

RNA Structure Genetic Code & Translation

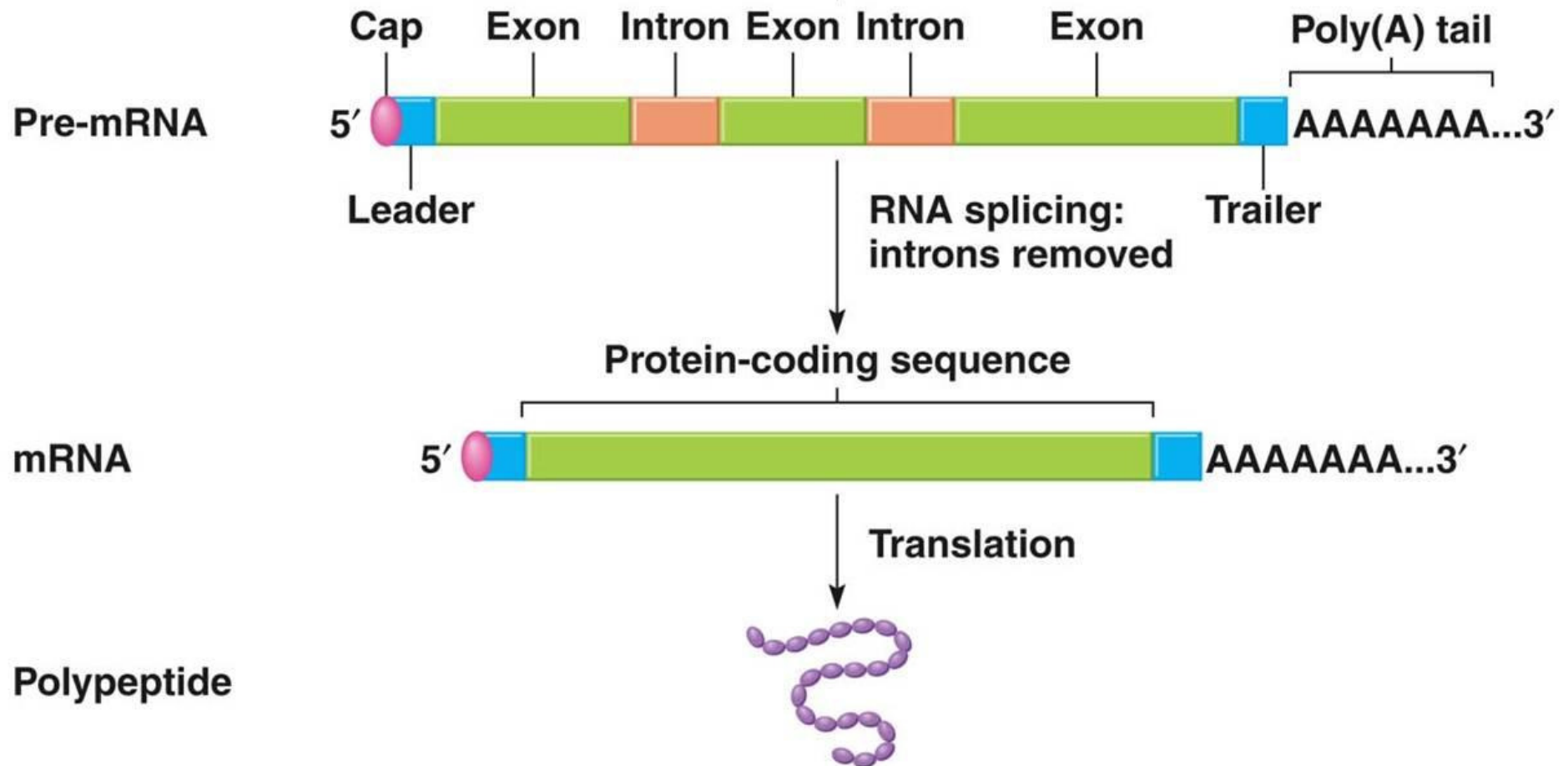


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Type of RNA

- 1. Ribosomal RNA (rRNA)**
 - **16S (small ribosomal subunit)**
 - **23S (large ribosomal subunit)**
 - **5S (large ribosomal subunit)**
- 2. Transfer RNA (tRNA)**
- 3. Messenger RNA (mRNA)**

hn-RNA (Pre-mRNA) & m-RNA



m-RNA

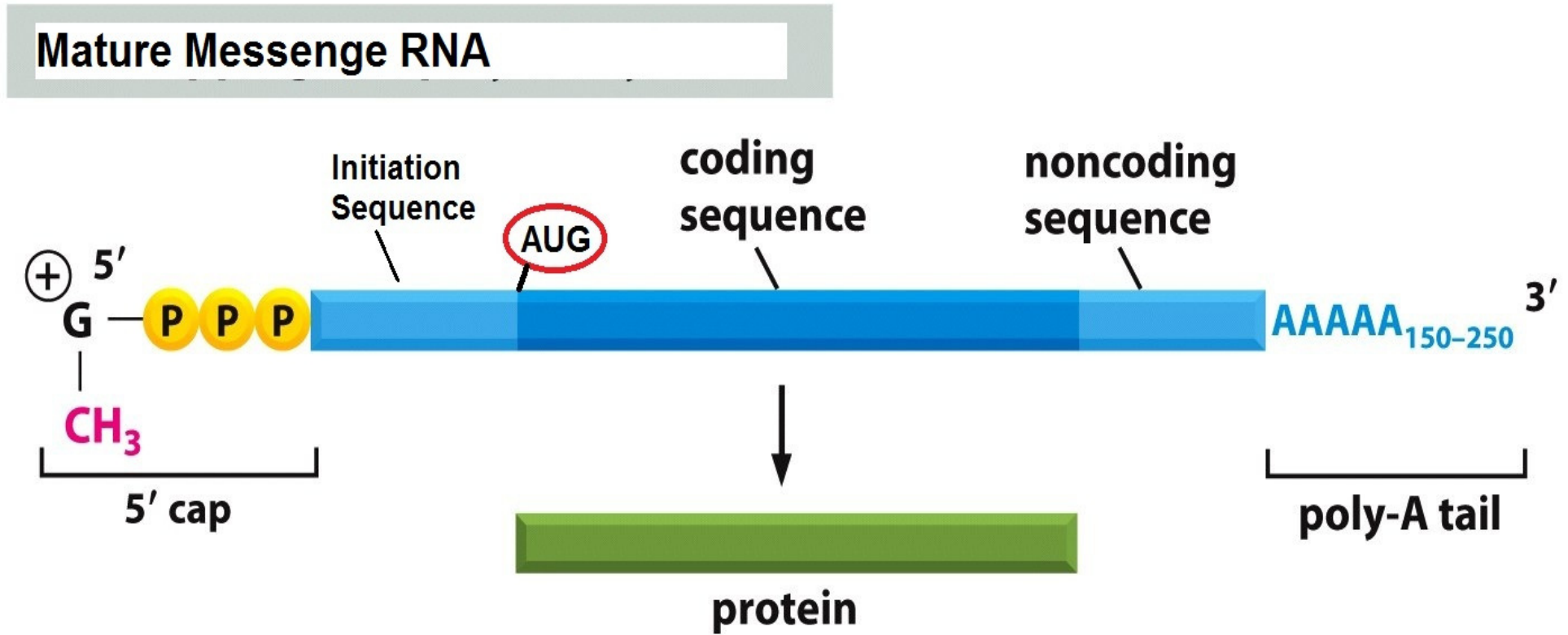
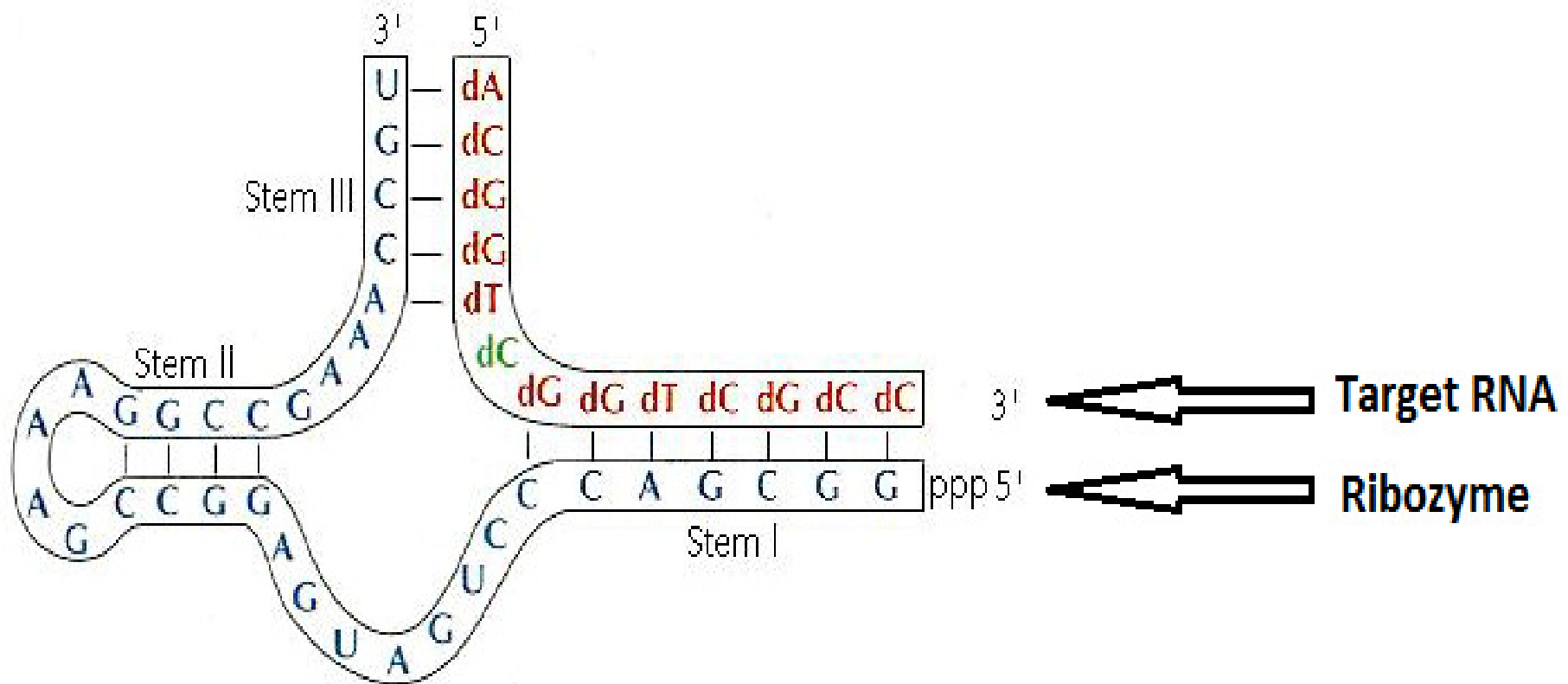
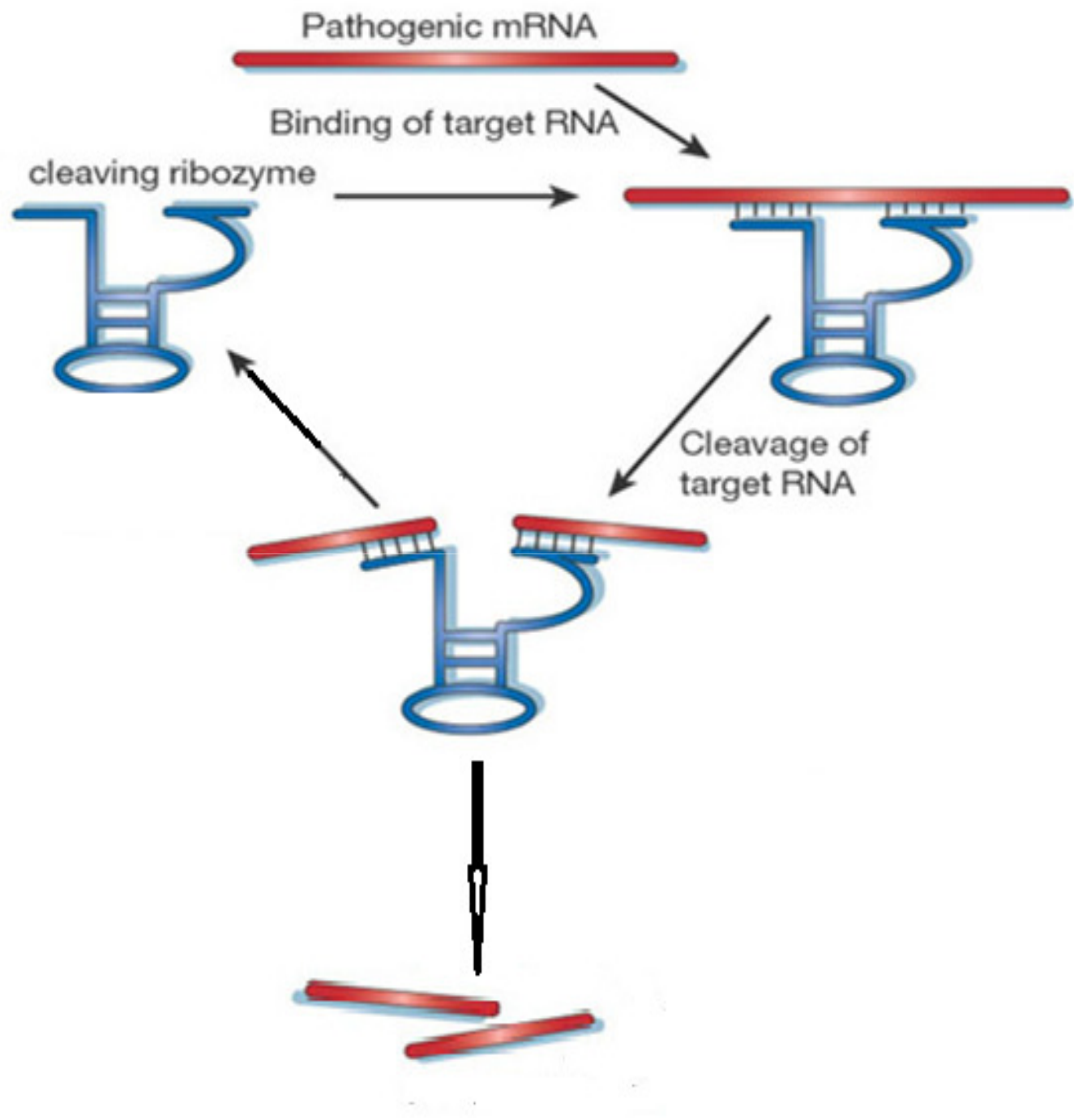


Figure 7-16a Essential Cell Biology 3/e (© Garland Science 2010)

