Protein synthesis

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Overview

- Genetic information stored in chromosome are finally translated into proteins and are the end products of most information pathway.

- The pathway of protein synthesis is called **translation** because the language of the nucleotide sequence on the mRNA is translated into language of aminoacid sequence.

- The process of translation requires a **genetic code** through which the information contained in nucleic acid sequence are expressed to produce a specific sequence of amino acid.

The Genetic Code

- The genetic code is a dictionary that defines the correspondance between a sequence of nucleotides bases and the sequence of aminoacid.
- Each individual word in the code is composed of 3 nucleotide base, these genetic words are called **codons.**

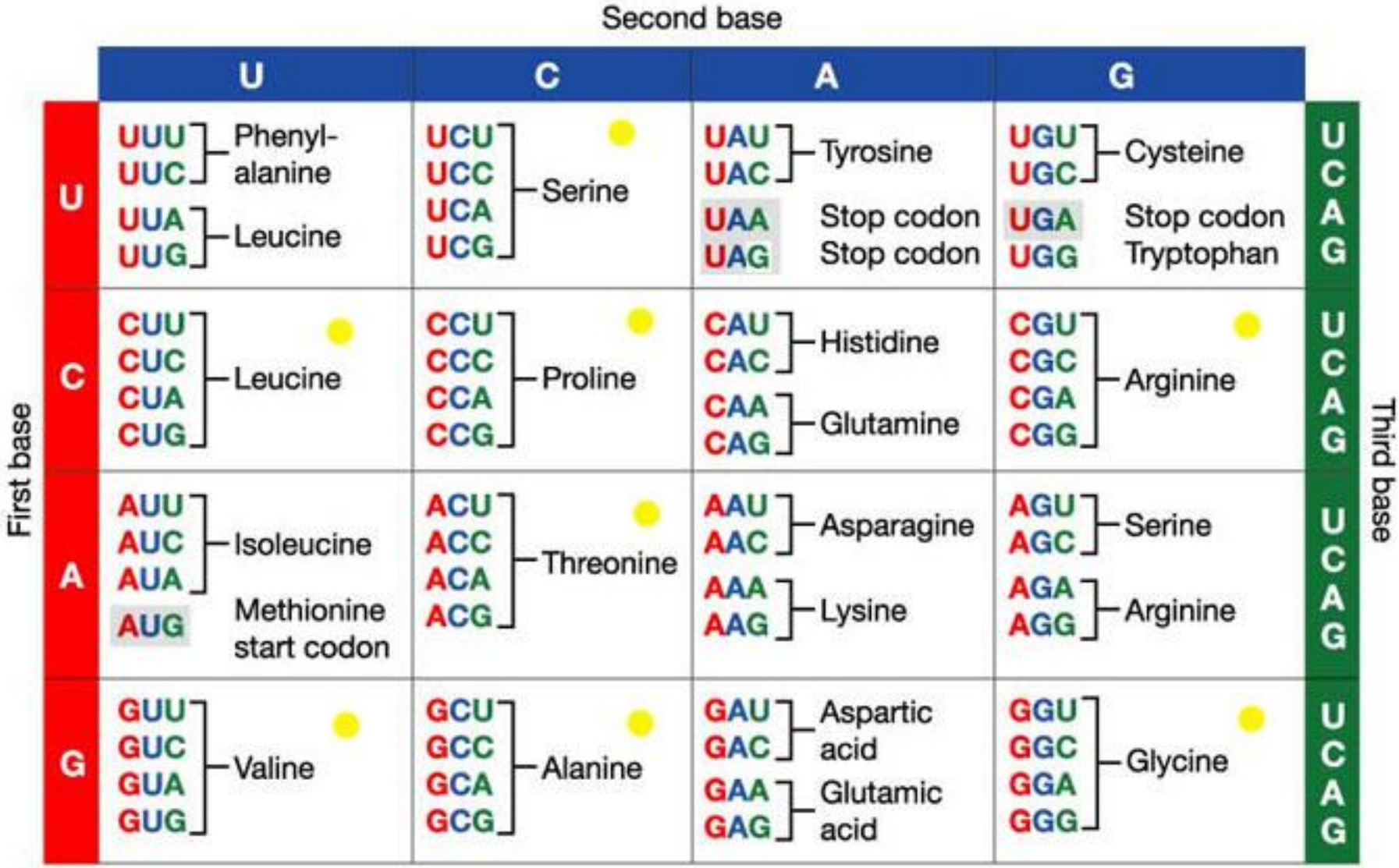
Codons

- Codons are presented in mRNA language of

  adenine (A) guanine (G) cytosine (C) uracil (U)

- Their nucleotide sequence are always in 5' – 3' direction
- 4 nucleotide bases are used to produce 3 base codons
- Therefore there are 64 different combinations of bases taking 3 at a time.

How to translate a codon?



There are 4 important codon which are named as :

a) **start codon**

AUG (methinonie)

b) **stop codon**

UAG

UGA

UAA

stop codon donot code for aminoacid, they terminate the codon.

Characterstics of genetic code

1) **Specificity :** code is specific (unambiguous), ie a particular codon always codes for the same amino acid.

2) **Universality :** they are universal, its specificity has been conserved from early stage of evolution with only slight difference(exception - mitochondria UGA codes for tryptophan)

3) **Degeneracy :** somtimes called **redundant** although each codon corresponds to a single aminoacid, a given aminoacid may have more than one codon , eg: leucine ,serine, arginine – 6 different codons , methionine and tryptophan – 1

4) **Nonoverlapping and commaless**

Consequences of altering the nucleotide sequence

1. **Silent mutation:** The codon containing the changed base may code for the same amino acid. E.g. if the serine codon UCA is given a different third base—U—to become UCU, it still codes for serine.

2. **Missense mutation:** The codon containing the changed base may code for a different amino acid. E.g. if the serine codon UCA is given a different first base—C—to become CCA, it will code for proline.

3. **Nonsense mutation:** The codon containing the changed base may become a termination codon. For example, if the serine codon UCA is given a different second base—A—to become UAA, the new codon causes termination of translation at that point

4. Other mutations: These can alter the amount or structure of the protein produced by translation.

a. ***Trinucleotide repeat expansion:*** sequence of three bases that is repeated in tandem will become amplified in number, so that too many copies of the triplet occur. E.g. amplification of the CAG codon leads to the insertion of many extra glutamine residues in the huntingtin protein, causing Huntington disease

b. ***Splice site mutations:*** Mutations at splice sites can alter the way in which introns are removed from pre-mRNA molecules, producing aberrant proteins.

c. ***Frame-shift mutations:*** If one or two nucleotides are either deleted from or added to the coding region of a message sequence, a frame-shift mutation occurs and the reading frame is altered. This can result in a product with a radically different amino acid sequence, or a truncated product due to the creation of a termination codon.

Components required for Translation

1. Aminoacid
2. Tranfer RNA
3. Aminoacyl tRNA synthetase
4. Messenger RNA
5. Ribosome
6. Protein factors
7. ATP and GTP as energy source

1) Amino acid

All amino acids that appear in finished protein must be present at the time of protein synthesis. If one amino acid is missing like if diet doesnot contain essential amino acid, translation stops.

2) Transfer RNA

Atleast one specific type of tRNA is required for each aminoacid In humans -- 50 species of tRNA

In bacteria -- 30-40 species of tRNA

Since there are only 20 amino acids, some amino acids have more than one specific tRNA molecule.

