

Topic 10

Translation

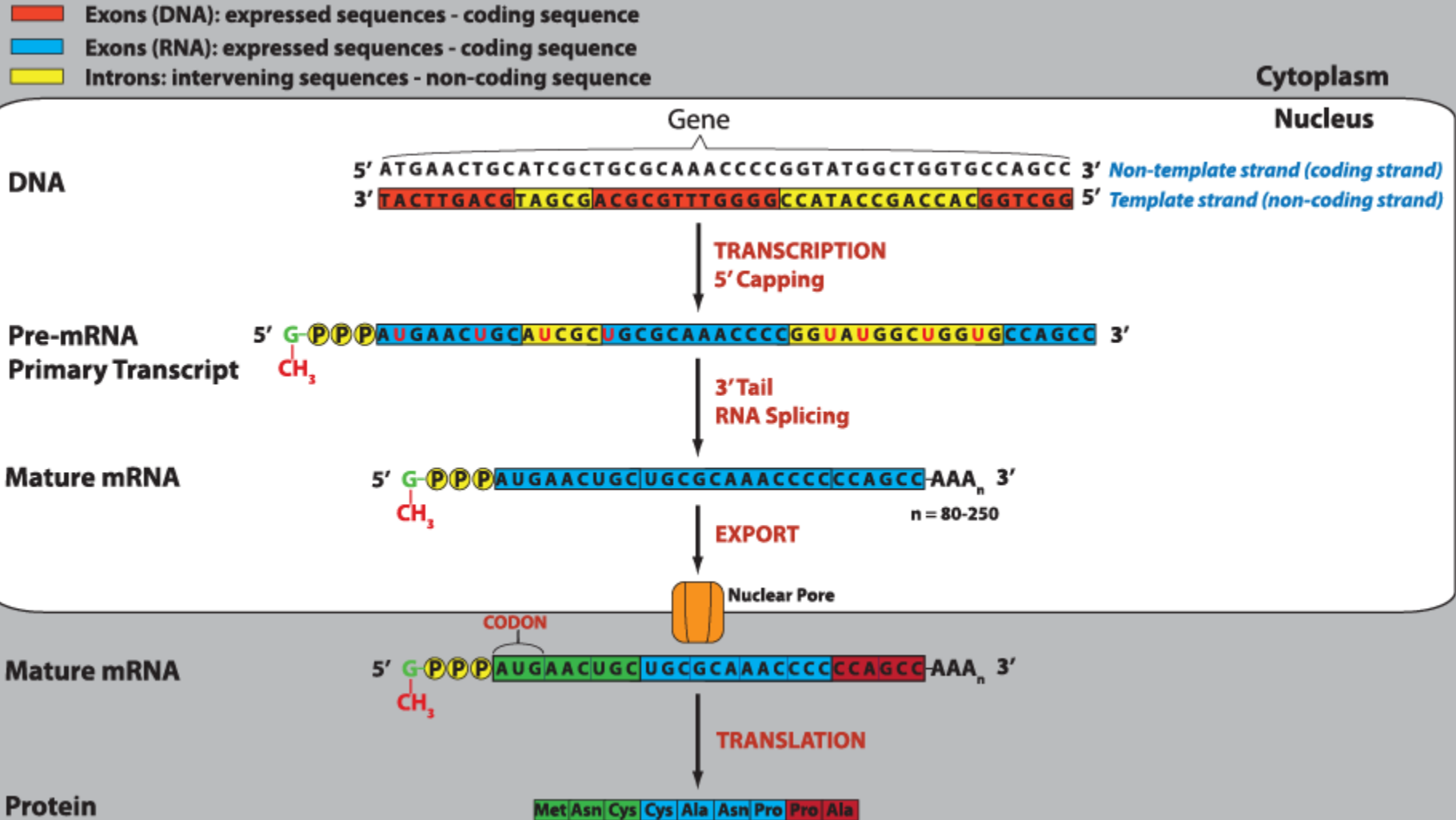
Nelson & Cox (2016)

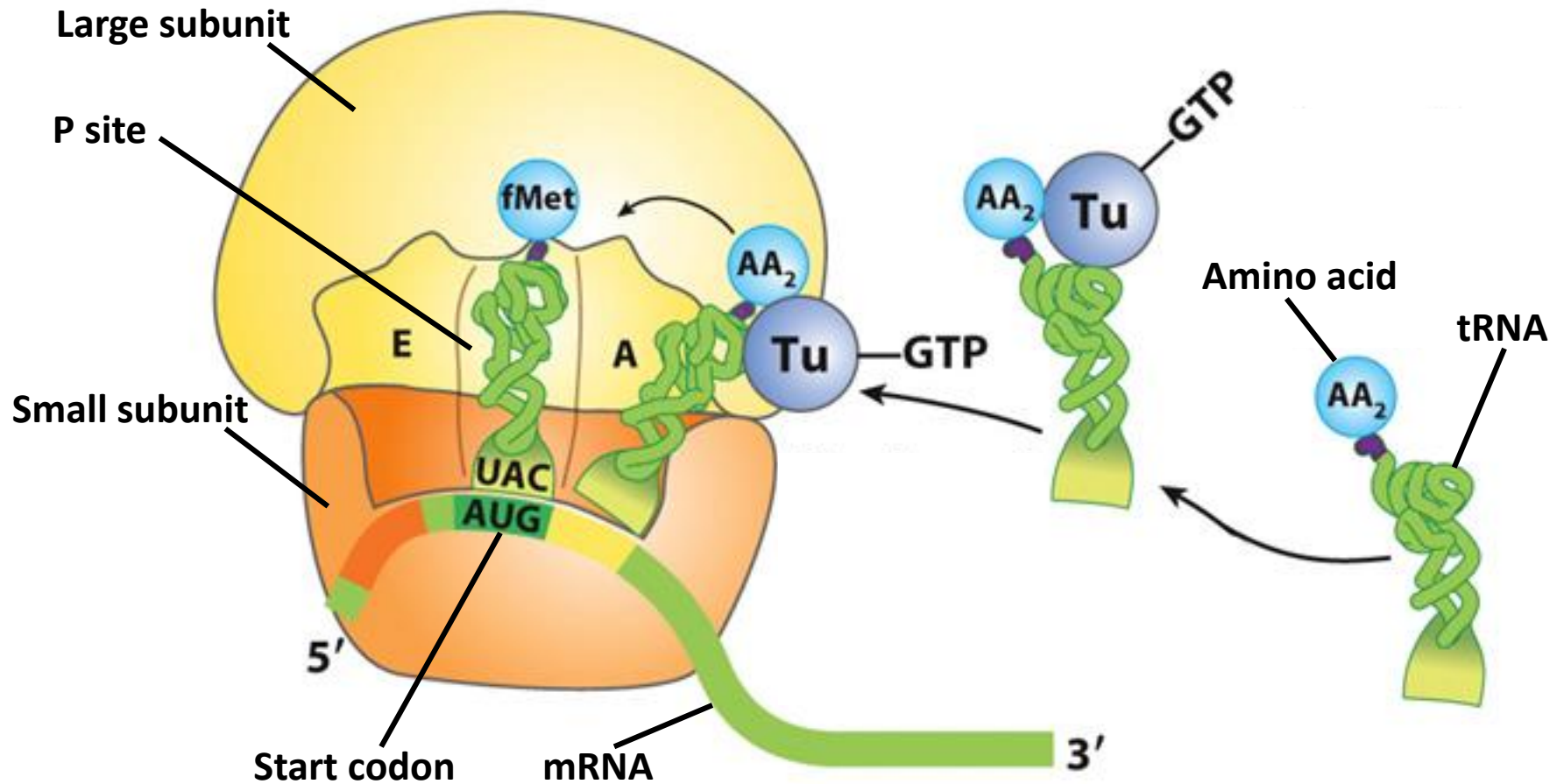
Chapter 26 pp. 1103-1139

Nelson & Cox (2017)

