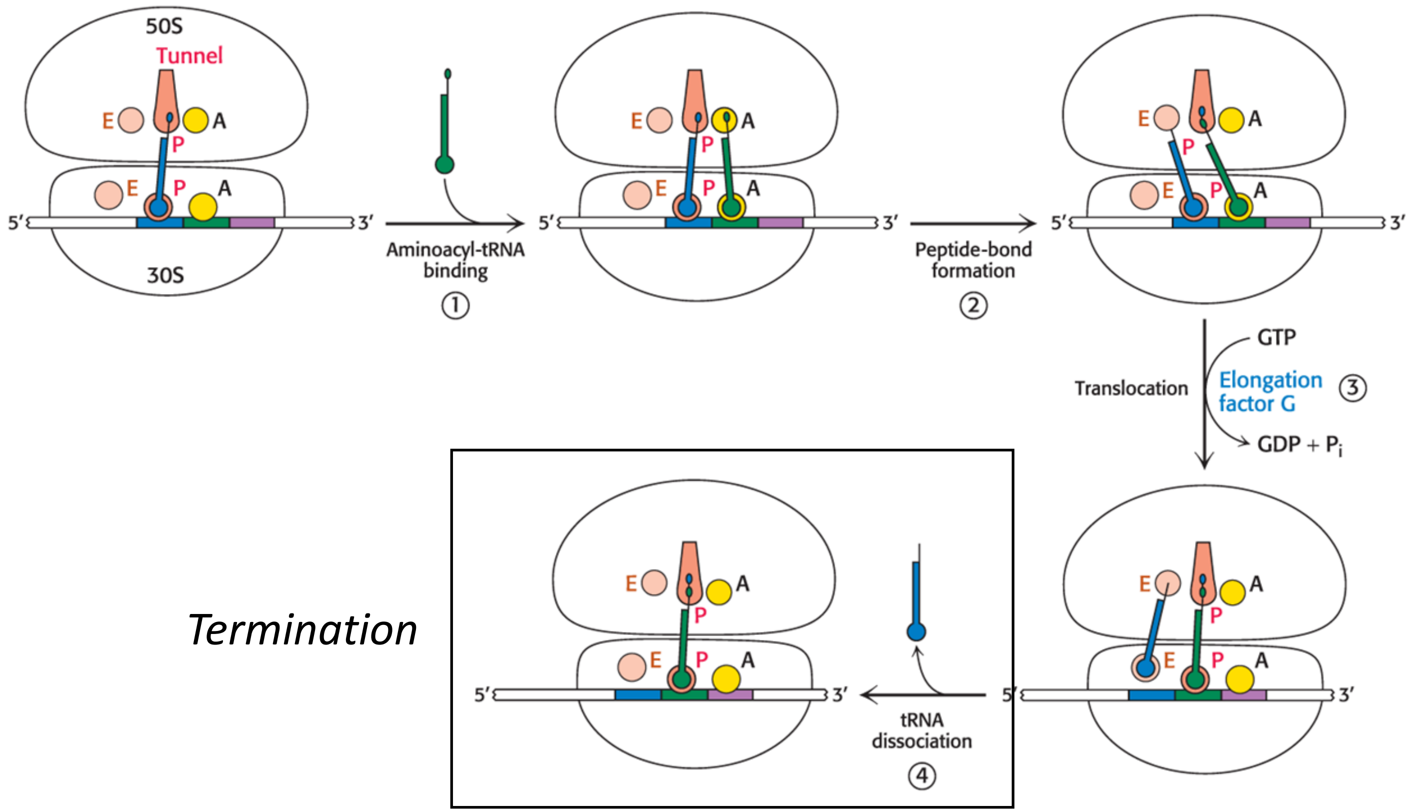
<u>elongation factor G (EF-G, translocase)</u> - catalyzes the movement of mRNA by one codon (requires GTP)

1) EF-G in the GTP form binds near the A site

2) GTP hydrolysis triggers a conformational change in EF-G that displaces the peptidyl-tRNA in the A site by one codon (peptidyl-tRNA move from A to P site)

