

# 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)**

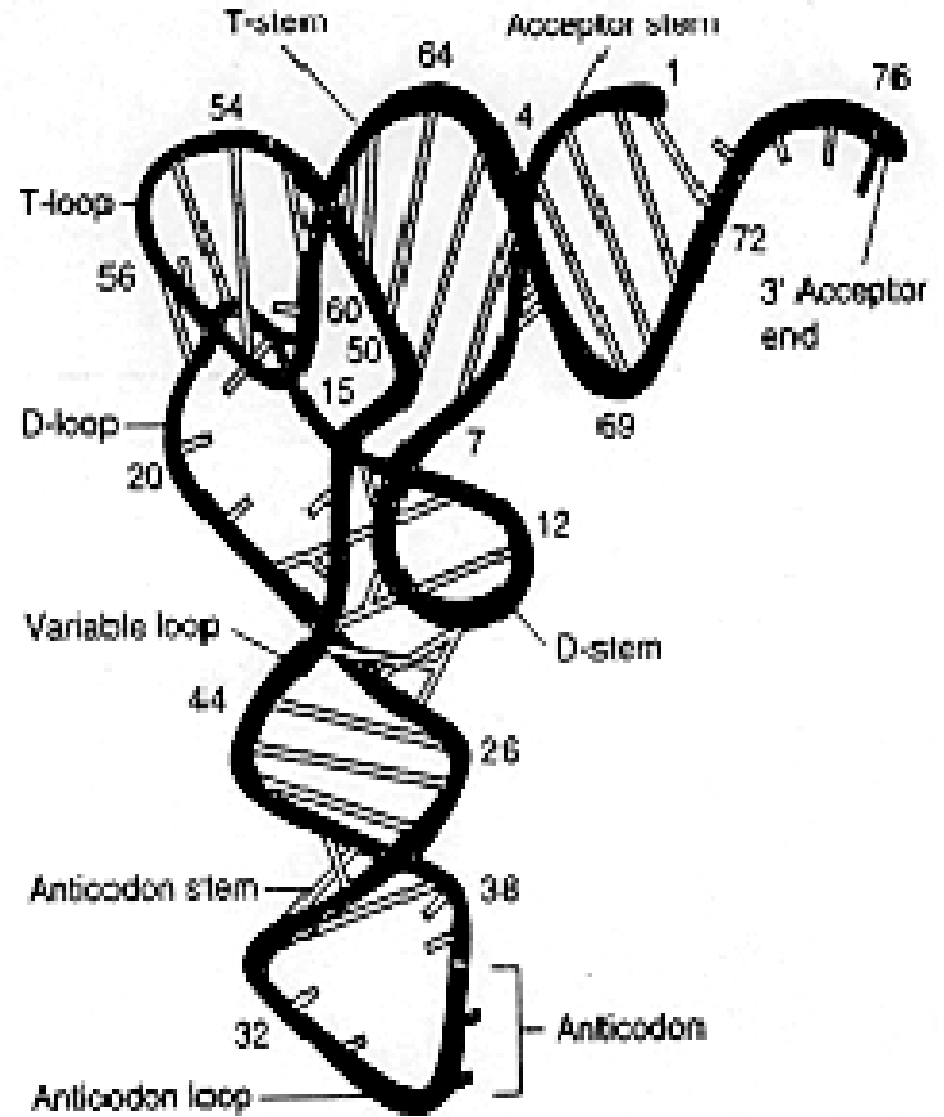
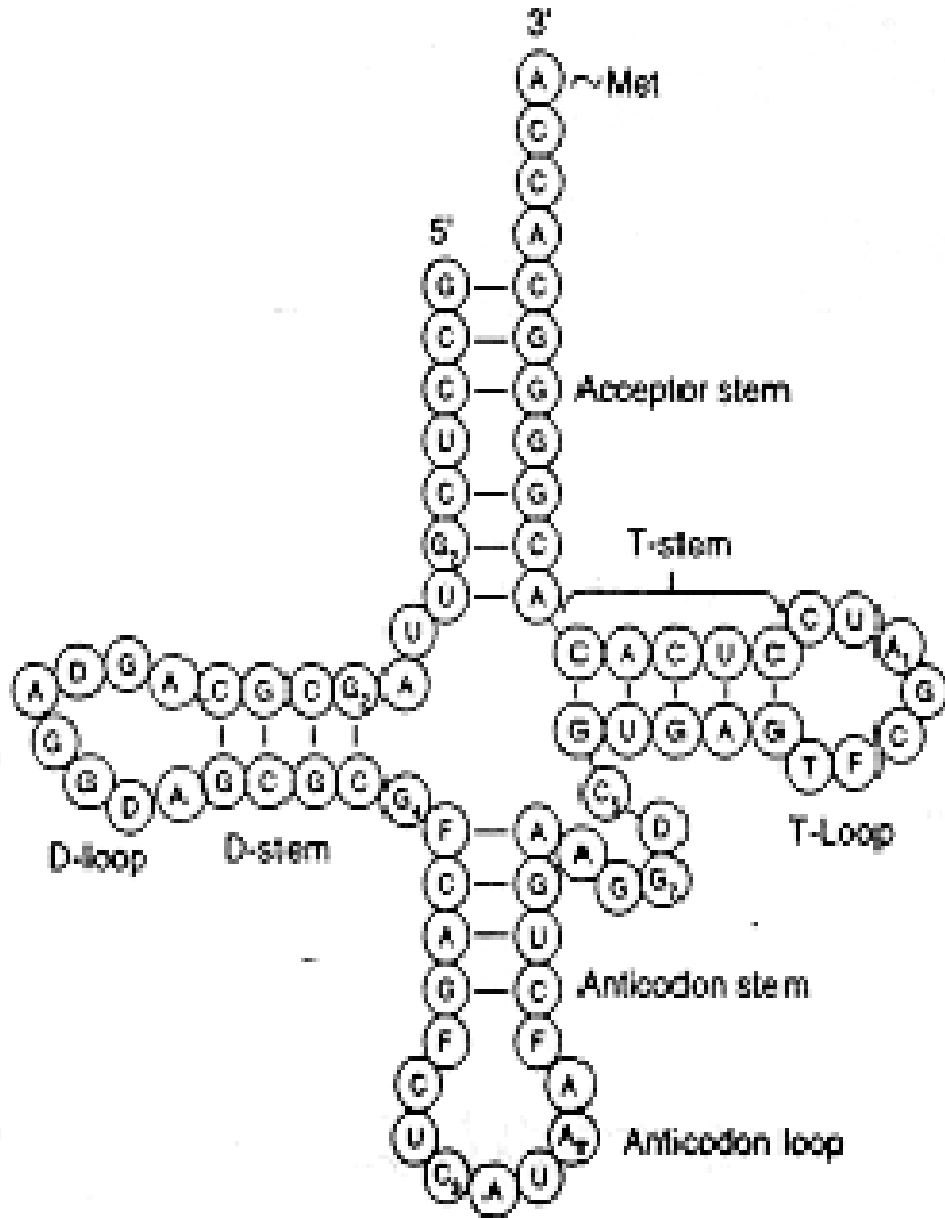
## **3. Messenger RNA (mRNA)**

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# 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”**.