Chapter 26 pp. 1077-1114

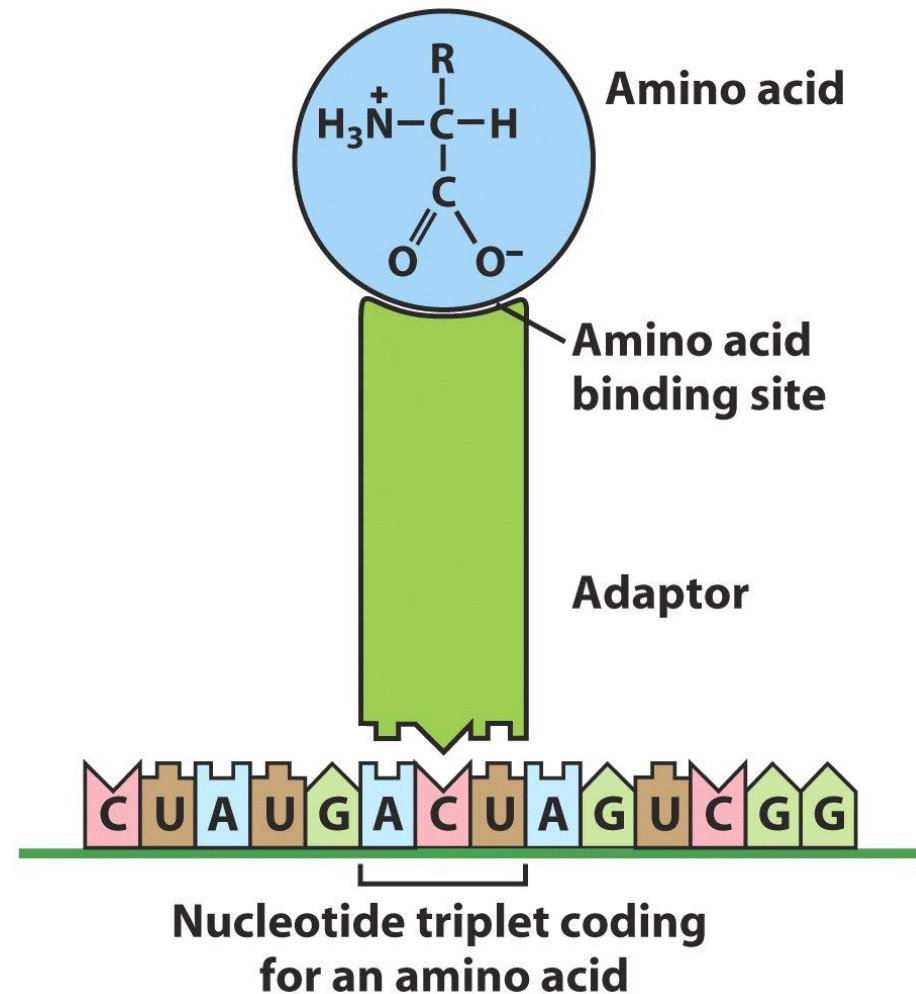
Overview of Translation





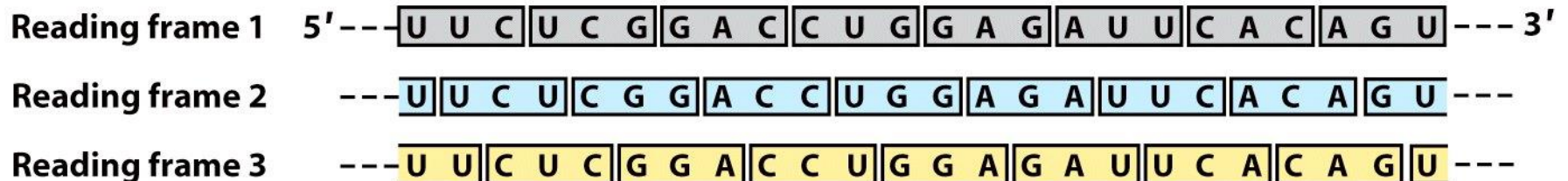
How do we Translate mRNA into Protein?

- Crick's adaptor hypothesis
 - How would you convert a 4 letter nucleic acid language (A,U,G,C) into 20 different amino acids?
 - $4^1 = 4$
 - $4^2 = 16$
 - $4^3 = 64$
- Hypothesis: an adaptor (RNA) molecule exists in which one part binds a specific amino acid, while the other recognises a specific nucleic acid sequence encoding that amino acid
- This adaptor molecule was later found to be a tRNA



Codon

- As Crick hypothesised, each amino acid molecule added to a growing peptide chain is encoded for by a triplet of nucleotides.
- This triplet is called a codon.
- Translation occurs through the successive reading of these triplets in a **non-overlapping** fashion.
- Therefore, the starting position of the first codon determines the reading frame and hence the amino acid sequence.
- In general, there are 3 possible reading frames for any one mRNA sequence.
- However, normally the reading frame is defined by a **start codon (AUG)**



First letter of codon (5' end)

Second letter of codon

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

Start Codon:

AUG

=Formylmethionine (prokaryote)

= Methionine (eukaryote)

Termination codons:

UAA

UAG

UGA

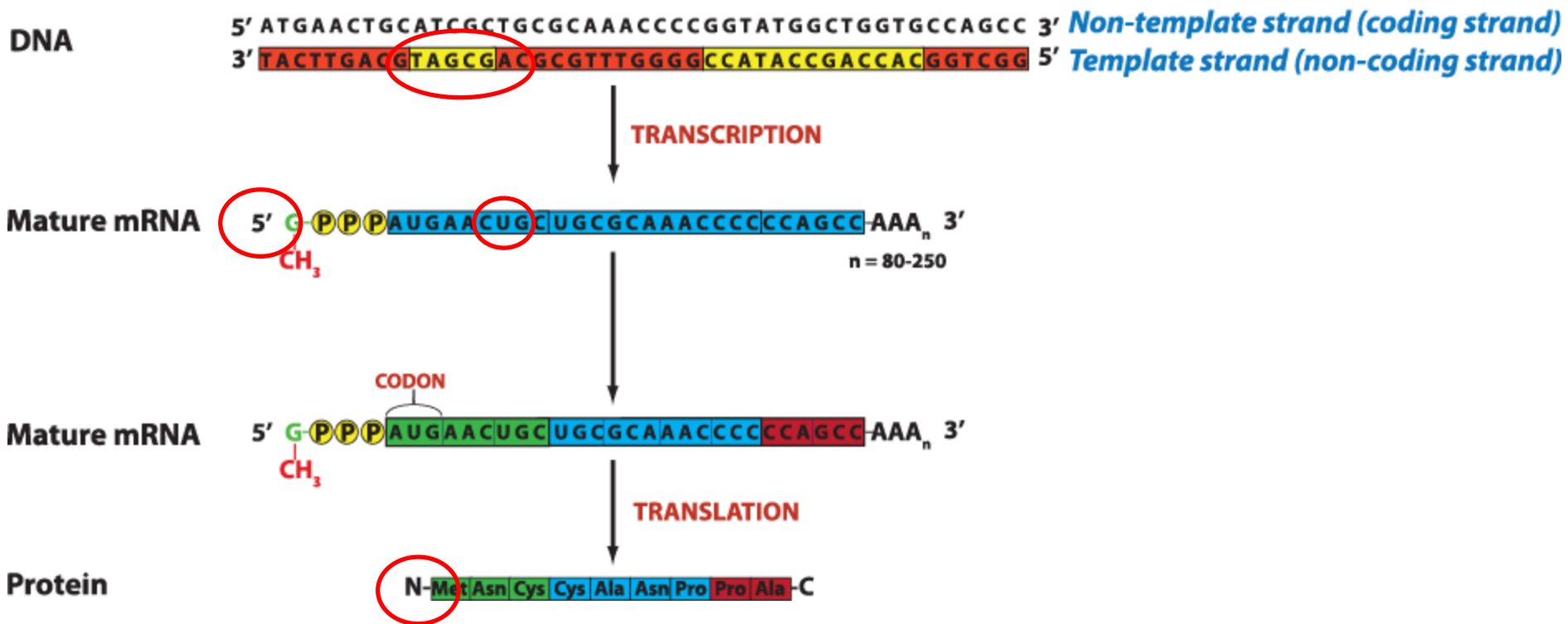
= Stop translation

TABLE 27–3 Degeneracy of the Genetic Code

<i>Amino acid</i>	<i>Number of codons</i>	<i>Amino acid</i>	<i>Number of codons</i>
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6