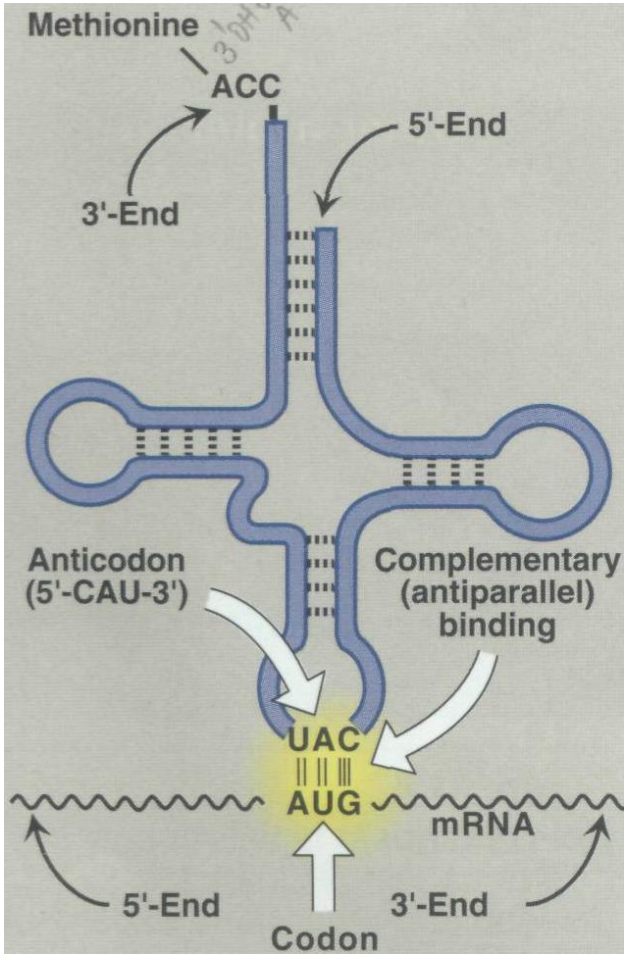
Two important sites in tRNA

a) **Amino acid attachement site** : present on 3’ end of tRNA.

when tRNA has covalently attached to amino acid - **charged tRNA** when it doesnot - **uncharged tRNA**

Amino acid attached to tRNA is said to be activated.

b) **Anticodon site** : each tRNA has 3 base nucleotide sequence k/a **anticodon** which pairs with specific codon on mRNA.



3) Aminoacyl tRNA synthetase

- Required for attachement of carboxyl group of aa to 3' end of its corresponding tRNA.
- Since there is no affinity of nucleic acids for specific aa, this recognition must be carried out by a protein molecule capable of recognizing both a specific tRNA and a specific aa.

- At least 20 specific enzymes are required for these specific recognition functions and for proper attachment.
- The process of **recognition and attachment (charging)** proceeds in two steps by one enzyme for each of the 20aa.
- These enzymes are termed **aminoacyltRNA synthetases.**
- Synthetases have a "proofreading" or "editing" activity that can remove **mischarged aa** from the tRNA molecule.

- Overall reaction requires ATP
- - The extreme specificity of the synthetase in recognizing both the amino acid and its cognate tRNA contributes to the high fedility of translation of genetic message
- - It also has proofreading activity

- synthetases have a "proofreading" or "editing" activity that can remove mischarged amino acids from the tRNA molecule.

4) Messenger RNA

- Specific mRNA that acts as a template is required for synthesis of polypetide chain.

5) Ribosomes

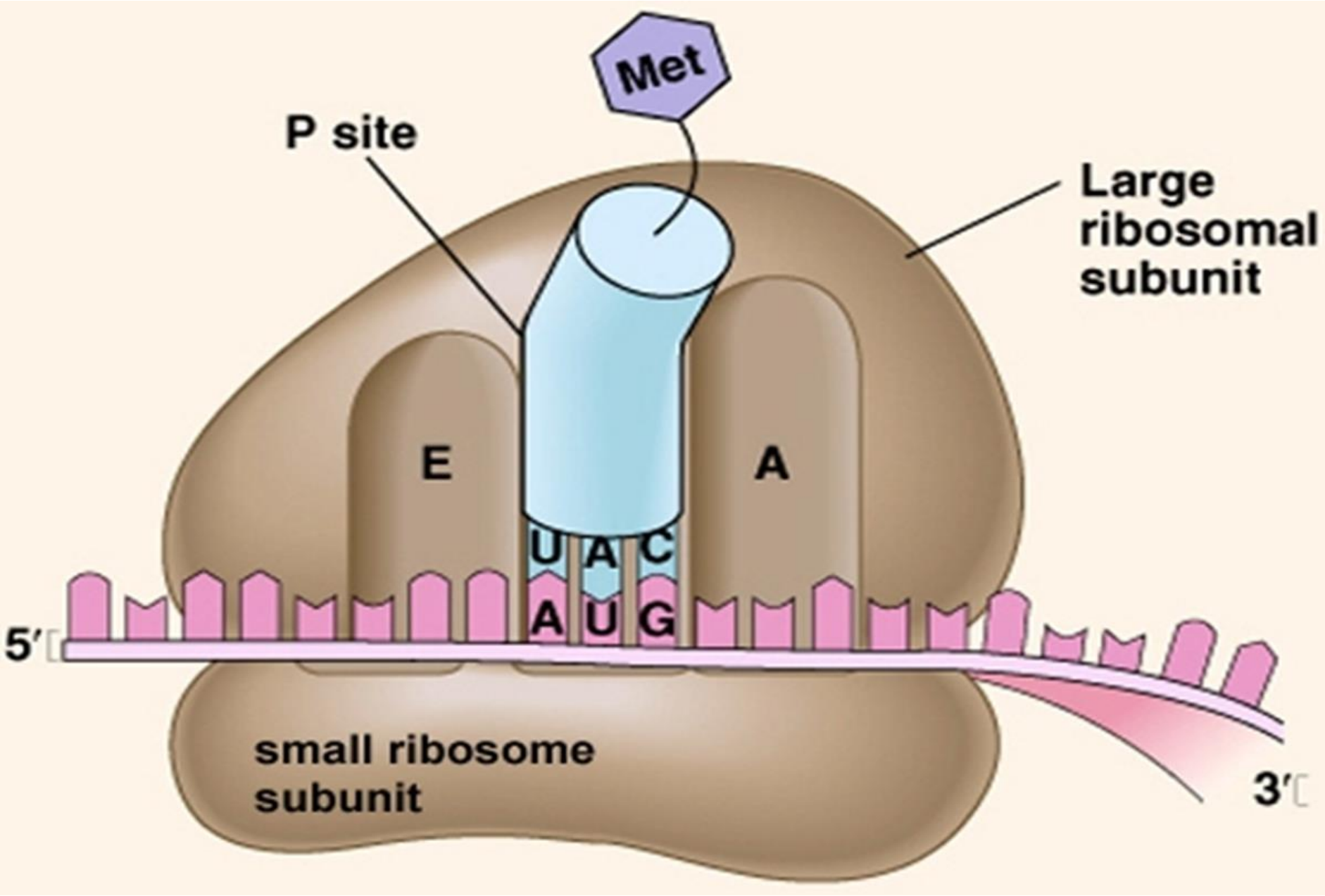
- Ribosomes are large complexes of protein and ribosomal RNA
- Consists of 2 subunits : 1 lagre and 1 small
- **Small ribosomal subunit** : binds mRNA and is responsible for the **accuracy**
- **Large ribosomal subunit** : catalyzes **formation of peptide bonds** that link amino acid residues in a protein.