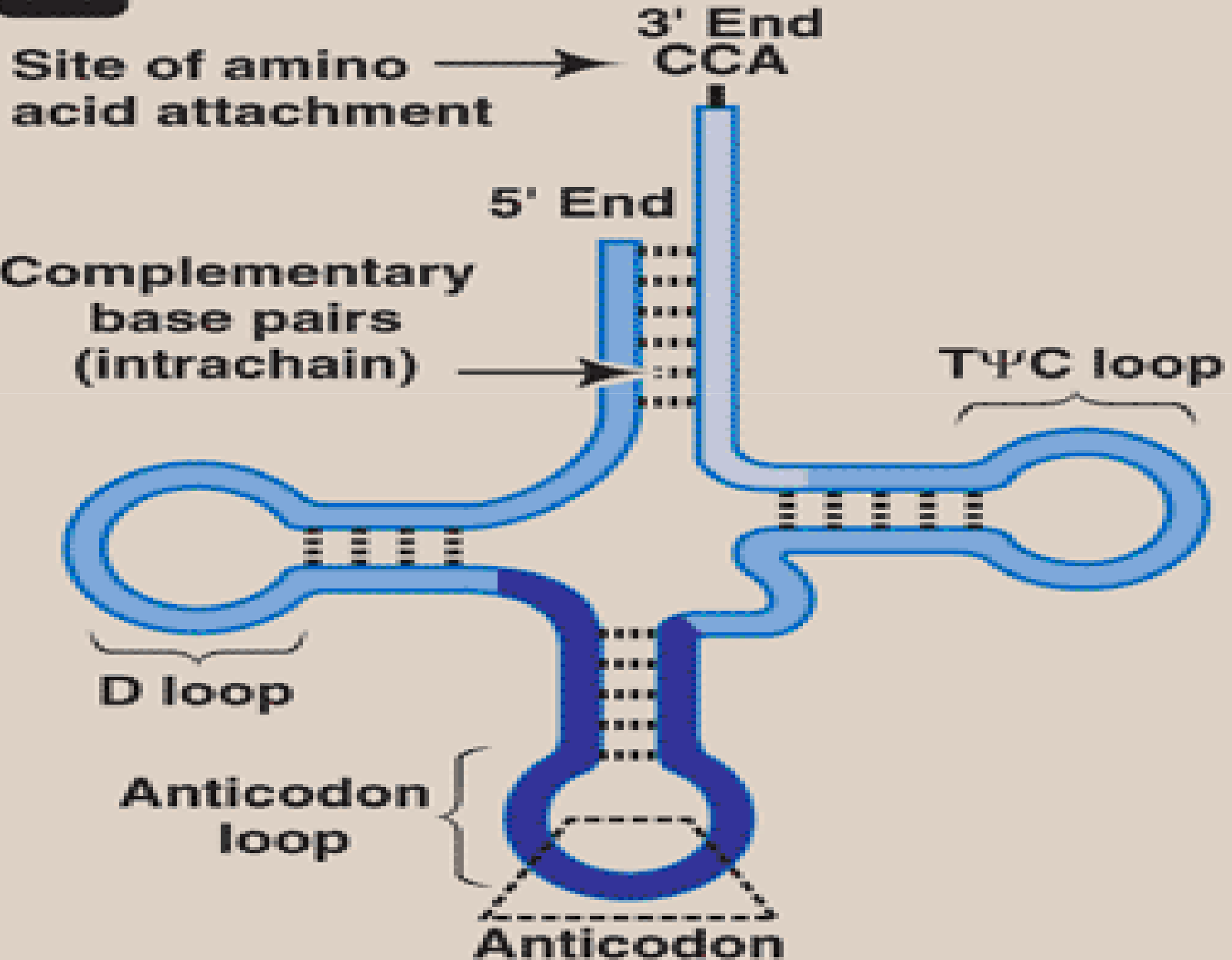
# Secondary structure



# Tertiary structure

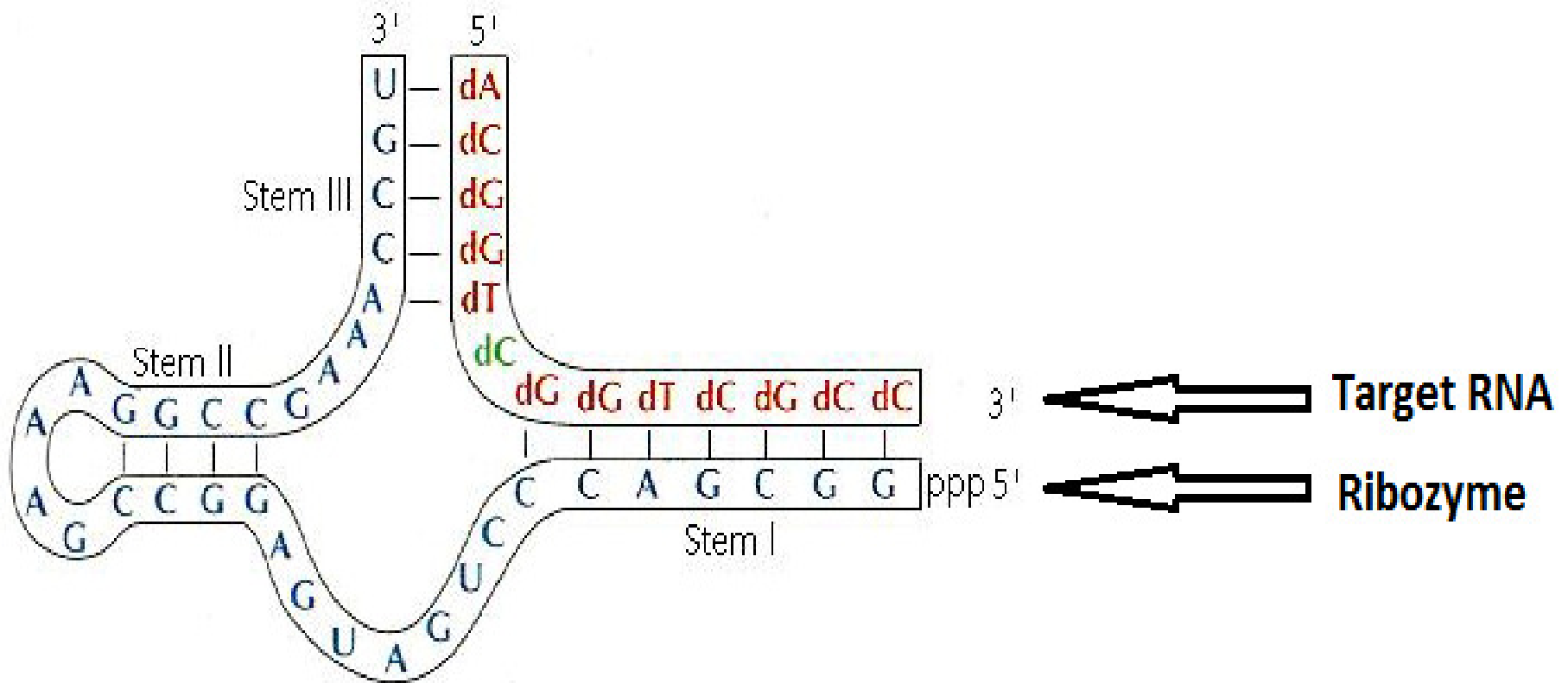
# t-RNA

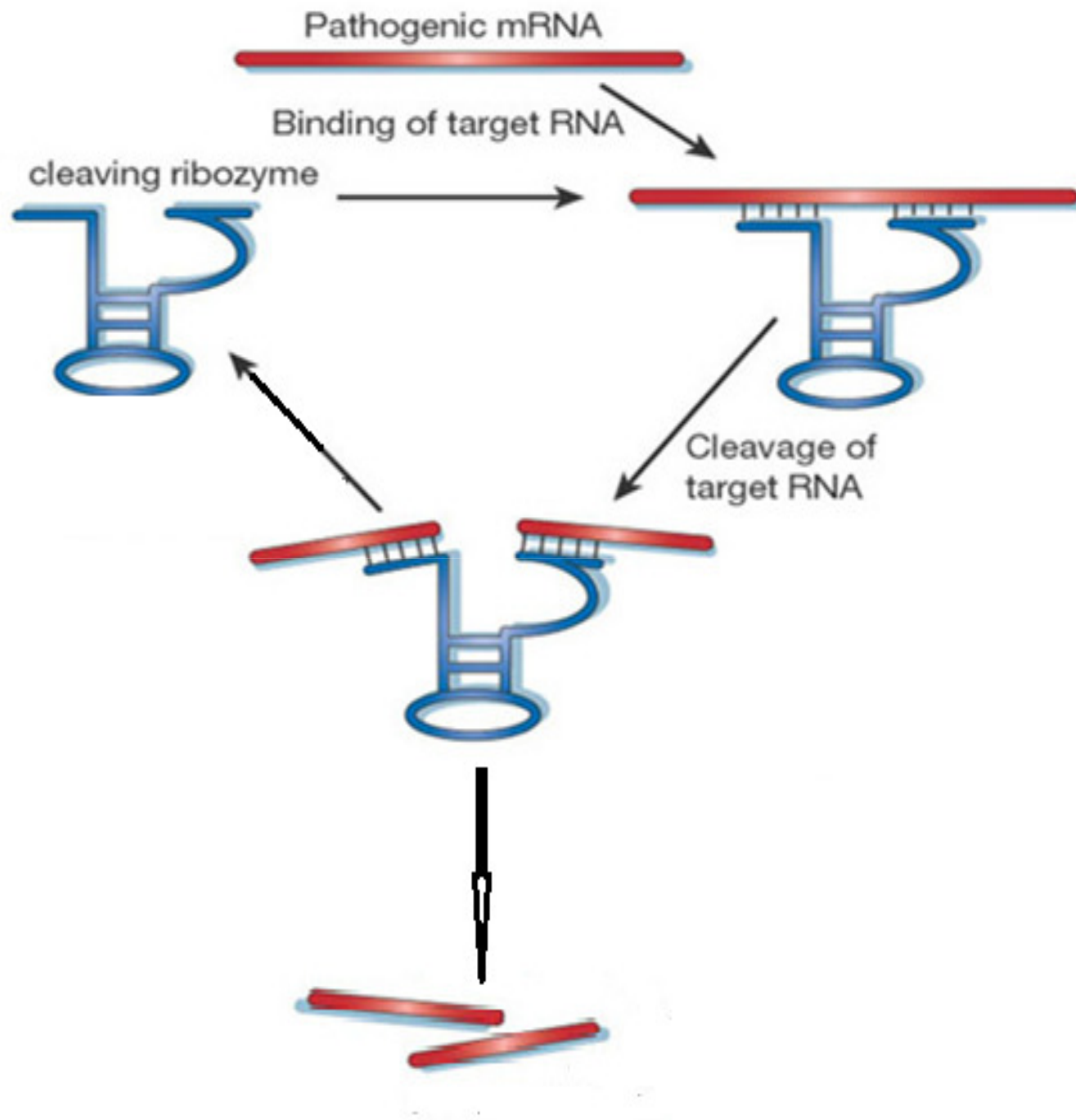
**A**



# 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
- 
- **Dihydrouracil 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

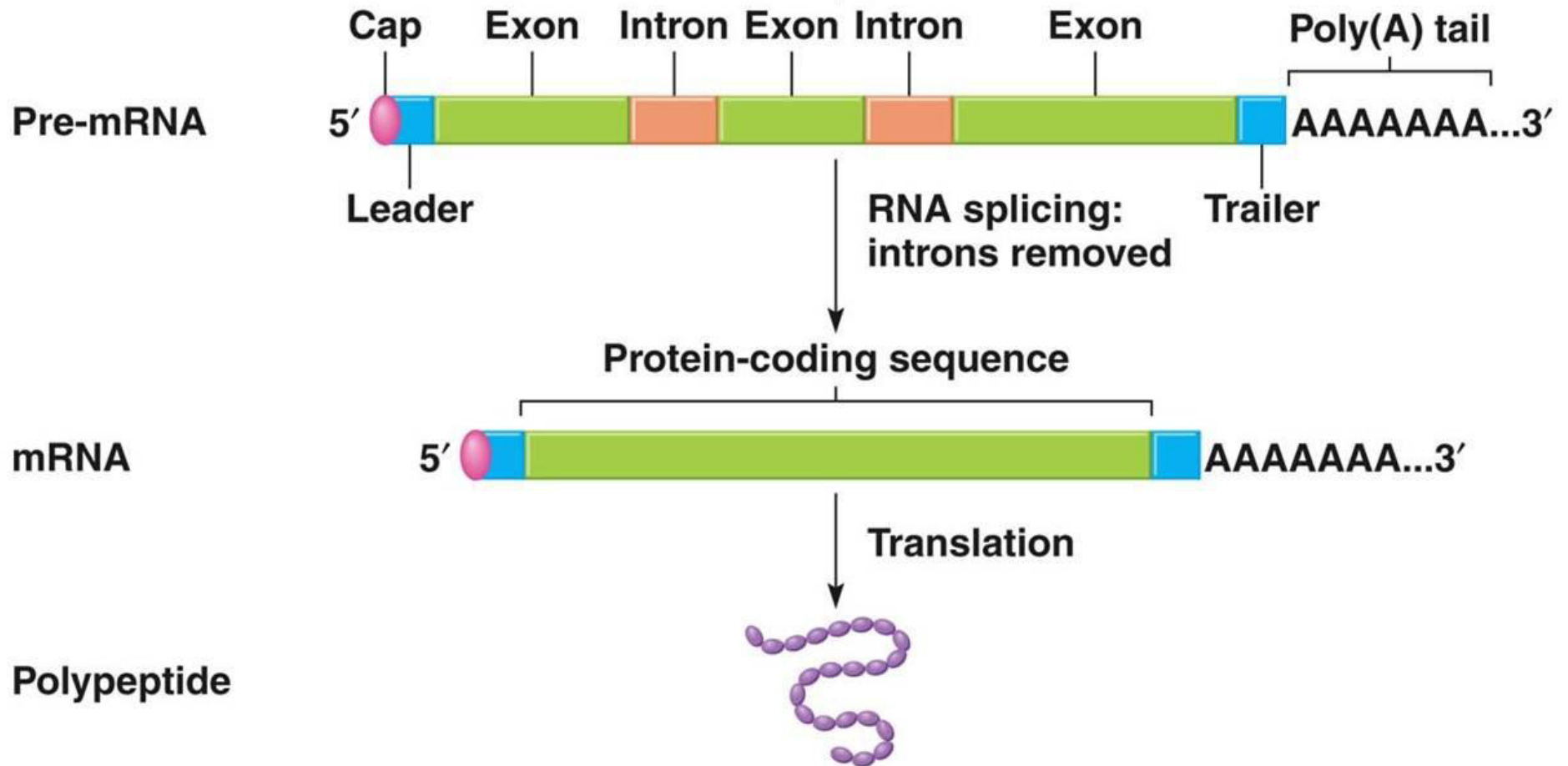




I



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



# m-RNA

## Mature Messenge RNA

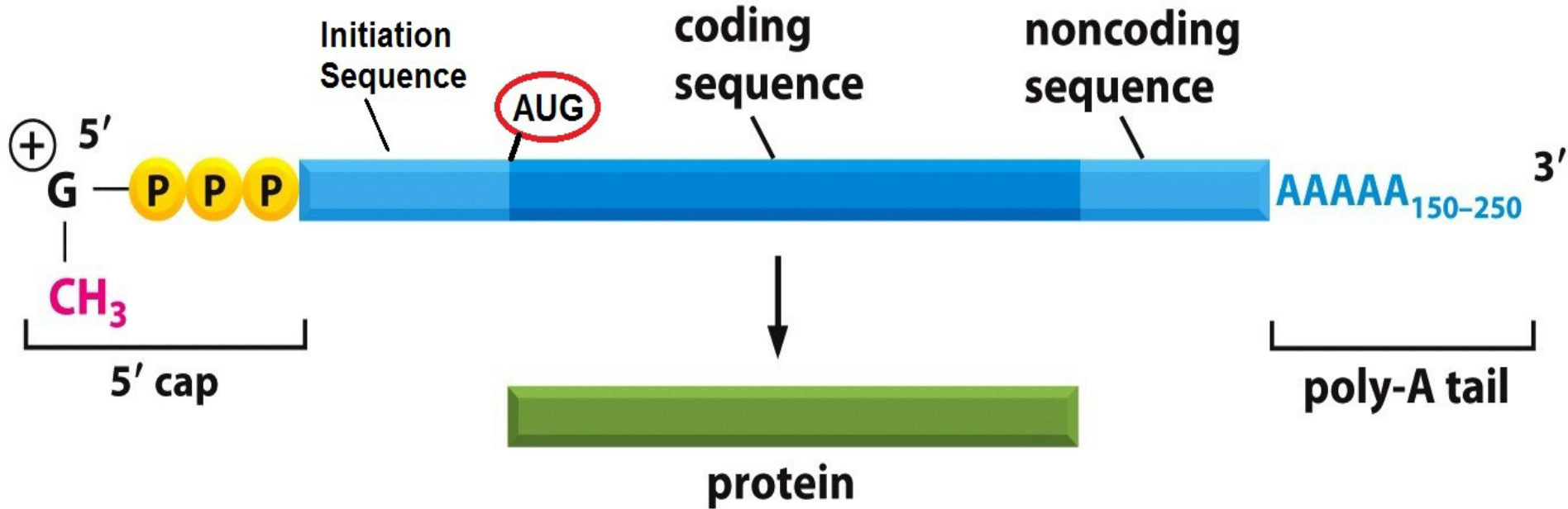
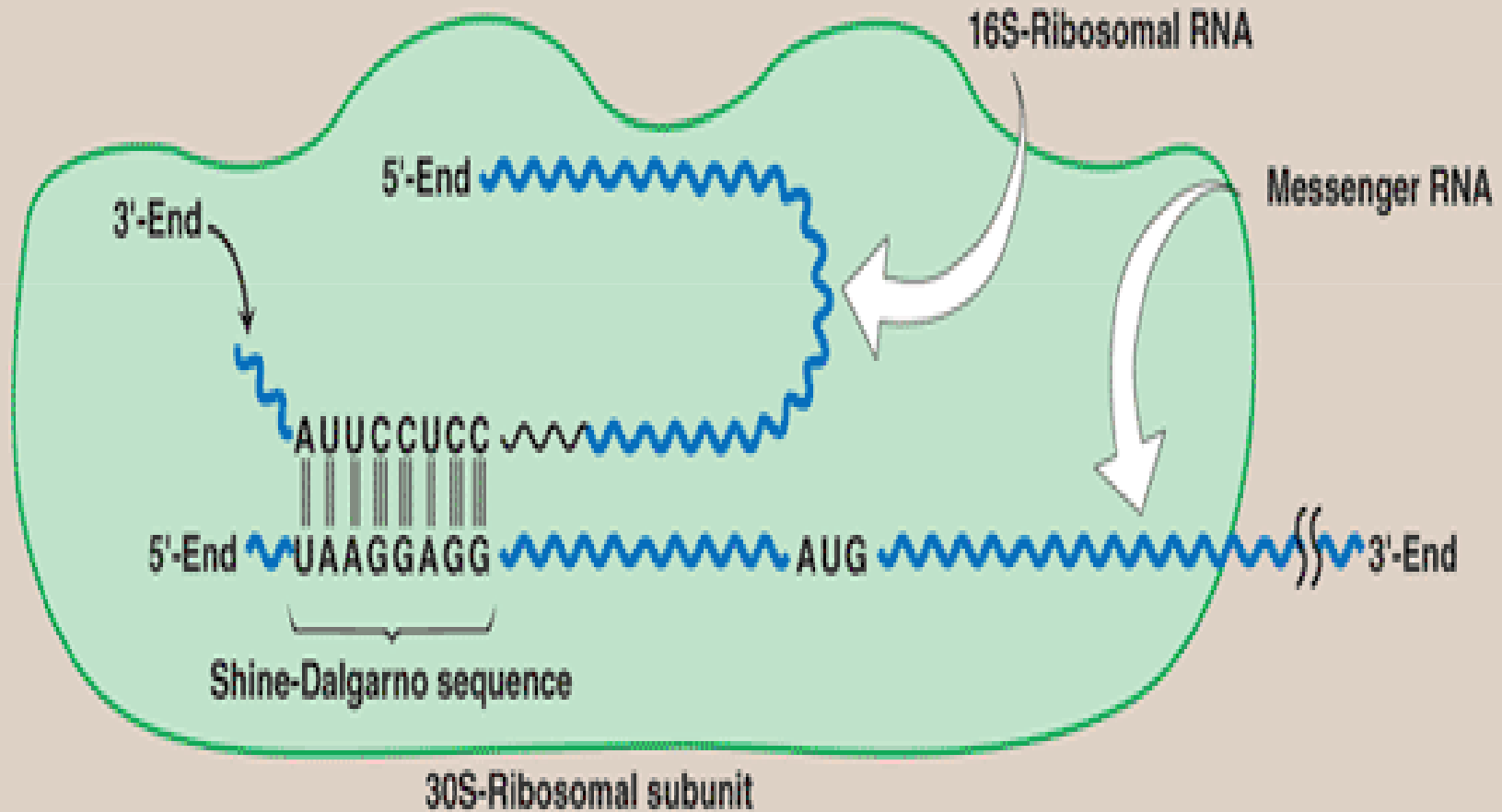


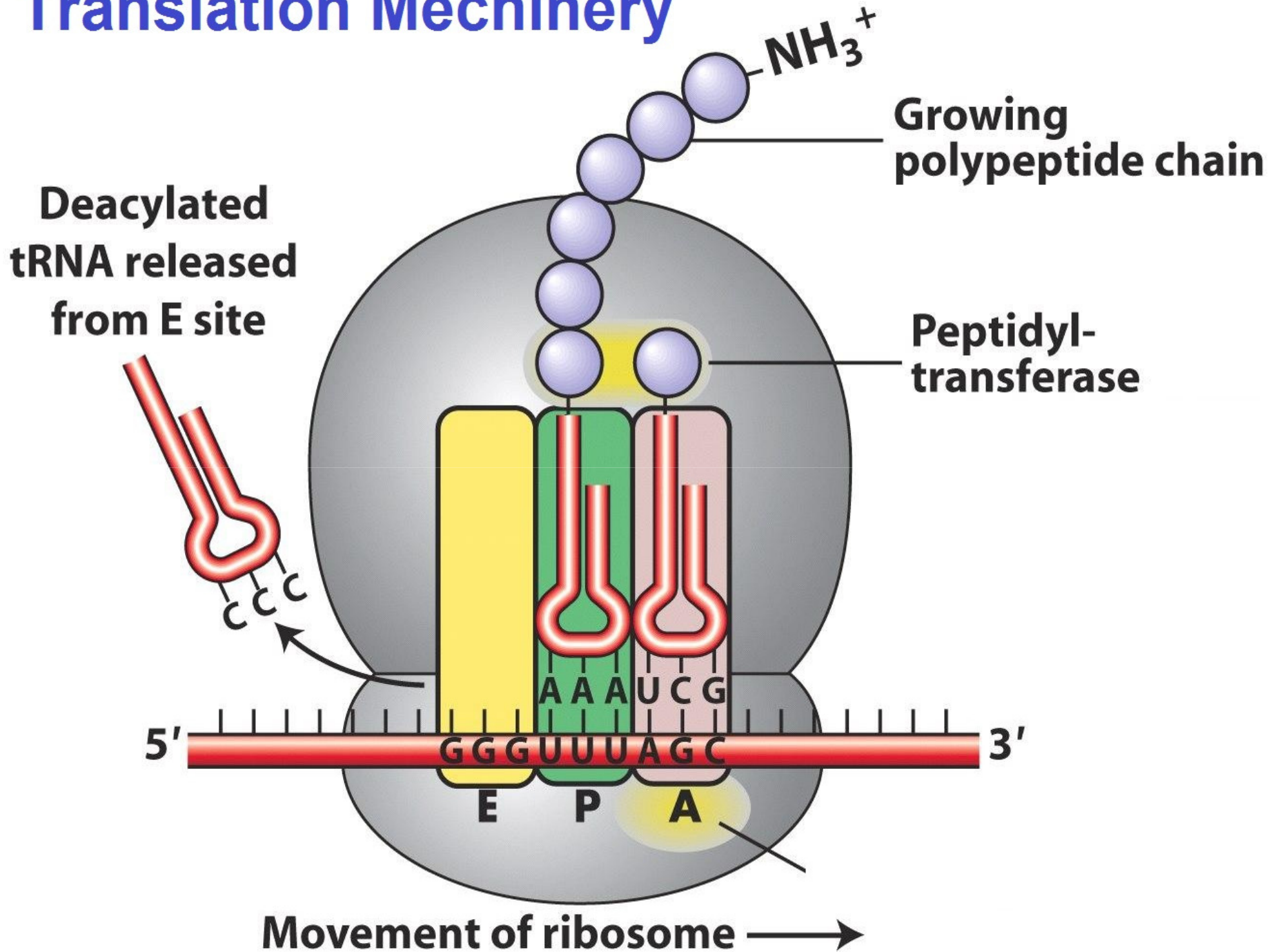
Figure 7-16a Essential Cell Biology 3/e (© Garland Science 2010)