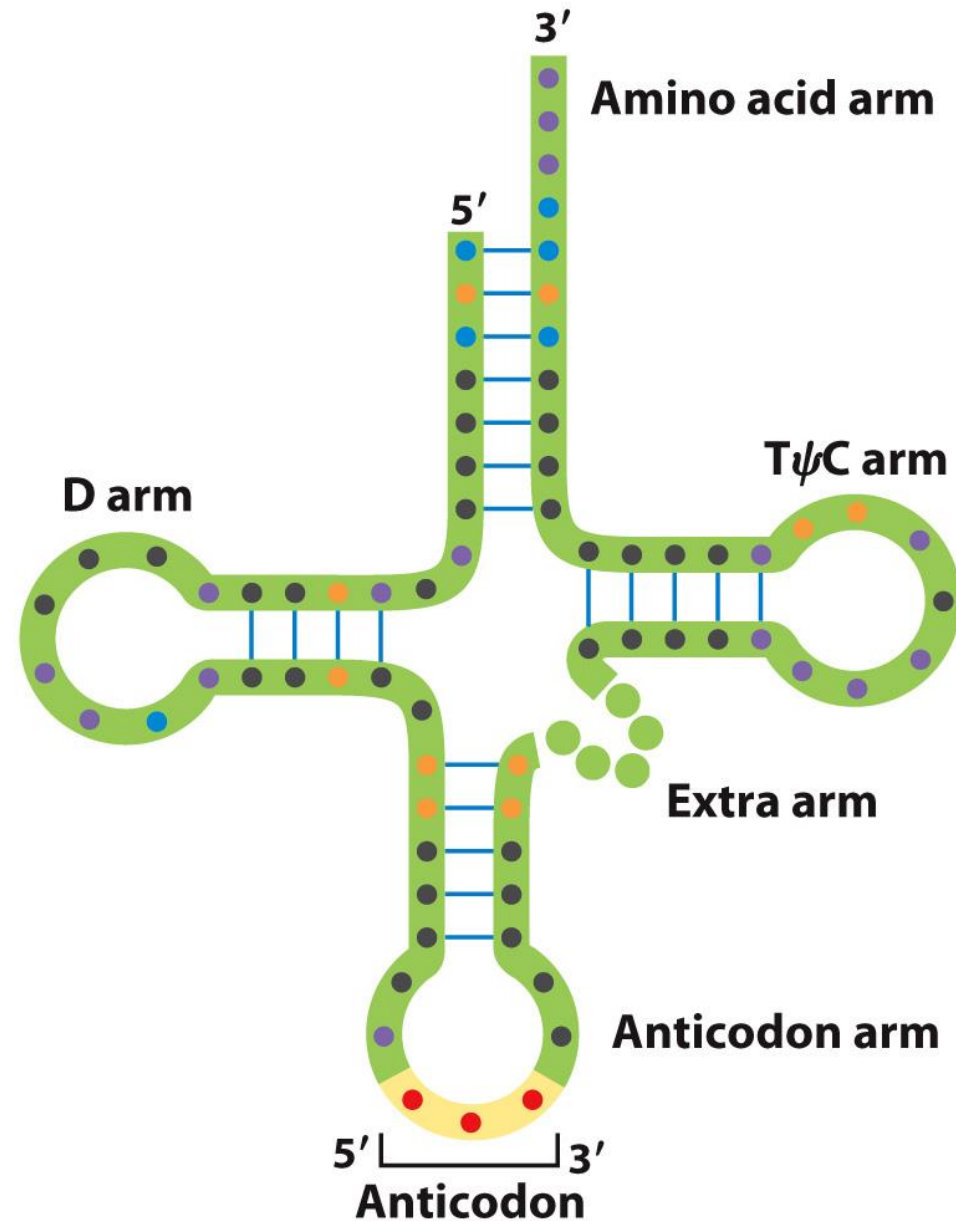
Transcription and Translation

- Exons (DNA): expressed sequences - coding sequence
- Exons (RNA): expressed sequences - coding sequence
- Introns: intervening sequences - non-coding sequence



Transfer RNA (tRNA)

- Small single stranded RNA molecule (73-93 nucleotides)
- Two-dimensional image shows a cloverleaf like structure with four major arms.
- Larger tRNAs have a fifth smaller arm
- One end of the tRNA carries the anticodon that recognises a specific triplet codon sequence in the mRNA during translation
- The other end (3') of the tRNA contains the acceptor stem which binds an amino acid to the free 2' or 3'-OH group



“Wobble”

- If each tRNA recognised only one codon, cells would have a different tRNA species for each amino acid
 - 64 codons (3 stop codons) = 61 coding codons
 - However, most cells have <45 species of tRNA
- Instead, relatively loose base pairing between 3' end of a codon with 5' end of some anti-codons allows multiple recognitions to occur
- This position is said to “wobble”
- The main wobble bases are;
 - Inosinate (I): **I-U**, **I-A** or **I-C**
 - Guanine (G): **G-C** or **G-U**
 - Uracil (U): **U-A** or **U-G**

TABLE 27-4

How the Wobble Base of the Anticodon Determines the Number of Codons a tRNA Can Recognize

1. One codon recognized:

Anticodon	(3') X - Y - C (5')	(3') X - Y - A (5')
Codon	(5') X' - Y' - G (3')	(5') X' - Y' - U (3')

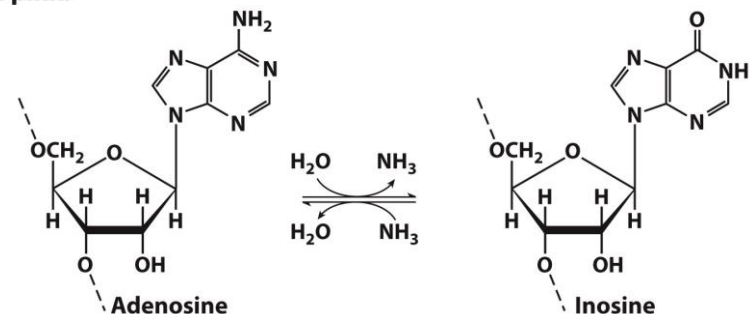
2. Two codons recognized:

Anticodon	(3') X - Y - U (5')	(3') X - Y - G (5')
Codon	(5') X' - Y' - A (3')	(5') X' - Y' - C (3')

3. Three codons recognized:

Anticodon	(3') X - Y - I (5')
Codon	(5') X' - Y' - A (3')

Note: X and Y denote bases complementary to and capable of strong Watson-Crick base pairing with X' and Y', respectively. Wobble bases—in the 3' position of codons and 5' position of anticodons—are shaded in pink.



“Wobble”

- Wobble is achievable because: first 2 letters are the primary determinants of specificity, e.g.
 - Arginine: **CGU**, **CGC**, **CGA**, **CGG**
- Rule: When the first base of the anticodon is C or A, only one codon is recognised. When G or U, two codons are recognised. When I, three codons are recognised

1. One codon recognized:

Anticodon (3') X–Y–C (5') (3') X–Y–A (5')

– – –
– – –
– – –

– – –
– – –
– – –

Codon (5') X'–Y'–G (3') (5') X'–Y'–U (3')

2. Two codons recognized:

Anticodon (3') X–Y–U (5') (3') X–Y–G (5')

– – –
– – –
– – –

– – –
– – –
– – –

Codon (5') X'–Y'–A_G (3') (5') X'–Y'–U_G (3')

3. Three codons recognized:

Anticodon (3') X–Y–I (5')

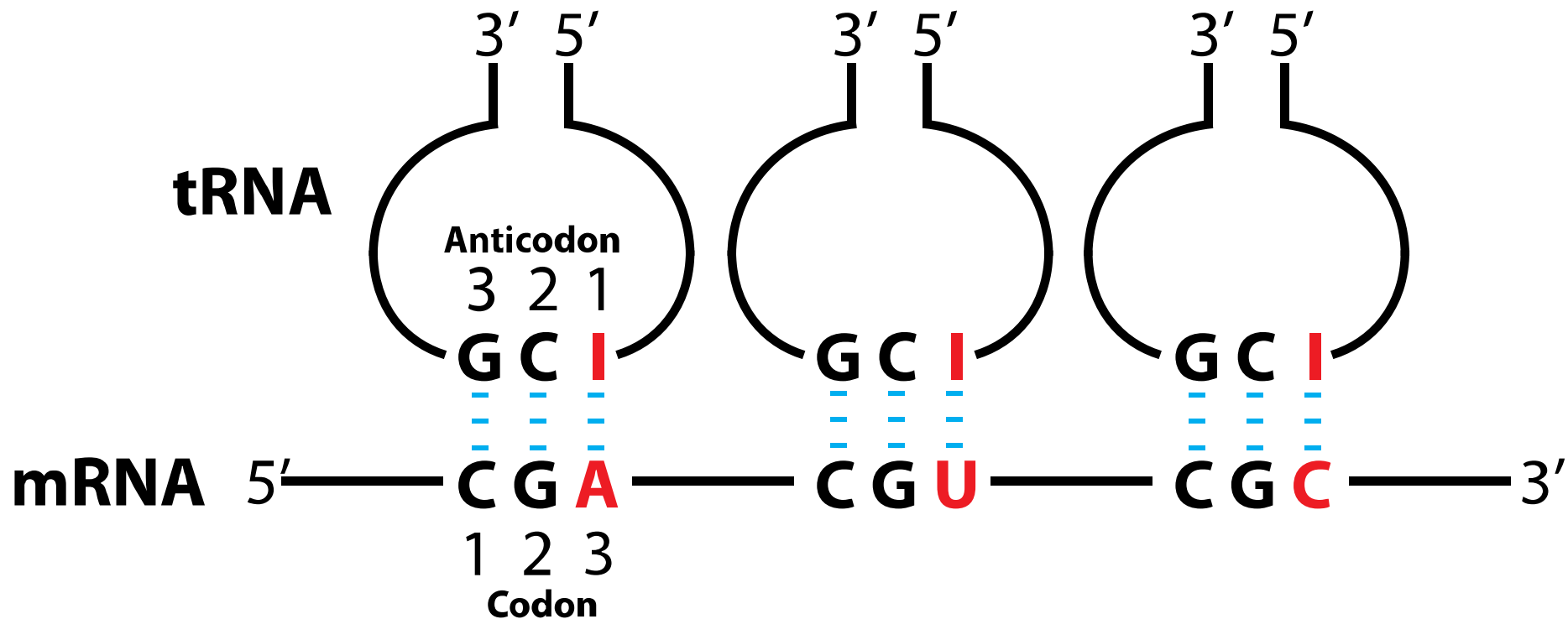
– – –
– – –
– – –

Codon (5') X'–Y'–A_U
C

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

“Wobble”