Different sites on Ribosome (3 binding sites of tRNA)

A site : binds aminoacyl tRNA

P site : occupied by peptidyl tRNA

E site : occupied by empty tRNA as it is about to exit the ribosome


Met
P site
Large ribosomal subunit
E
A
U A C
A U G
5′
3′
small ribosome subunit

6) Protein factors

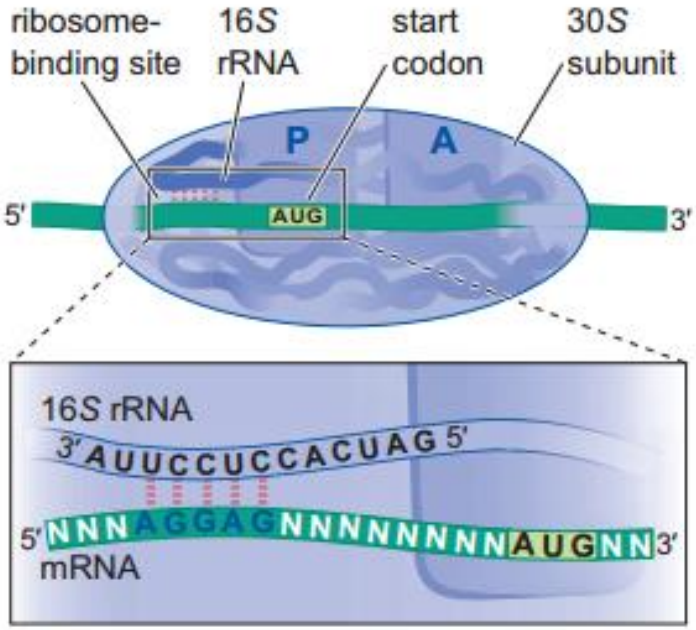
- Initation , elongation and termination factors are required for peptide synthesis

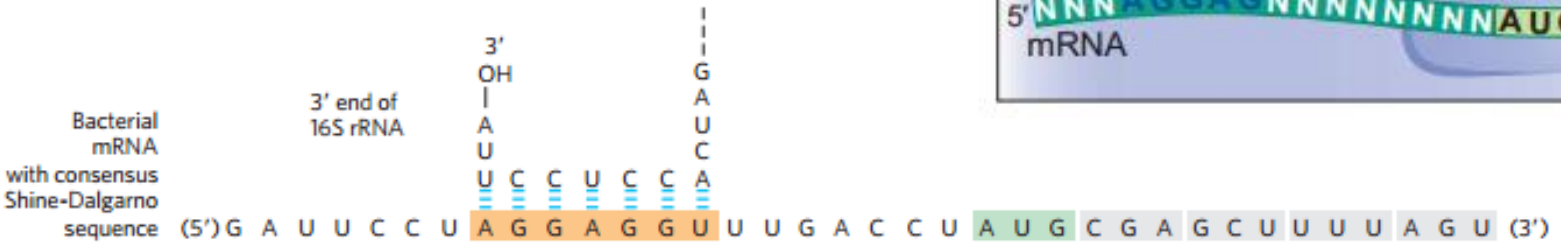
7) ATP and GTP

- Cleavage of four high-energy bonds is required for the addition of one amino acid to the growing polypeptide chain
- two from ATP in the aminoacyl-tRNA synthetase and two from GTP—one for binding the aminoacyl-tRNA to the A site and one for the translocation step
- Additional ATP and GTP molecules are required for initiation in eukaryotes, whereas an additional GTP molecule is required for termination in both eukaryotes and prokaryotes.

8)Ribosomal RNA

- Prokaryotic mRNAs Are Initially Recruited to the Small Subunit by Base Pairing to rRNA





Wobble hypothesis

- There are 64 possible codons. For translation, each of these codons requires a tRNA molecule with a complementary anticodon.

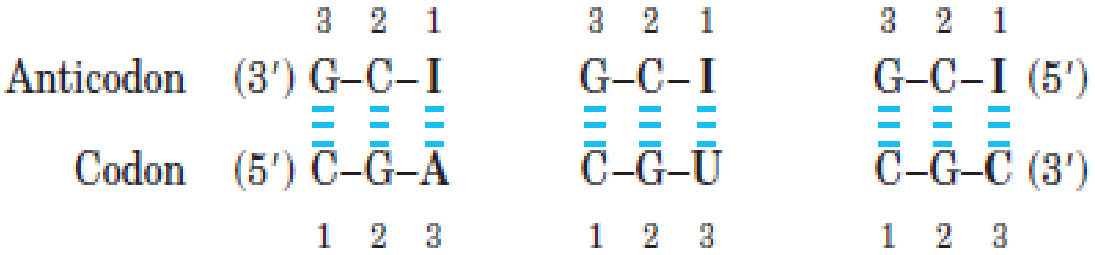
- If each tRNA molecule paired with its complementary mRNA codon using Watson-Crick base pairing, then 64 types of tRNA molecule would be required.

- Since most organisms have fewer than 45 species of tRNA, some tRNA species must pair with more than one codon.

Wobble hypothesis

- The anticodons in some tRNAs include the nucleotide inosinate (designated I), which contains the uncommon base hypoxanthine.

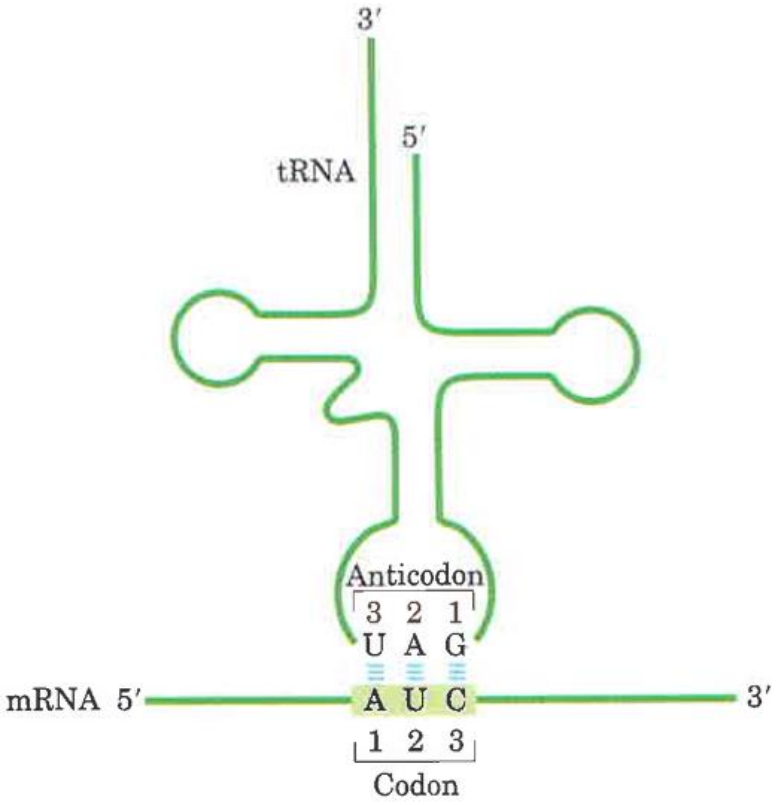
- Inosinate can form hydrogen bonds with three different nucleotides (U, C, and A).