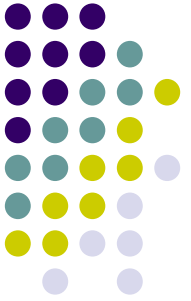
# Shine – Dalgarno Sequence

“Facilitate Initiation of Translation”



# Translation Machinery





# 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											
		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	U	C	A	G	U
	U	C	A	G	C
	U	C	A	G	A
	U	C	A	G	G
C	C	C	A	G	U
	C	C	A	G	C
	C	C	A	G	A
	C	C	A	G	G
A	A	C	A	G	U
	A	C	A	G	C
	A	C	A	G	A
	A	C	A	G	G
G	G	C	A	G	U
	G	C	A	G	C
	G	C	A	G	A
	G	C	A	G	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.

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

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

**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,

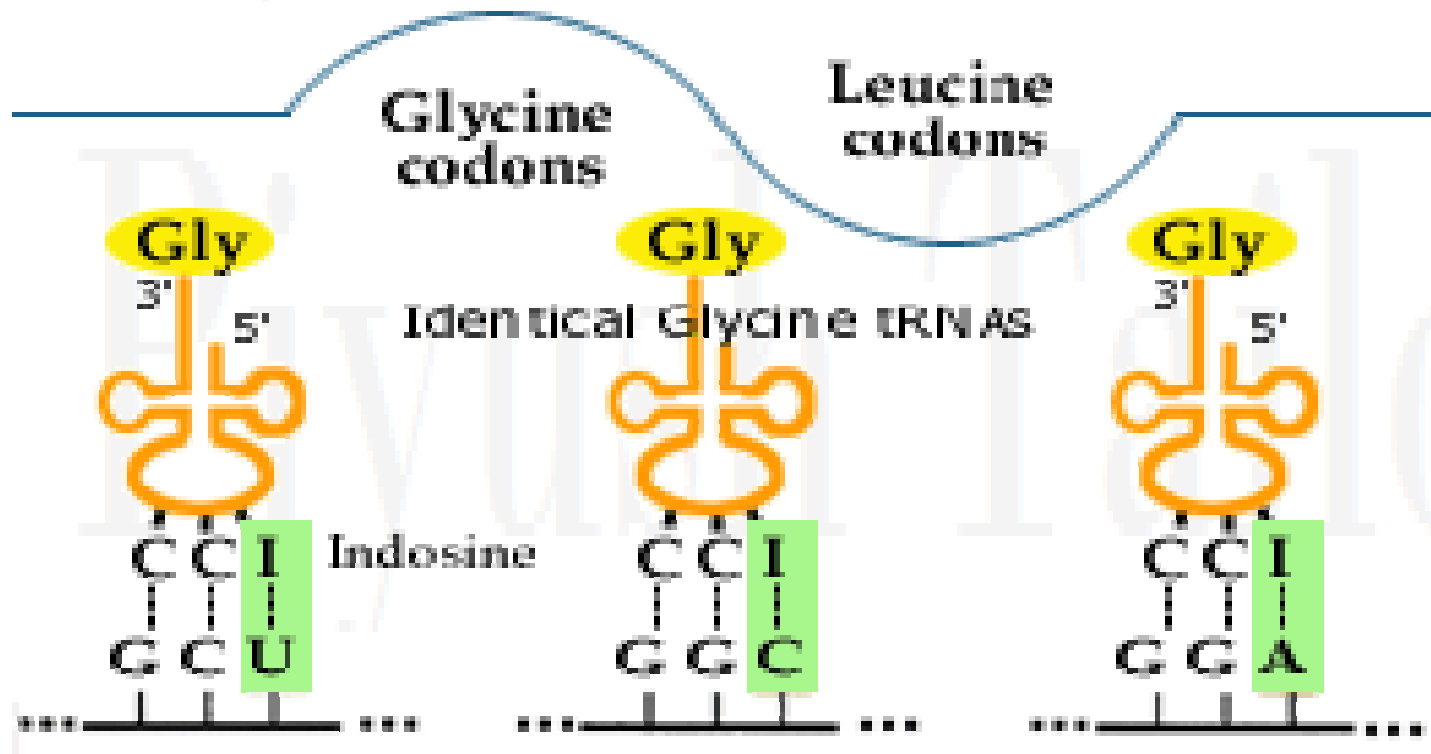
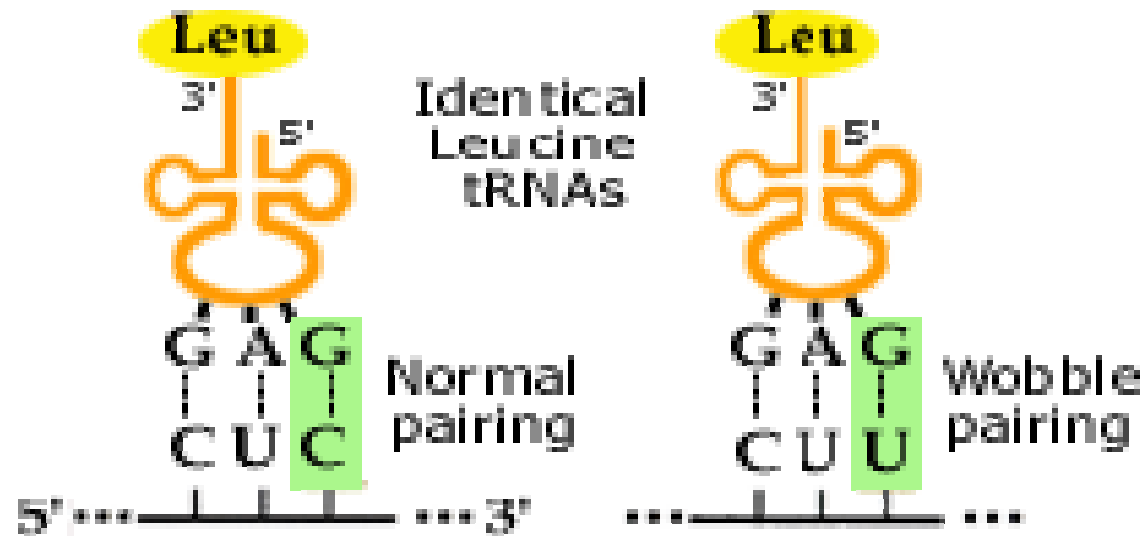
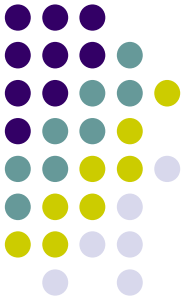
Mitochondrial DNA = AGG, AGA,

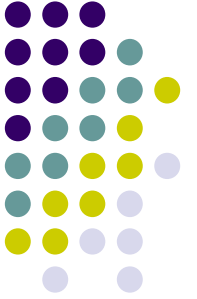
## 9. **Wobbling Phenomenon :**

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.

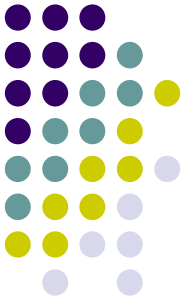
# Wobbling Phenomenon





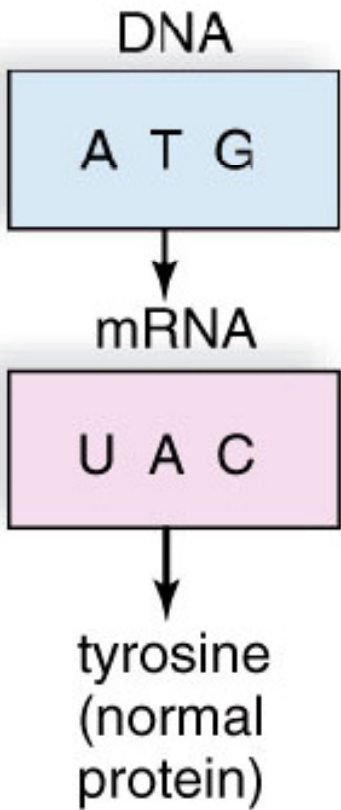
# Mutation

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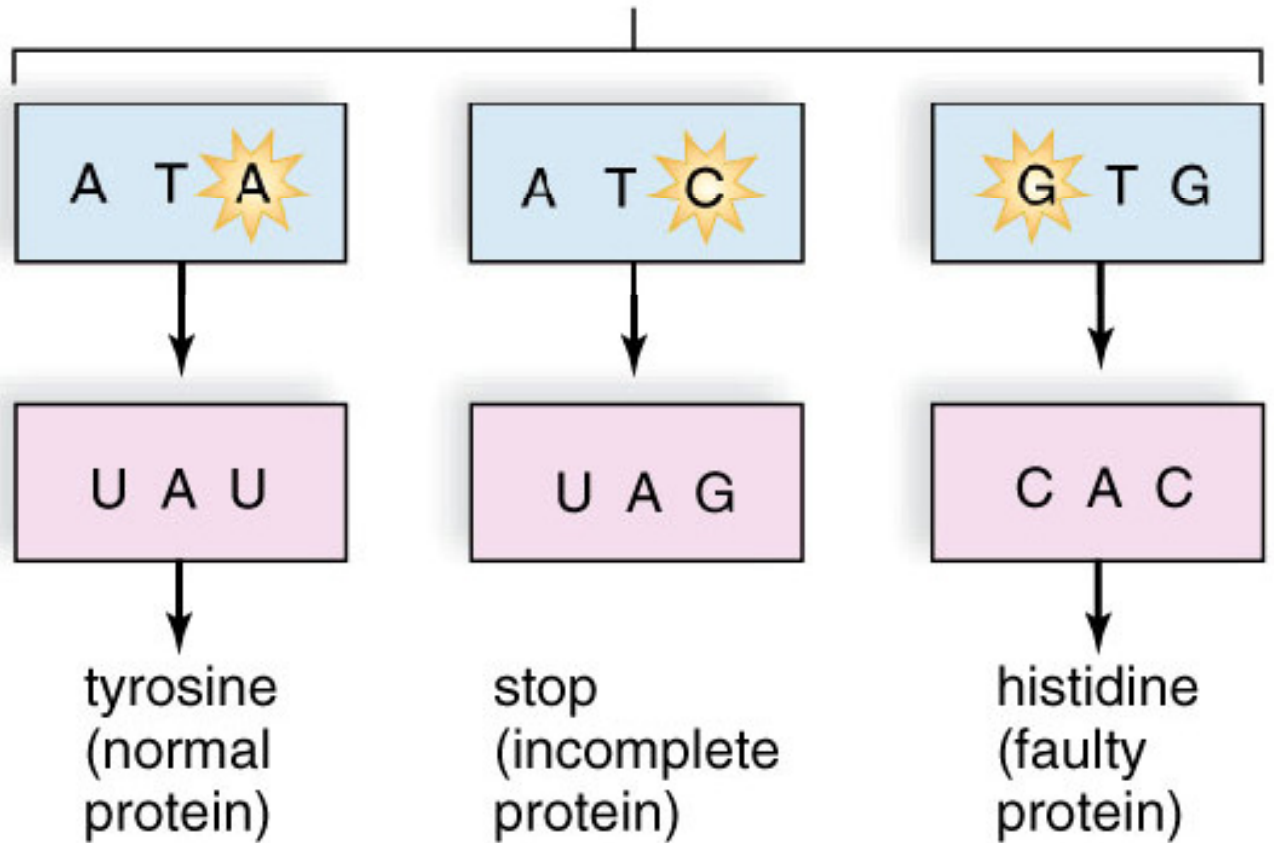


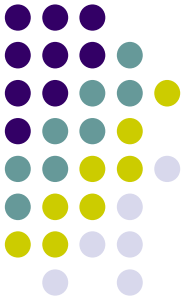
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### No mutation



### Point mutations





## DNA Sequence

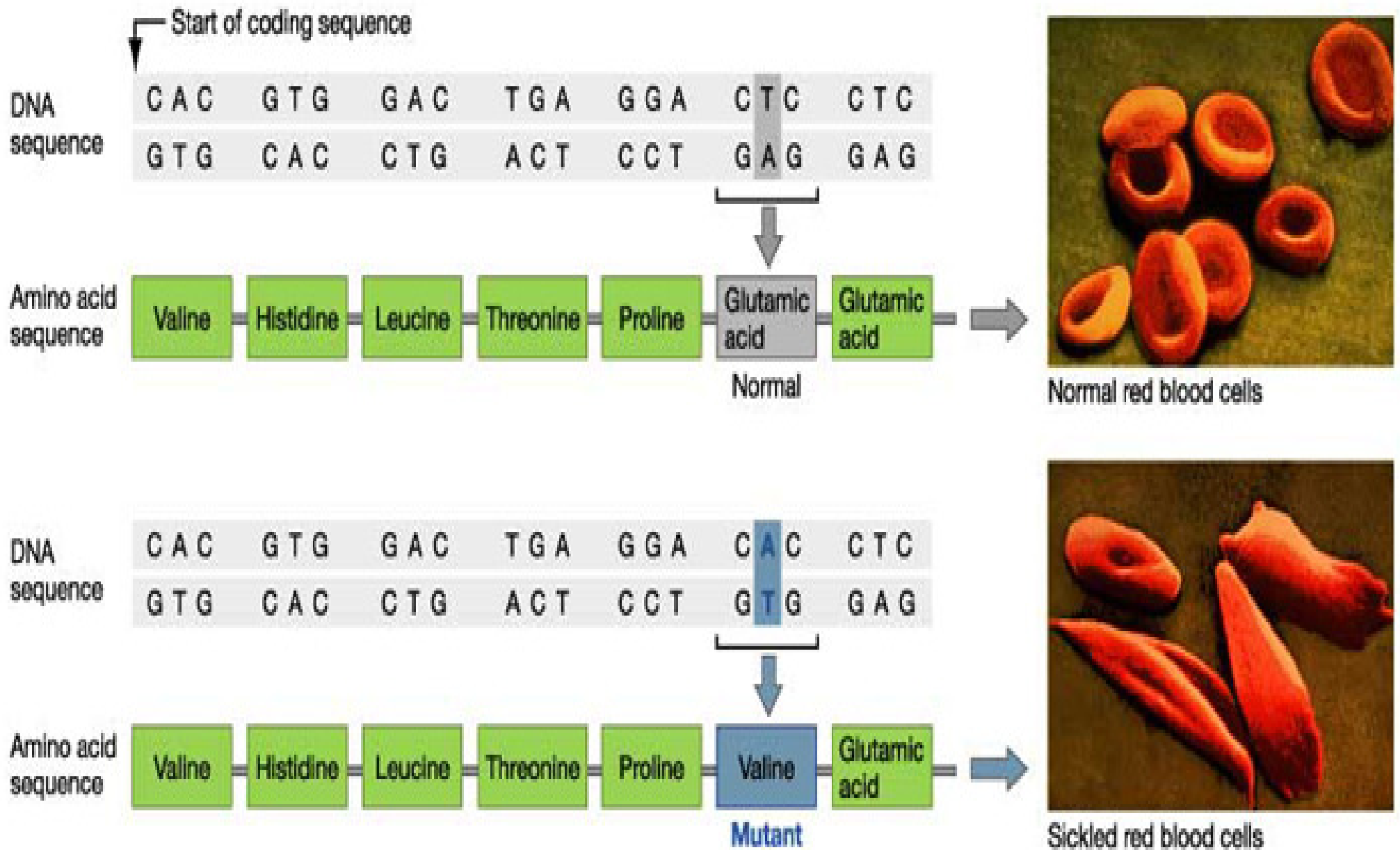
## Amino Acid Sequence

**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

DNA  
Translator



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.