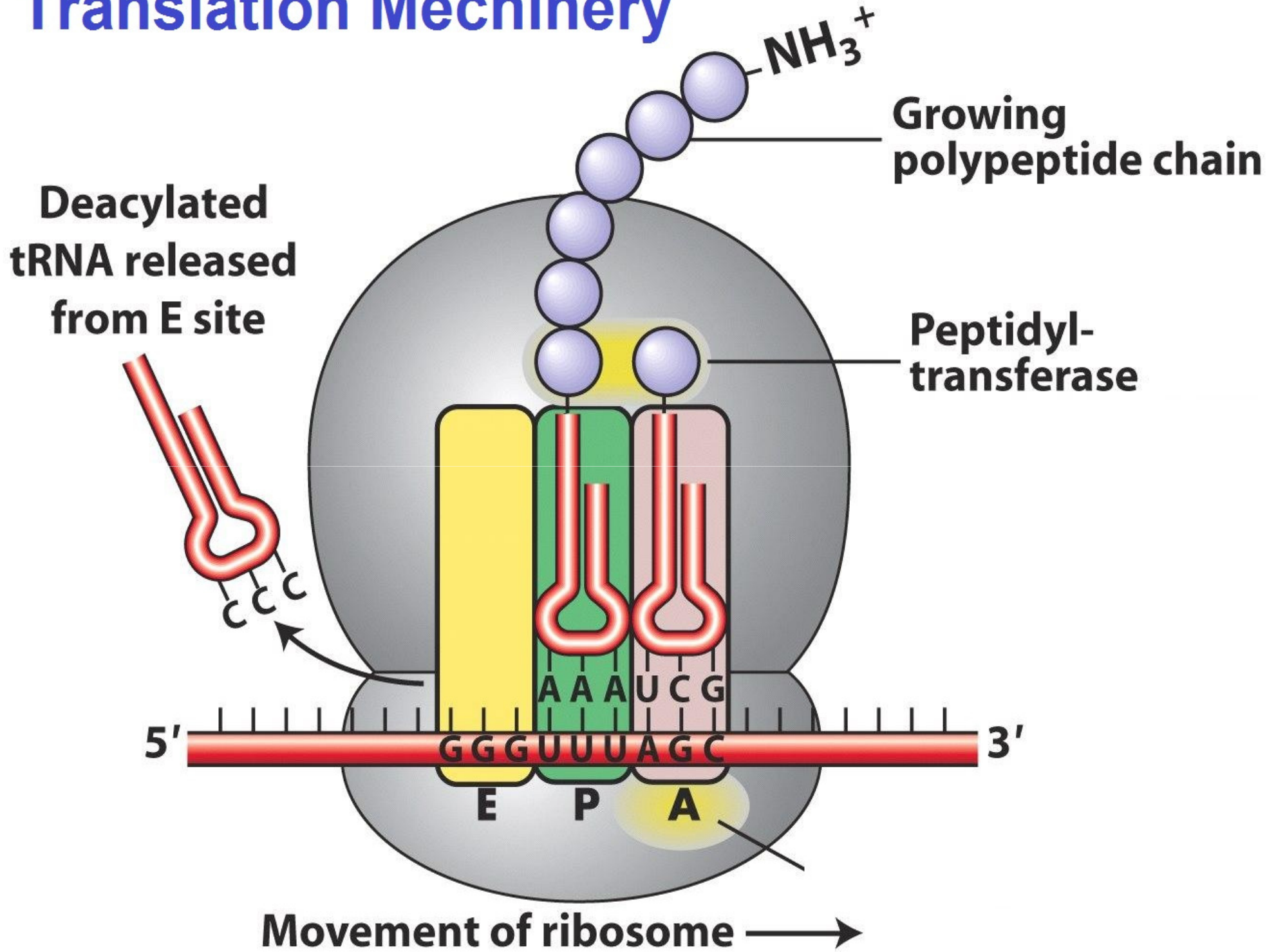
r-RNA (Ribosomal RNA)

- ⊙ Association with several proteins
- ⊙ Type :
 - In Prokaryots = 23S, 16S, and 5S
 - In Eukaryots = 28S, 18S, 5.8S, and 5S
- ⊙ **“S” = Svedberg unit**
Related to the molecular weight and shape.
- ⊙ Function :
 - ⊙ Sites for protein synthesis.
 - ⊙ Catalysts in protein synthesis.
 - ⊙ E.g. **“Ribozyme”**.

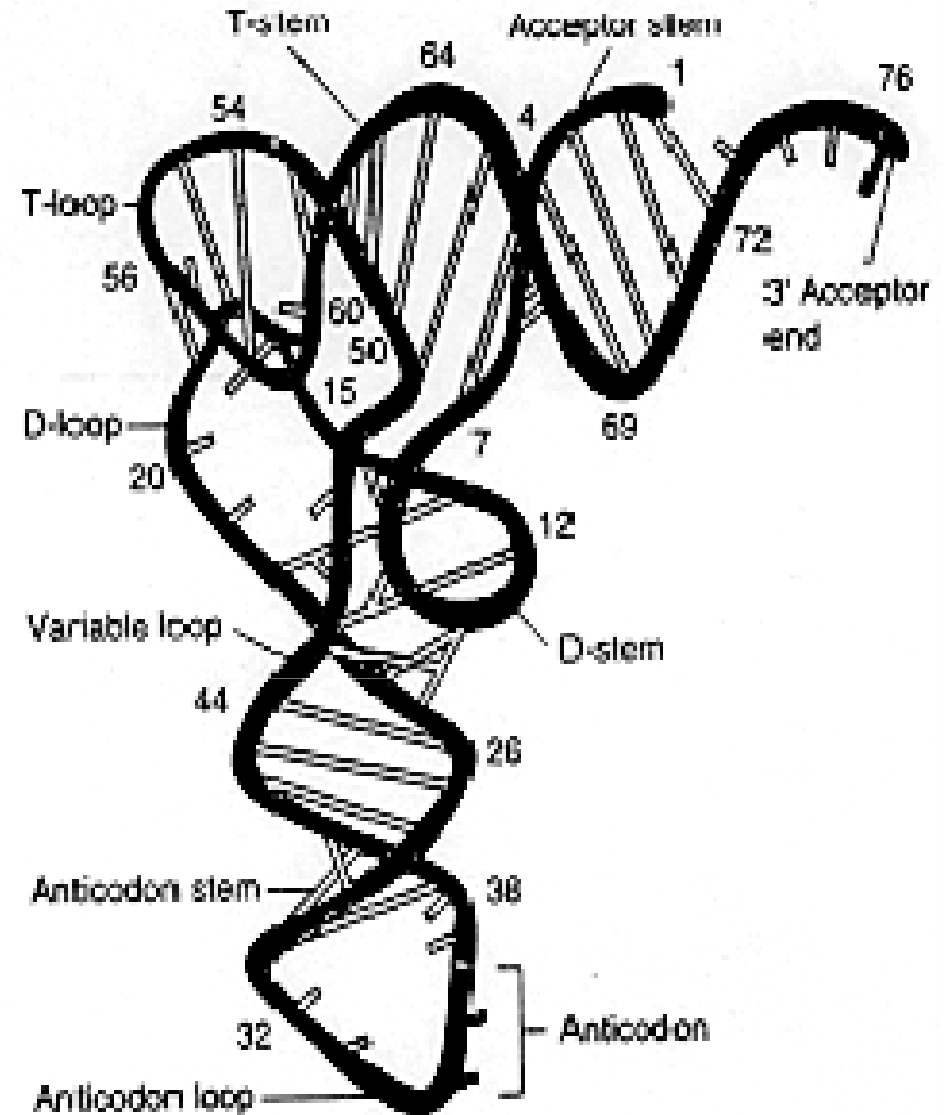
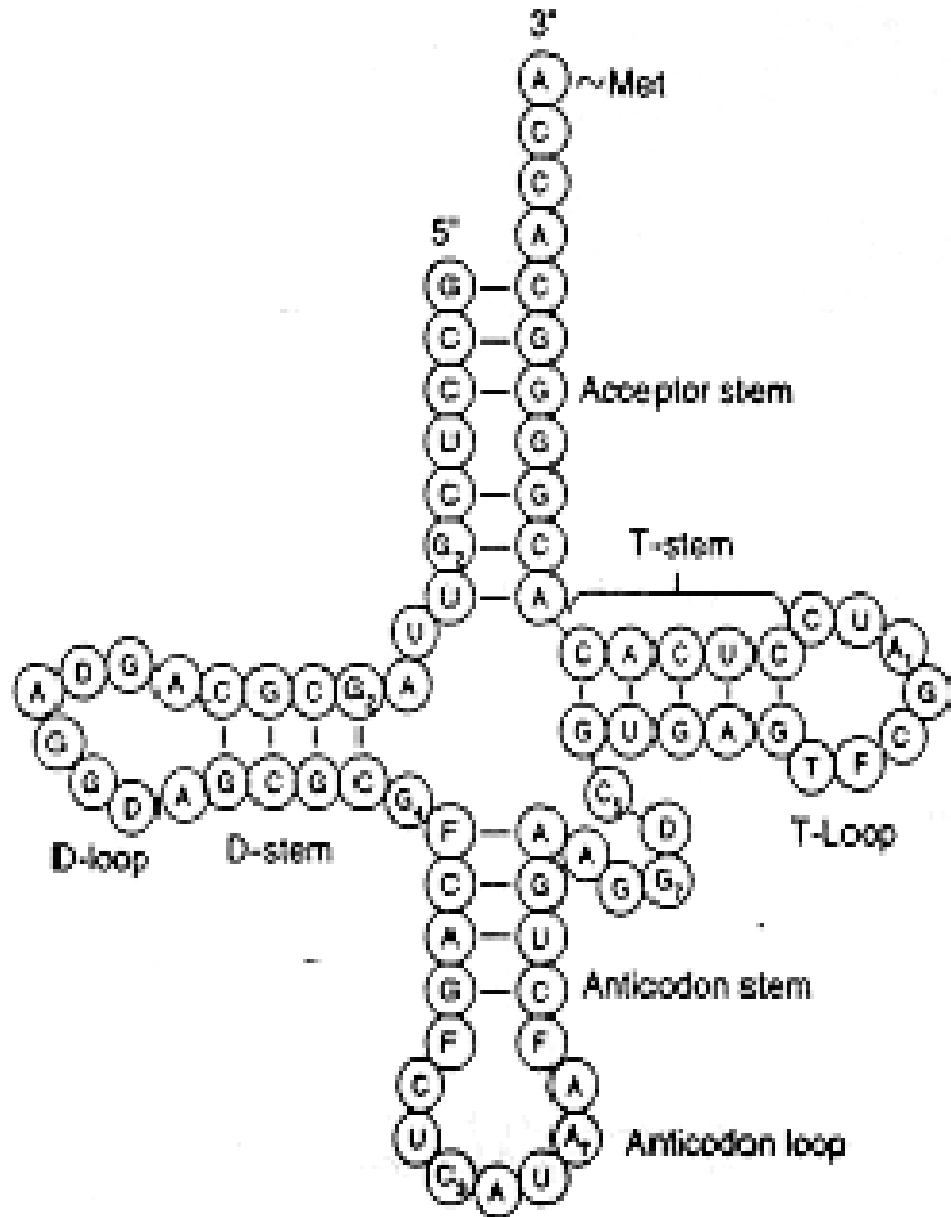




Translation Machinery



Secondary structure



Tertiary structure

t-RNA

A

Site of amino acid attachment

3' End
CCA

5' End

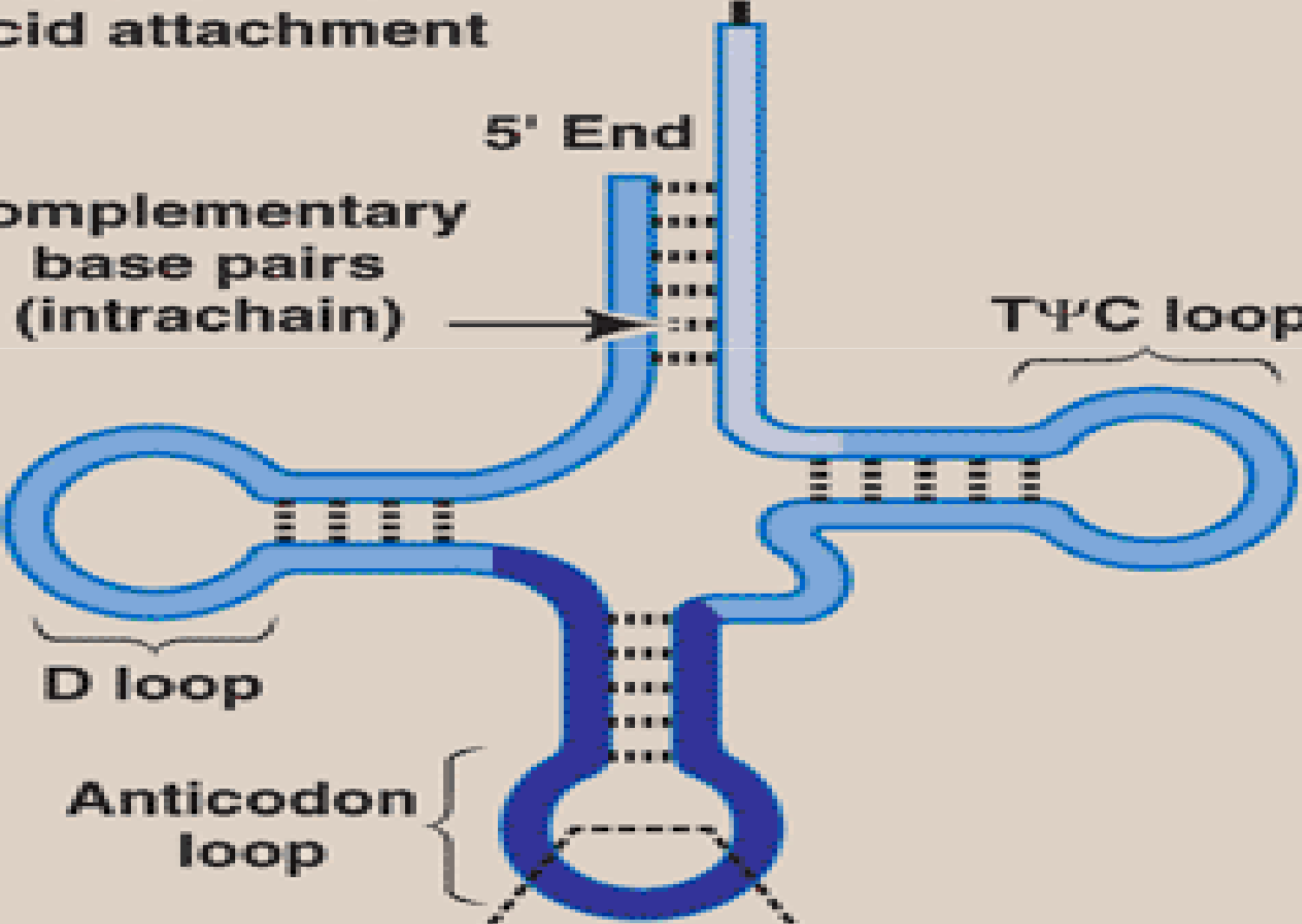
Complementary base pairs (intrachain)

TΨC loop

D loop

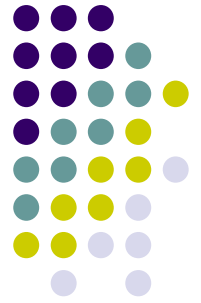
Anticodon loop

Anticodon



t- RNA (transfer RNA)

- Smallest (73 – 93 ns)
 - Easily soluble = s-RNA
 - Specific tRNA for All 20 amino acids.
 - Clover leaf like structure.
 - Unusual bases (for example, dihydrouracil,)
 - Intrachain base-pairing = Looks secondary & tertiary structure.
 - Serves as an “adaptor” molecule
-
- **Dihydrouacil arm** = Recognition Enzyme to add amino acid
 - **Pseudouridine arm** = Binding t-RNA to ribosome
 - **Anticodon arm** = Recognize triplet codon on m-RNA
 - **Acceptor arm** = carries amino acid



Genetic Code

- Nucleotides read in triplet “codons”
 - 5' → 3'
- Each codon translates to an amino acid
- 64 possible codons
 - 3 positions and 4 possibilities (AGCU) makes 4^3 or 64 possibilities
 - Degeneracy or redundancy of code
 - Only 20 amino acids
 - Implications for mutations

Genetic Code



second position

		second position							
		U	C	A	G				
first position	U	UUU	Phe (F)	UCU	Ser (S)	UAU	Tyr (Y)	UGU	Cys (C)
		UUC	Phe (F)	UCC	Ser (S)	UAC	Tyr (Y)	UGC	Cys (C)
		UUA	Leu (L)	UCA	Ser (S)	UAA	STOP	UGA	STOP
		UUG	Leu (L)	UCG	Ser (S)	UAG	STOP	UGG	Trp (W)
	C	CUU	Leu (L)	CCU	Pro (P)	CAU	His (H)	CGU	Arg (R)
		CUC	Leu (L)	CCC	Pro (P)	CAC	His (H)	CGC	Arg (R)
		CUA	Leu (L)	CCA	Pro (P)	CAA	Gln (Q)	CGA	Arg (R)
		CUG	Leu (L)	CCG	Pro (P)	CAG	Gln (Q)	CGG	Arg (R)
	A	AUU	Ile (I)	ACU	Thr (T)	AAU	Asn (N)	AGU	Ser (S)
		AUC	Ile (I)	ACC	Thr (T)	AAC	Asn (N)	AGC	Ser (S)
		AUA	Ile (I)	ACA	Thr (T)	AAA	Lys (K)	AGA	Arg (R)
		AUG	Met (M)	ACG	Thr (T)	AAG	Lys (K)	AGG	Arg (R)
	G	GUU	Val (V)	GCU	Ala (A)	GAU	Asp (D)	GGU	Gly (G)
		GUC	Val (V)	GCC	Ala (A)	GAC	Asp (D)	GGC	Gly (G)
		GUA	Val (V)	GCA	Ala (A)	GAA	Glu (E)	GGA	Gly (G)
		GUG	Val (V)	GCG	Ala (A)	GAG	Glu (E)	GGG	Gly (G)

5' - BASE	MIDDLE BASE			3' - BASE	
	U	C	A		G
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

1 These four rows show sixteen amino acids whose codons begin (5') with A.

2 This column shows sixteen amino acids whose codons have the middle base U.

4 The codon 5'-AUG-3' designates methionine (Met).

3 These four, separated rows show sixteen amino acids whose codons end (3') with G.

Use of the genetic code table to translate the codon AUG

Genetic Code



1. **Triplet Codons** :

Each codon is a consecutive sequence of three bases

2. **Non-overlapping** :

Codes are always read one after another.