- So in 1966, Francis Crick proposed the Wobble hypothesis to account for this

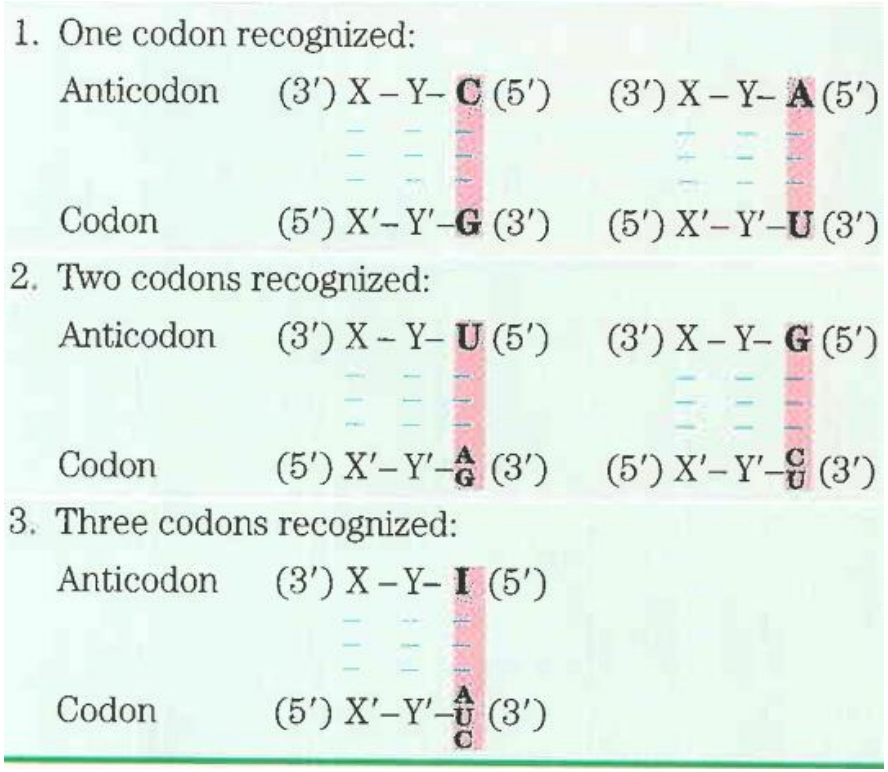
Wobble hypothesis

1. The first two bases of an mRNA codon always form strong Watson-Crick base pairs with the corresponding bases of the tRNA anticodon and confer most of the coding specificity.



2. The first base of the anticodon determines the number of codons recognized by the tRNA.

- When the first base of the anticodon is C or A, base pairing is specific and only one codon is recognized by that tRNA.
- When the first base is U or G, binding is less specific and two different codons may be read.
- When inosine (I) is the first (wobble) nucleotide of an anticodon, three different codons(U,C&A) can be recognized the maximum number for any tRNA.



- 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.

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′–**C** / **U** (3′)

3. Three codons recognized:

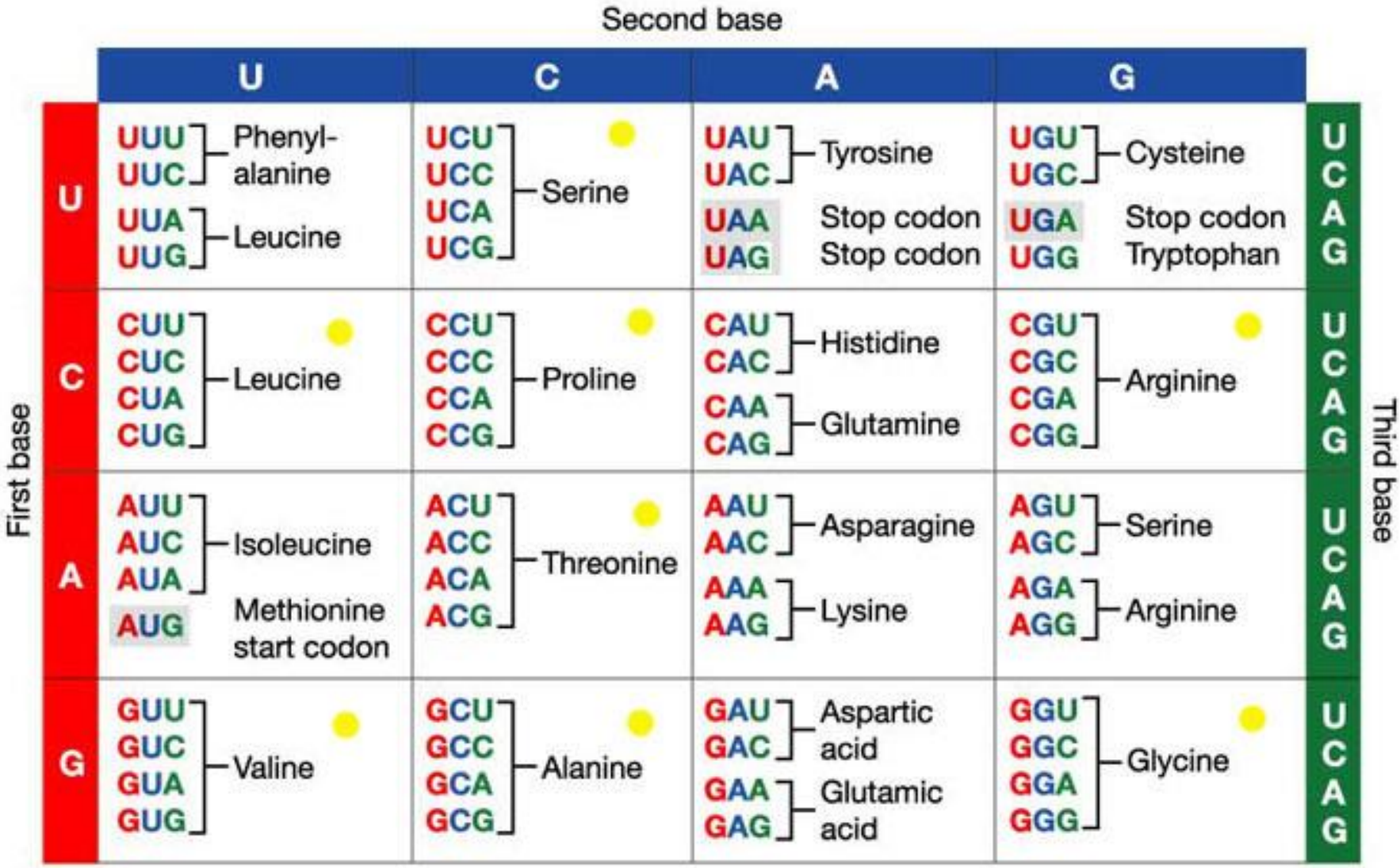
Anticodon	(3′) X – Y– **I** (5′)
Codon	(5′) X′– Y′–**A** / **U** / **C** (3′)

3. When an amino acid is specified by several different codons, the codons that differ in either of the first two bases require different tRNAs.

4. A minimum of 32 tRNAs are required to translate all 61 codons (31 to encode the amino acids and 1 for initiation).

- Since the third base of the codon pairs loosly with anticodon, it permits rapid dissociation of tRNA from its codon during protein synthesis
- If all three bases of a codon engaged in strong Watson-Crick pairing with the anticodon, tRNAs would dissociate too slowly and this would severely limit the rate of protein synthesis
- Codon-anticodon interactions balance the requirements for accuracy and speed

How to translate a codon?



Steps in Protein Synthesis

- The process of protein synthesis translates the 3 letter alphabet of nucleotide sequence on mRNA into 20 letter alphabet of amino acid.