CODON        139 | 140 | 141 | 142 |

m-RNA    ....AAA UAC CGU **UAA** GCU GGA ...

..... lys ..... tyr ..... arg ... **STOP**

**α globin**



CODON        139 | 140 | 141 | 142 | 143 | 144 | ..... 172 | 173

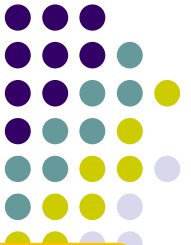
m-RNA    ....AAA UAC CGU **C**AA GCU GGA ..... GAA **UAA**

..... lys ..... tyr ..... arg ..... **gln** .....ala .....gly ..... glu ..... **STOP**

CODON        139 | 140 | 141 | 142 |

m-RNA    ....AAA UAC CGU **UAA** GCU GGA ...

**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

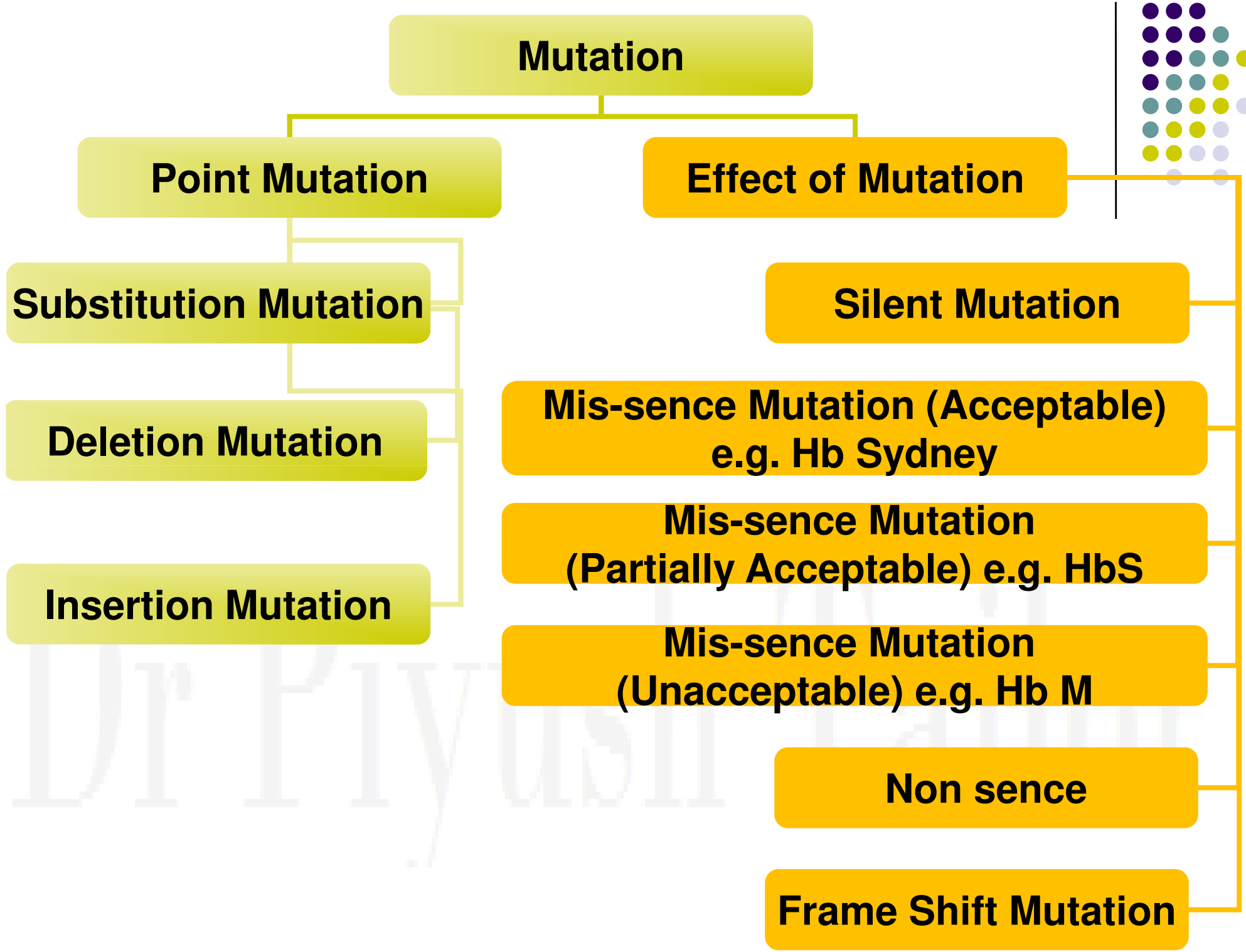
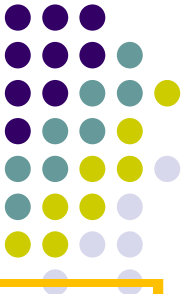
#### Codon Insertion

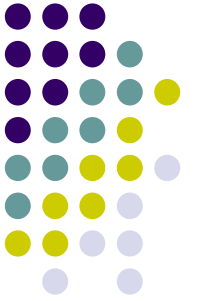
Huntington's chorea

#### Base deletion

Frame Shift Mutation







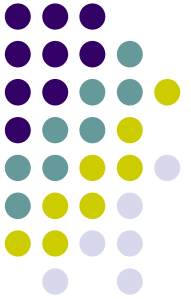
# Substitution Mutation

## ● Transition

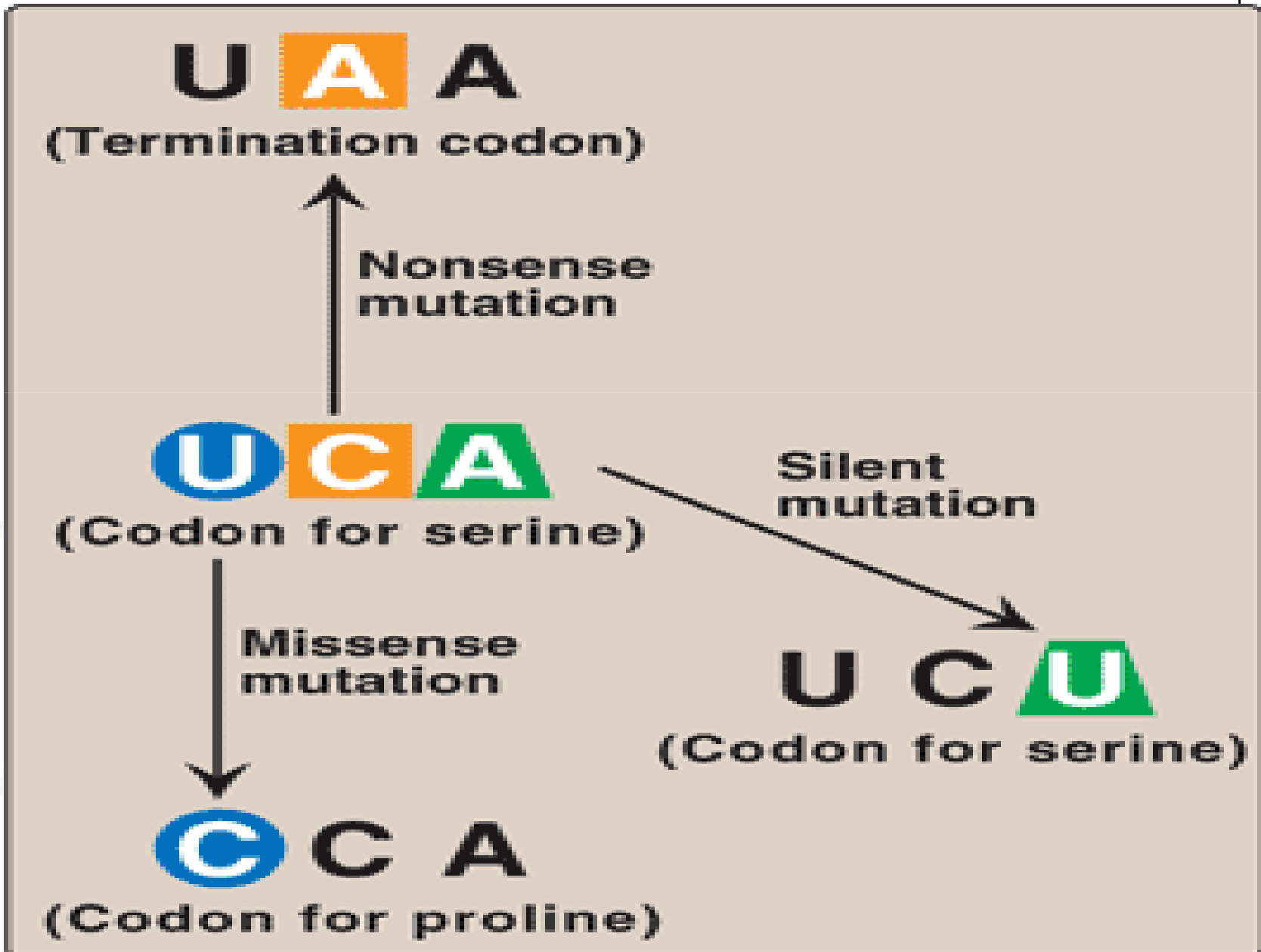
- Purine replace by Purine  
or
- Pyrimidine replace by Pyrimidine

## ● Transversion

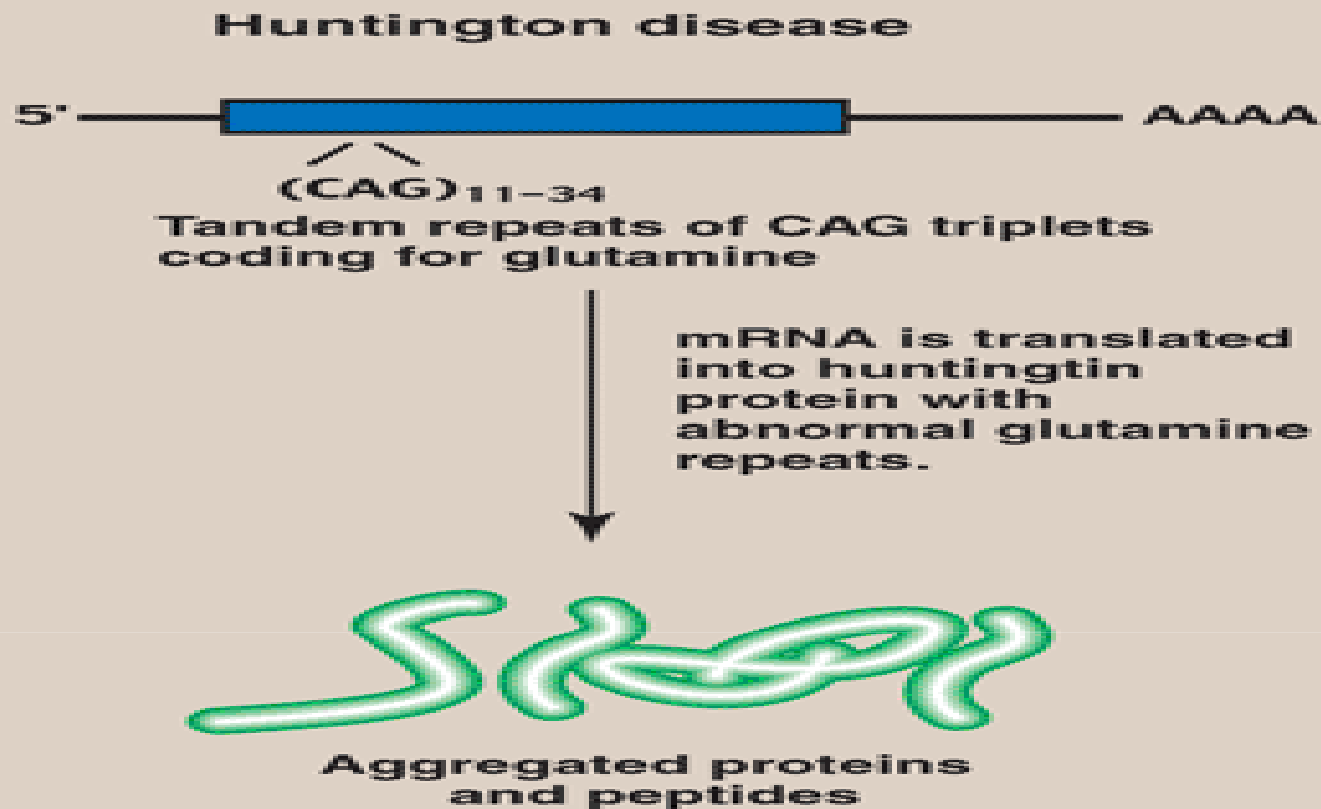
- Purine replace by Pyrimidine  
Or
- Pyrimidine replace by Purine
- E.g. Sickle cell anaemia
- GAG (glutamic acid) = GUG (valine)