**tRNA^{Arg} (arginine) includes inosinate:
Therefore, recognises 3 codons**

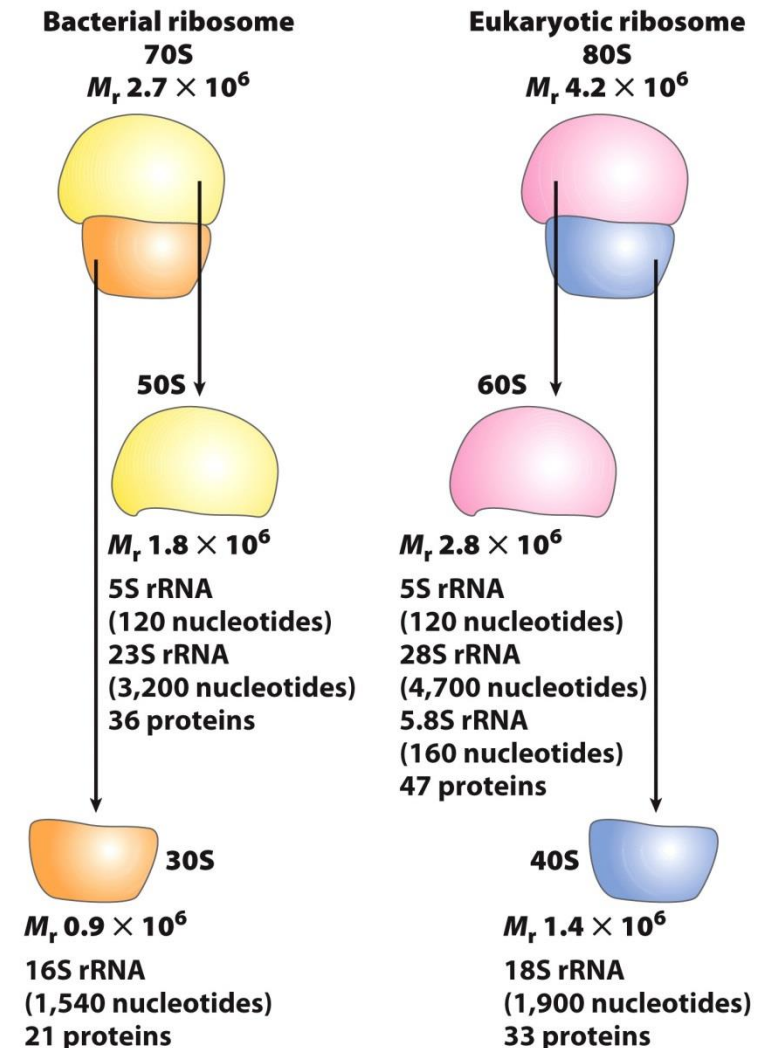


Protein Synthesis

- Occurs on ribosomes
 - Supramolecules: composed of 65% ribosomal RNA (rRNA), and 35% proteins
- Uses activated transfer RNA (tRNA) molecules and messenger RNA (mRNA)

Ribosomes

- Ribosomes are supramolecules composed of two unequal subunits consisting of RNA and protein.
 - Bacterial ribosome (**70S**)
 - = **50S** (5S & 23S rRNA) + **30S** (16S rRNA)
 - Eukaryotic ribosome (**80S**)
 - = **60S** (5S, 5.8S & 28S rRNA) + **40S** (18S rRNA)
- S = sedimentation coefficient, relates to the molecular weight, volume and diameter of the molecule
- Interestingly**, ribosomal RNA forms the structural core, with proteins acting as secondary elements
- There is no protein within the active site of the ribosome
- Therefore, rRNA has **enzymatic properties** (ribozyme)
- The 2 ribosomal subunits come together to form a cleft that the mRNA will pass through as the ribosome moves

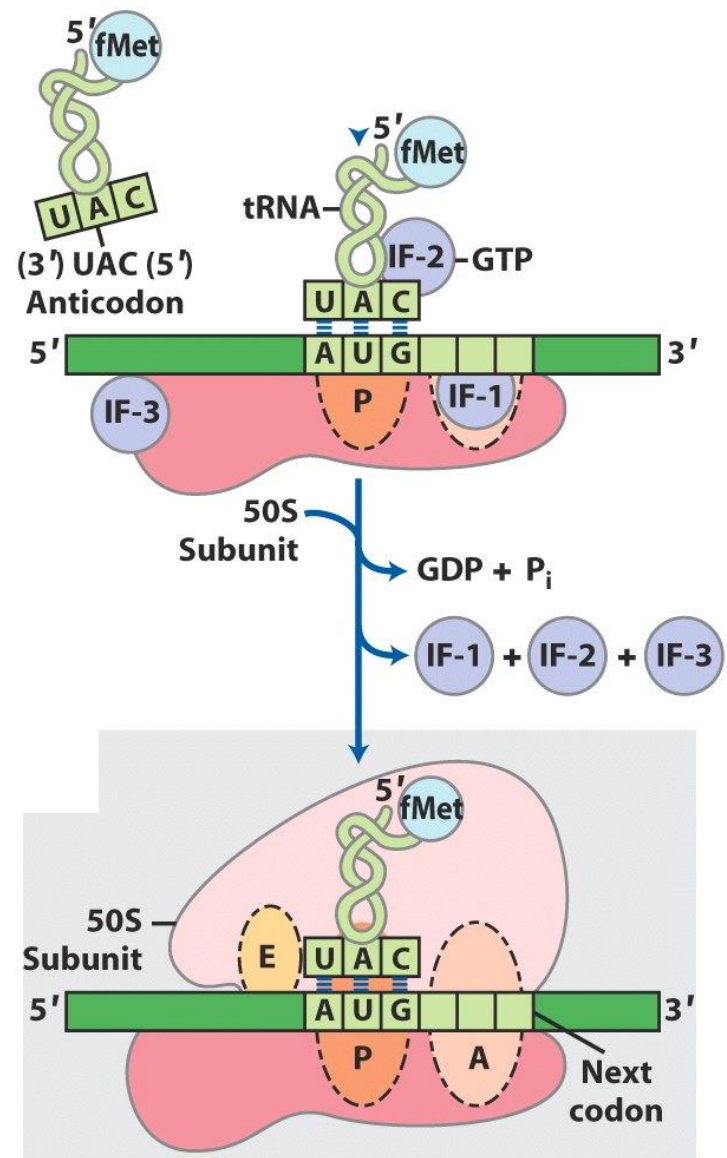


Five Stages of Protein Synthesis

1. Activation of amino acids
2. Initiation
3. Elongation
4. Termination and release
5. Folding and post-translational processing

Protein Synthesis

- **Stage 1: Activation of amino acids.** This is achieved by attaching the correct amino acid (critical) to its appropriate tRNA (aminoacyl-tRNA).
- **Stage 2: Initiation.** mRNA binds to small ribosomal subunit, followed by the initiating aminoacyl-tRNA (fMet or Met) binding to the start codon (AUG). Finally, the larger ribosomal subunit binds to form the initiation complex
- **Stage 3: Elongation.** The newly forming polypeptide chain is lengthened by the continuous binding of further tRNA molecules to their corresponding codons on the mRNA sequence within the ribosomal complex.



Protein Synthesis

- **Stage 4: Termination.** Completion of the new polypeptide chain is signalled by a termination codon (UAA, UGA, UAG). Peptide is released and ribosomal complex is recycled for another round of synthesis
- **Stage 5: Folding and post-translational modifications.** New polypeptide chain folded into its three dimensional shape. May also undergo modifications (e.g. trimming of peptide chain, acetylation, phosphorylation etc.)

