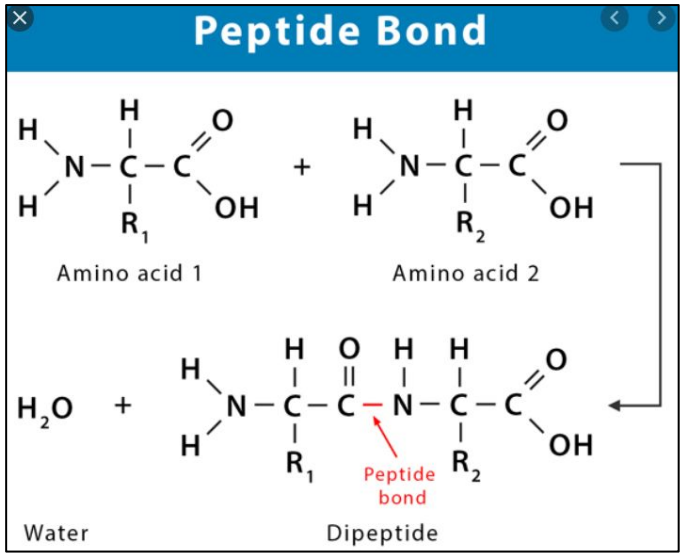
Module 3:
TRANSLATION

SYLLABUS:

Mechanism of translation, activation of amino acid initiation, elongation and termination of protein synthesis. Post-translational modification and protein targeting, protein splicing. Differences between prokaryotic and eukaryotic protein synthesis, inhibitors of translation.

Translation

- **Translation** is the final step in the transfer of genetic information from the DNA to its ultimate product- the Protein
- In genes, information is stored in the form of sequence of 4 deoxynucleotides. It is finally converted to sequence of amino acids by a coded language known as **genetic code**
- The synthesis of proteins takes place at the **ribosome**
- A cellular mechanism is required for reading the genetic code and bringing the appropriate amino acid to the site of protein synthesis. Both of these roles are played by tRNA
- The ribosome facilitates **decoding** of mRNA by inducing the binding of complementary tRNA anticodon sequences to mRNA codons.
- The tRNAs carry specific amino acids that are chained together into a polypeptide as the mRNA passes through and is "read" by the ribosome.
- The polypeptide later folds into an active protein and performs its functions in the cell



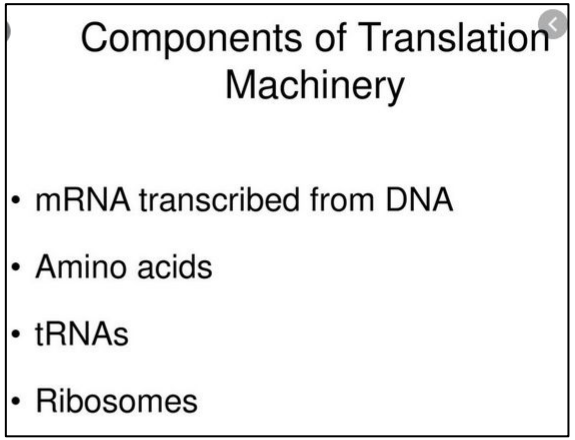
- **Amino acids contain two active groups: -COOH group** and **-NH2 group (carboxyl** And **amino group)**
- **Condensation of amino acid** takes place by interaction between these groups
- **The bond between -COOH group of 1st amino acid and -NH2 group of next amino acid is known as PEPTIDE BOND (-CONH bond)**
- During this condensation, a **water molecule is released**.
- Protein synthesis takes place from **N terminal to C terminal.**

PROTEIN SYNTHESIS MACHINERY

- **mRNA:** carries genetic information in the form of codons, acts as template for translation
- **Ribosomes:** site of synthesis
- **tRNA:** contains mechanism to read codons present in mRNA (in the form of anticodons)and brings amino acids to the site of synthesis.
- **Enzyme aminoacyl tRNA synthetase:** mediates the attachment of amino acid to tRNA.