- Important difference between prokaryotes and eukaryotes is in prokaryotes - translation and transcription are coupled ie translation starting before transcription is completed , this is due to lack of nuclear membrane in prokaryotes.

- Actual steps in protein synthesis

1. **Initiation**
2. **Elongation**
3. **Termination**

Initiation

- 1st assembly of component of translation occurs which include - 2 ribosomal subunits , mRNA to be translated , aminoacyl tRNA , GTP , initation factors

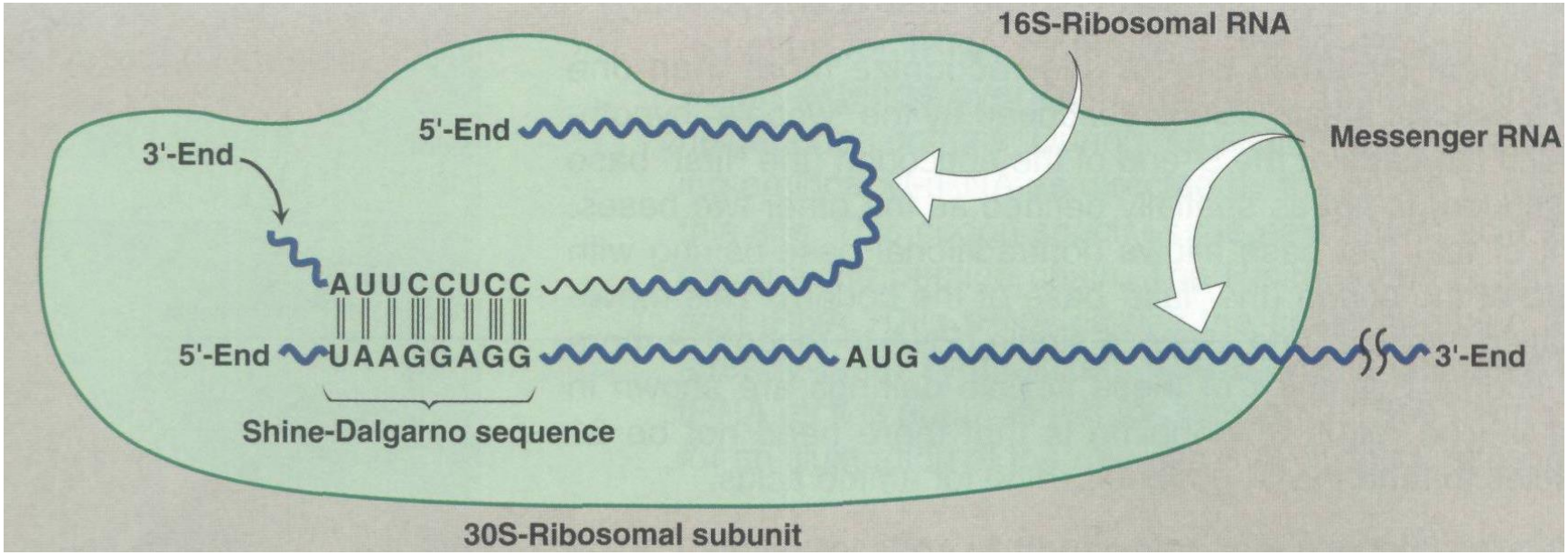
- Initiation factors in prokaryotes **IF 1 , IF 2 , IF 3**

- Initiation factors in eukaryotes – more than 10 – **eIF**

- There are 2 mechanism by which ribosome recognizes AUG that initiates translation.