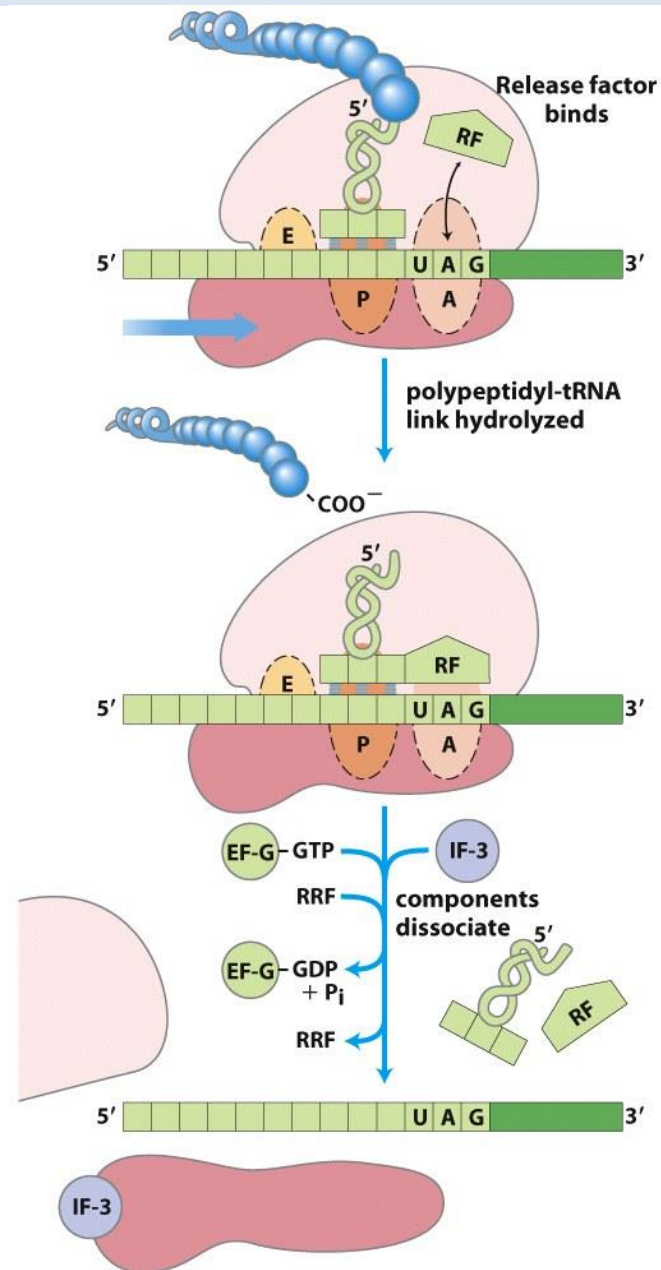
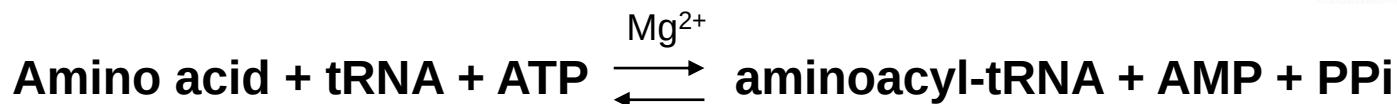
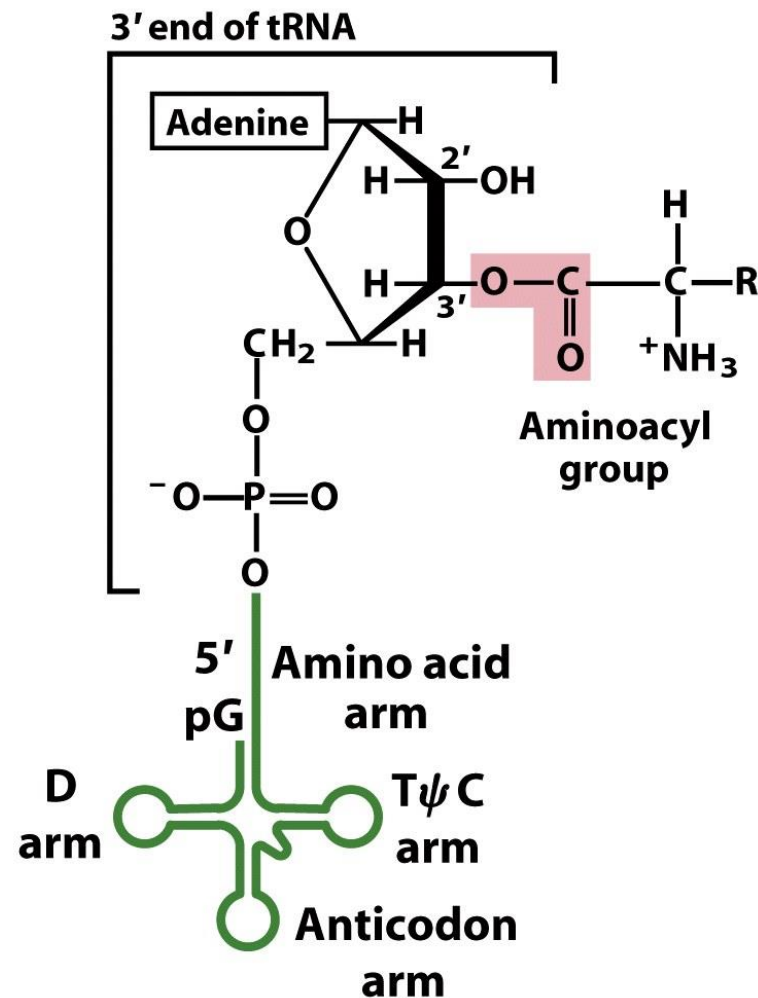


TABLE 27-5**Components Required for the Five Major Stages of Protein Synthesis in *E. Coli***

Stage	Essential components
1. Activation of amino acids	20 amino acids 20 aminoacyl-tRNA synthetases 32 or more tRNAs ATP Mg ²⁺
2. Initiation	mRNA N-Formylmethionyl-tRNA ^{fMet} Initiation codon in mRNA (AUG) 30S ribosomal subunit 50S ribosomal subunit Initiation factors (IF1, IF2, IF3) GTP Mg ²⁺
3. Elongation	Functional 70S ribosomes (initiation complex) Aminoacyl-tRNAs specified by codons Elongation factors (EF-Tu, EF-Ts, EF-G) GTP Mg ²⁺
4. Termination and ribosome recycling	Termination codon in mRNA Release factors (RF1, RF2, RF3, RRF) EF-G IF3
5. Folding and posttranslational processing	Chaperones and folding enzymes (PPI, PDI); specific enzymes, cofactors, and other components for removal of initiating residues and signal sequences, additional proteolytic processing, modification of terminal residues, and attachment of acetyl, phosphoryl, methyl, carboxyl, carbohydrate, or prosthetic groups

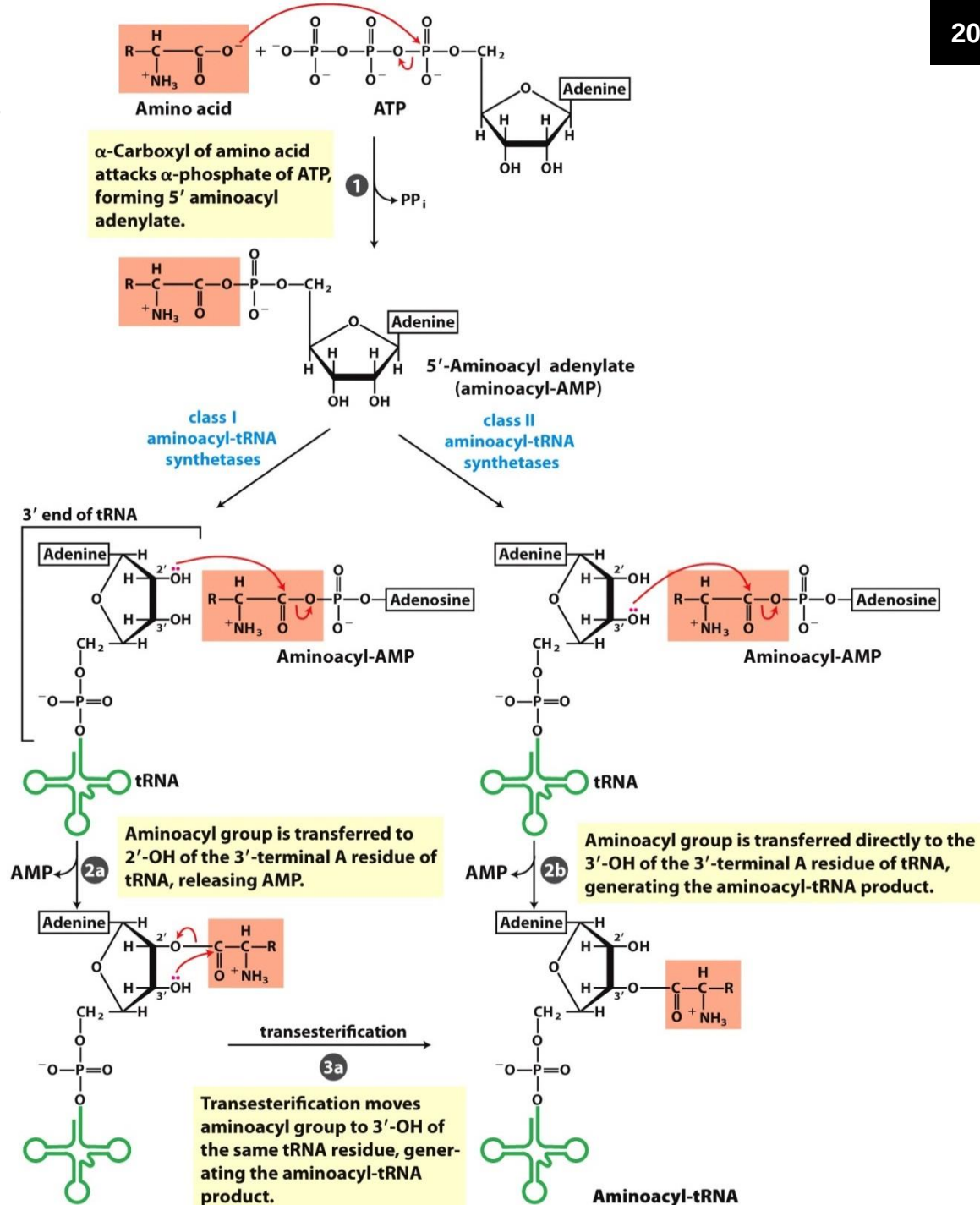
1. Activation of Amino Acids

- **Aminoacyl-tRNA synthetases** attach (esterify) the amino acids onto their corresponding tRNA, creating an **aminoacyl-tRNA**
- 20 amino acids, 20 aminoacyl-tRNA synthetases
- For amino acids that have more than one tRNA, the same aminoacyl-tRNA synthetase is used
- Two major classes of aminoacyl-tRNA synthetases that differ only in how they enzymatically attach the amino acid