Overview of mechanism of protein biosynthesis

Initiation, Elongation, Translocation and Termination



Termination

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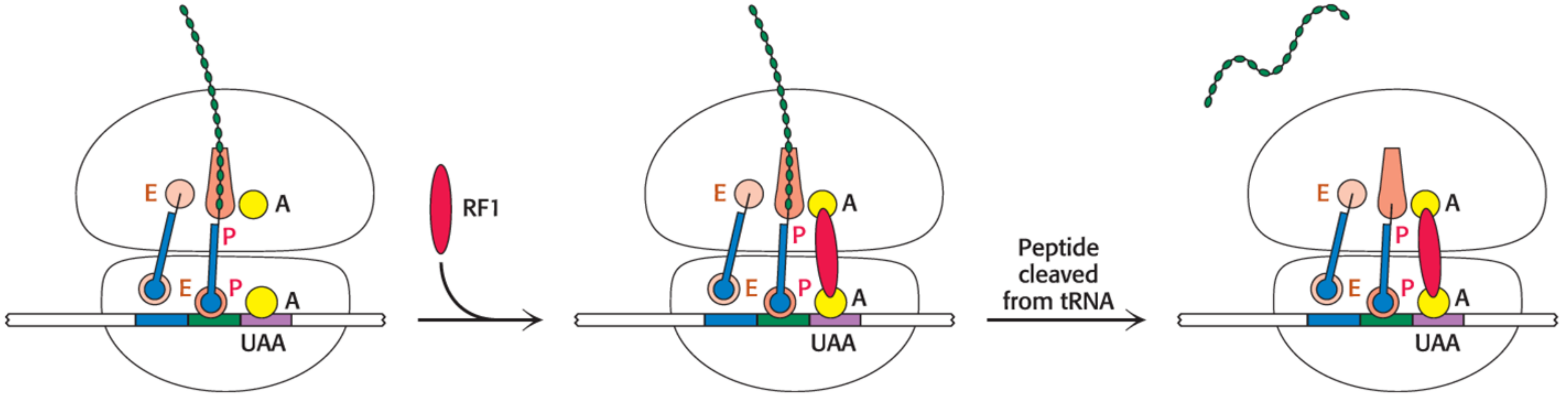
Termination is catalyzed by release factors that read stop codons

Release factors (RFs)

RF1 and RF2 are proteins recognize stop codons (UAA, UGA, or UAG)