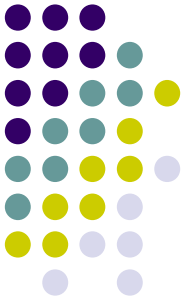
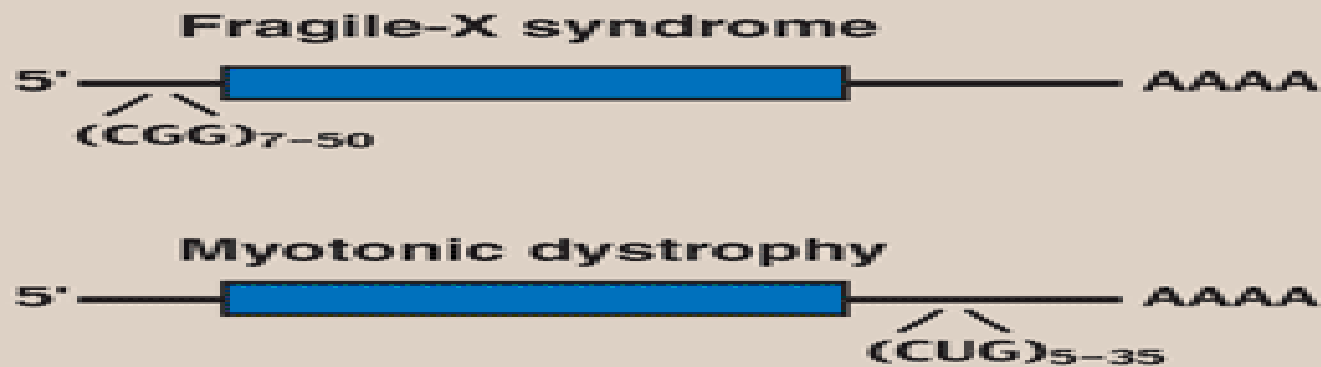
# Effect Of Mutation



# Huntington disease



## Other triplet expansion diseases



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# Addition of base

mRNA

U C A U C C U A U G G G C U

Ser Ser Tyr Gly

U

Addition of U

U C A C C U A U G G G C U

Ser Pro Met Ala

5'-End

3'-End

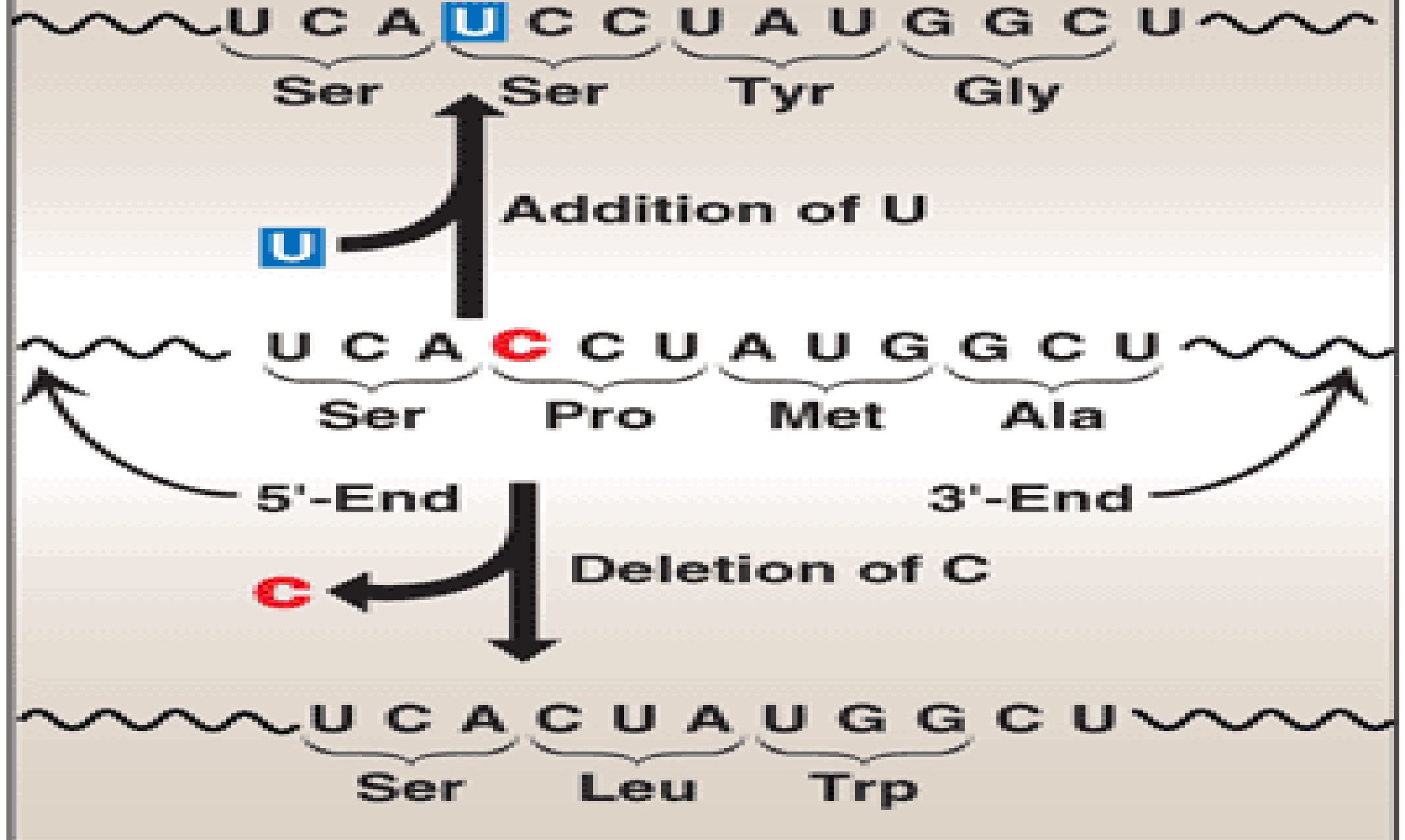
Deletion of C

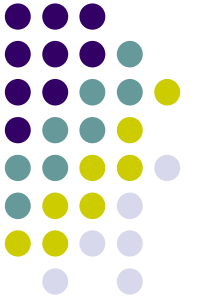
C

U C A C U A U G G G C U

Ser Leu Trp

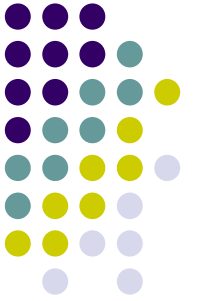
# Deletion of base





# Translation (Protein Synthesis)

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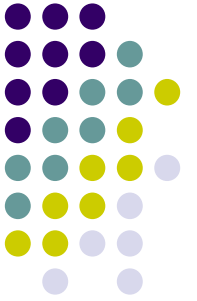


# Translation

- Steps:
  - Initiation
  - Elongation
  - Termination
  - Post - Translation

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# Translation Process



## ● Requires

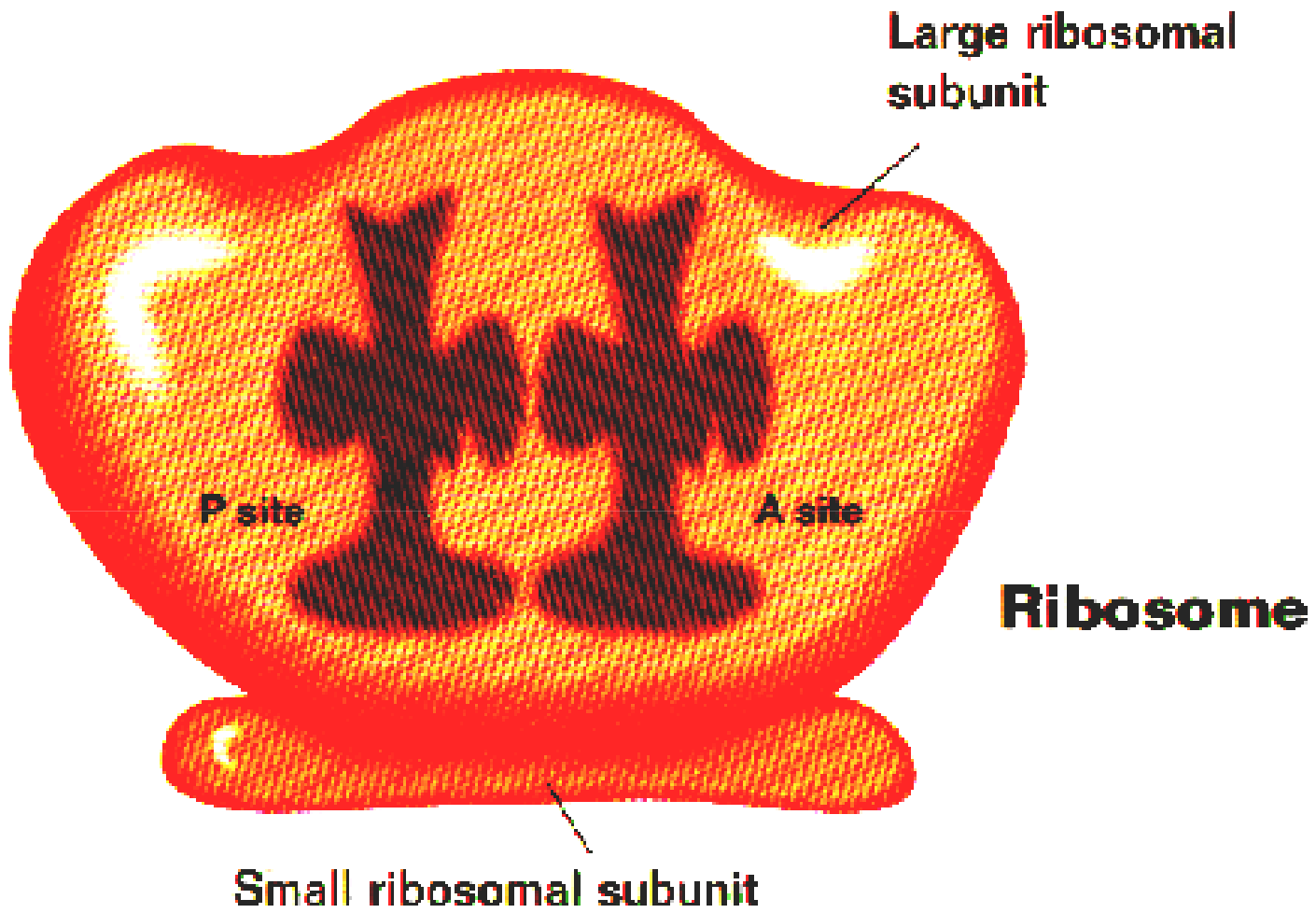
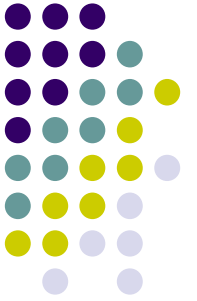
- Ribosomes (50s + 30s)
- r-RNA
- t-RNA
- m-RNA
- Amino acid

## ● 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.

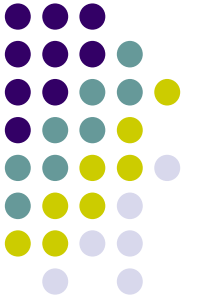


# Ribosome



DIAGRAM

# Translation



## ● Initiation

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

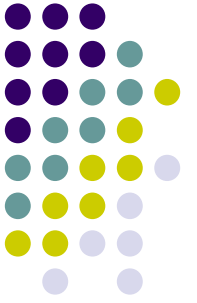
## ● Elongation

- **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

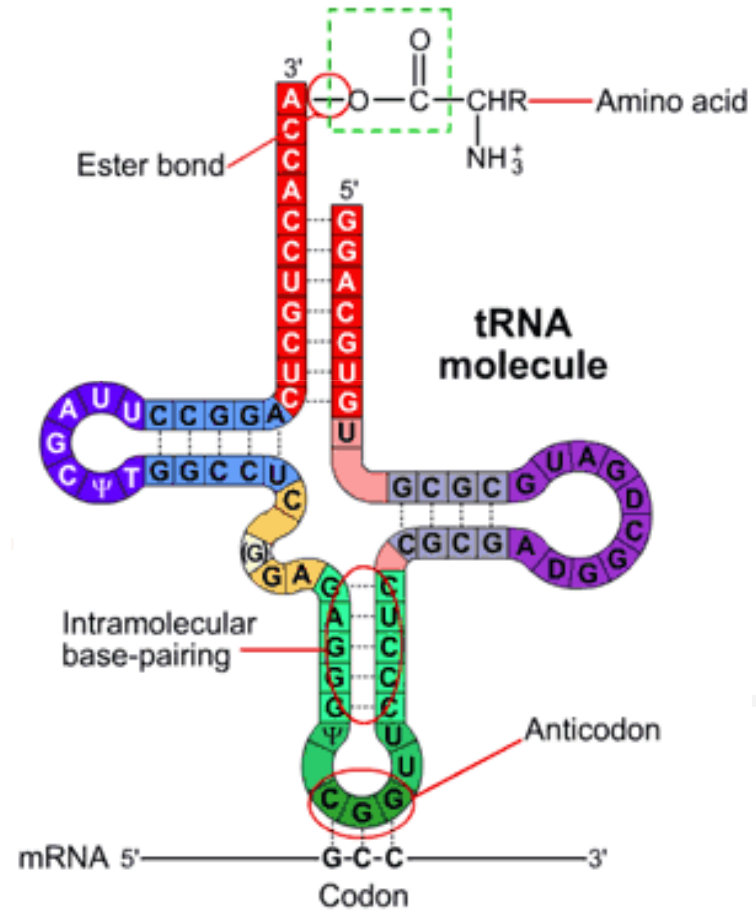
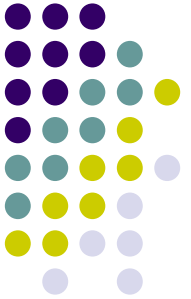
- t-RNA with **stop codon** binds into ribosome
- No amino acid attached to t-RNA
- Complex falls apart

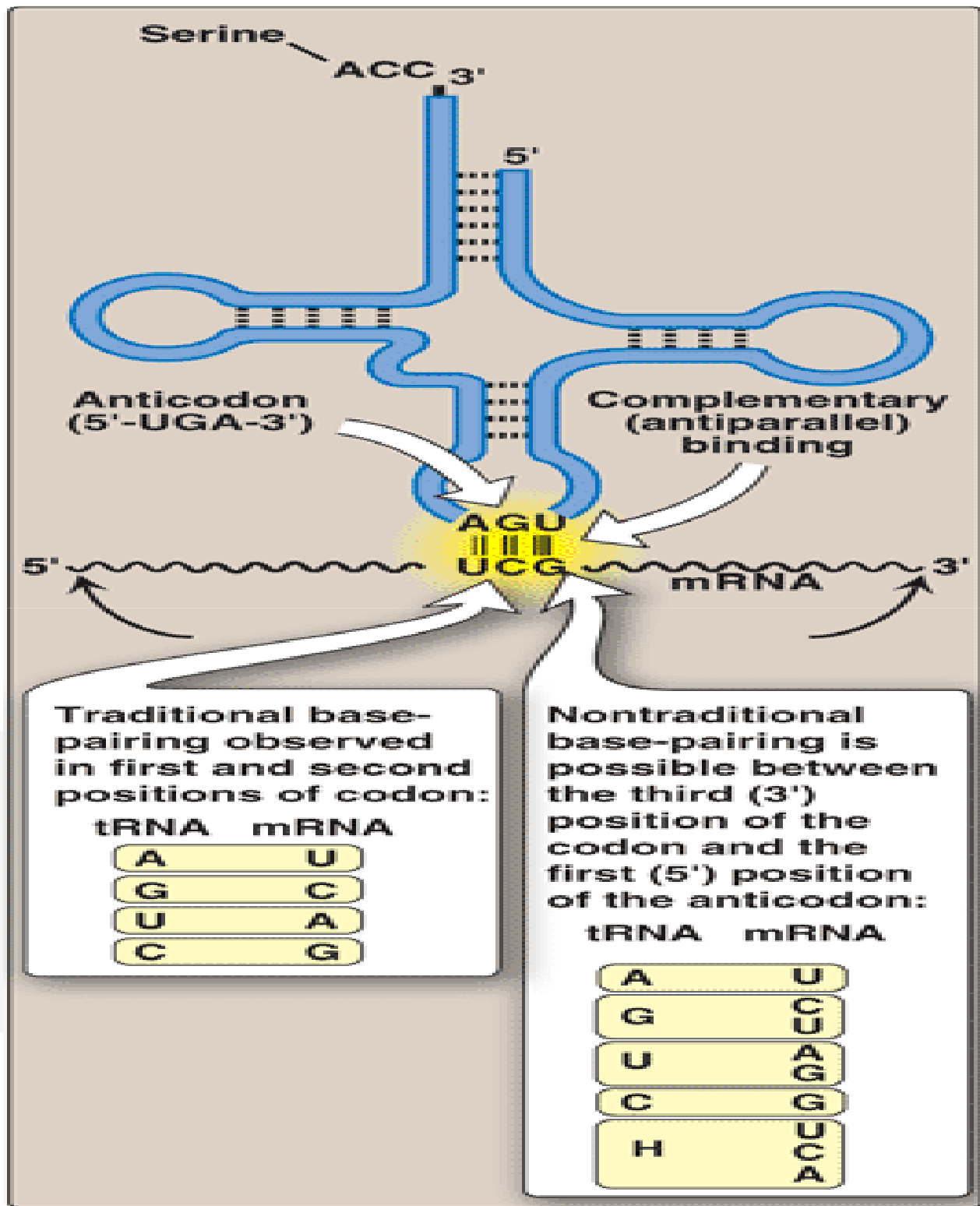
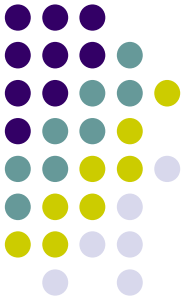
## A – P site on ribosome