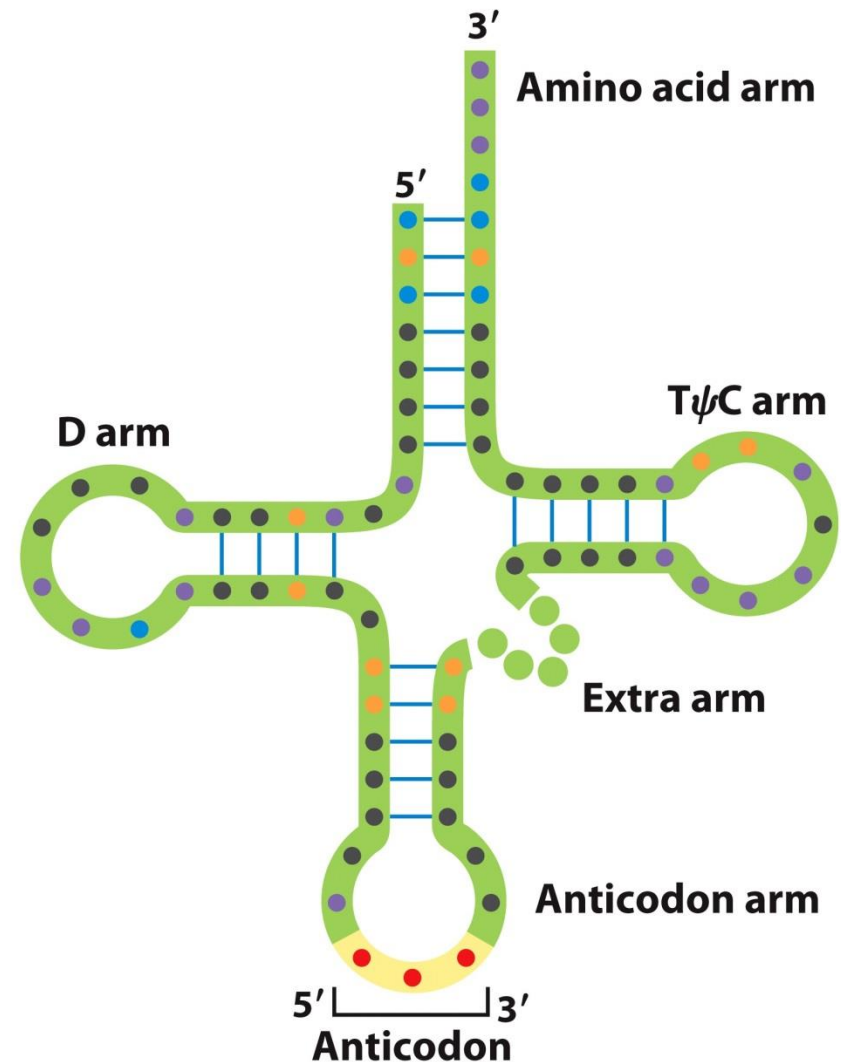
Aminoacyl-tRNAs

- Step 1:** activation of the amino acid through addition of AMP (AA + ATP → aminoacyl-AMP + PP_i)
- Step 2:** aminoacyl group is transferred to its corresponding tRNA (aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP)
 - Depends on class of enzyme. Class 2: directly attached to 3' OH group; Class 1: attached initially to 2' OH, before being attached to 3' OH group.



Aminoacyl-tRNA Synthetase Proofreading

- It is critical that the correct amino acid is added to the tRNA, as this is not checked by the ribosome
- Three processes allow the correct amino acid to be added by the aminoacyl-tRNA synthetase:
 - The correct amino acid fits within the active site
 - Recognises specific nucleotides within the correct tRNAs (orange and blue dots in figure)
 - Proofreading ability

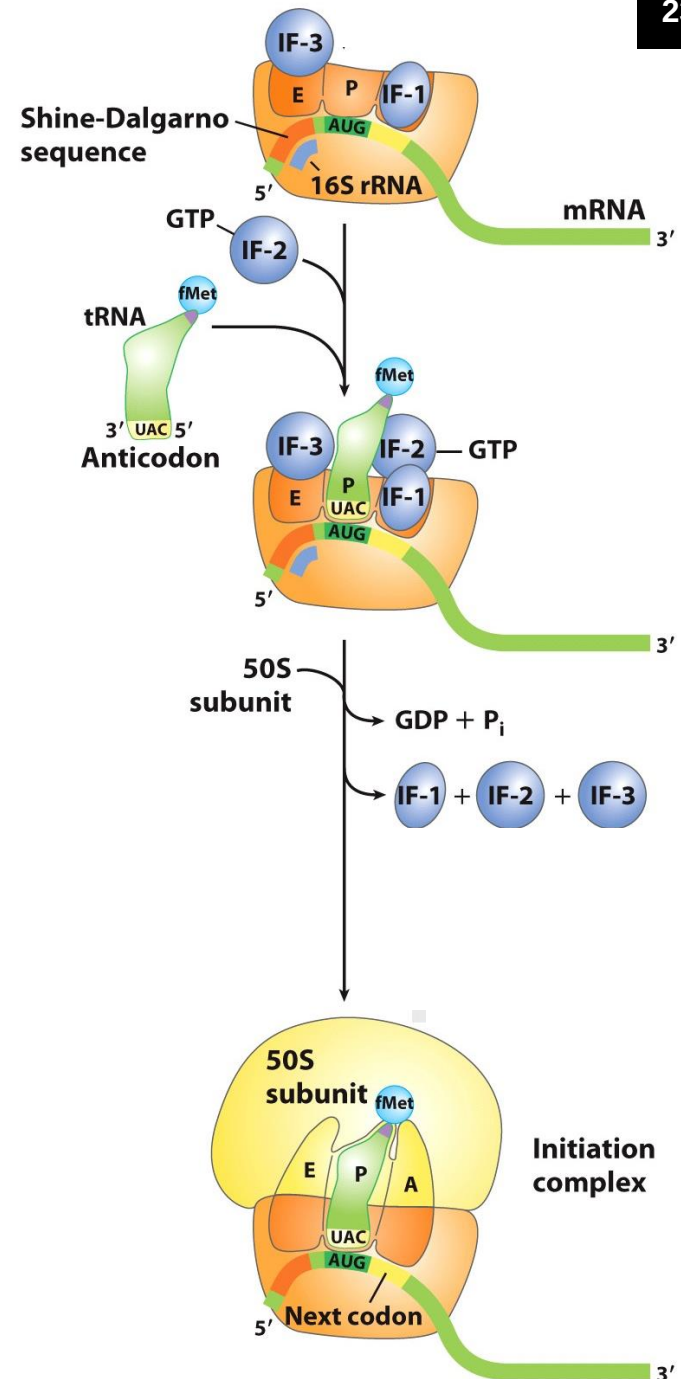


2. Initiation of Synthesis

- Formation of initiation complex
 - Contains: 30S ribosomal subunit, 50S ribosomal subunit, mRNA, GTP, initiating tRNA, initiation factors (IF) and magnesium
 - Three step process
 1. 30S ribosomal subunit binds IF's 1 & 3. mRNA then binds to the 30S+IF subunit. In bacteria, correct binding of the mRNA is guided by the Shine-Dalgarno sequence.
 2. Initiation aminoacyl-tRNA (fMet or Met) and GTP-bound-IF2 binds to start codon (AUG) on 30S+IF+mRNA complex
 - Even though there is only 1 codon for Met, all organisms have 2 tRNAs for Met
 - tRNA^{fmet}- used exclusively for the initiation Met
 - tRNA^{met}- used for all other Met residues
 3. 50S ribosomal subunit now binds to the complex, while at the same time all three IFs disassociate, producing a functional 70S ribosome (**initiation complex**).

Initiation Complex

- Bacterial ribosomes have 3 sites that bind tRNA:
 - Aminoacyl (A) site:** binds incoming aminoacyl-tRNA
 - Peptidyl (P) site:** contains the extending peptide chain attached to a tRNA
 - Exit (E) site:** binds uncharged tRNAs that have finished
- tRNA^{fmet}** binds to **P site**.
- IF-3** prevents premature binding of 50S subunit.
- IF-1** Prevents binding of tRNA at the **A site** (prevents elongation)



Eukaryotic Initiation

TABLE 27-8 Protein Factors Required for Initiation of Translation in Bacterial and Eukaryotic Cells