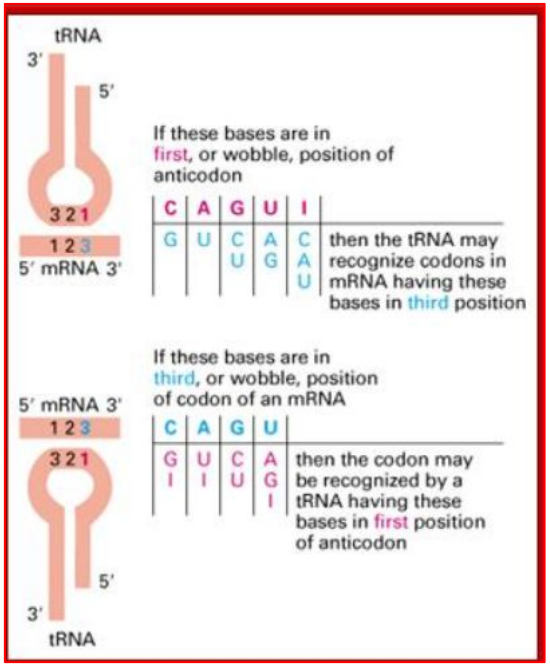
(TOPICS ALREADY DISCUSSED)

- **Structure of mRNA**
- **Genetic code**
- **Structure of tRNA**



Wobble theory:

- Each tRNA is specific for one amino acid, to which it will bind
- As there are multiple codons for some of the amino acids more than one tRNA may recognise a single amino acid. These tRNA's are called as **iso acceptor tRNA**
- If one a.a: one codon: one tRNA
- if one a.a : 2 codons : 2 tRNA's
- Also there are 61 codons for 20 amino acids but tRNA's are only about 50 different kinds.
- Therefore some TRNA's should be able to recognise more than one codons
- **How?** The degeneration of genetic code is at the 3rd base in majority of cases. Usually the 1st two bases do not vary in the multiple codons of an amino acid
- Eg: **Isoleucine Ile: AUU, AUC, AUA.**
- It was found that in many cases only 1st two bases of the codon participate in strong codon- anticodon interaction. The interaction with 3rd base can be loose or even unconventional like G:U pairing. This phenomenon is known as **wobble effect.**
- **Wobbling is between codons of same amino acid. Hence** A single tRNA will never recognise the codons for two different a.a even if 1st 2 bases are same and differ in 3rd nase



List of amino acids with their possible codon options. In red are the only two amino acids which have only one possible codon

Alanine	GCT, GCC, GCA, GCG
Arginine	CGT, CGC, CGA, CGG, AGA, AGG
Asparagine	AAT, AAC
Aspartate	GAT, GAC
Cysteine	TGT, TGC
Glutamine	CAA, CAG
Glutamate	GAA, GAG
Glycine	GGT, GGC, GGA, GGG
Histidine	CAT, CAC
Isoleucine	ATT, ATC, ATA
Leucine	TTA, TTG, CTT, CTC, CTA, CTG
Lysine	AAA, AAG
Methionine	ATG
Phenylalanine	TTT, TTC
Proline	CCT, CCC, CCA, CCG
Serine	TCT, TCC, TCA, TCG, AGT, AGC
Threonine	ACT, ACC, ACA, ACG
Tryptophan	TGG
Tyrosine	TAT, TAC
Valine	GTT, GTC, GTA, GTG
STOP	TAG, TAA, TGA

OVERVIEW OF TRANSLATION:

- Ribosomes bind to messenger RNAs and use their sequences for determining the correct sequence of amino acids to generate a given protein.
- The base triplets of transfer RNA (tRNA) pair with those of mRNA and at the same time deposit their amino acids on the growing protein chain.
- Amino acids are selected and carried to the ribosome by transfer RNA (tRNA) molecules, which enter the ribosome and bind to the messenger RNA chain via an anti-codon stem loop.
- For each coding triplet (codon) in the messenger RNA, there is a transfer RNA that matches and carries the correct amino acid for incorporating into a growing polypeptide chain.
- Once the protein is produced, it can then fold to produce a functional three-dimensional structure.

RIBOSOMES

- Ribosomes are macromolecular machines, found within all living cells, that perform biological protein synthesis (mRNA translation).
- Ribosomes link amino acids together in the order specified by the codons of messenger RNA (mRNA) molecules to form polypeptide chains.
- Ribosomes consist of two major components: the small and large ribosomal subunits. Each subunit consists of one or more ribosomal RNA (rRNA) molecules and many ribosomal proteins (RPs or r-proteins).
- The ribosomes and associated molecules are also known as the ***translational apparatus*.**
- Ribosomes occur both as free particles in prokaryotic and eukaryotic cells and as particles attached to the membranes of the endoplasmic reticulum in eukaryotic cells.