- 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

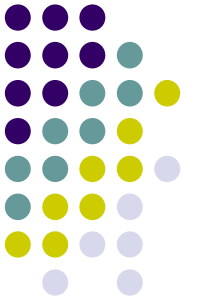




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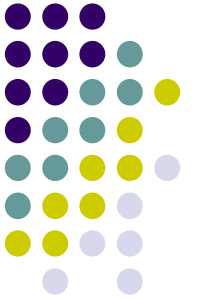
# Aminoacyl-tRNA synthetases



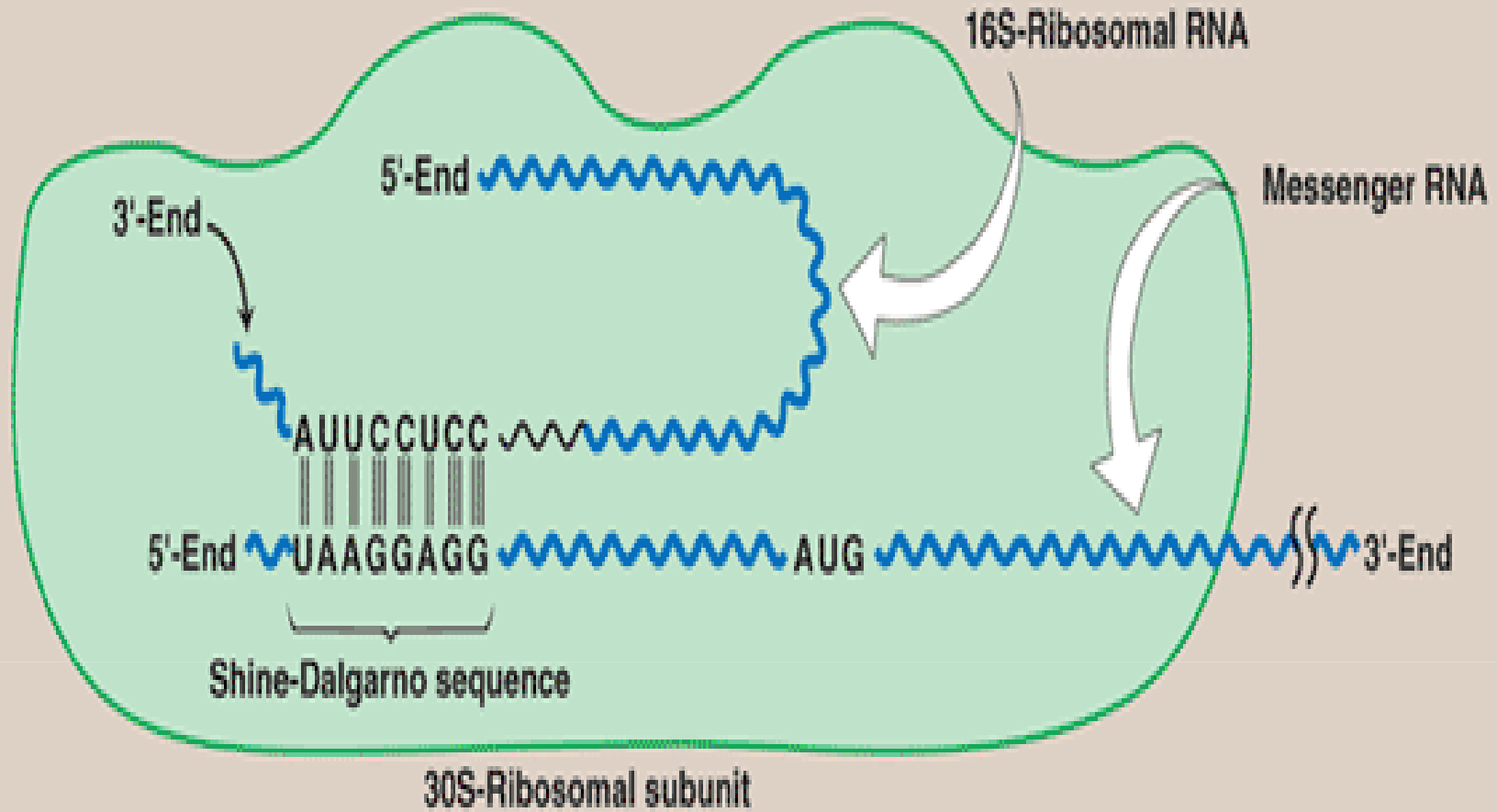
- Required for attachment of amino acids to their corresponding t-RNA.
- Carboxyl group of an amino acid to the 3'-end of t-RNA.
- Require ATP.
- Extreme specificity
- High fidelity of translation of the genetic message.
- Enzyme have “proofreading” or “editing” activity

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# Initiation

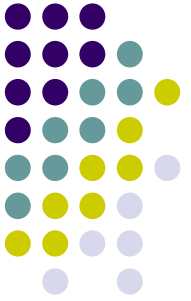


- Involves the assembly of the components
- Components include
  - Two ribosomal subunits.
  - m-RNA to be translated.
  - Aminoacyl t-RNA specified by the first codon
  - GTP
  - Initiation factors
    - In Prokaryotes, Initiation factors (**IF-1, IF-2, IF-3**)
    - In eukaryotes, more than 10 (designated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



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





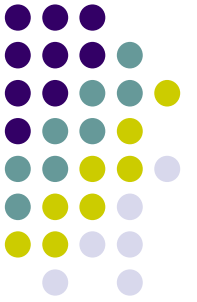
## ● Shine - Dalgarno (SD) sequence

- 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

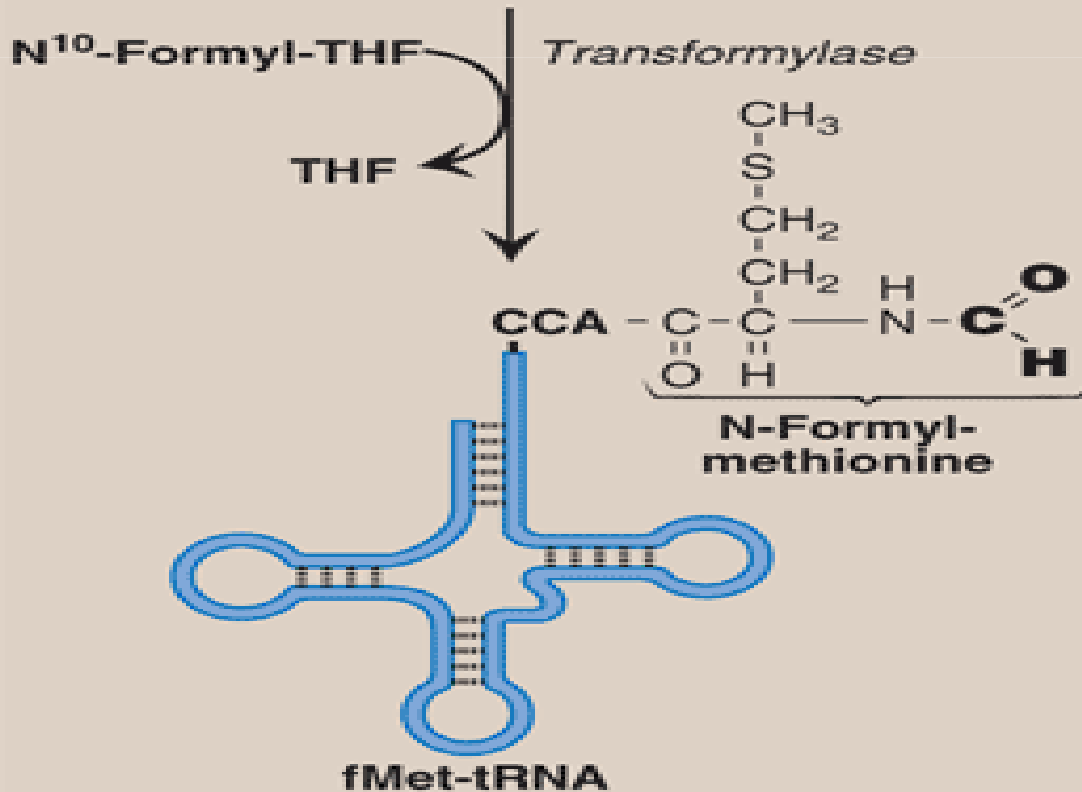
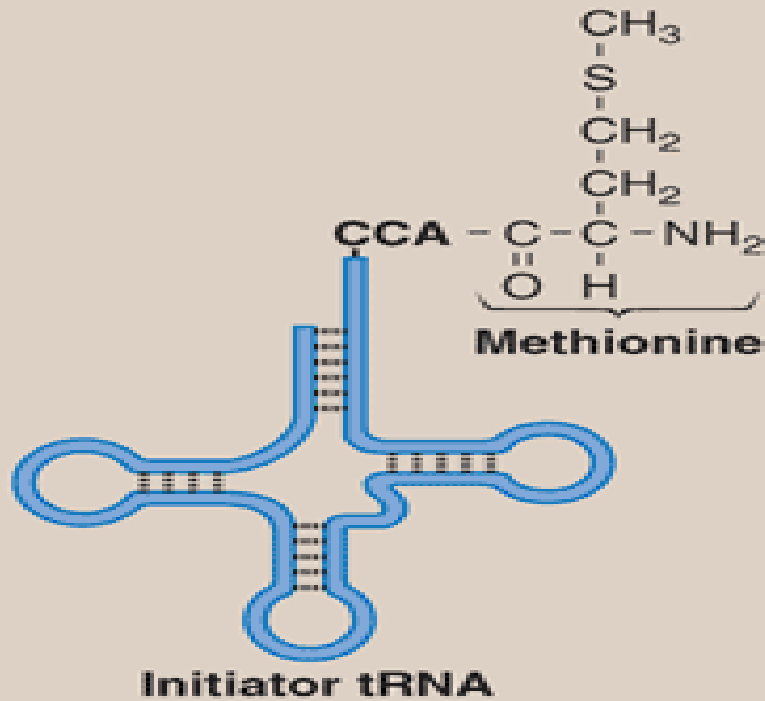
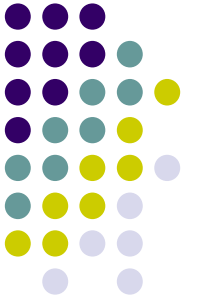
## ● In eukaryotes

- 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



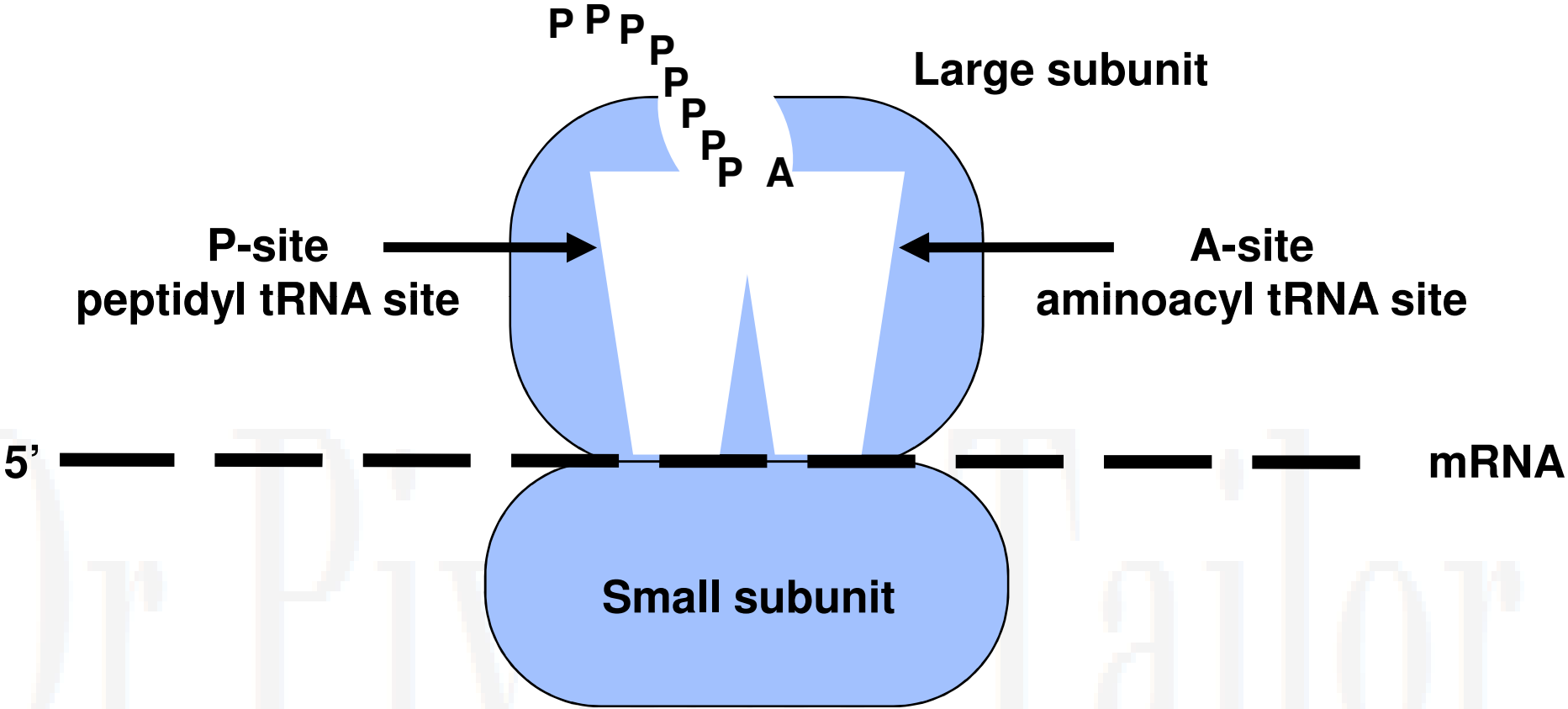
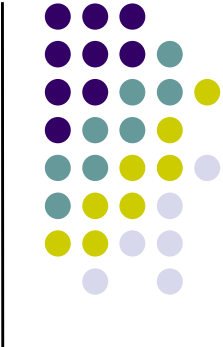
- **AUG** is recognized by initiator t-RNA.
- **Recognition** is facilitated by **IF-2** (bound to **GTP**).
- Initiator **t-RNA** enters the ribosomal **P** site
- **GTP** is hydrolyzed to **GDP**.
- Initiator t-RNA carries **N-formylated methionine**.
- N10-formyl tetrahydro**folate** as the carbon donor.
- In eukaryotes, the initiator tRNA carries a methionine that is not formylated.