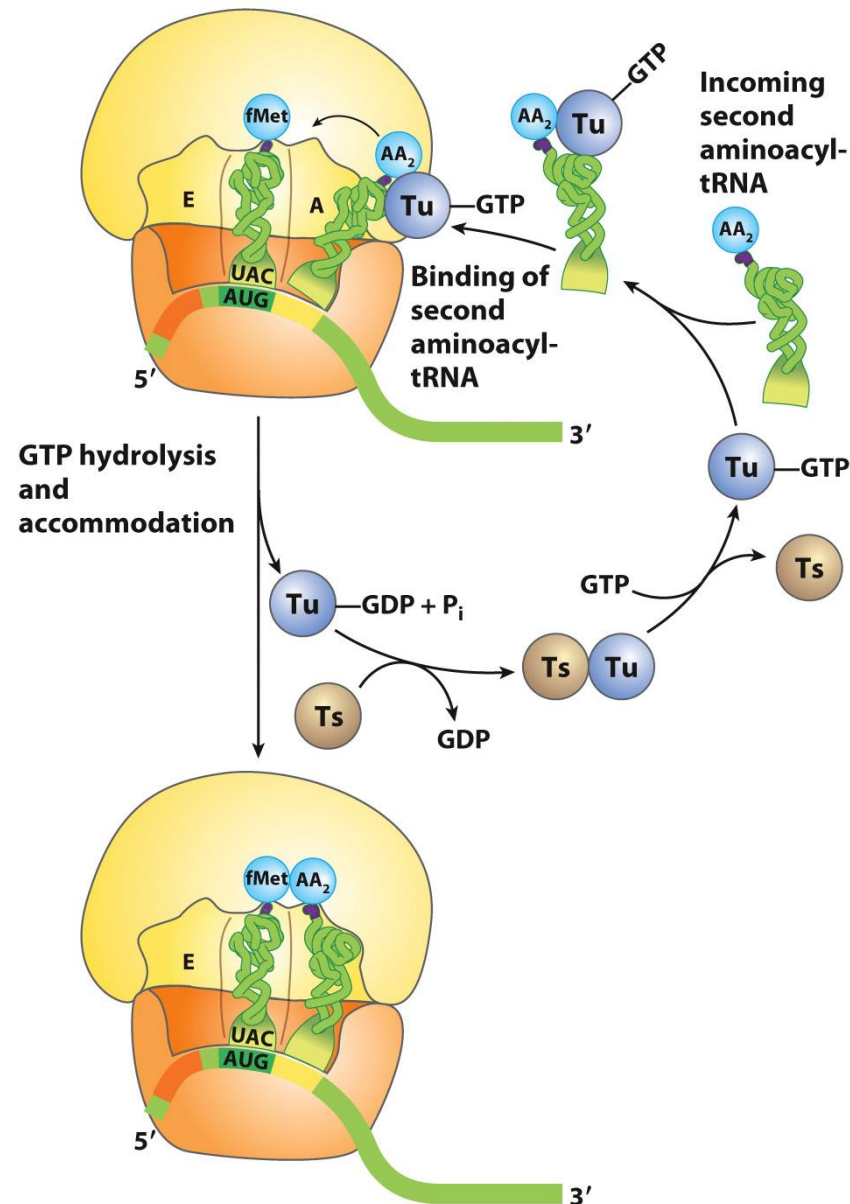
<i>Factor</i>	<i>Function</i>
Bacterial	
IF-1	Prevents premature binding of tRNAs to A site
IF-2	Facilitates binding of fMet-tRNA ^{fMet} to 30S ribosomal subunit
IF-3	Binds to 30S subunit; prevents premature association of 50S subunit; enhances specificity of P site for fMet-tRNA ^{fMet}
Eukaryotic*	
eIF2	Facilitates binding of initiating Met-tRNA ^{Met} to 40S ribosomal subunit
eIF2B, eIF3	First factors to bind 40S subunit; facilitate subsequent steps
eIF4A	RNA helicase activity removes secondary structure in the mRNA to permit binding to 40S subunit; part of the eIF4F complex
eIF4B	Binds to mRNA; facilitates scanning of mRNA to locate the first AUG
eIF4E	Binds to the 5' cap of mRNA; part of the eIF4F complex
eIF4G	Binds to eIF4E and to poly(A) binding protein (PAB); part of the eIF4F complex
eIF5	Promotes dissociation of several other initiation factors from 40S subunit as a prelude to association of 60S subunit to form 80S initiation complex
eIF6	Facilitates dissociation of inactive 80S ribosome into 40S and 60S subunits

*The prefix “e” identifies these as eukaryotic factors.

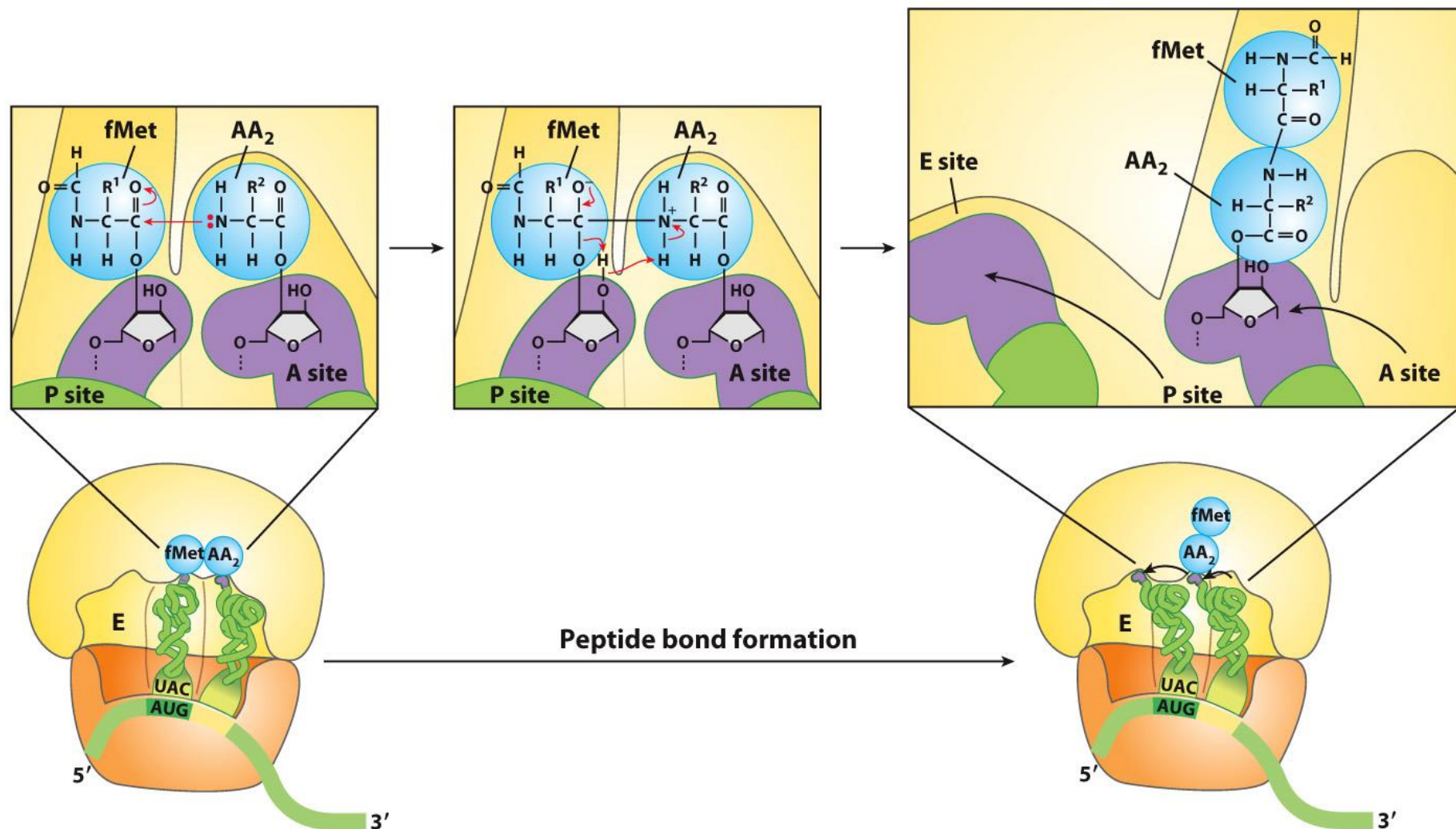
3. Elongation

- In bacteria, elongation requires: ribosomal initiation complex, aminoacyl-tRNAs, elongation factors (EF: EF-Tu, EF-Ts, EF-G) and GTP.
- Three step process
 1. **Binding of an appropriate incoming aminoacyl-tRNA:** Incoming aminoacyl-tRNA binds to a GTP-bound-EF-Tu, before binding to the **A site** of the 70S complex, hydrolysing the GTP.
 2. **Peptide bond formation:** occurs between two amino acid residues attached to the tRNAs occupying site A and P on the ribosome. This occurs through the transfer of the amino acid group in site P to the second amino acid group in site A. Enzymatic formation of the peptide bond is achieved by peptidyl transferase (23S rRNA ribozyme)
 3. **Translocation:** ribosome moves one codon towards the 3' end of the mRNA sequence. This shifts the now-empty tRNA from site P to E, and the growing peptide chain and corresponding tRNA from site A to P. This movement requires the hydrolysis of GTP which is attached to EF-G

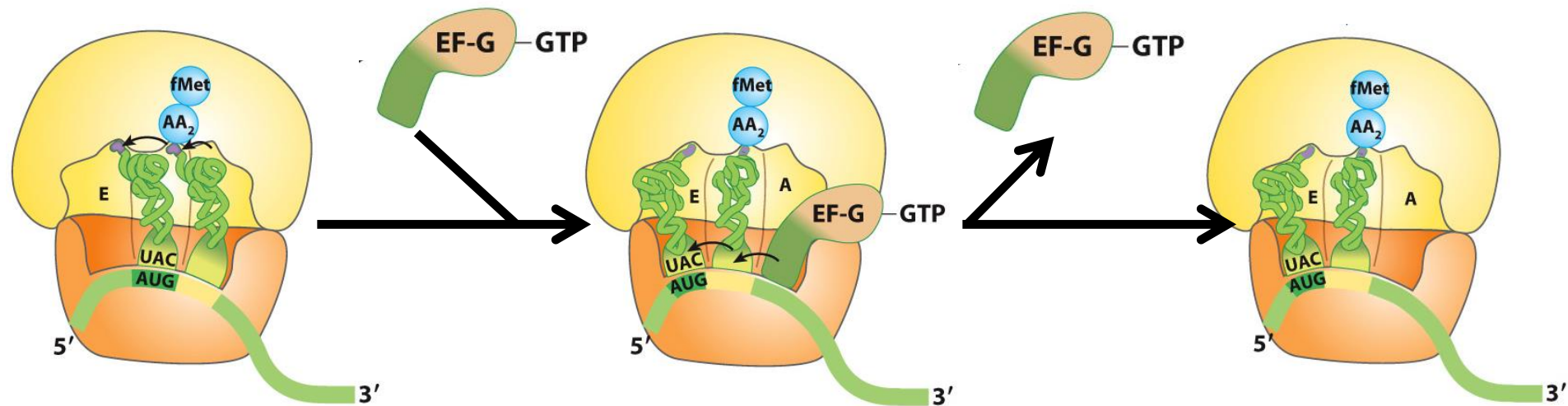
Step 1: Binding of an incoming aminoacyl tRNA