RF3 is a GTPase that catalyzes the removal of RF1 or RF2 from the ribosome



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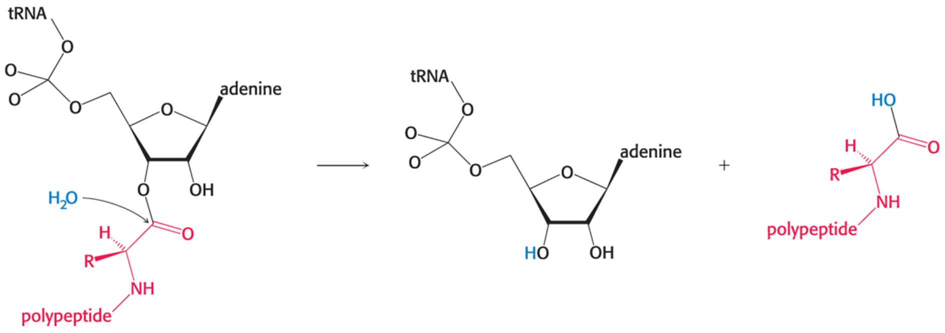
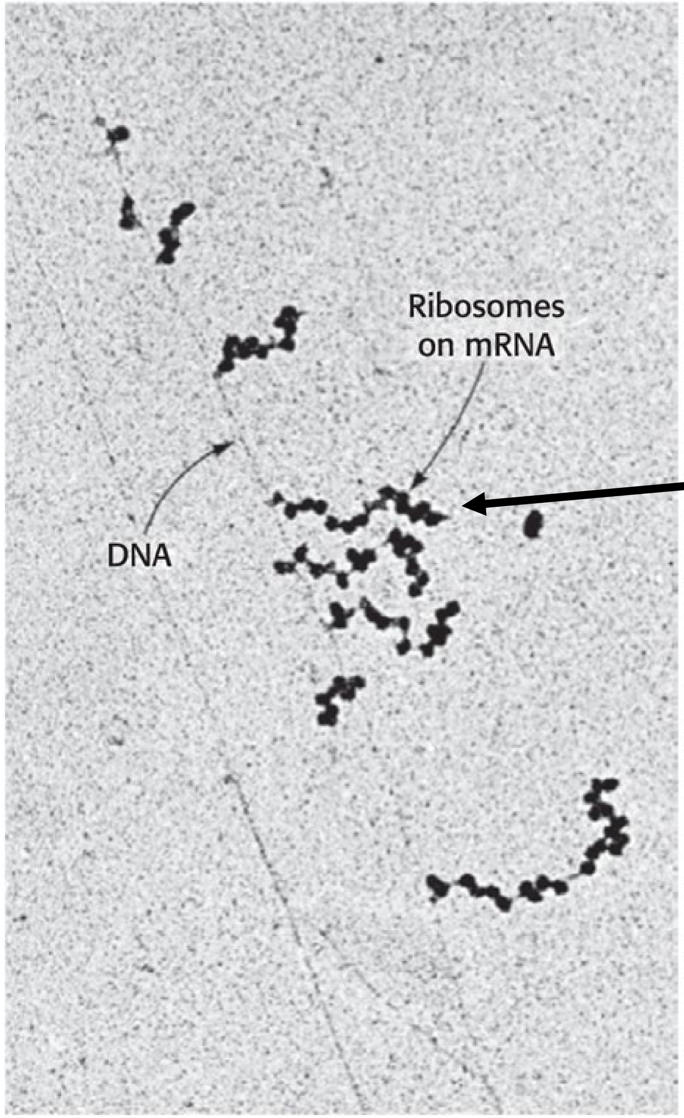
RF1 or RF2 bind stop codon at site A of the mRNA in the ribosome

- RF contains a highly conserved GGQ sequence, with a methylated glutamine (Q) that promotes an attack of the ester linkage between the tRNA and the polypeptide chain



In Bacteria: transcription and translation are coupled in space and time



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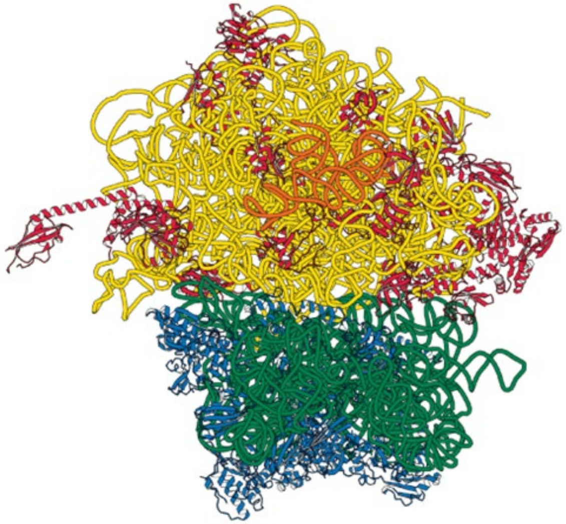
- In bacteria, there is minimal time between transcription and translation
- Multiple ribosomes can simultaneously translate one mRNA