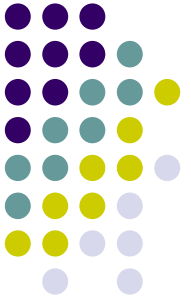
Generation of the  
initiator  
N-formylmethionyl-  
tRNA  
(fMet-tRNA)

Taylor

# Ribosome structure



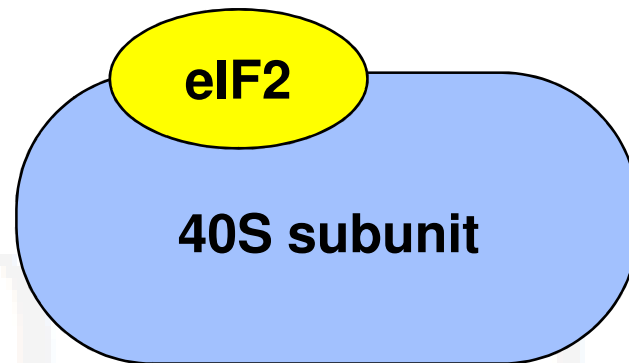
Ribosome with bound tRNAs and mRNA



# Initiation of protein synthesis: mRNA binding

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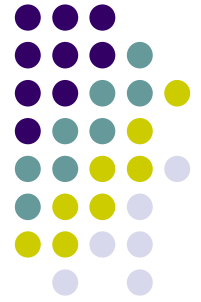
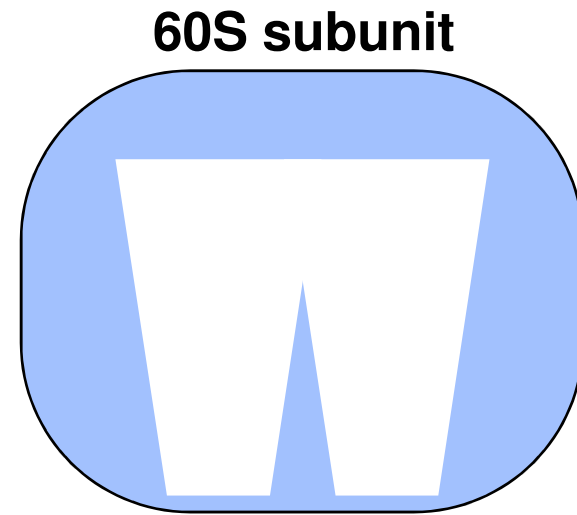
Initiator tRNA bound to the small ribosomal subunit with the eukaryotic initiation factor-2 (eIF2)



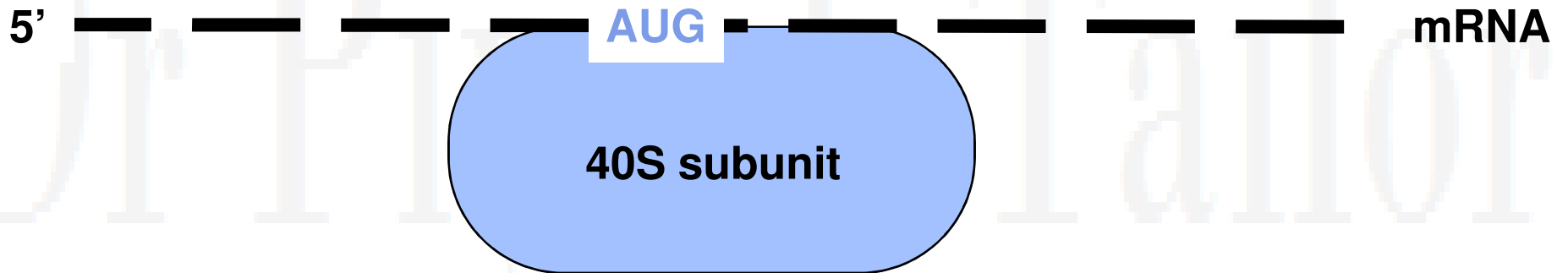
The small subunit finds the 5' cap and scans down the mRNA to the first AUG codon



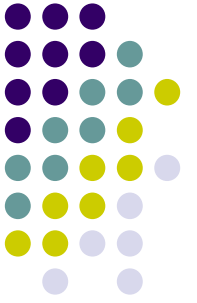
- the initiation codon is recognized
- eIF2 dissociates from the complex
- the large ribosomal subunit binds



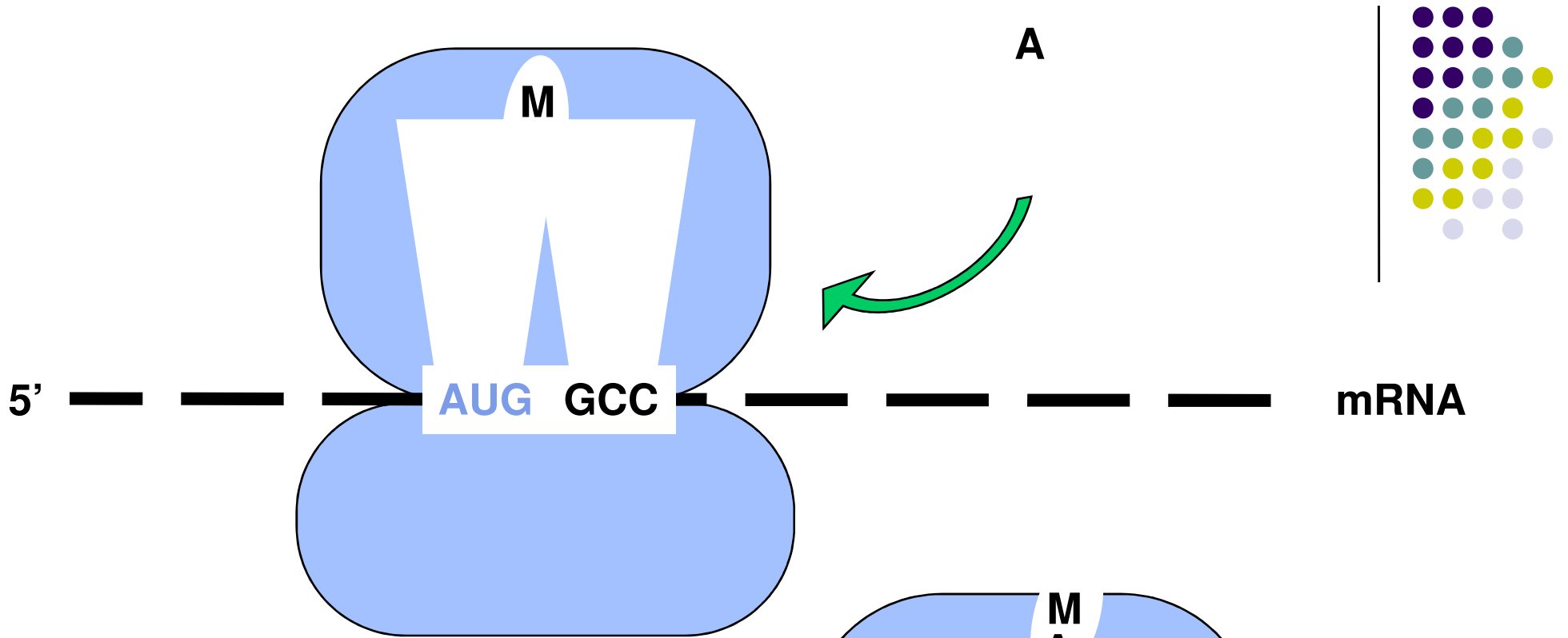
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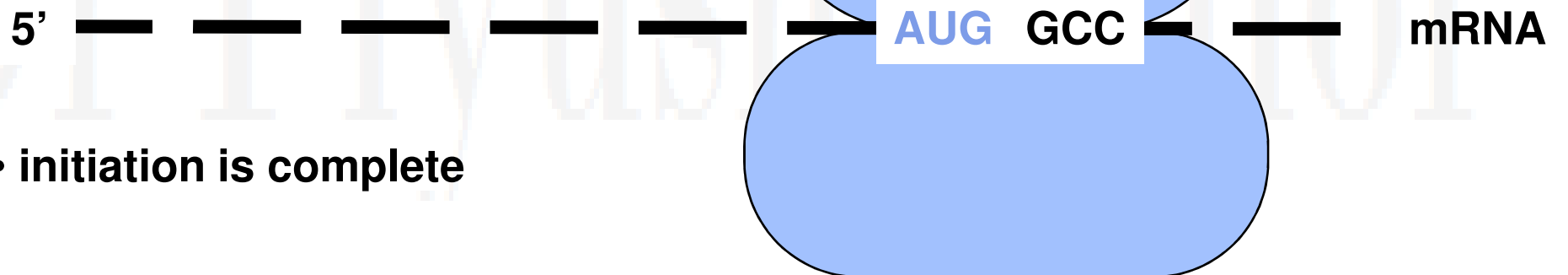
# Elongation



- 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.

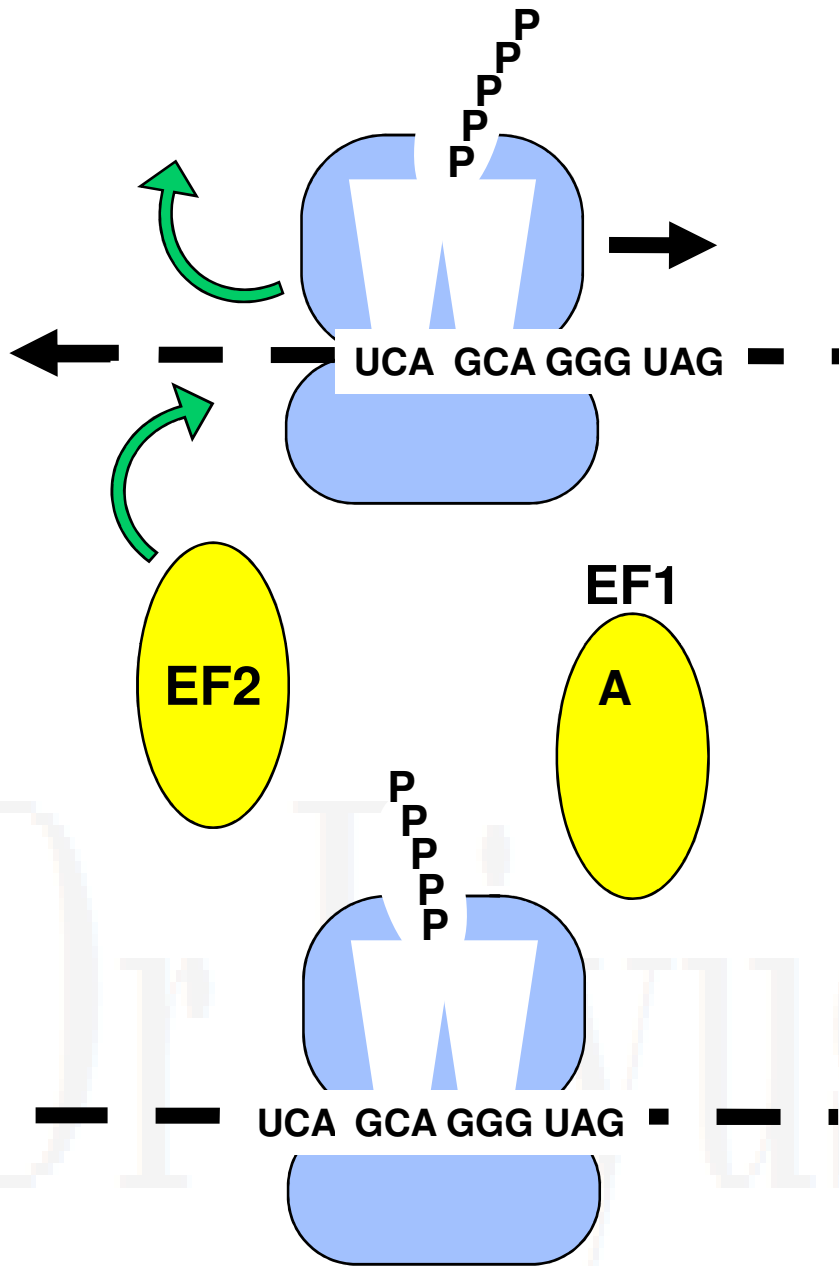


- aminoacyl tRNA binds the A-site
- first peptide bond is formed

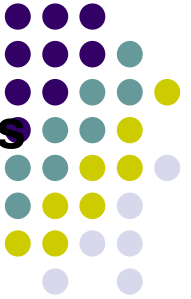


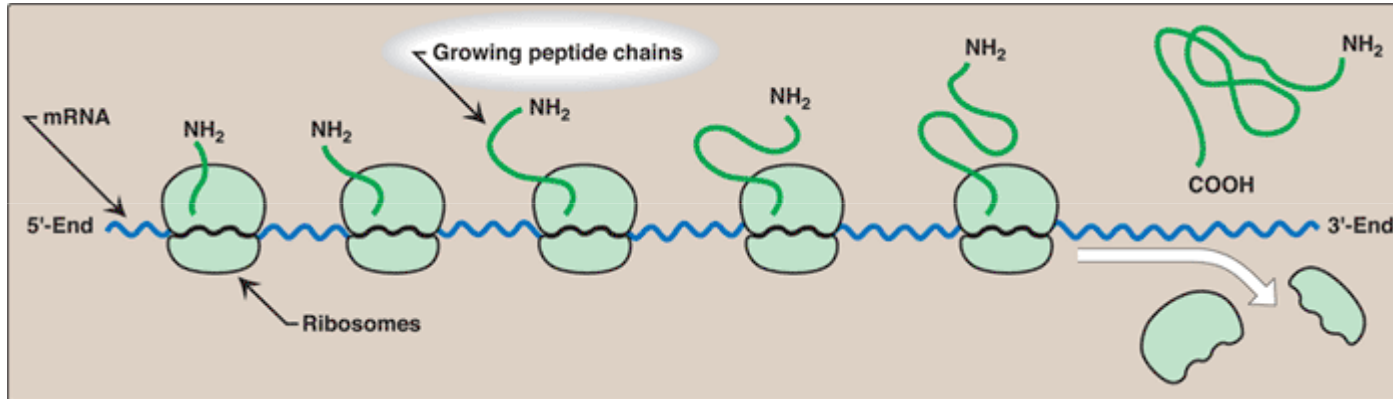
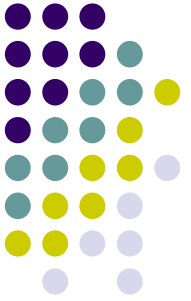