Step 2: Peptide bond formation

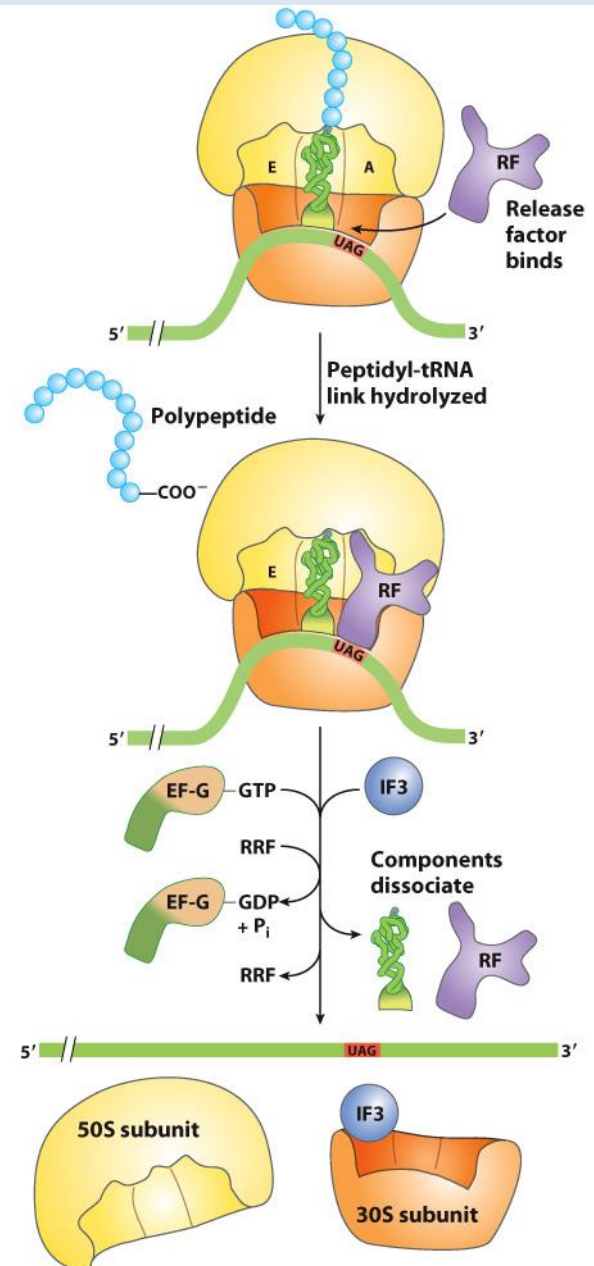


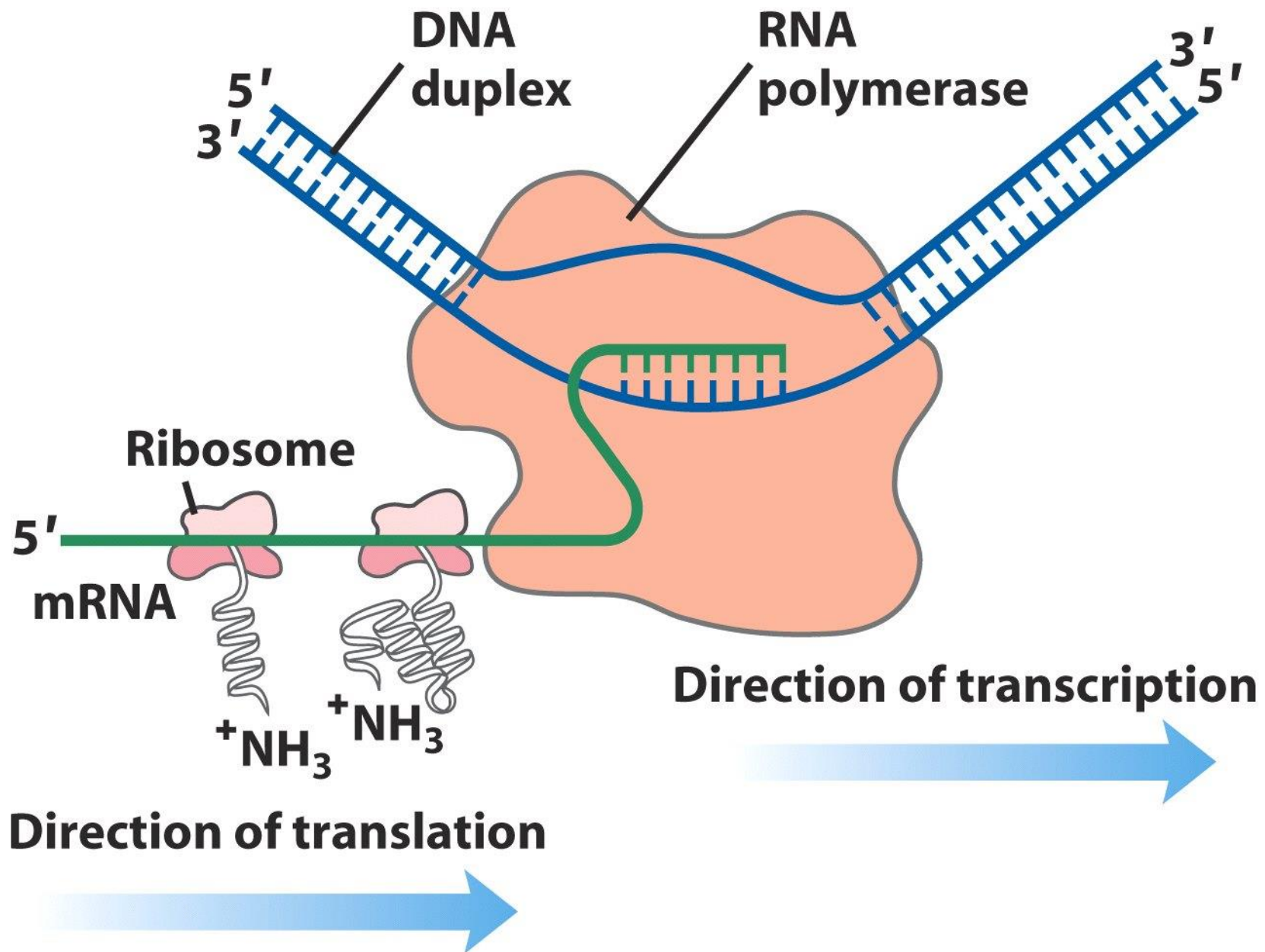
Step 3: Translocation



4. Termination

- Termination is signalled by one of three termination codons (UAA, UAG, UGA)
- In bacteria, once the termination codon enters the A site, three termination/release factors (RF-1, RF-2 & RF-3) bind.
- This causes:
 - Hydrolysis of terminal peptidyl-tRNA bond
 - Release of the free polypeptide and last tRNA
 - Dissociation of the 70S ribosome into its corresponding subunits (30S & 50S)





Transcription and Translation are coupled in bacteria

5. Post-Translational Modifications

- Needs to assume its native conformation and become functionally active
- Many proteins require **post-translational modifications**:
 - Amino and carboxyl-terminal modifications
 - Removal of fMet from N-terminal
 - 50% Eukaryotic proteins are acetylated at the amino group of the N-terminal
 - Loss of signal sequences
 - Sequence used to guide proteins to the correct cellular location
 - Modification of individual amino acids
 - Phosphorylation, acetylation, methylation etc.
 - Attachment of carbohydrate side chains (glycoproteins) or other macromolecules (e.g. lipids - Lipoproteins)
 - Addition of prosthetic groups
 - Proteolytic cleavage (activation)
 - Formation of disulphide bonds

You should be able to...

- Describe and use the terms: translation, peptide bond, genetic code, codon, anti-codon, wobble;
- Understand the structure of rRNA and tRNA
- Appreciate the complexity of the translation process;
- Describe the translation process;
- Understand the difference in translation complexity between eukaryotes and prokaryotes;
- Appreciate the function of base-pairing in translation;
- Describe and understand the role of ribosomes and tRNAs in translation.

What's next.....nothing, we've finished

- ✓ • Topic 1 – Foundations in Biochemistry
- ✓ • Topic 2 – Amino Acids
- ✓ • Topic 3 – Proteins
- ✓ • Topic 4 – Lipids and Membranes

- ✓ • Topic 5 – Signal Transduction
- ✓ • Topic 6 – Enzymes
- ✓ • Topic 7 – Nucleotides and Nucleic Acids
- ✓ • Topic 7 – DNA Replication
- ✓ • Topic 8 – Transcription
- ✓ • Topic 9 – Translation

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