3. **Non-punctuated** :

Codes are always continues.

4. **Specificity (unambiguous)** :

One codon always codes for specific amino acid

5. **Universal** :

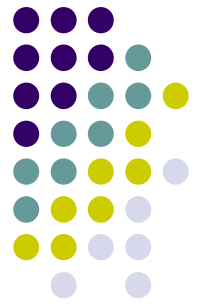
Codons represent same amino acid in all species.

Except in Mitochondria

6. **Degenerate** :

Each codon corresponds to a single A.A.

But A.A. may have more than one triplet codon.



7. **Initiator codon** :

AUG is start codon

8. **Termination codons**

Nuclear DNA = UAA, UGA, UAG,

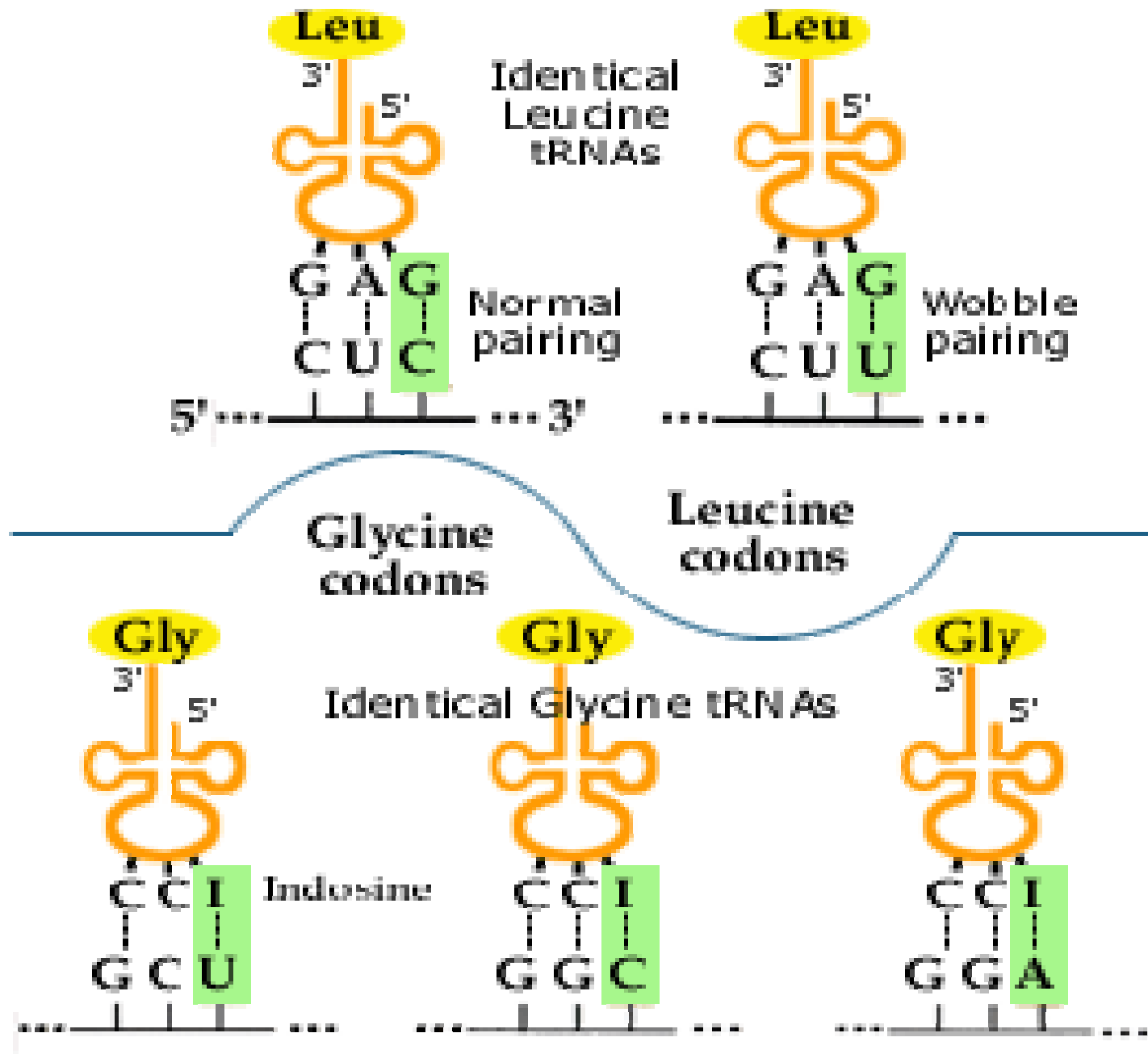
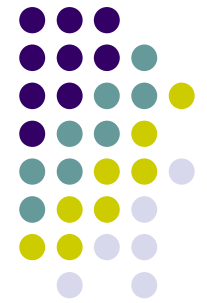
Mitochondrial DNA = AGG, AGA,

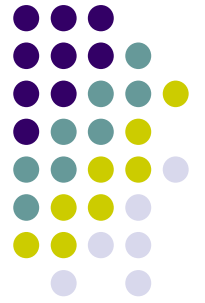
9. **Wobbling Phenomenon** :

The reduced stringency between the third base of the codon and the complementary nucleotide in the anticodon is called wobble.

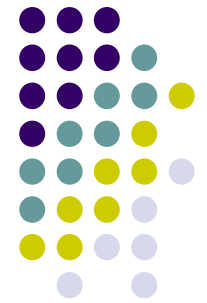
It reduce effect of mutation.

Wobbling Phenomenon



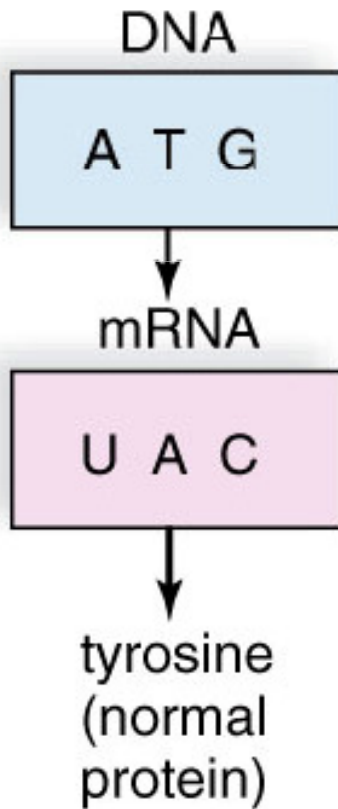


Mutation

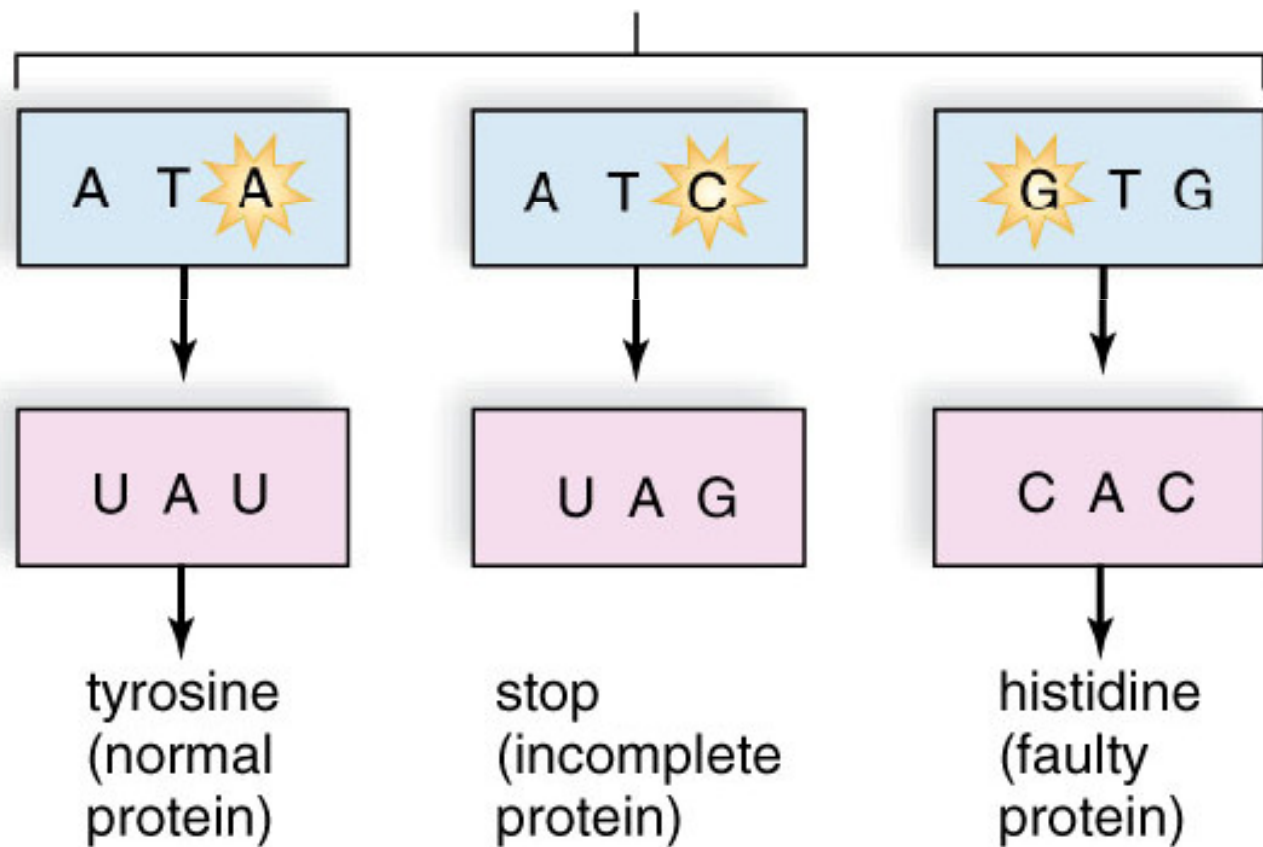


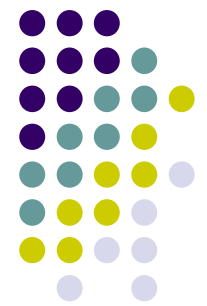
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No mutation



Point mutations





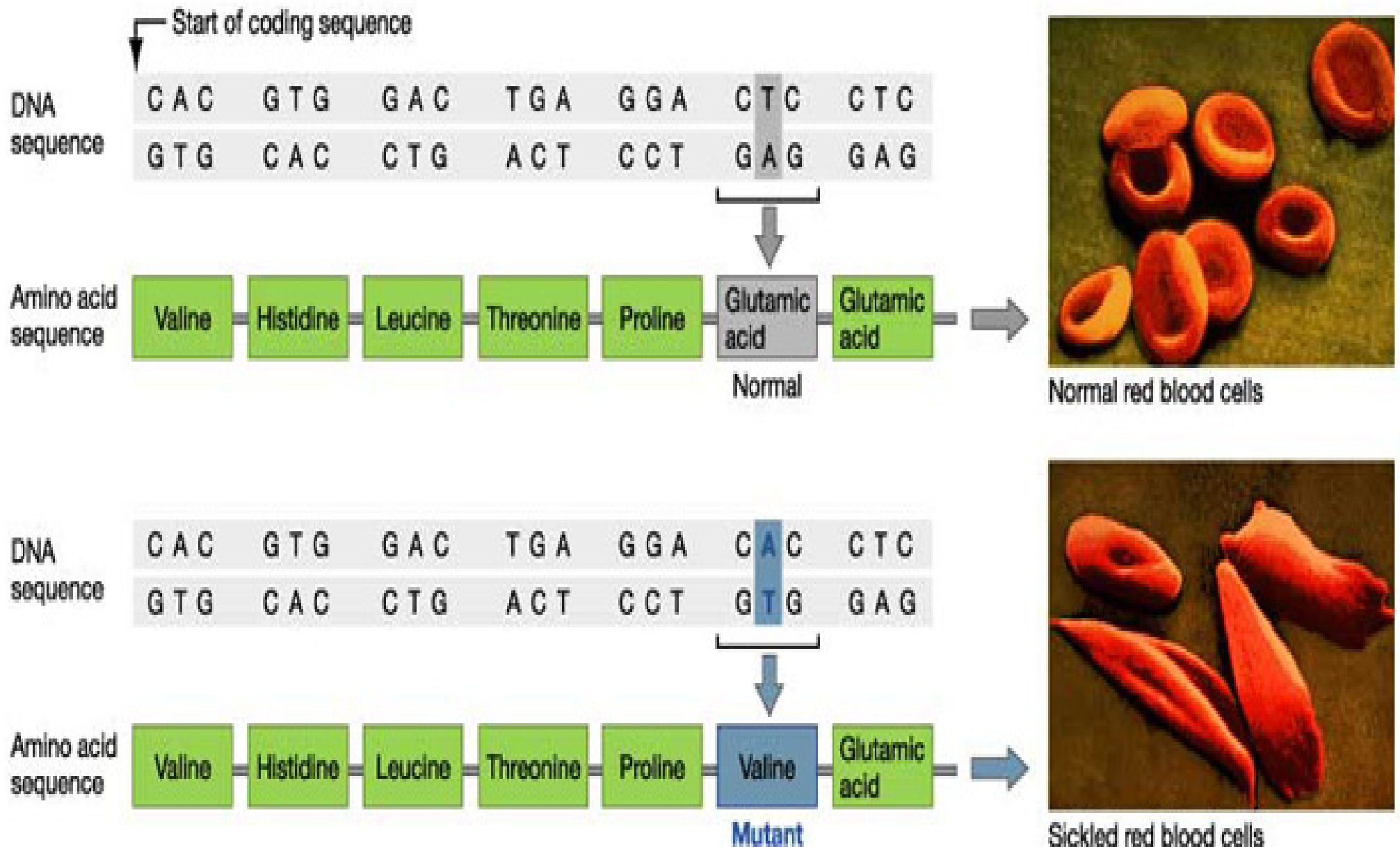
DNA Sequence

Amino Acid Sequence

Normal: CAG CCC ACT → Gln-Pro-Thr
Codon 1 Codon 2 Codon 3

Insertion Mutation (Frameshift): CAG TCC CAC T → Gln-Ser-His ?
Codon 1 Codon 2 Codon 3 Codon 4

Insertion Mutation (Non-frameshift): CAG TTT CCC ACT → Gln-Phe-Pro-Thr
Codon 1 Codon 2 Codon 3 Codon 4



The change in amino acid sequence causes hemoglobin molecules to crystallize when oxygen levels in the blood are low. As a result, red blood cells sickle and get stuck in small blood vessels.