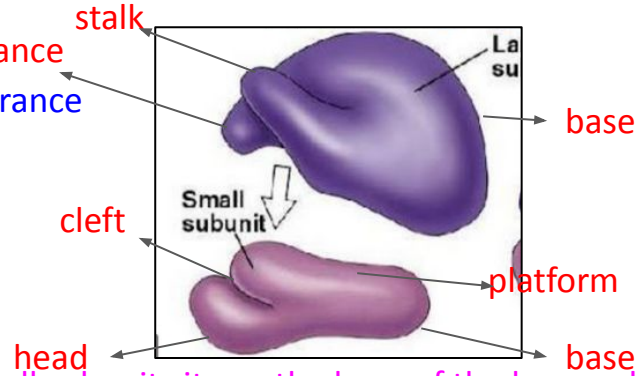
- Ribosomes are one of the largest cellular entities
- **Size of ribosomes:**
  - Prokaryotes: 70S
  - Eukaryotes: 80S

S= Swedgberg unit OR sedimentation coefficient in ultra centrifugation

- They have complex nucleoprotein structure and made of **2 subunits: small subunit and large subunit**
  - the small ribosomal subunits- these read the mRNA
  - the large ribosomal subunits- they form polypeptide chains of amino acids
- **In Prokaryotes:**
  - **Small subunit:** 30S
    - Has one RNA (16S rRNA) and 21 proteins
  - **Large subunit:** 50S
    - Has two RNA's (23S rRNA, 5S rRNA) and 34 proteins
- **In Eukaryotes:**
  - **Small subunit:** 40S
    - Has one RNA (18S rRNA) and 33 proteins
  - **Large subunit:** 60S
    - Has 3 RNA's (28S rRNA, 5.8S rRNA, 5S rRNA) and 49 proteins

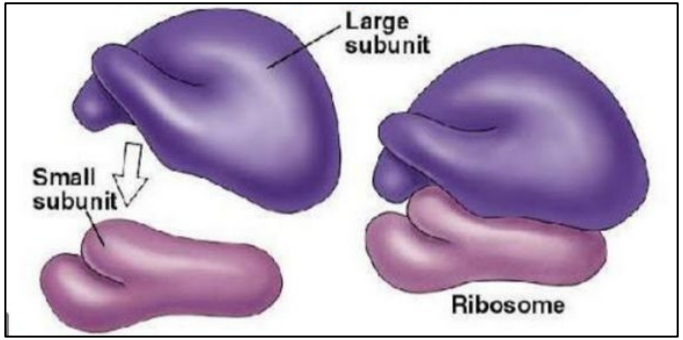
3D structure of prokaryotic ribosome:

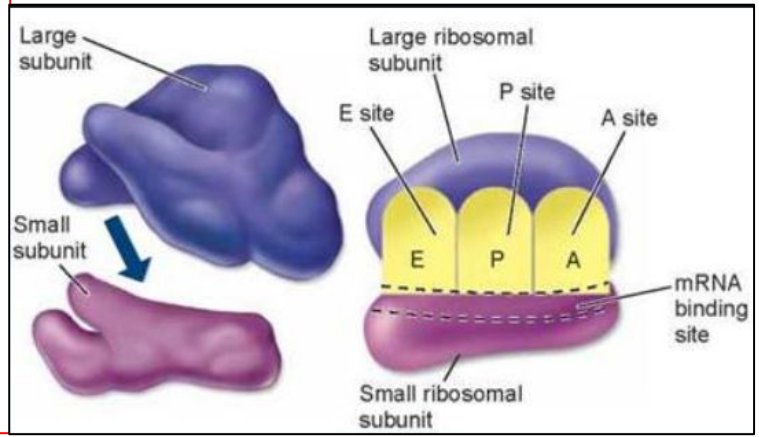
- **Small subunit**: elongated and asymmetrical having platform like structure on one side and base and a head on other side. Depressed cleft between head and platform
- **Large subunit:** more compact having a base, a stalk and a protuberance



- **During assembly of ribosomes:** the platform like region of the small subunit sits on the base of the large subunit and the mRNA fits in between the 2 subunits
- The size of the ribosome is big enough to bind with 2 tRNA molecules and about 40 bases of mRNA at the same time
- The complete ribosome has **3 functional sites:**
    - **Acceptor site (** also known as **aminoacyl tRNA site or A site or entry site)**
    - **Donor site (**also known as **Peptidyl tRNA site or P site)**
    - **Exit site (** also known as **deaminoacyl tRNA site or E site)**