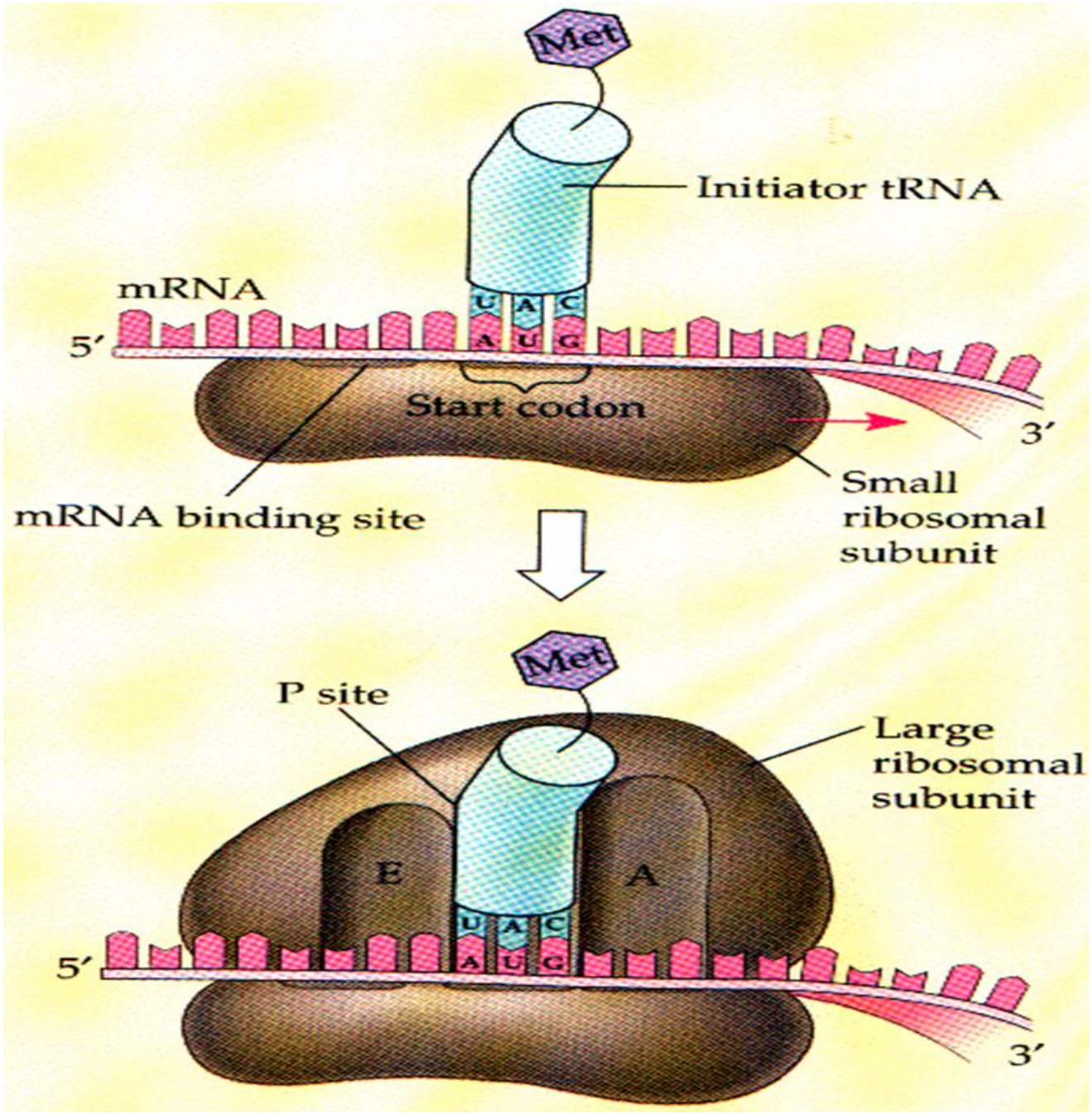
1) First mechanism

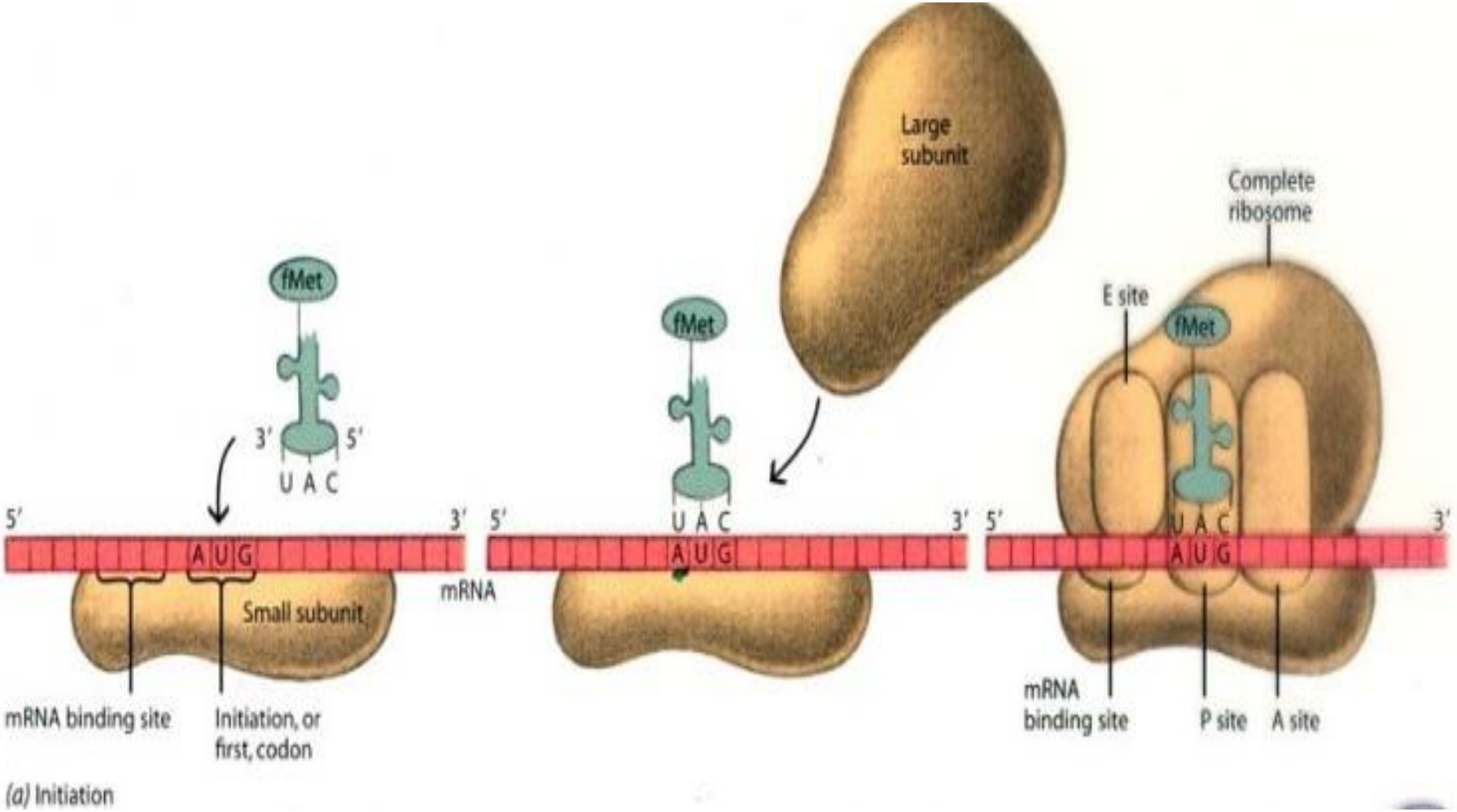
a) **Prokaryotes** ： **Shine Dalgarno sequence** located 6-10 bases upstream of initiating AUG codon base pair with 16S RNA thus facilitating the positioning of small (30S) ribosomal subunit in mRNA in close proximity to AUG.



2) **Second mechanism (Initiation codon)**

- Initating AUG is recognized by a special **initiator tRNA**
- Recognization facilitated by IF2GTP in prokaryotes and eIF-2GTP + other eIF in eukaryotes
- The charged initiator tRNA directly enters P site
- In bacteria and mitochondria initiator tRNA carries N –formylated methionine
-In eukaryotes initiator tRNA carries methionine that is not formylated
- The large ribosomal subunit then joins the complex and a functional ribosome is formed with charged initiating tRNA in P-site


Met
Initiator tRNA
mRNA
5′
U A C
A U G
Start codon
3′
mRNA binding site
Small ribosomal subunit
Met
P site
Large ribosomal subunit
E
A
U A C
A U G
5′
3′