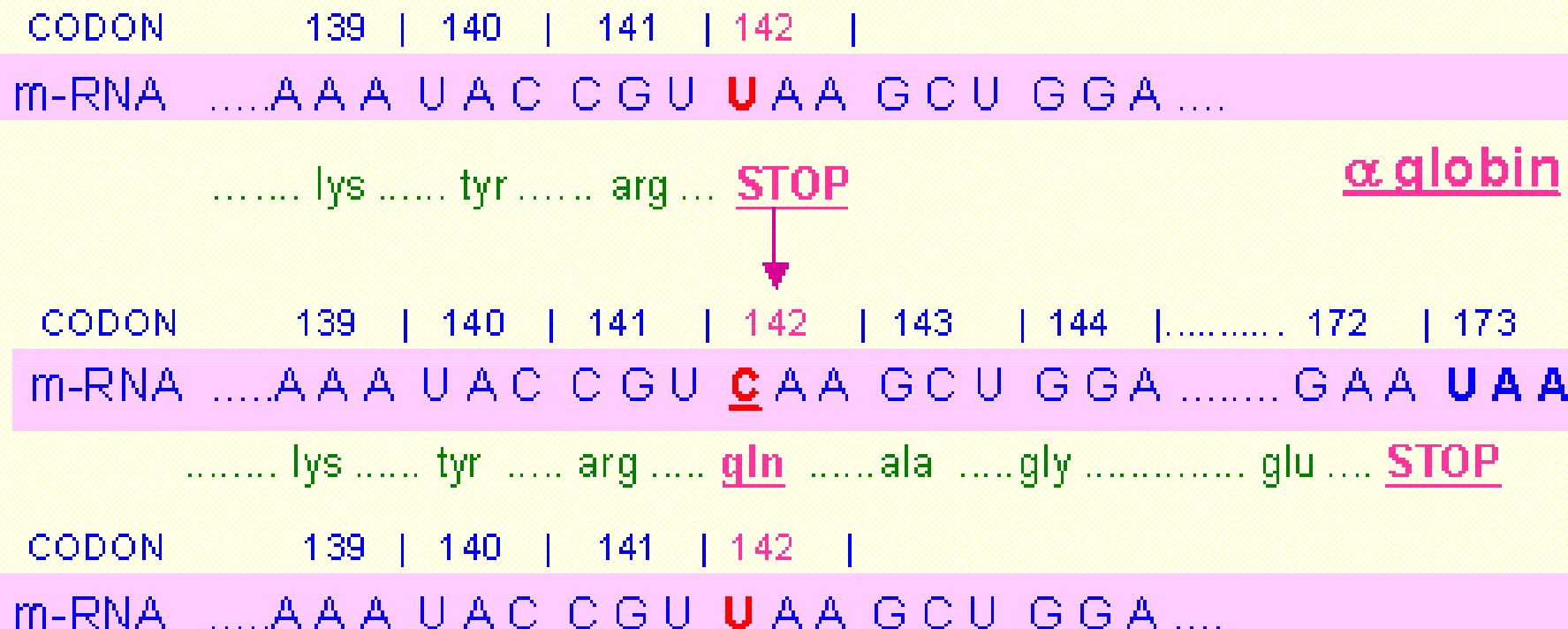
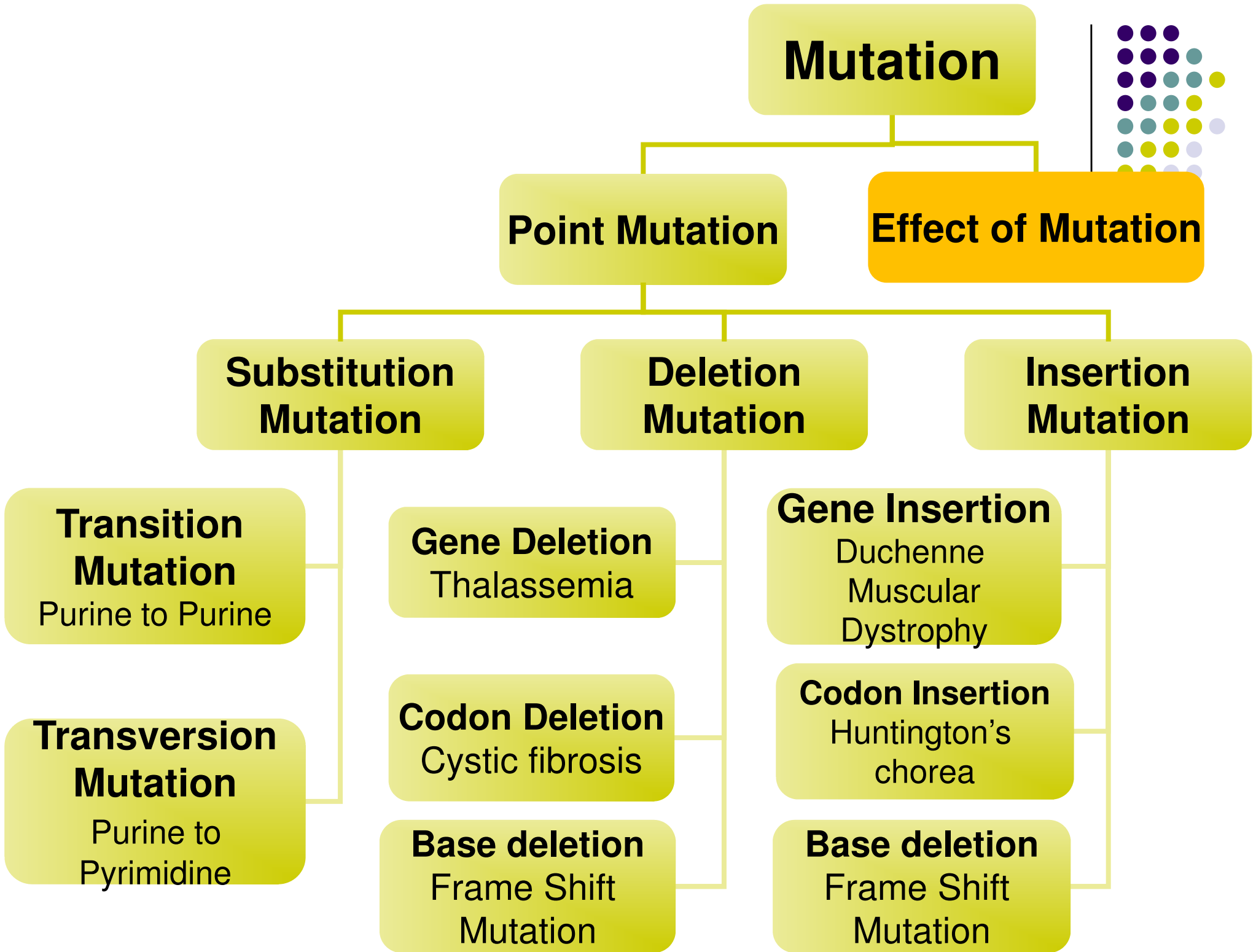


Fig 5.3. Point mutation of U to C alters the stop codon UAA at position 142, resulting in α-globin chain elongation and formation of a variant haemoglobin, Hb Constant Spring.



Mutation

Point Mutation

Effect of Mutation

Substitution Mutation

Deletion Mutation

Insertion Mutation

Transition Mutation

Purine to Purine

Transversion Mutation

Purine to Pyrimidine

Gene Deletion

Thalassemia

Codon Deletion

Cystic fibrosis

Base deletion

Frame Shift Mutation

Gene Insertion

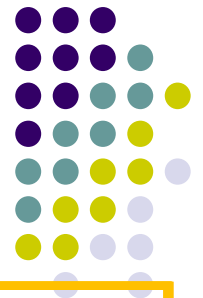
Duchenne Muscular Dystrophy

Codon Insertion

Huntington's chorea

Base deletion

Frame Shift Mutation



Mutation

Point Mutation

Substitution Mutation

Deletion Mutation

Insertion Mutation

Effect of Mutation

Silent Mutation

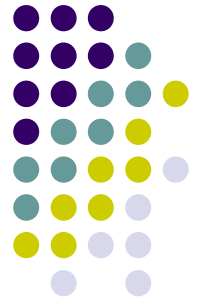
Mis-sence Mutation (Acceptable)
e.g. Hb Sydney

Mis-sence Mutation
(Partially Acceptable) e.g. HbS

Mis-sence Mutation
(Unacceptable) e.g. Hb M

Non sence

Frame Shift Mutation



Substitution Mutation

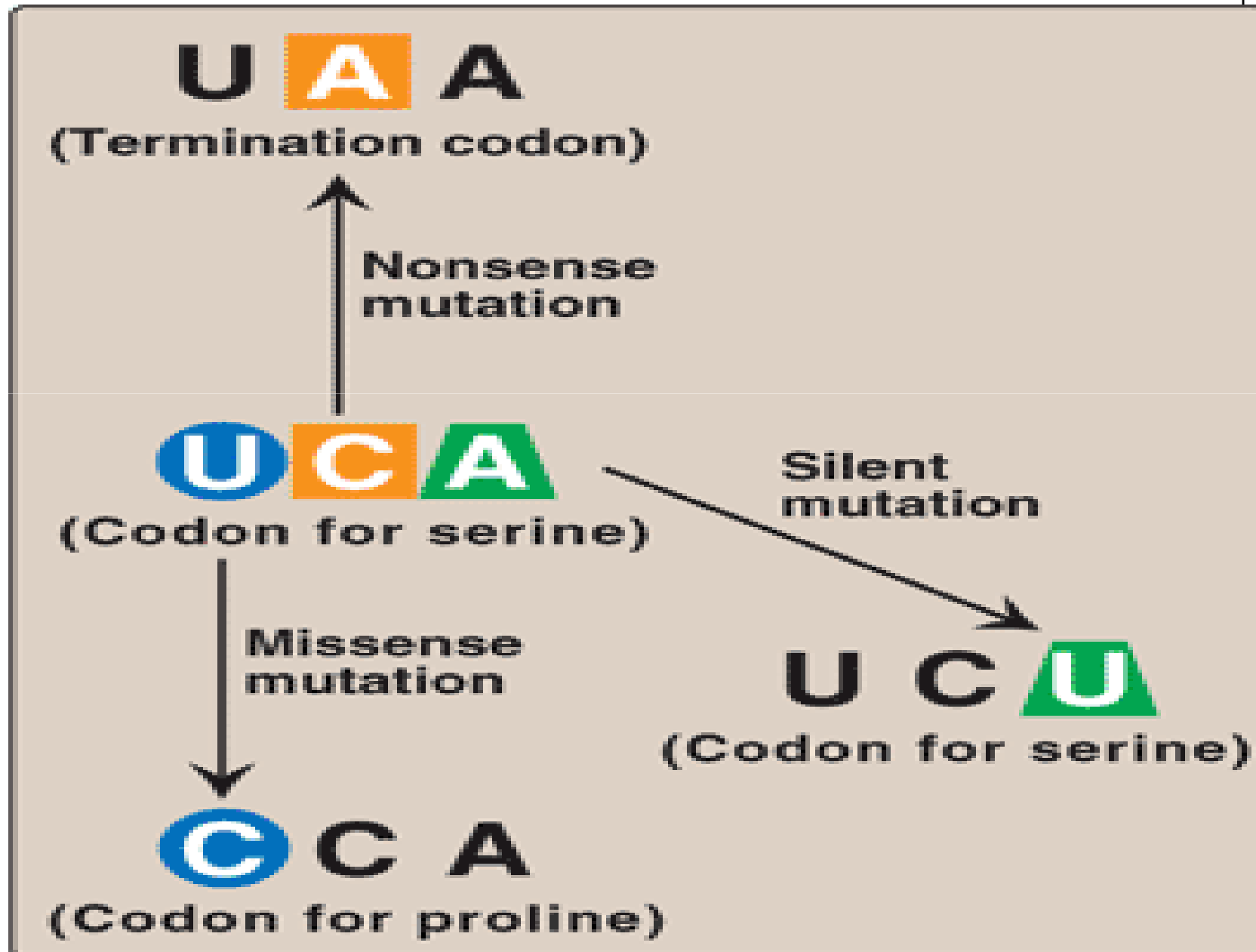
● Transition

- Purine replace by Purine
or
- Pyrimidine replace by Pyrimidine

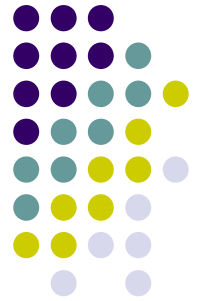
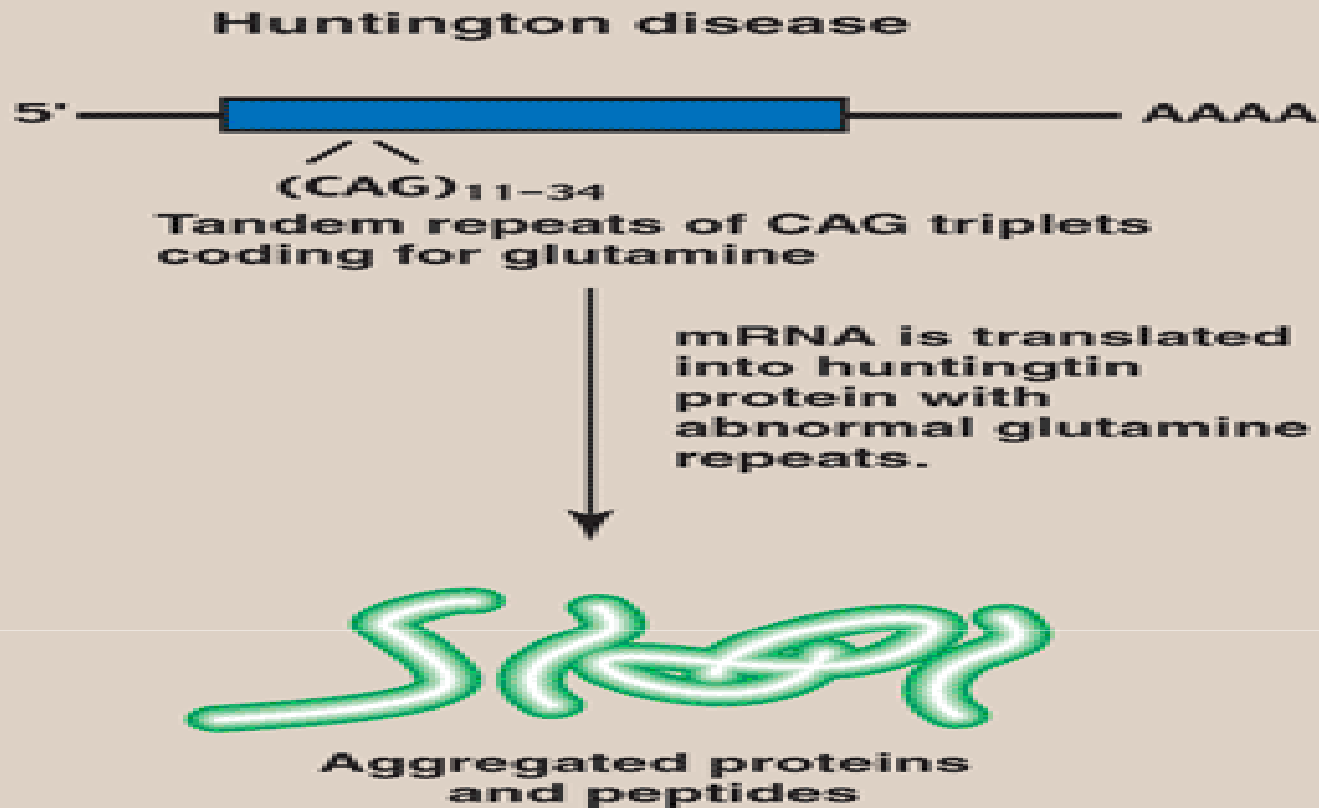
● Transversion