# Elongation



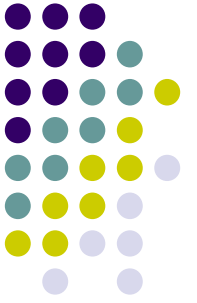
- the uncharged tRNA dissociates from the P-site
- **translocation** = the ribosome shifts one codon along mRNA, moving peptidyl tRNA from the A-site to the P-site. **requires EF2**
- **next aminoacyl tRNA** then binds within the **A-site**; tRNA binding requires EF1
- **energy for elongation is provided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





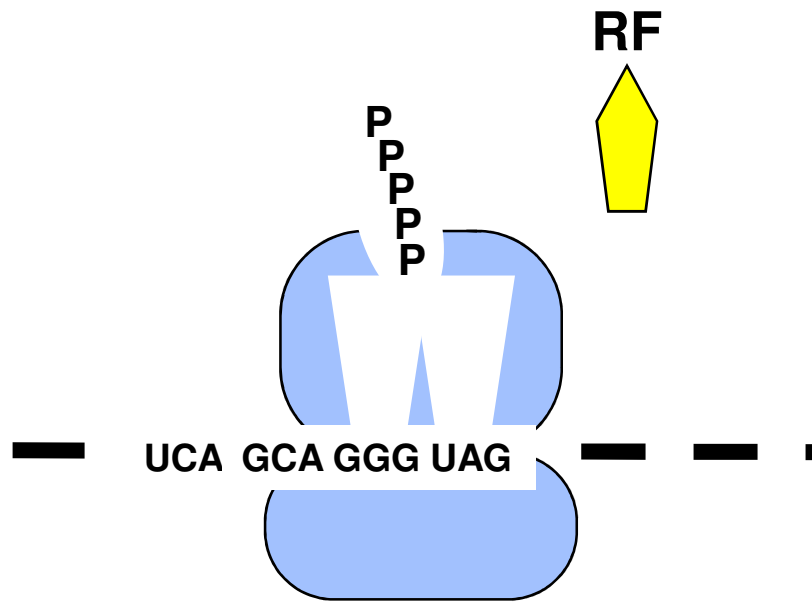
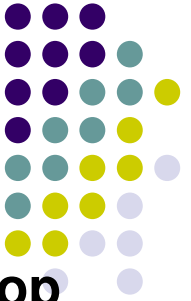
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# Termination

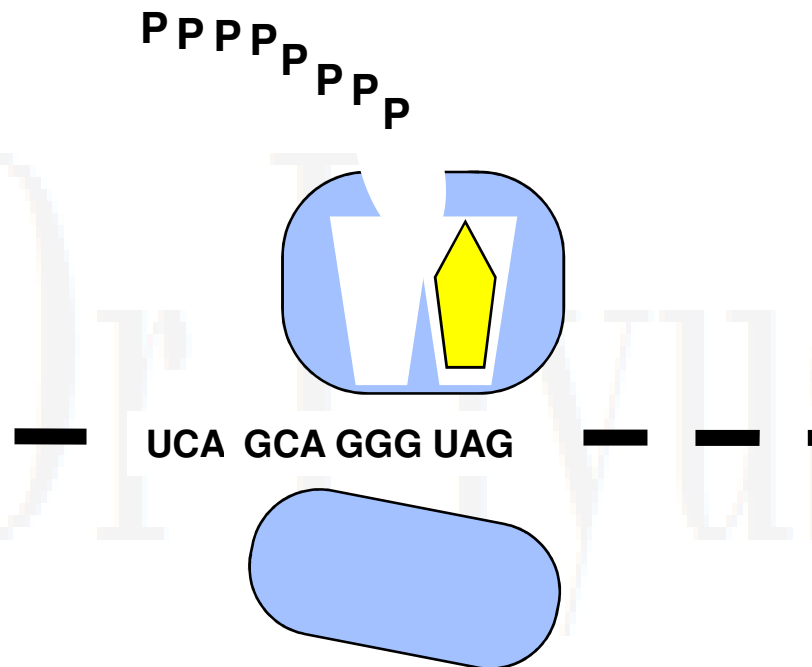


- Termination occurs when one of the three termination codons moves into the A site.
- Termination codons are **recognized** in by
  - **RF-1 = UAA and UAG**
  - **RF-2 = UAA and UGA**
- RF induces peptidyltransferase to **hydrolyze the bond** linking the **peptide to the tRNA** at the A site, causing 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



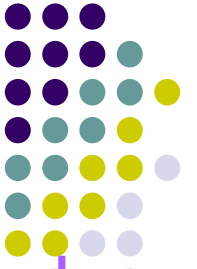
- when translation reaches the stop codon, **RF binds within the A-site**, recognizing the stop codon



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

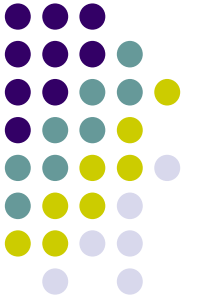
# Post-translation modification

## 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

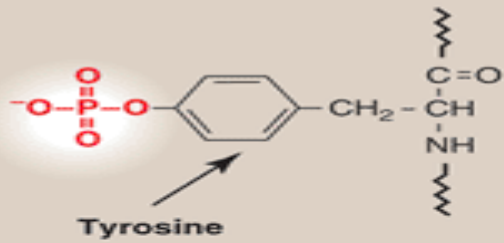
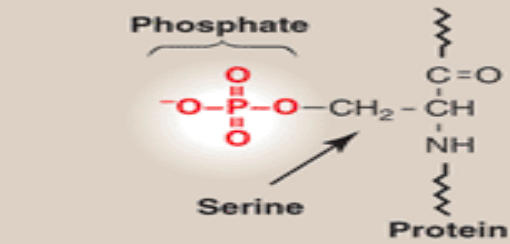
## Covalent Alterations



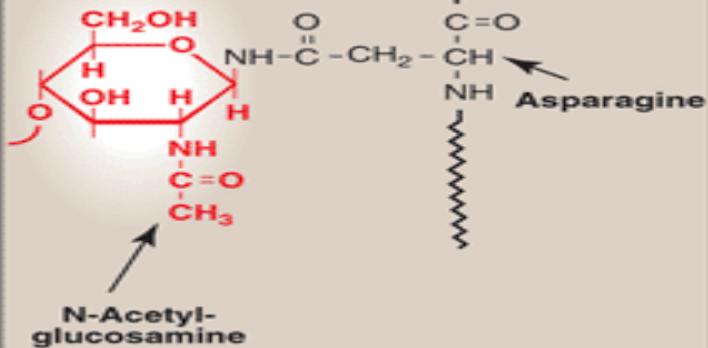
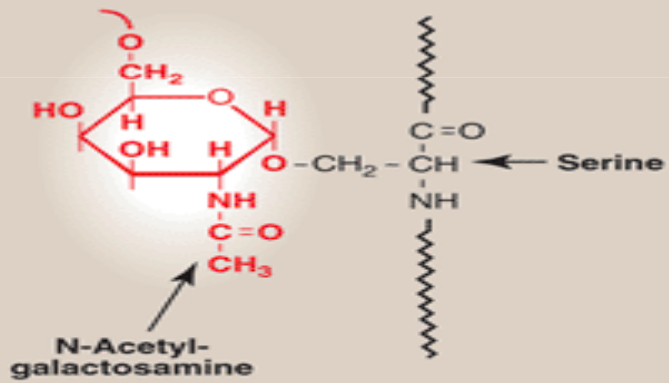
### 1. Other covalent modification :

- **Vitamin K–dependent carboxylation** of Glutamate residues of **clotting factors**.
- **Biotin** =  $\epsilon$ -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

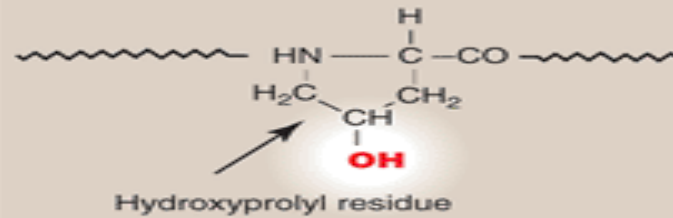
### Phosphorylation



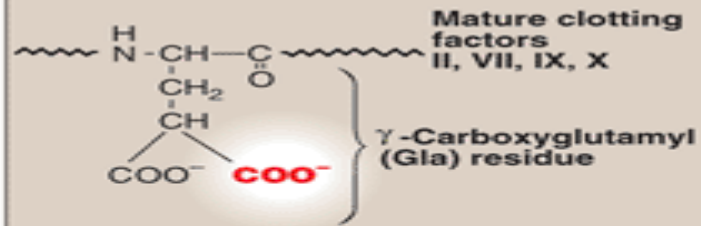
### Glycosylation



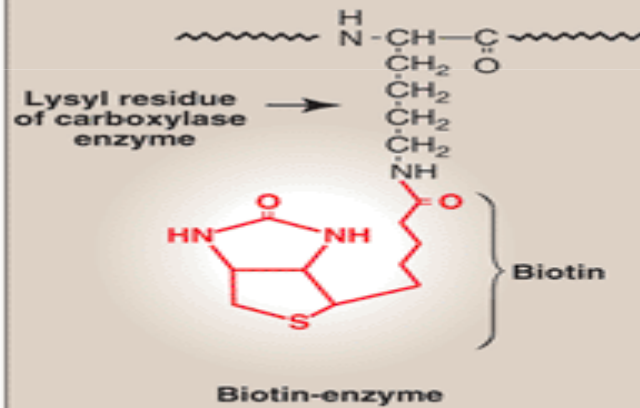
### Hydroxylation



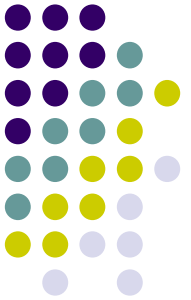
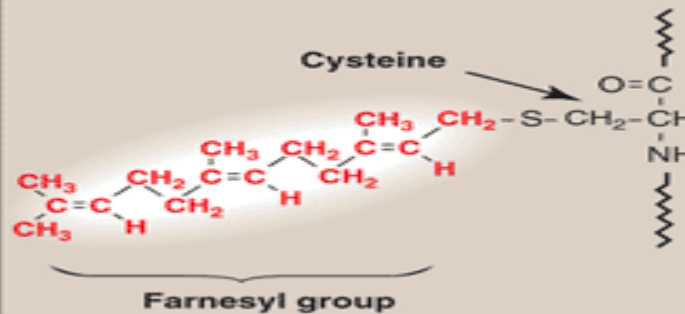
### Carboxylation



### Biotinylated enzyme



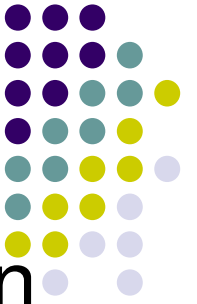
### Farnesylated protein



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# 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**”.

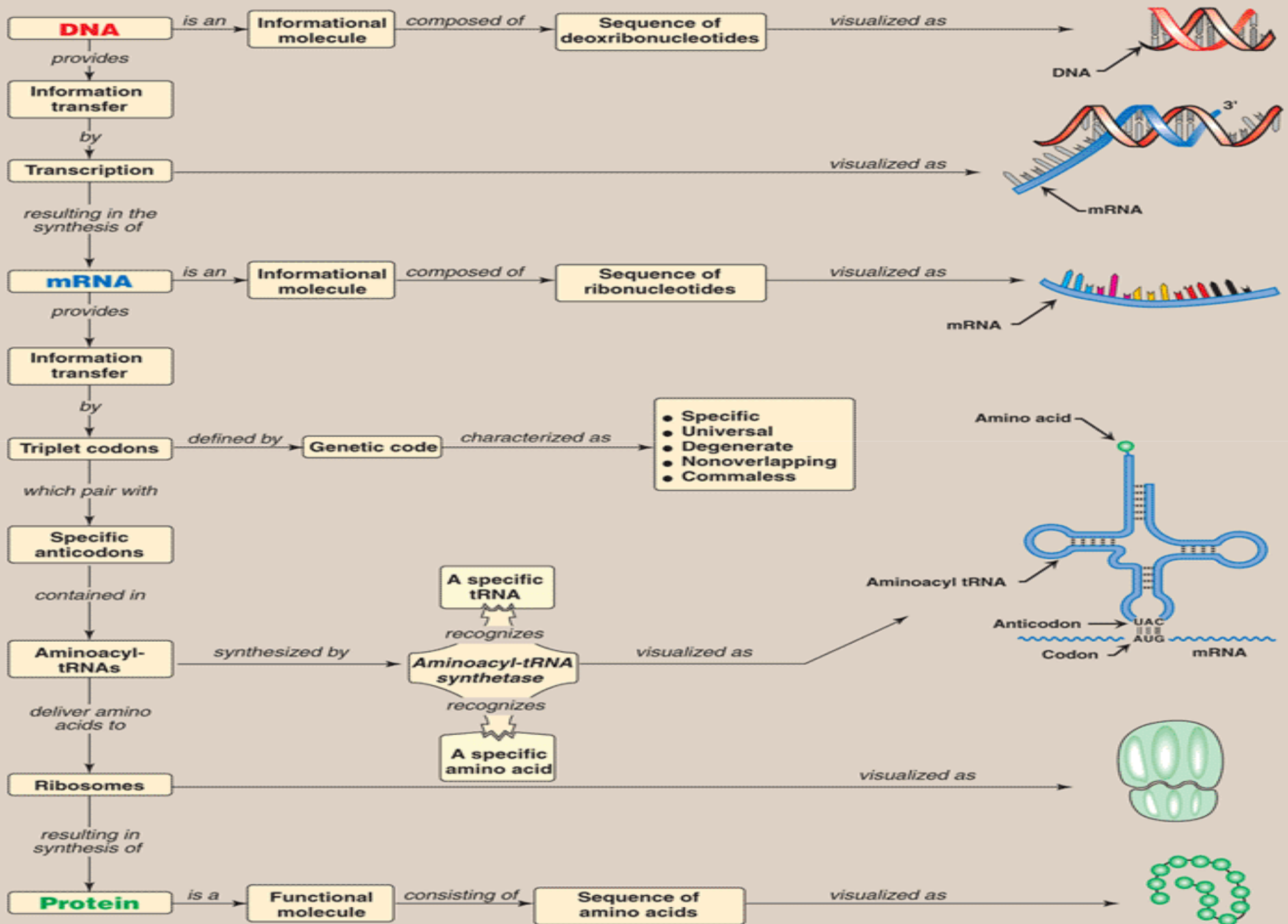
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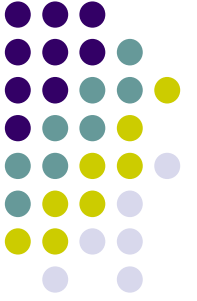
# Inhibitor of Translation



<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>
<b>Puramycin</b>	<b>Amino-acyl t-RNA</b>
<b>Chloramphenicol</b>	<b>Peptidyl Transferase</b>
<i>Diphtheria Toxin</i>	<i>eEF-2</i>

# Flow of genetic information





**Thanks**

Dr Piyush Tailor