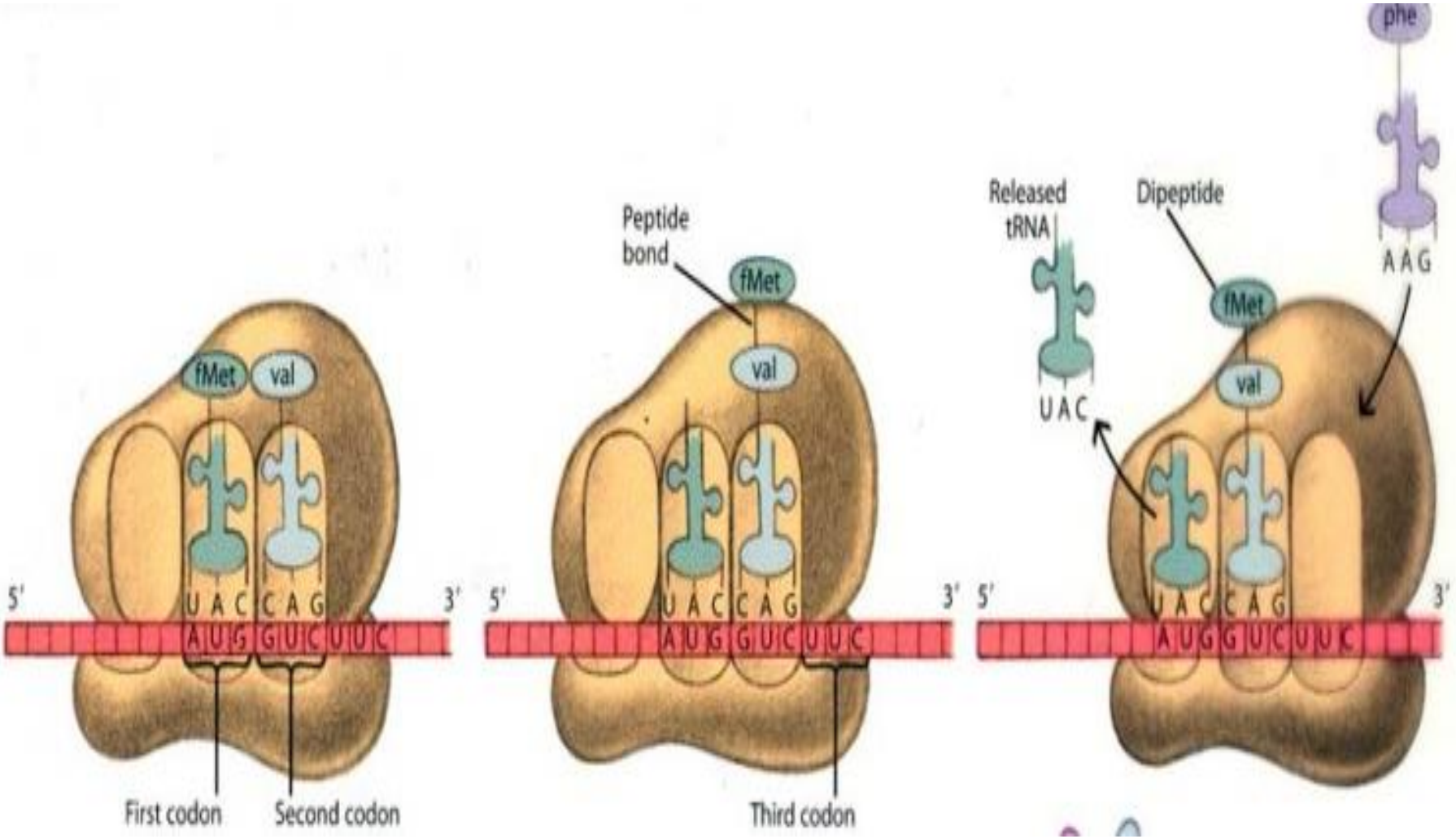
(a) Initiation

Elongation

- It involves addition of amino acid to the carboxyl end of the growing chain
- For this ribosome moves from 5’ – 3’ end
- Then aminoacyl tRNA whose codon apperas next on mRNA template carrying the second aminoacid moves to site A
- This is facilitated by

  in prokaryotes : EF-Tu GTP and EF-Ts

  in eukaryotes : EF-1α GTP and EF-1βγ
- Formyl methionine carried by tRNA in P site is then joined to the amino acid carried by tRNA that has just entered the A site, by a peptide bond catalyzed by peptidyl transferase (ribozyme)


fMet
val
5'
U A C C A G
A U G G U C U U C
3'
First codon
Second codon
Peptide bond
fMet
val
5'
U A C C A G
A U G G U C U U C
3'
Third codon
Released tRNA
Dipeptide
phe
A A G
fMet
val
U A C
5'
U A C C A G
A U G G U C U U C
3'

Translocation

- Ribosome then advances 3 nucleotides towards the 3’ end of mRNA known as translocation
- For translocation:

prokaryotes – requires EF-G-GTP

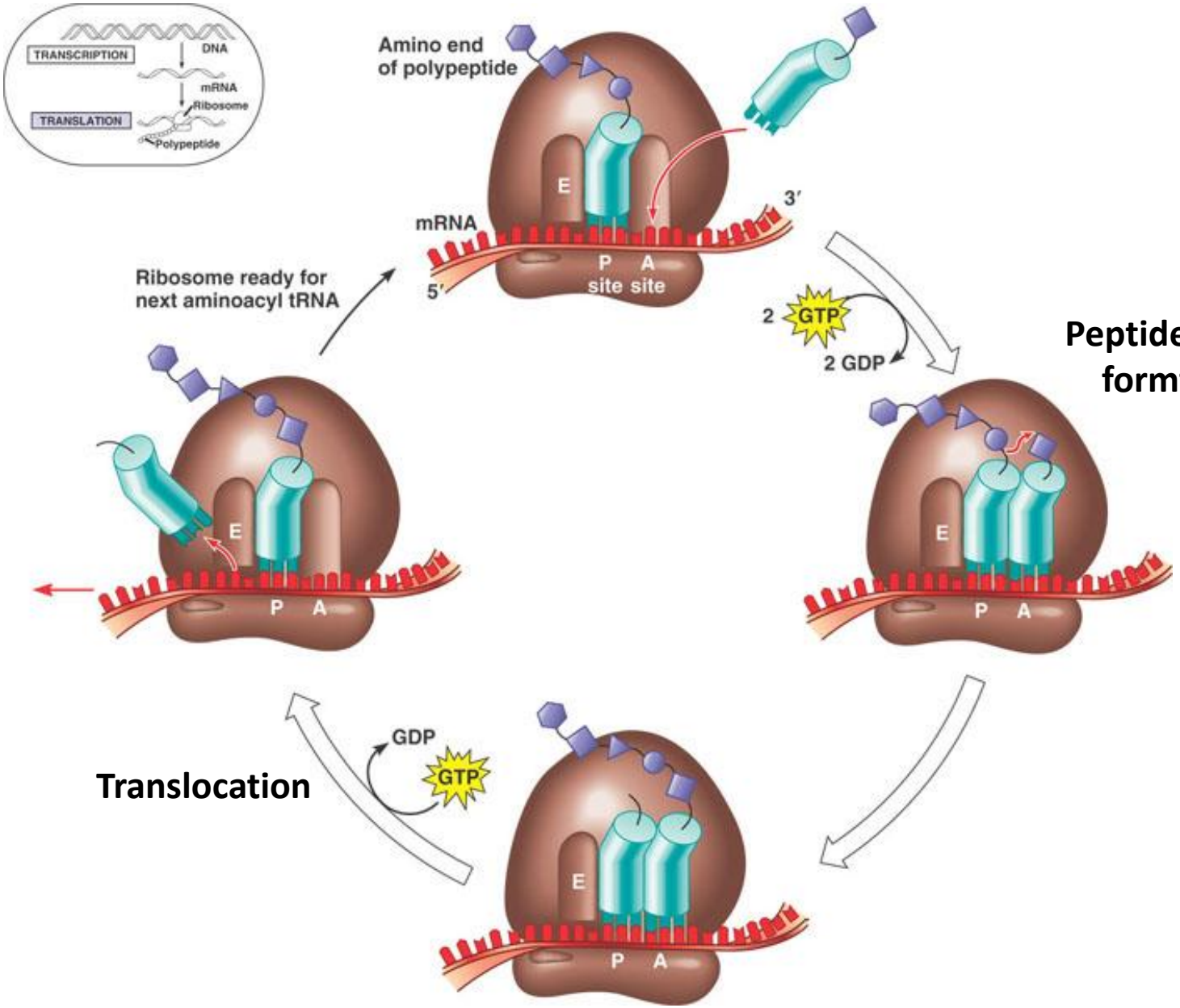
eukaryotes – requires EF-2-GTP

- Translocation causes

1) movement of uncharged tRNA from P to E site

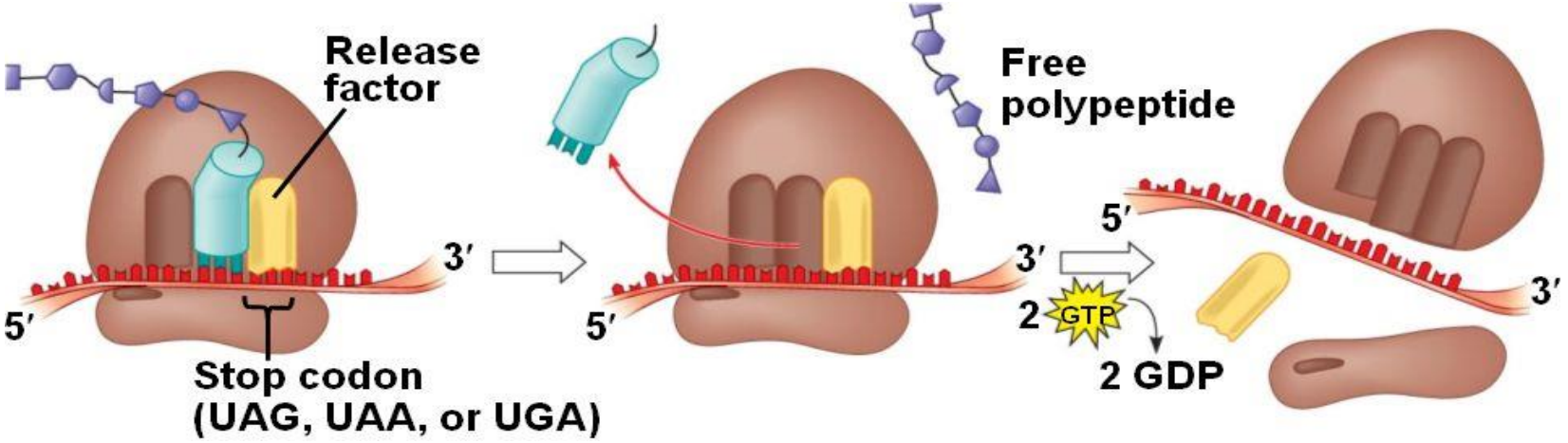
2) movement of peptidyl tRNA from A to P site