- Purine replace by Pyrimidine
Or
- Pyrimidine replace by Purine
- E.g. Sickle cell anaemia
- GAG (glutamic acid) = GUG (valine)

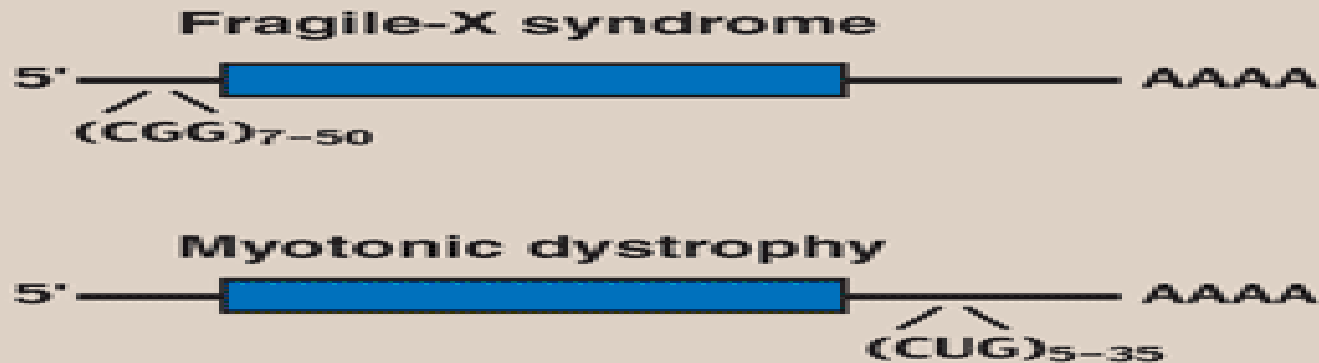
Effect Of Mutation



Huntington disease

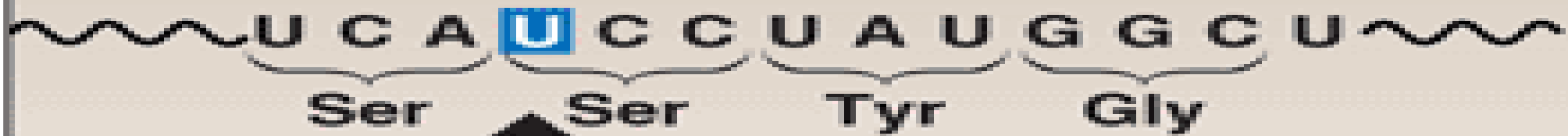


Other triplet expansion diseases



Addition of base

mRNA



Addition of U

U

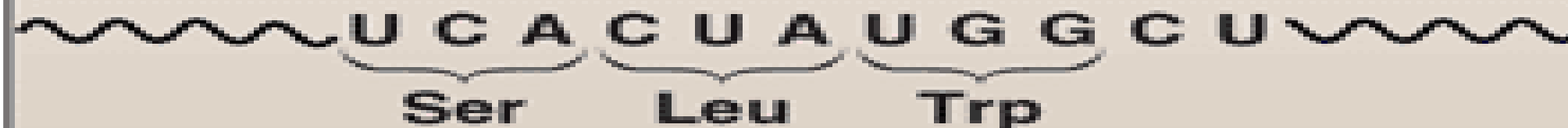


5'-End

3'-End

Deletion of C

C

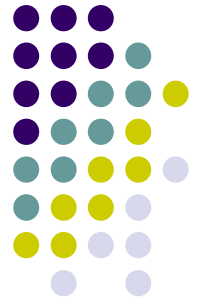


Deletion of base



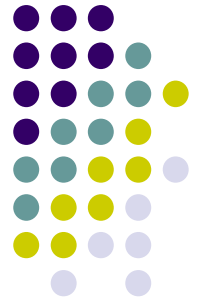
Translation (Protein Synthesis)

Translation



- Steps:
 - Intiation
 - Elongation
 - Termination
 - Post - Translation

Translation Process



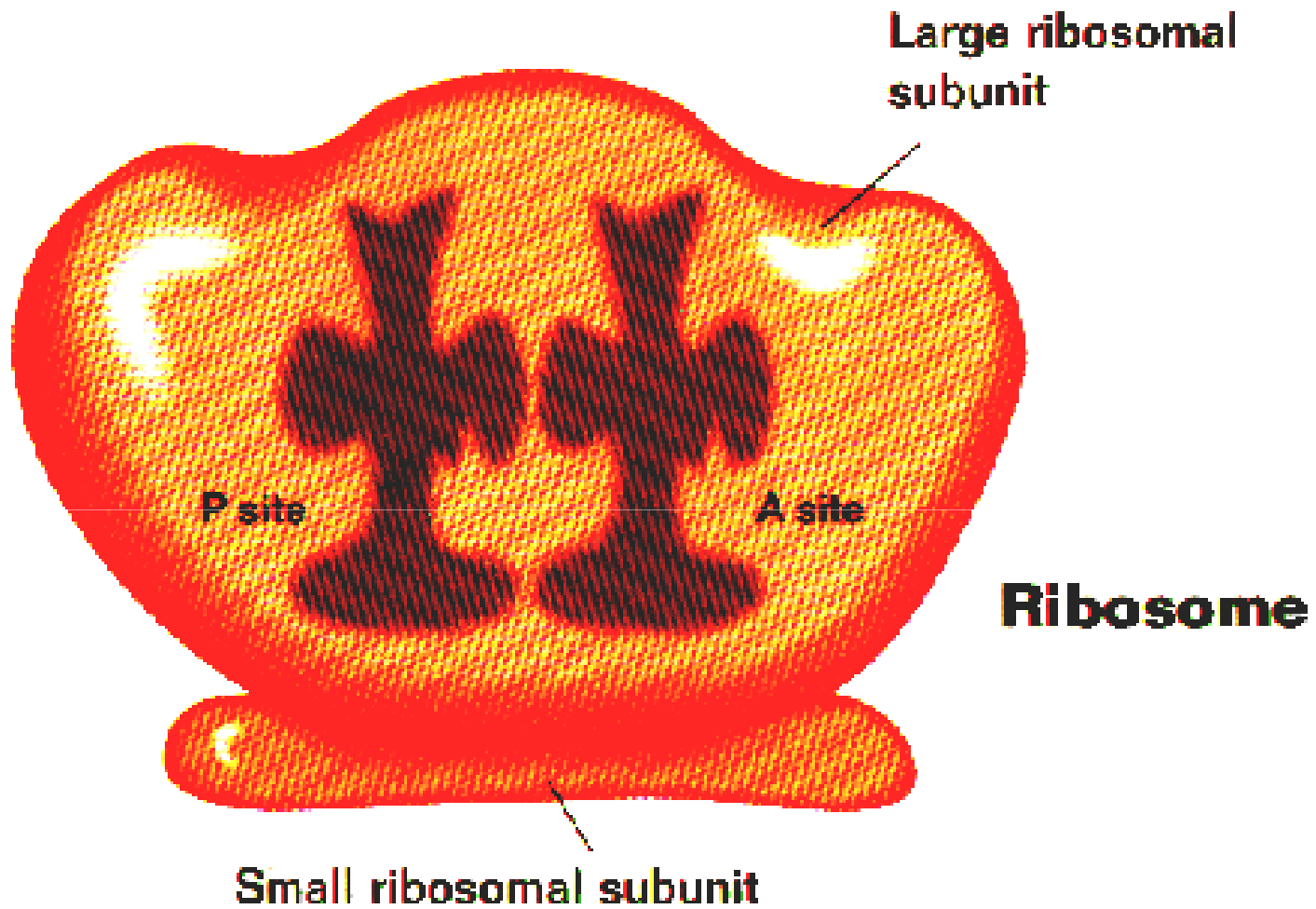
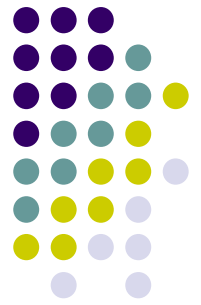
● Requires

- Ribosomes (50s + 30s)
- r-RNA
- t-RNA
- m-RNA
- Amino acid

● Ribosome

- Made of protein and r-RNA (Nucleo-protein)
- Has internal sites for 2 t-RNA molecules.
- Two subunit
 - Prokaryotic 50S + 30S subunits = form a 70S.
 - Eukaryotic 60S + 40S subunits = form an 80S.

Ribosome



Translation



● Initiation

- Ribosomal subunits **assemble** on mRNA
- r-RNA aids in binding of mRNA

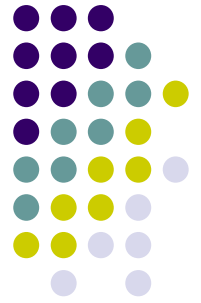
● Elongation

- **t-RNAs** with appropriate anticodon loops **bind to complex**
- have **amino acid attached** (done by other enzymes)
- **Amino acids transfer** from t-RNA 2 to t-RNA 1
- Process repeats

● Termination

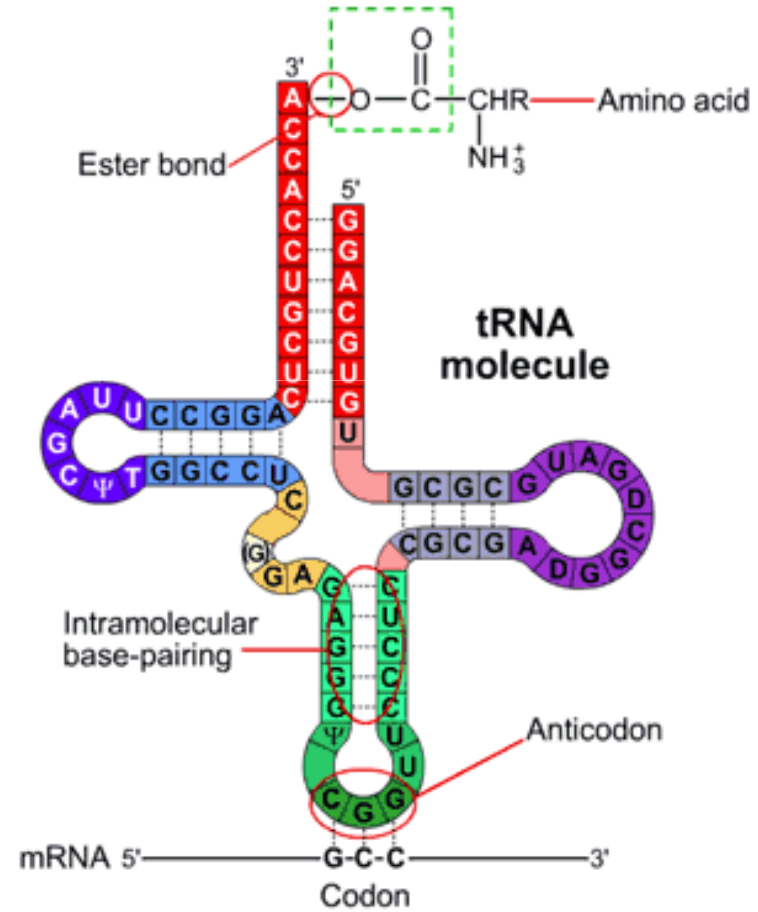
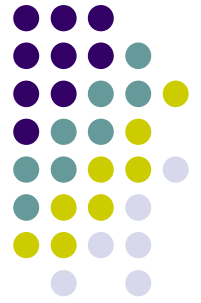
- t-RNA with **stop codon** binds into ribosome
- No amino acid attached to t-RNA
- Complex falls apart

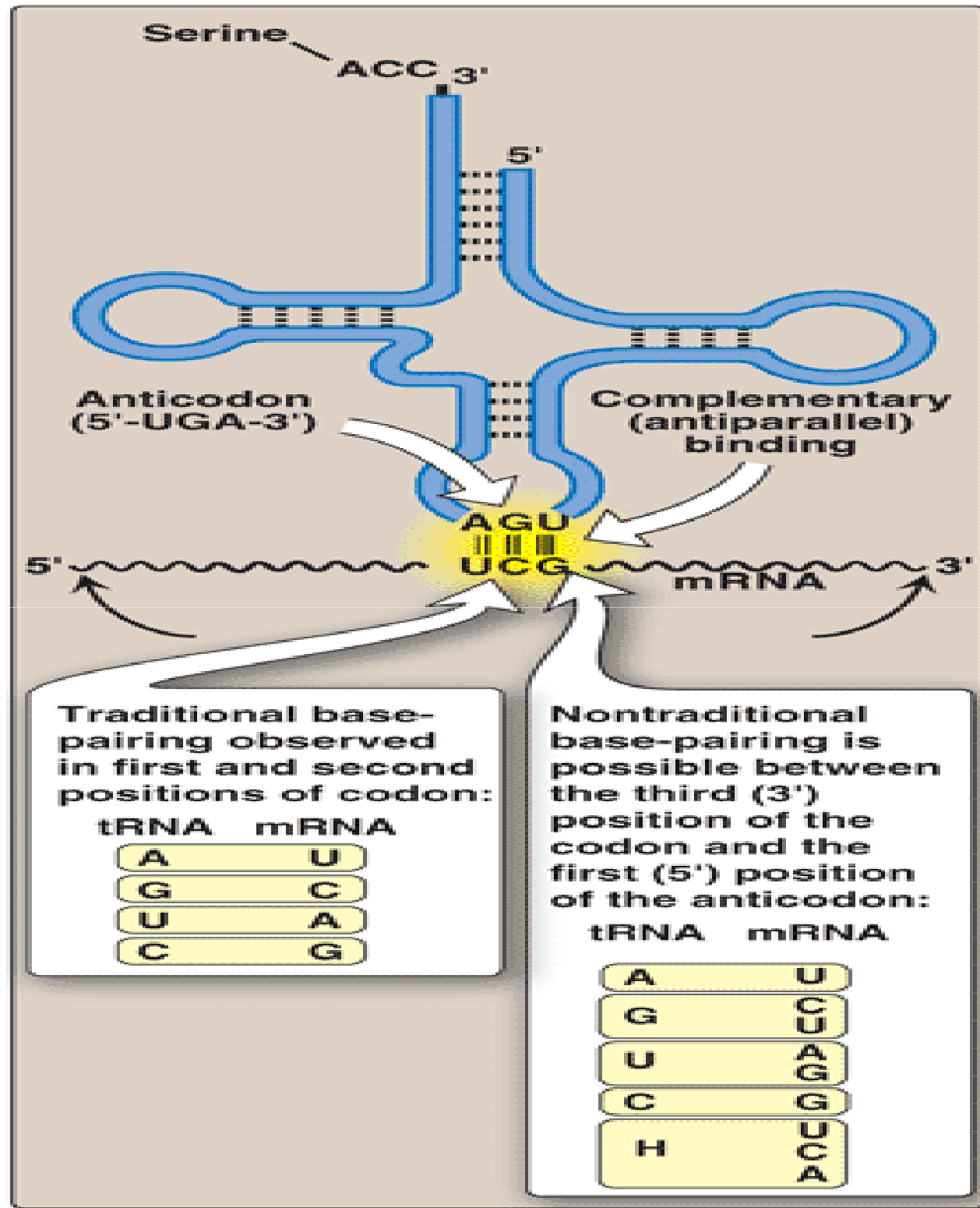
A – P site on ribosome



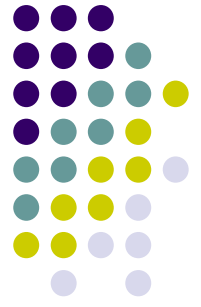
- Ribosome has two binding sites for t-RNA
— **P & A** sites —
- Together, they cover two neighboring codons.
- **P-site** binds
 - codon is occupied by **Peptidyl t-RNA**.
 - This t-RNA carries the **chain of amino acids** that has already been synthesized.
- **A site** binds
 - incoming **Aminoacyl-tRNA** as directed by the codon.
 - This codon **specifies the next amino acid** to be added to the growing peptide chain.