<u>Polysome:</u> a group of ribosomes bound to the same mRNA molecule

Difference between bacterial and eukaryotic protein biosynthesis

Bacteria

70S ribosome



50S subunit

34 proteins

23S rRNA

5S rRNA

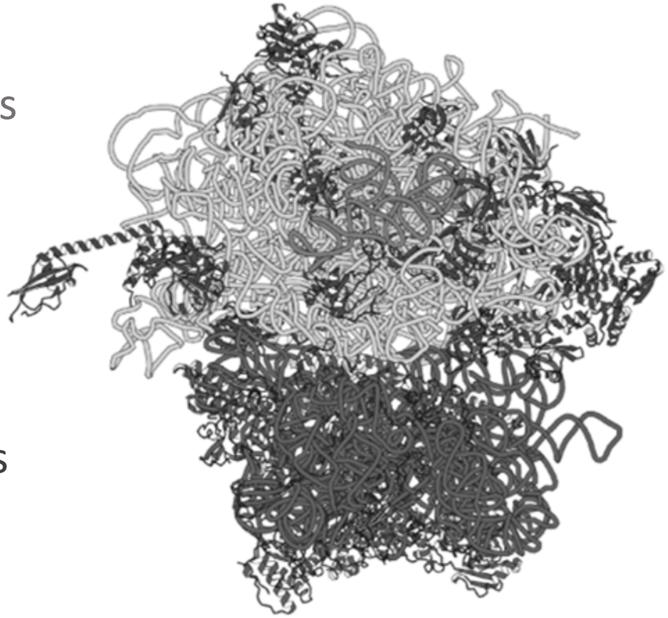
30S subunit

21 proteins

16S rRNA

Eukaryotic

80S ribosome



60S subunit

47 proteins

5S rRNA

5.8S rRNA

28S rRNA

40S subunit

33 proteins

18S rRNA

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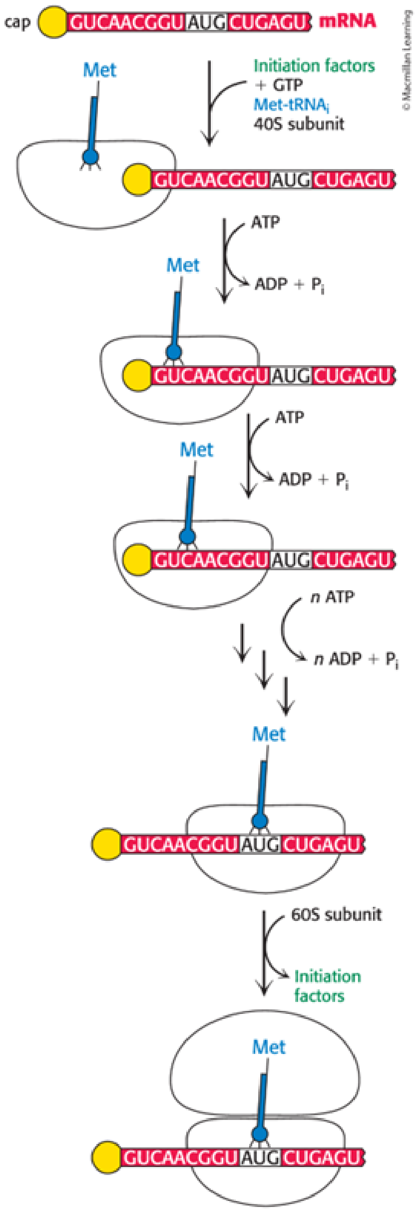
Difference between bacterial and eukaryotic protein biosynthesis



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- The initiating amino acid is methionine rather than N-formylmethionine

 - a special tRNA called Met-tRNAi^{Met} participates in initiation

- No Shine–Dalgarno sequence is present on the 5′ side to distinguish initiator AUGs from internal ones