- **A site and P site** extend to both the small and the large subunits and are adjacent to each other
- **The movement of ribosome** along the mRNA (one codon at a time) during the elongation of translation process shifts the tRNA occupying the A site to P site and a new tRNA comes to join the A site.
- The small subunit has the site for binding of mRNA which lies near the cleft of the small subunit
- There is also a site for the enzyme **peptidyl transferase** which transfers the growing peptide which is associated with the peptidyl tRNA at the P site to the amino acid bound to aminoacyl tRNA that is present at the A site and forms a new peptide bond during the polypeptide elongation
- These binding sites are jointly known as **translation domain.** This constitutes ⅔ rd of total area of ribosomes.
- Other 1/3rd part of ribosome contains the E site is referred to as **Exit domain**





Charging of tRNA molecule:

- The binding of amino acid to tRNA molecule is called as **Charging** and a charged tRNA is known as **aminoacyl tRNA.**
- After **tRNA is charged with an amino acid,** the name of that amino acid is written before tRNA in **superscript.**
- To **differentiate between different tRNA molecules** the name of specific amino acid is written as **subscript**
- **Example:**
  - **$tRNA_{Met}$: tRNA is specific for Methionine**
  - **$^{Met}tRNA$: tRNA that is specific for Methionine has a methionine molecule bound to it**
- Charging of tRNA is an energy dependent process: carried out in 2 steps. catalyzed by **aminoacyl-tRNA synthetases** (aaRSs)

- **1st step: activation of amino acid**
  - **Enzyme** binds ATP to amino acid to form **aminoacyl AMP**.
  - Inorganic pyrophosphate is released.
  - **aa + ATP ⟶ aa-AMP + PP, (pyrophosphate)**
- **2nd step: charging**
  - activated amino acid reacts with its specific tRNA to form **aminoacyl tRNA.**
  - AMP is released
  - **aa-AMP + tRNA ⟶ aa-tRNA + AMP**

Aminoacyl tRNA synthetase:

- M.W : varies between 40-100kD
- 20 different aminoacyl tRNA synthetases are present
- Each enzyme is specific for an amino acid
- Single enzyme recognizes all isoacceptor tRNA for particular amino acid
- All tRNA s which are recognized by an aminoacyl tRNA synthetase are known as **Cognate tRNA's** for that enzyme

MECHANISM OF TRANSLATION:

4 basic steps:

1. **Activation of amino acid and formation of aminoacyl tRNA**
2. **Initiation of protein synthesis**
3. **Elongation of polypeptide chain**
4. **Termination of translation and release of nascent polypeptide chain**

1. Activation of amino acid and formation of aminoacyl tRNA

- Enzymatic process catalysed by aminoacyl tRNA synthetase
- Aminoacyl tRNA synthetase binds with its cognate tRNA, the right amino acid and an ATP molecule at different sites.
- 1st step: activation: **aa + ATP ⟶ aa-AMP (aminoacyl AMP) + PP (pyrophosphate)**
- If amino acid is not the correct one, the hydrolysis of aminoacyl AMP takes place and a.a is dissociated
- 2nd step: charging: **aa-AMP + tRNA ⟶ aa-tRNA (aminoacyl tRNA) + AMP**
- If pairing is wrong, and wrong a.a is esterified with tRNA then aminoacyl tRNA dissociates and aa is released.
- If the combination is correct only then the aminoacyl tRNA leaves the enzyme and is transported to ribosome
- **One ATP is utilized for the charging of each tRNA**

2. Initiation:

- All the steps and reactions which precede the formation of 1st peptide bond are referred as initiation of translation
- The most complex process and requires many initiation factors (IF)

In PROKARYOTES:

- **30S subunit** of ribosome binds to the mRNA at **RBS sequences**
- Requires **IF-3:** plays 2 roles
    a. Acts as anti-association factor: prevents the binding of free 30S to free 50S subunit
    b. Facilitates binding of 30S to mRNA
- This binding positions the small subunit in such a way that **initiation codon of mRNA is at the 30S subunit part of the P site** of ribosome. (partial P site)
- In prokaryotes initiation aa is always formylated methionine fMet
- On other side, The charged initiator tRNA ^{fMet}**tRNA$_i$,** reacts with **IF-2,** forms a **binary complex.**
- IF-2 is specific for Initiator tRNA
- The ^{fMet}tRNA$_i$:IF-2 **binary complex reacts with GTP** and forms a **ternary complex.**