Transfer RNA



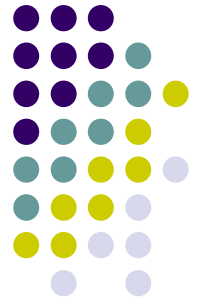


Aminoacyl-tRNA synthetases

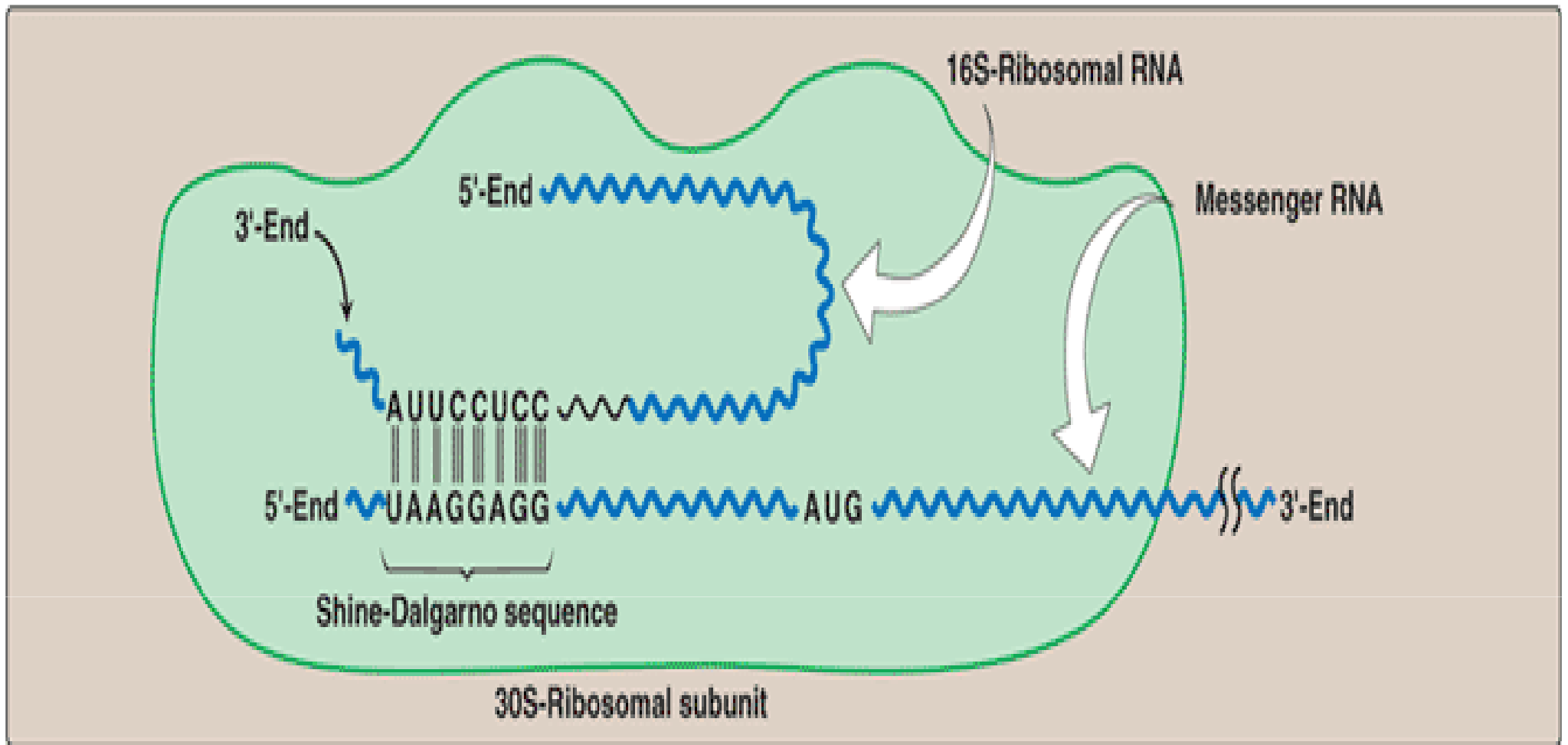


- Required for attachment of amino acids to their corresponding t-RNA.
- Carboxyl group of an amino acid to the 3'-end of t-RNA.
- Require ATP.
- Extreme specificity
- High fidelity of translation of the genetic message.
- Enzyme have “proofreading” or “editing” activity

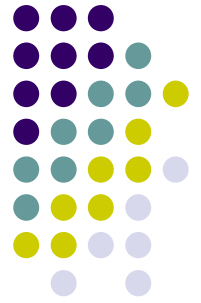
Initiation



- Involves the assembly of the components
- Components include
 - Two ribosomal subunits.
 - m-RNA to be translated.
 - Aminoacyl t-RNA specified by the first codon
 - GTP
 - Initiation factors
 - In Prokaryotes, Initiation factors (**IF-1, IF-2, IF-3**)
 - In eukaryotes, more than 10 (designated eIF).



Complementary binding between prokaryotic mRNA Shine-Dalgarno sequence and 16S rRNA.



● Shine - Dalgarno (SD) sequence

- Purine rich sequence (e.g. 5'-UAAGGAGG-3')
- 6 - 10 bases upstream of the initiating AUG codon
- Near m-RNA 5'-end.
- 16S r-RNA of the 30S ribosomal subunit has complementary base pair at 3'-end.
- Facilitating the binding of the 30S ribosomal subunit on the mRNA

● In eukaryotes

- the 40S ribosomal subunit binds to the cap structure at the 5'-end of the mRNA
- By eIF-4 and moves down the mRNA until it encounters the initiator AUG.
- This “scanning” process requires ATP.

Initiation codon

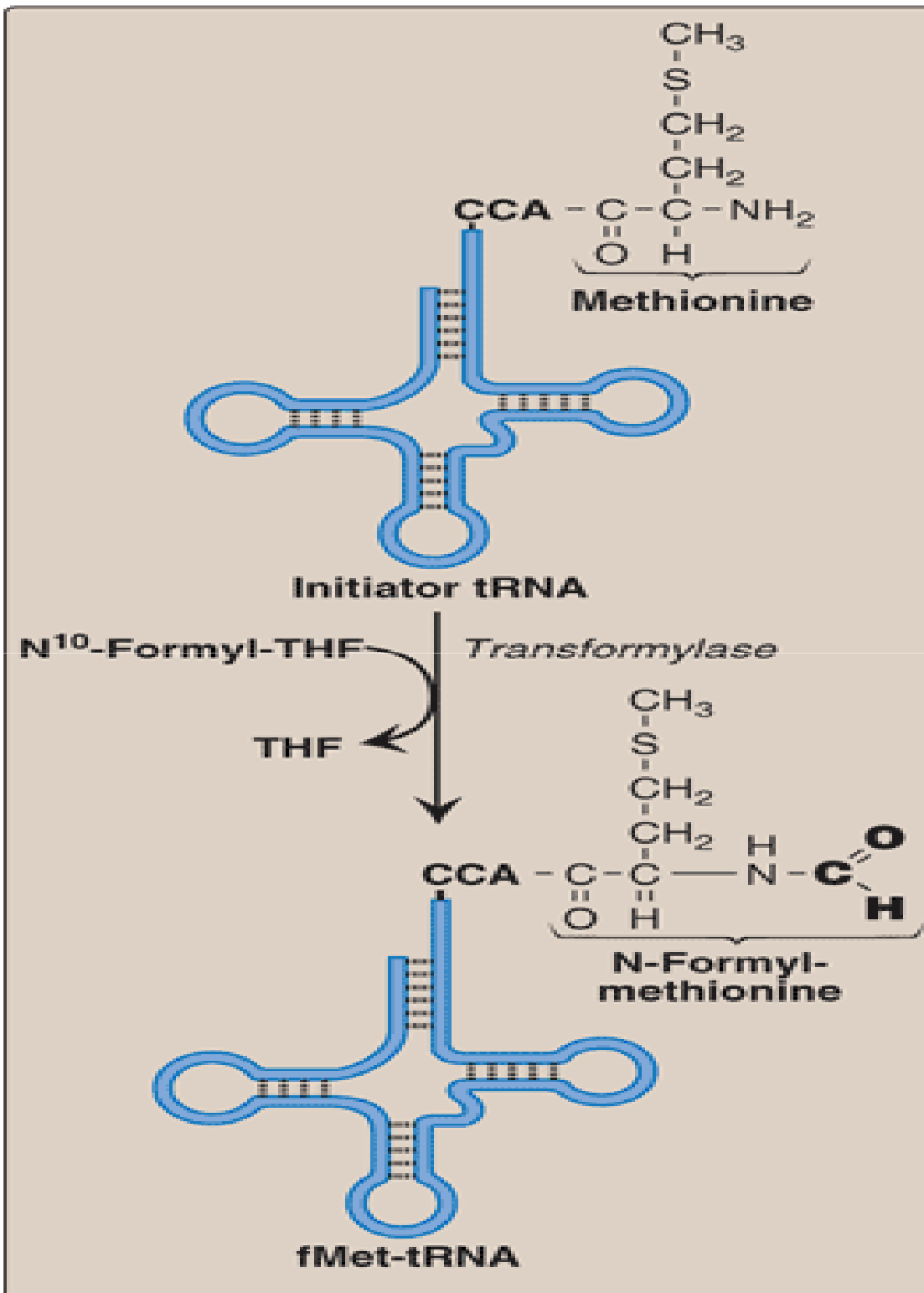
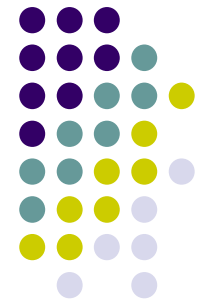


- **AUG** is recognized by initiator t-RNA.
- **Recognition** is facilitated by **IF-2** (bound to **GTP**).

- Initiator **t-RNA** enters the ribosomal **P** site
- **GTP** is hydrolyzed to **GDP**.

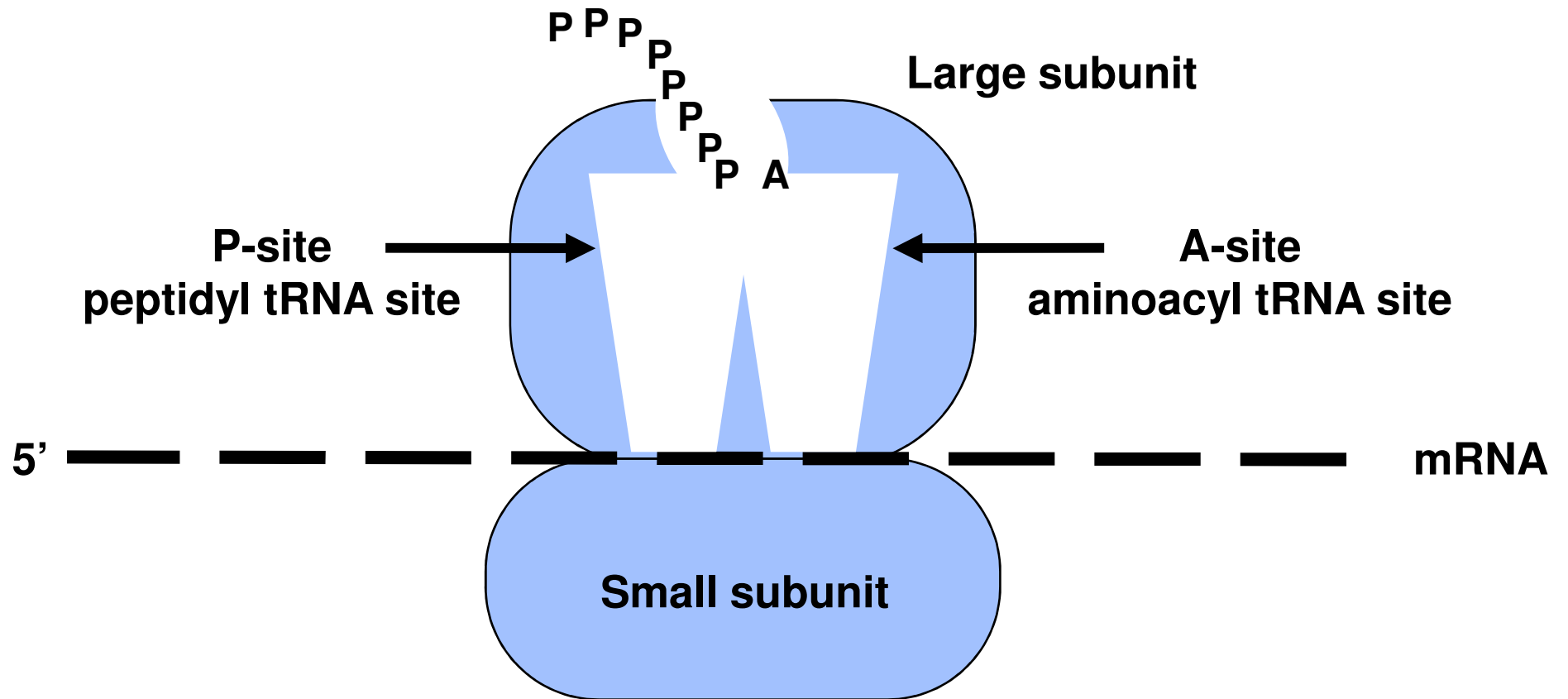
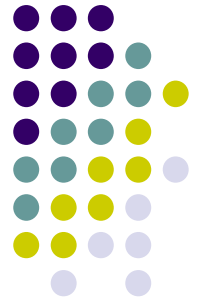
- Initiator t-RNA carries **N-formylated methionine**.
- N10-formyl tetrahydro**folate** as the carbon donor.

- In eukaryotes, the initiator tRNA carries a methionine that is not formylated.