- The singular start site is usually the AUG nearest to the 5′ end

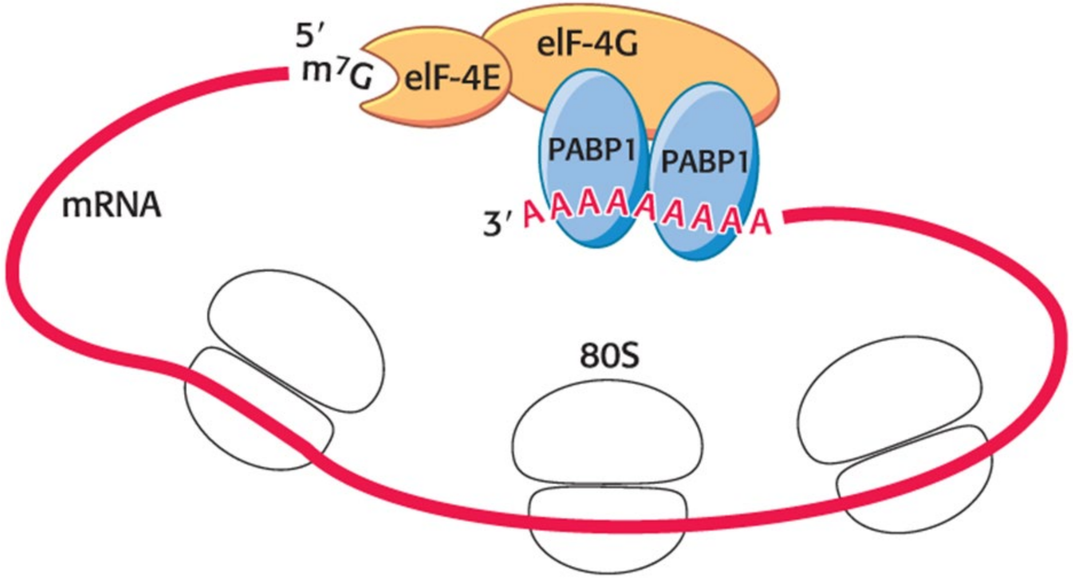
- Eukaryotes use many more initiation factors (eIF) with more complex interplay.

Note: Codon AUG encodes for the amino acid methionine

Difference between bacterial and eukaryotic protein biosynthesis

Protein interactions circularize eukaryotic mRNA

Eukaryotic proteins bound to the 5′ cap and poly(A)-binding protein 1 (PABP1) bound to the 3′ poly(A) tail come together to create a circular mRNA.



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This circularization may regulate translation of certain mRNAs

- prevents translation of mRNA molecules that have lost their poly(A) tails

A variety of antibiotics and toxins inhibit protein synthesis

TABLE 30.4 Antibiotic inhibitors of protein biosynthesis

Antibiotic	**Action**
Streptomycin and other aminoglycosides	Inhibit initiation and cause the misreading of mRNA (bacteria)
Tetracycline	Binds to the 30S subunit and inhibits the binding of aminoacyl-tRNAs (bacteria)
Chloramphenicol	Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (bacteria)
Cycloheximide	Inhibits translocation (eukaryotes)
Erythromycin	Binds to the 50S subunit and inhibits translocation (bacteria)
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA (bacteria and eukaryotes)

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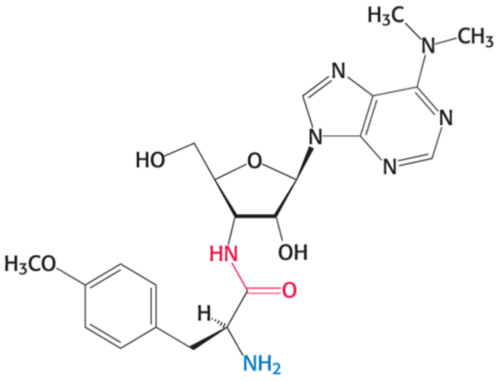
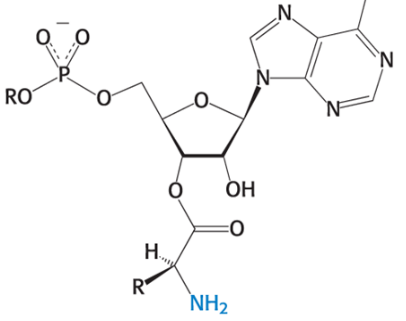


Puromycin

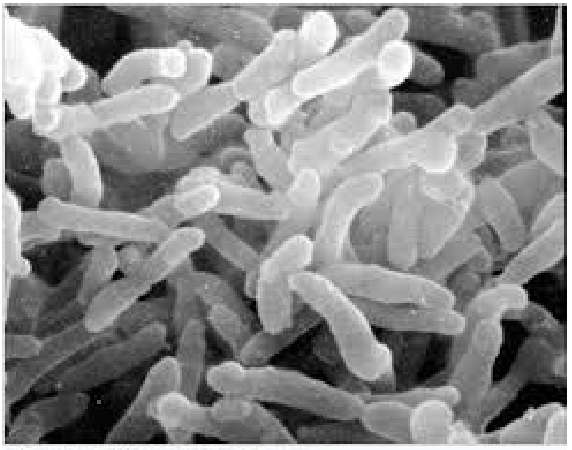


Aminoacyl-tRNA

puromycin: analog of the terminal part of aminoacyl-tRNA that binds to the A site and forms a peptide bond with the growing peptide chain, leading to release of the nascent peptide chain