- This process repeats until termination codon is encountered


TRANSCRIPTION
DNA
mRNA
Ribosome
TRANSLATION
Polypeptide
Amino end
of polypeptide
E
3′
mRNA
5′
P A
site site
Ribosome ready for
next aminoacyl tRNA
2
GTP
2 GDP
Peptide bond
formtion
E
P A
E
P A
GDP
GTP
Translocation
E
P A

Termination

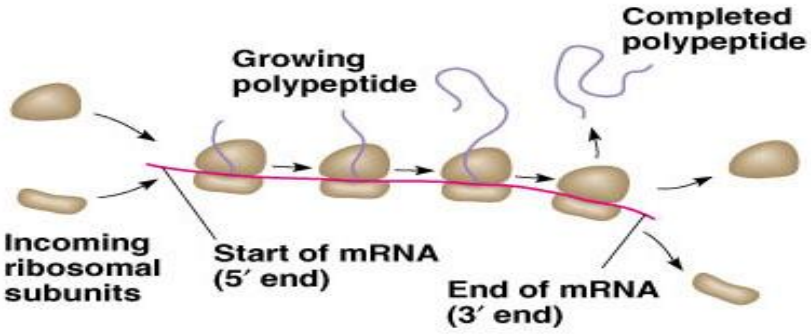
- It occurs when one of the **three termination codons** moves into A site
- These codons are recognized by **release factor (RF)**
- which causes hydrolysis of the bond between peptide and tRNA at P site releasing the nascent protein from ribosome
- In prokaryotes - RF1 recognizes UAA and UAG
  - RF2 recognizes UGA and UAA
  - RF-3-GTP releases RF1 and RF2



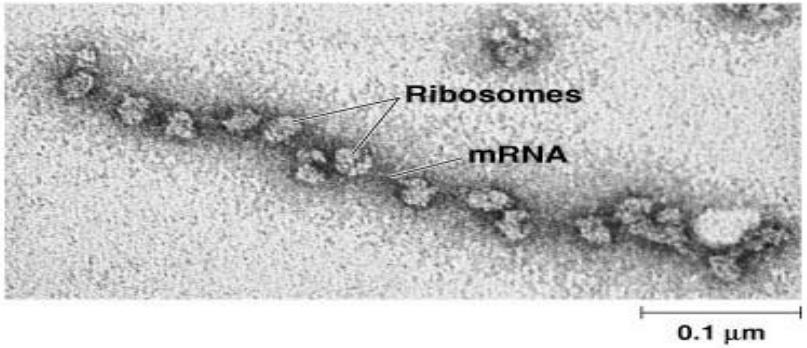
- Once the nascent protein is released other components like ribosomal subunits , mRNA, tRNA and protein factor are also released and recycled for synthesis of another polypeptide chain

Role of polyribosome

- Multiple ribosome on the same mRNA forms polyribosome or polysome.
- Because of their large size, ribosome particles can not attach to an mRNA any closer than 80 nucleotides apart.
- Single mammalian ribosome is capable of synthesizing about 400 peptide bonds per minutes.
- Thus a polyribosome helps in formation of multiple copies of protein that are synthesizes.



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

Co and posttranslational modification

- Many proteins synthesized from mRNA template as a precursor molecule must be modified to achive the active protein.

- These polypeptide chains covalently modified, either **while they are still attached to ribosome (cotranslational) or after their synthesis has been completed (posttranslational)**

- These modification may include removal of part of translated seg or covalent addition of 1 or more chemical group.

1. Amino and carboxy terminal modification

- The amino terminal Met residue, additional aa terminal, and some carboxyterminal are removed enzymatically in formation of functional proteins.

2. Loss of signal sequence

- 15-30 residues at the aminoterminal end of some proteins play a role in directing the protein to its ultimate destination in cell. Such signal segments are eventually removed by specific peptidase.

3. Modification of individual aa

a. The hydroxyl grp of certain Ser, Thr, Tyr residues of some proteins are enzymatically phosphorylated by ATP.

such modification varies from one protein to next.

Eg. Milk protein casein has many phosphoserine group that binds calcium. So Ca, phosphate and amino acids, which are valuable for baby are provided by casein.

b. Extra carboxyl grp may be added to Glucose residues by vit K dependent carboxylation. The resulting γ-carboxyglutamate residues are essential for activity of several blood clotting ptoteins like prothrombine.

4. **Attachment of carbohydrate side chain**

- Those proteins that are destined to become part of plasma membrane or those that function extracellularly or act as lubricating proteoglycans that coat the mucus mem, **contain carbohydrate chains.**
- Carbohydrate chain is attached to amide nitrogen of asparagine(N-linked) or hydroxyl group of serine, threonine or hydroxylysine (O-linked).

5. **Hydroxylation**

- Proline and lysine residues of α chain of collagen are extensively hydroxylated by vit C dependent hydroxylase in Endoplasmic reticulumn.

6. **Addition of Isoprenyl grp**

- Isoprenyl grp is an intermediate in cholesterol synthesis such as **fernesyl pyrophosphate.**
- A **thioether bond** is formed between isoprenyl grp added to eukaryotic protein and cystein residue.
- Protein modified in such way includes **Ras protein**, products of **ras oncogene** and **proto-oncogene**, and G-protein and lamins.
- Isoprenly group helps to **anchor** the protein in mem.
- Carcinogenic activity of ras oncogene is lost when isoprenylation of Ras protein is **blocked**.
- So identifying the inhibitors of such post translational modification can help in **cancer chemotherapy**.

7. **Proteolytic processing**

- Many proteins are initially synthesized as large, inactive precursor, which are trimmed to smaller and active form.
- Trimming occurs at various site:

Eg: **proinsulin –** in developing secretory vesicles

Collagen – are cleaved after secretion from cells

Zymogens – are the inactive precursors of enzyme become active only when they reach their proper sites of action.

Eg- pancreatic zymogen, trypsinogen becomes activated to trypsine in SI.

- pepsinogen is activated to pepsine only in stomach in acidic pH.

Protein synthesis inhibited by many antibotics and toxins

- Streptomycine
- Tertracycline

30S → inhibit initiation

- Puromycin
- Chloramphenicol

inhibits peptidyl transferase

- Clindamycine
- Erythromycine

50S → inhibits translocation

- Diptheria toxin → inhibits EF-2 – prevents translocation

REFERENCES

- Harper's biochemistry 25th-edition
- Lippincott's illustrated reviews 5th-edition.
- Lehninger's principles of biochemistry 5th-edition

THANK YOU