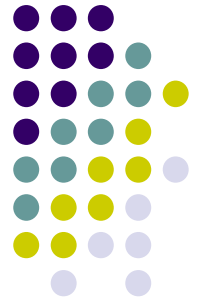
Generation of the
initiator
N-formylmethionyl-
tRNA
(fMet-tRNA)

Ribosome structure



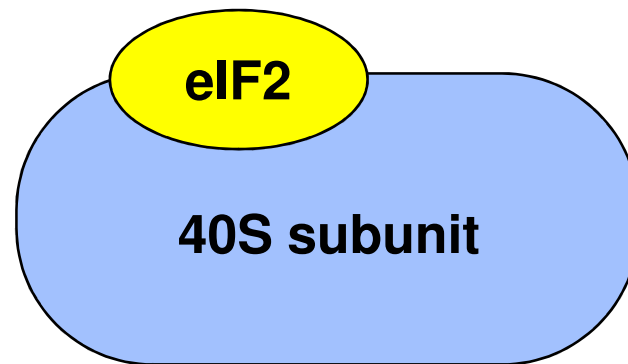
Ribosome with bound tRNAs and mRNA

Initiation of protein synthesis: mRNA binding



M

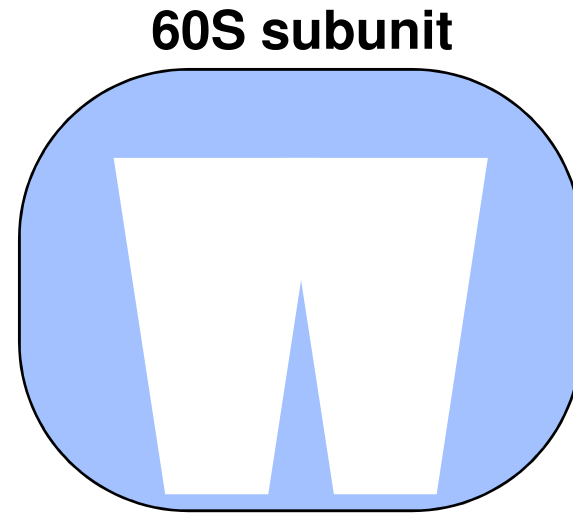
Initiator tRNA bound to the small ribosomal subunit with the eukaryotic initiation factor-2 (eIF2)



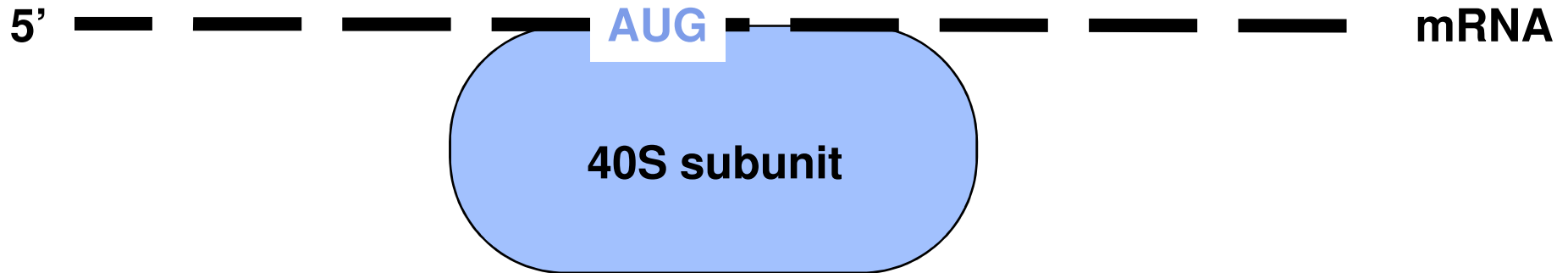
The small subunit finds the 5' cap and scans down the mRNA to the first AUG codon



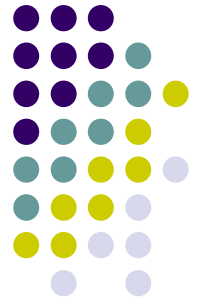
- the initiation codon is recognized
- eIF2 dissociates from the complex
- the large ribosomal subunit binds



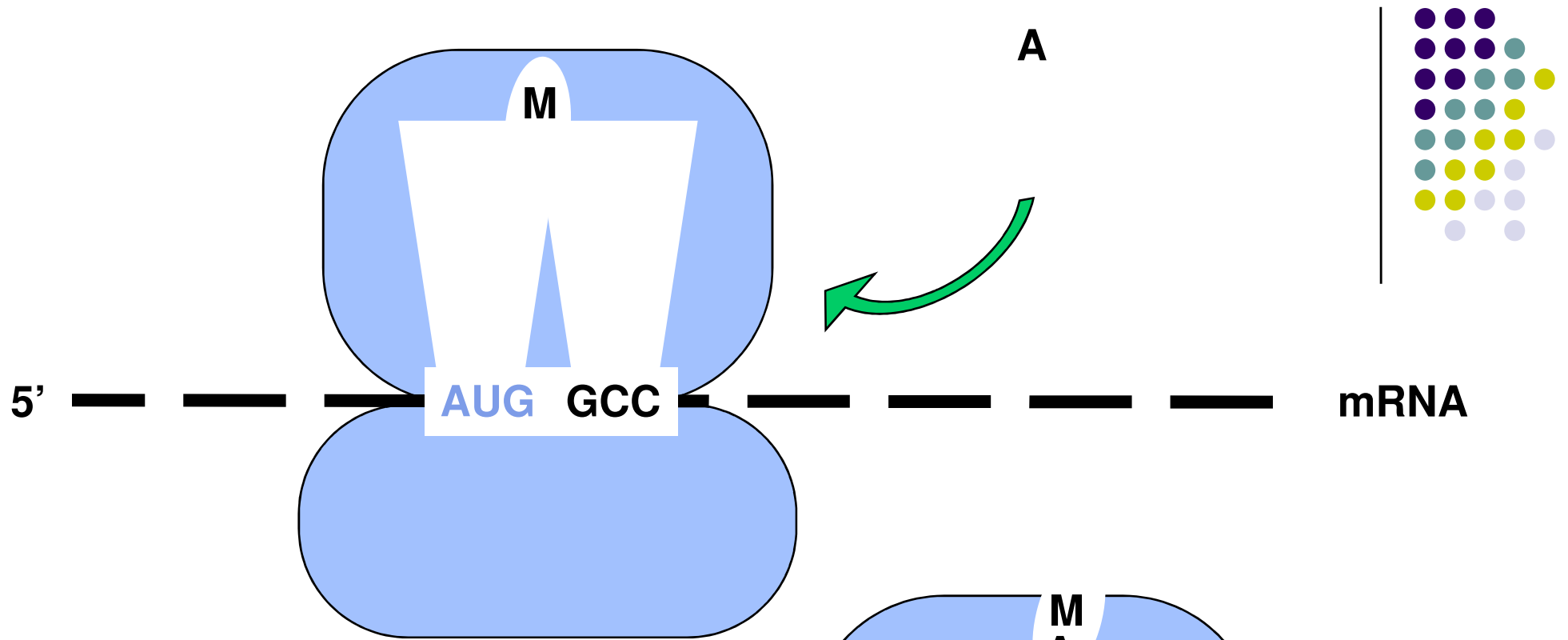
M



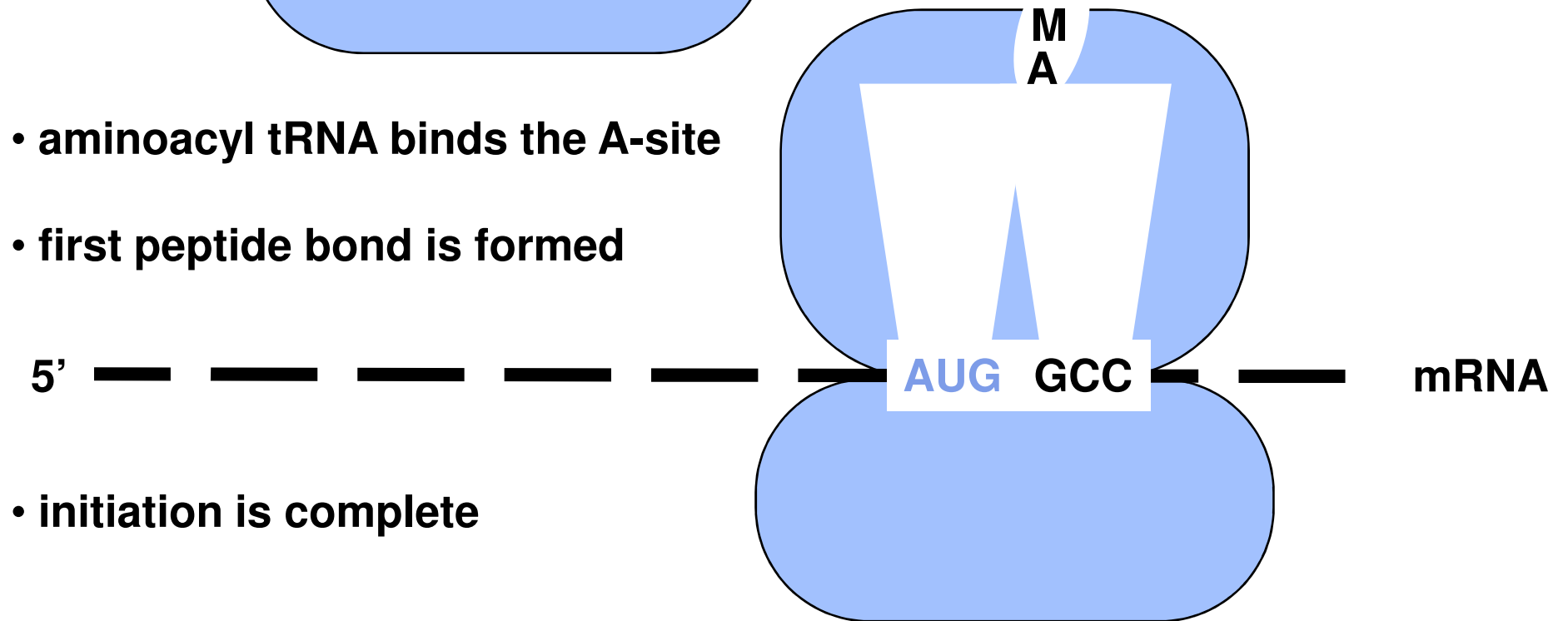
Elongation



- Addition of amino acids to the carboxyl end of the growing chain.
- Ribosome moves from the 5'-end to the 3'-end of mRNA
- New aminoacyl-tRNA for next triplet codon appears in ribosomal A site
- Facilitated by EF-Tu, EF-Ts & EF-G & requires GTP.
- After the peptide bond has been formed, the ribosome moves to next triplet codon toward the 3'-end of the mRNA. = **Translocation**
- This causes movement of the uncharged tRNA into the ribosomal E site and movement of the peptidyl-tRNA into the P site.

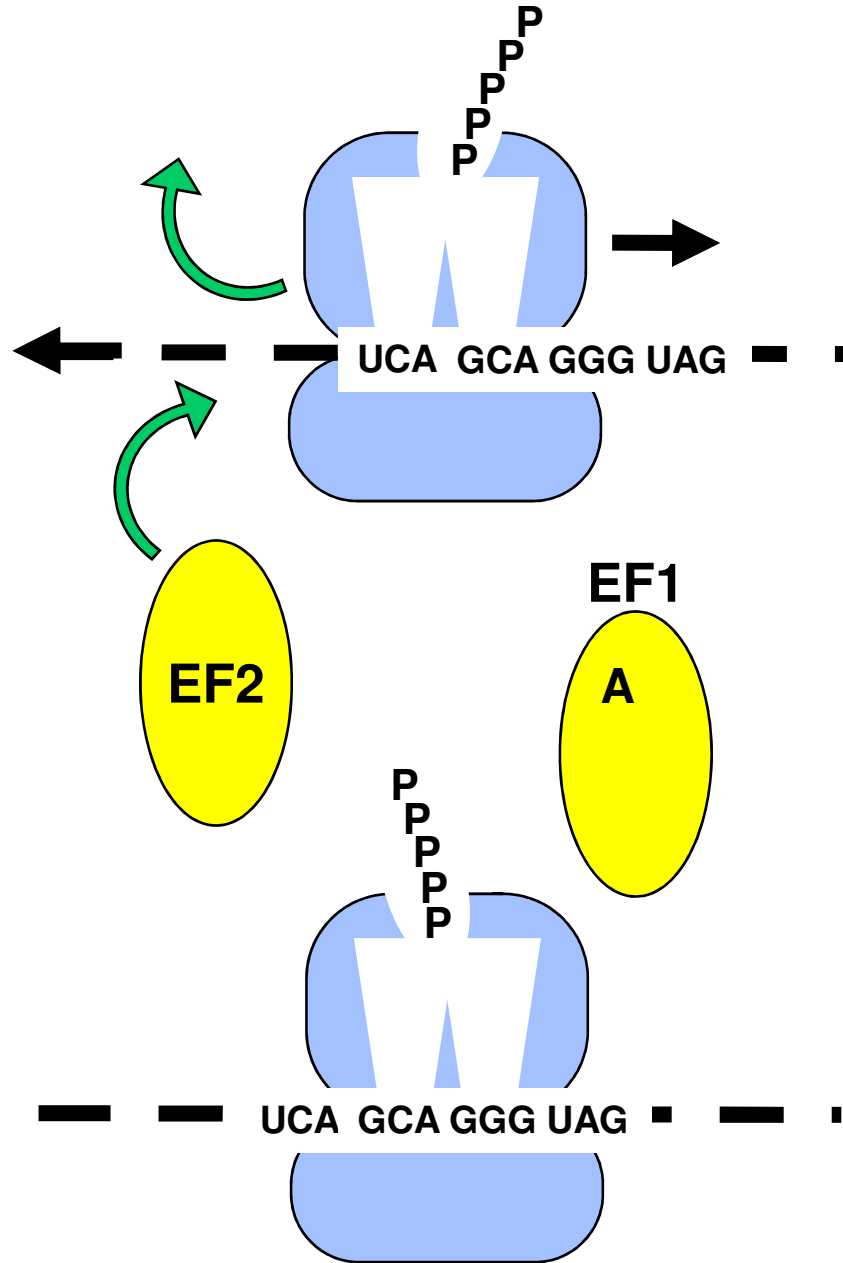


- aminoacyl tRNA binds the A-site
- first peptide bond is formed



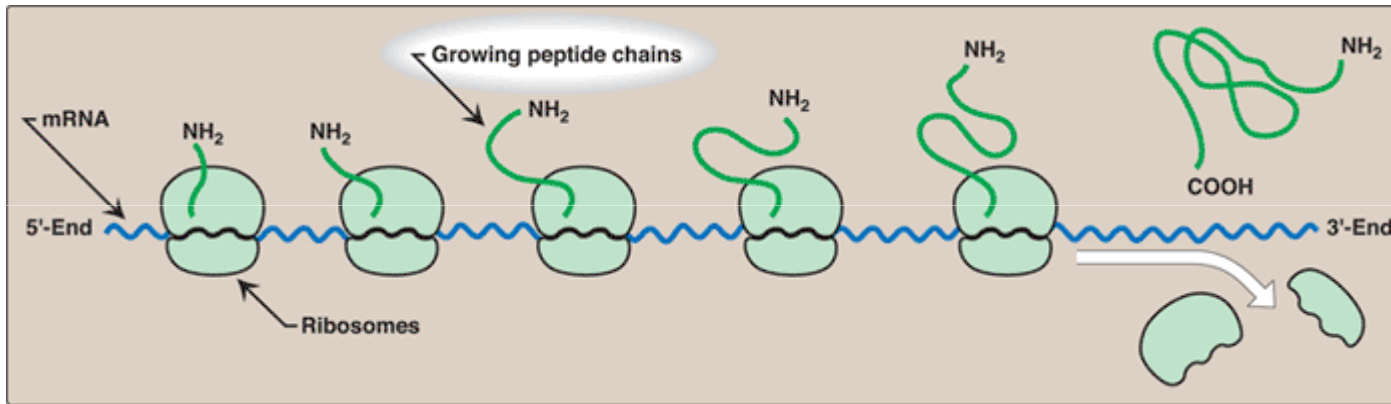
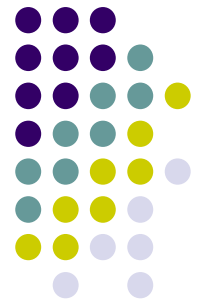
- initiation is complete

Elongation

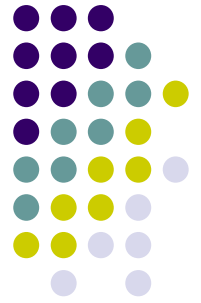


- the uncharged tRNA dissociates from the P-site
- **translocation** = the ribosome shifts one codon along mRNA, moving peptidyl tRNA from the A-site to the P-site. **requires EF2**
- **next aminoacyl tRNA** then binds within the **A-site**; tRNA binding requires EF1
- **energy for elongation is provided by the hydrolysis of two GTPs:**
 - one for translocation
 - one for aminoacyl tRNA binding