A variety of antibiotics and toxins inhibit protein synthesis

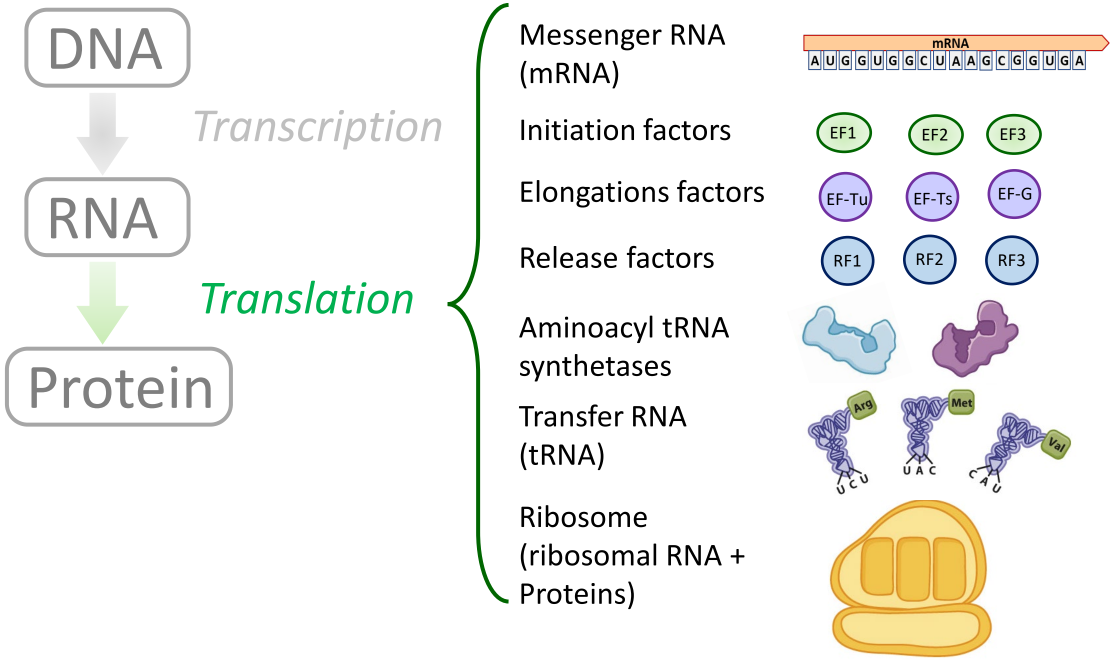
Diphtheria toxin blocks protein biosynthesis in eukaryotes by inhibiting translocation



- *Corynebacterium diphtheriae* = the bacterial cause of <u>diphtheria</u>
  - infects the upper respiratory tract of infected individuals
  - produces the diphtheria toxin
  - in unimmunized individuals, infection can be fatal.

- The toxin covalently prevents translocation

Concepts



DNA

Transcription

RNA

Translation

Protein

Messenger RNA (mRNA)

Initiation factors

Elongations factors

Release factors

Aminoacyl tRNA synthetases

Transfer RNA (tRNA)

Ribosome (ribosomal RNA + Proteins)

- Genetic code
- Degeneracy
- Codon
- Anticodon
- Transfer RNA
- ’Wobble’ effect
- Aminoacyl-tRNA synthetases
- Aminoacyl-tRNA
- Aminosylation
- Aminoacyl-tRNA synthetases activation site
- Aminoacyl-tRNA synthetases editing site
- Ribosome
- Ribosomal RNA
- Ribosome E site, P site and A site
- Shine-Dalgarno sequence
- Initiation, elongation, translocation, termination
- Initiation factors
- Elongation factors
- Termination factors
- Polysome
- Inhibition of trancription