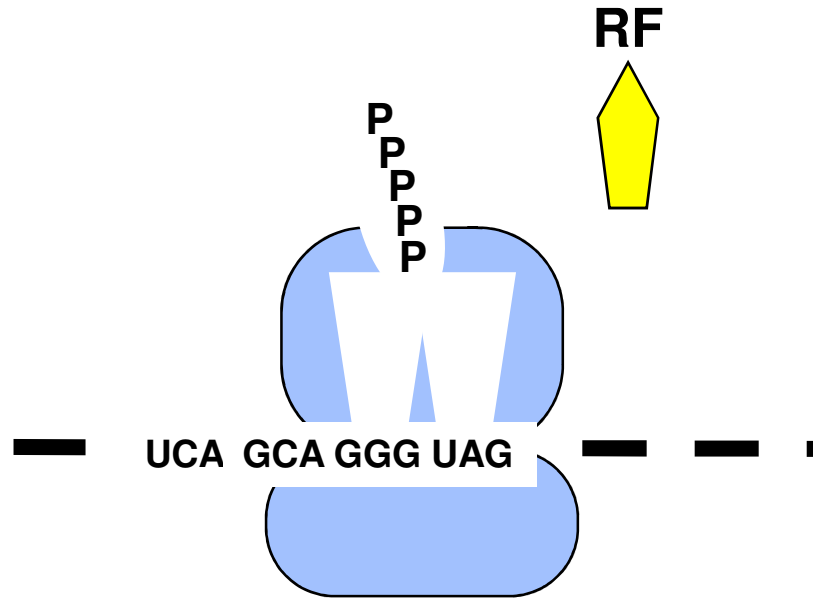
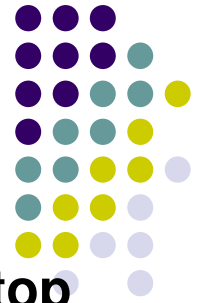


Termination

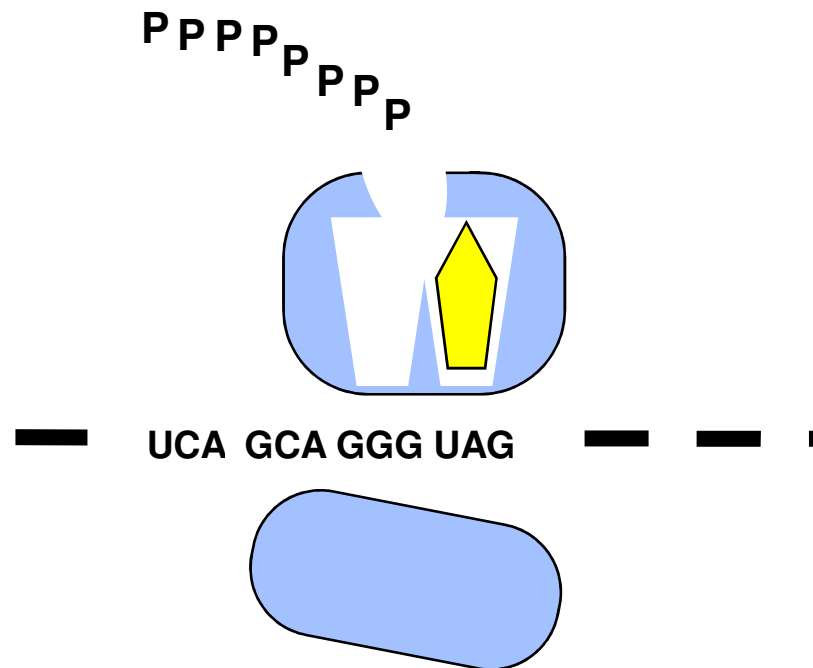


- Termination occurs when one of the three termination codons moves into the A site.
- Termination codons are **recognized** in by
 - **RF-1 = UAA and UAG**
 - **RF-2 = UAA and UGA**
- RF induces peptidyltransferase to **hydrolyze the bond** linking the **peptide to the tRNA** at the A site, causing the **nascent protein to be released** from the ribosome.
- **RF-3** (bound to GTP) cause **release of RF-1 or RF-2** as GTP is hydrolyzed.

Termination



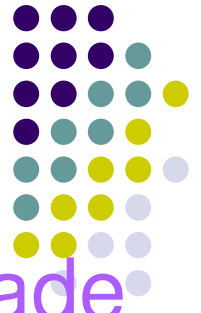
- when translation reaches the stop codon, **RF binds within the A-site**, recognizing the stop codon



- RF catalyzes the **hydrolysis** of the completed polypeptide from the peptidyl tRNA, and the **entire complex dissociates**

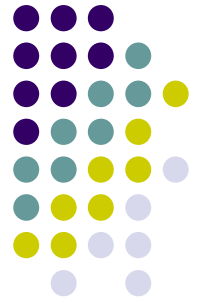
Post-translation modification

Trimming



- Many proteins are initially made as **initially made as large**, precursor molecules that are **not functionally active**.
- Precursor proteins are **cleaved in the endoplasmic reticulum or the Golgi apparatus**, others are cleaved in developing secretory vesicles.
- **Zymogens** are inactive secreted enzymes (including the proteases required for digestion).
- They **become activated through cleavage** when they reach their proper sites of action.
- E.g. Pancreatic zymogen, trypsinogen, becomes activated to in the small intestine.

Post-translation modification



Covalent Alterations

1. Phosphorylation :

- On hydroxyl groups of serine, threonine, tyrosine.
- increase or decrease the functional activity of the protein.

2. Glycosylation :

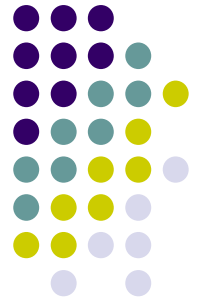
- Proteins of a plasma membrane.
- Carbohydrate attached to serine or threonine hydroxyl groups (O-linked) or the amide nitrogen of asparagine (N-linked).

3. Hydroxylation :

- Proline and lysine residues = collagen

Post-translation modification

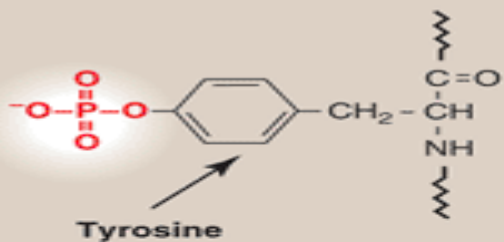
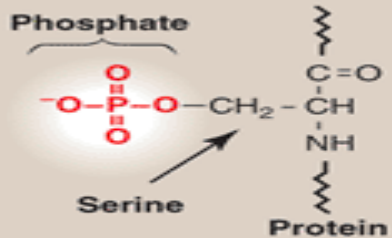
Covalent Alterations



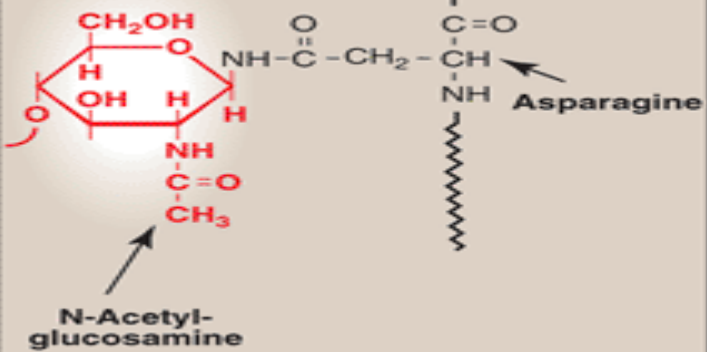
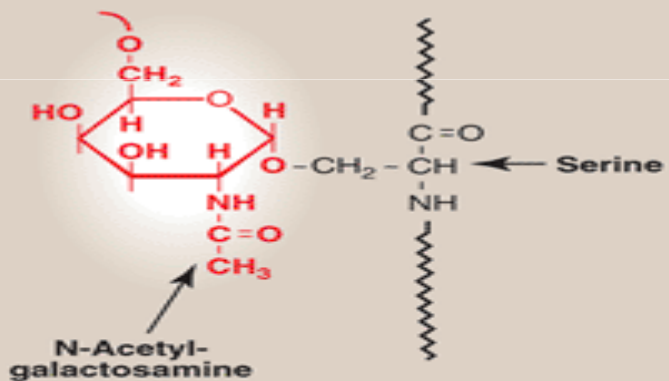
1. Other covalent modification :

- **Vitamin K–dependent carboxylation** of Glutamate residues of **clotting factors**.
- **Biotin** = ϵ -amino groups of lysine residues of biotin-dependent enzymes
= carboxylation reactions.
e.g. **pyruvate carboxylation**
- Attachment of lipids, such as farnesyl groups, can help anchor proteins in membranes.
- Acetylated

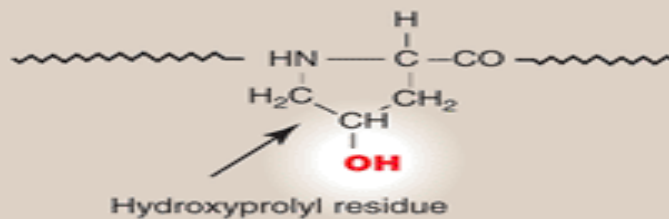
Phosphorylation



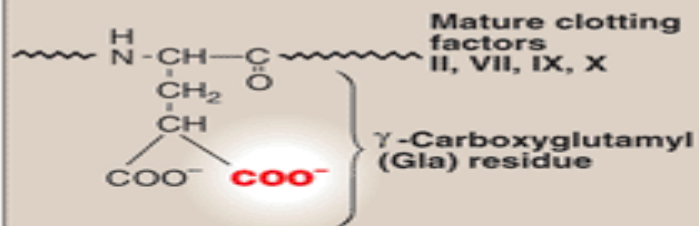
Glycosylation



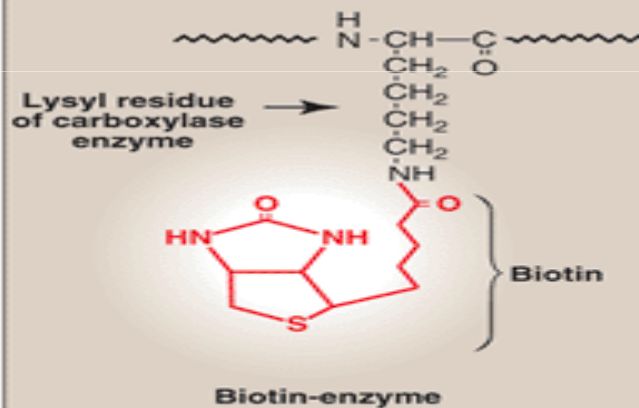
Hydroxylation



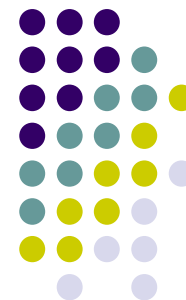
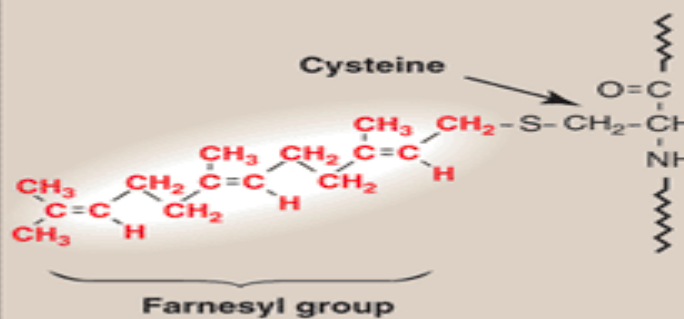
Carboxylation



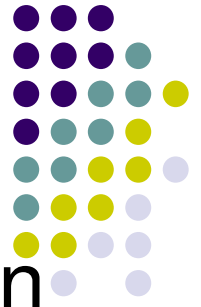
Biotinylated enzyme



Farnesylated protein



Post-translation modification



Protein degradation

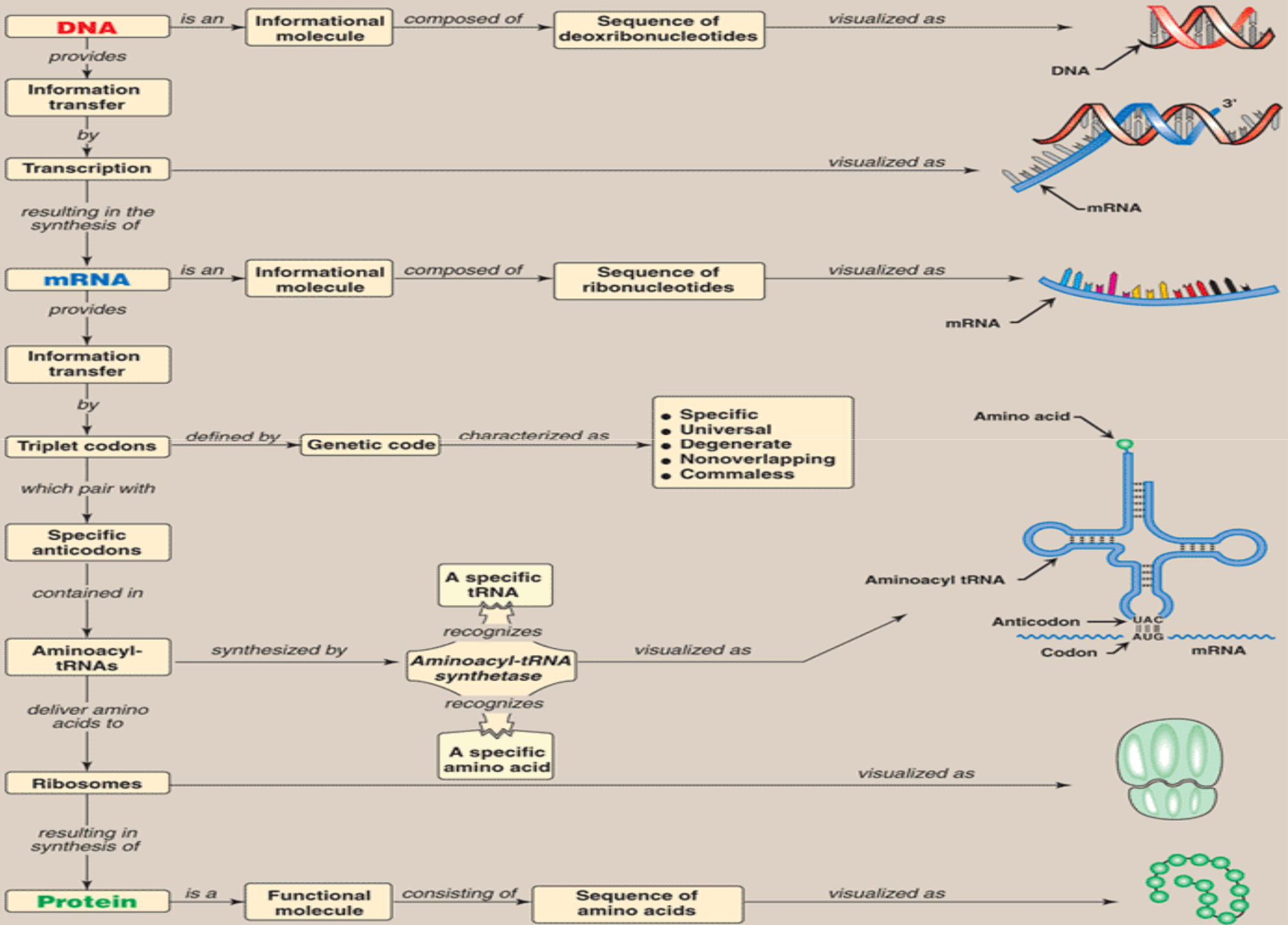
- **Defective Protein**, for rapid turnover are often marked for destruction by **ubiquitination**
- The attachment of a small, highly conserved protein, called **ubiquitin**.
- Proteins **marked** in this way are rapidly degraded by a cellular component known as the “**proteasome**”.

Inhibitor of Translation



<u>Inhibitor (Antibiotic)</u>	
<i>Erythromycin</i>	<i>50 s ribosomal subunit</i>
<i>Clindamycin</i>	<i>50 s ribosomal subunit</i>
<i>Tetracycline</i>	<i>30 s ribosomal subunit</i>
Puramycin	Amino-acyl t-RNA
Chloramphenicol	Peptidyl Transferase
<i>Diphtheria Toxin</i>	<i>eEF-2</i>

Flow of genetic information



Thanks