- **Ternary complex moves to mRNA:30S:IF-3 complex** and occupies the partial P site
- The **codon-anticodon interaction** takes place between mRNA and tRNA. This results in the formation of **initiation complex** of **mRNA:30S:IF-3:^{fMet}tRNA$_i$:IF-2:GTP**
- This causes the association of large subunit i.e **50S subunit binds to initiation complex**.
- **GTP is hydrolyzed** to GDP.
- **IF-2 and IF-3 are released** and forms the **complete ribosome:mRNA complex** which consists of **mRNA:70S:^{fMet}tRNA$_i$**
- **IF-1** gets associated with 30S subunit and provides **stability for initiation complex**
- Now the complex is ready to accept subsequent aa and elongation process

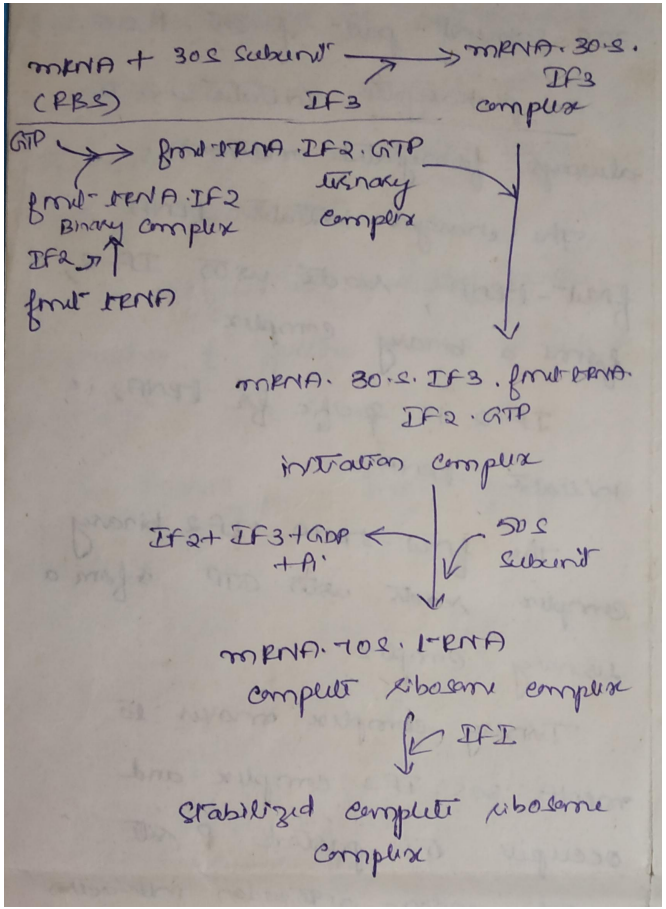
In eukaryotes:

- There are no **RBS or SD sequence**
- **Cap** at the 5’ end of euk mRNA helps in **positioning of small subunit of ribosome to initiation codon**
- 40S subunit binds to cap region of mRNA with the help of **cap binding proteins (CBP)**
- Ribosomal subunit slides through mRNA until it finds initiation codon AUG
- **9 different Initiation Factors IF’s** are required which are referred to as **eIF’s**

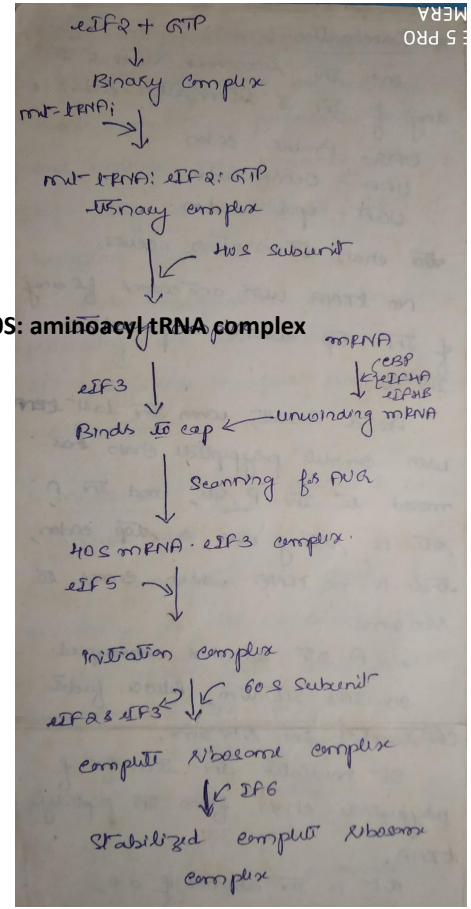
Steps:

- **CBP’s bind to cap** structure at 5’end of mRNA
- **eIF-4A and eIF-4B** associate with cap region to help in unwinding any tertiary structure which may present at 5’end
- On the other hand, **eIF-2 and GTP** associate to form **binary complex**
- ^{Met}**tRNA**$_i$ binds with binary complex and forms **ternary complex**
- Ternary complex is now **transferred to 40S** subunit and **40S: aminoacyl tRNA complex** is formed
- This complex **moves to mRNA** and binds to cap region. **eIF-3** is required for this binding

- This entire complex then slides through mRNA and **scans for initiation codon**
- eIF-4A and eIF-4B maintains mRNA in the unwound configuration.
- Once the complex has found initiation codon, scanning stops and complex positions itself in a manner **AUG occupies partial P site.**
- This results in the formation of **initiation complex.** This requires **eIF-5**
- **60S subunit** now associates to the **mRNA:40S:^{fMet}tRNA$_i$ Complex**
- **eIF-2 and eIF-3** are **released** and **complete ribosome complex** is formed
- GTP is hydrolysed providing energy
- **eIF-6** provides stability to the initiation complex



Initiation in prokaryotes



Initiation in eukaryotes

2. Elongation of polypeptide chain:

- **Once the initiation is successful** and complete initiation complex is formed, the **A site and P site are now complete.**
- At this stage, **P site is occupied by aminoacyl $tRNA_i$, while the A site of Ribosome is free**
- The **second aminoacyl tRNA can now bind to the A site.**
- **Elongation factor** is required for bringing the aminoacyl tRNA to the A site of the ribosome.
- **In prokaryotes:** This elongation factor is **EF-Tu**
- During elongation of polypeptide chain **EF-Tu** binds to **GTP** and forms the **binary complex (Tu:GTP)**
- This complex reacts with **aminoacyl tRNA** to form **ternary complex (Tu:GTP:tRNA)**
- Ternary complex **moves to the vacant A site** of the Ribosome
- Once the ternary complex is in the A site, a ribosomal protein carries **hydrolysis of GTP** bound to EF-Tu giving GDO and free Pi
- This GDP-Tu complex lacks the ability of GTP-Tu complex to bind to aminoacylated tRNA and hence **GDP-Tu gets dissociated from tRNA**, leaving **aminoacylated tRNA at the A site**

- At this stage **both A site and P site are occupied and a peptide bond can form**
- Formation of peptide bond is accompanied by the **cleavage of bond between fMet and $^{fMet}tRNA_i$**
- **Deacylated tRNA binds very poor to the P site,** so this tRNA **leaves the ribosome immediately** after peptide bond formation through **E site.**
- In addition, the **binding of peptidyl tRNA to the A site is weakned** because the binding of peptidyl tRNA to the P site is always strong
- Therefore, this **peptidyl tRNA should move from A site to P site.** This movement is called as **translocation** and elongation factor EF-G controls this process
- **During translocation** Ribosome moves forward on mRNA by one codon so that the next codon will now occupy the vacant A site
- Movement of Ribosome is a **2 step process:**
    - **In 1st step: peptidyl tRNA** which is still at A site is **moved to the P site**
    - **2nd step: entire ribosome translocates** to the next codon
- Once translocated, **EF-G is released**
- Another factor EF-Ts is required to make EF-Tu available for recirculation

In eukaryotes:

- **The basic events of Elongation are similar between prokaryotes and eukaryotes**
- Elongation factors are different
- In eukaryotes elongation is carried by the factor **eEF 1. (** Similar function to EF-Tu)
- Translocation is facilitated by **eEF 2 (**similar function to EF-G)

3. Termination of translation:

- Once the **ribosome reaches** to any of the **3 stop codons:**
  - **UAG: amber codon,**
  - **UAA: ochre codon,**
  - **UGA: opal codon,**

the **polypeptide chain termination occurs**

- There are **no tRNA with anticodons** for any of the stop codons present in the cell
- As a result **when the last tRNA with complete polypeptide chain has moved to the P site, A site is sitting over the stop codon.**
- There is **no tRNA** which **comes to Ribosome**.
- **Therefore A site remains unoccupied**
- In this situation **release factor (RF) enters the Ribosome**
- It mediates the **transfer of polypeptide chain from the peptidyl tRNA**
- Because of the absence of amino acid, only **water molecule is incorporated**. This results in the **dissociation of peptide from the peptidyl tRNA molecule** and **release of complete polypeptide chain** to the cell cytosol.

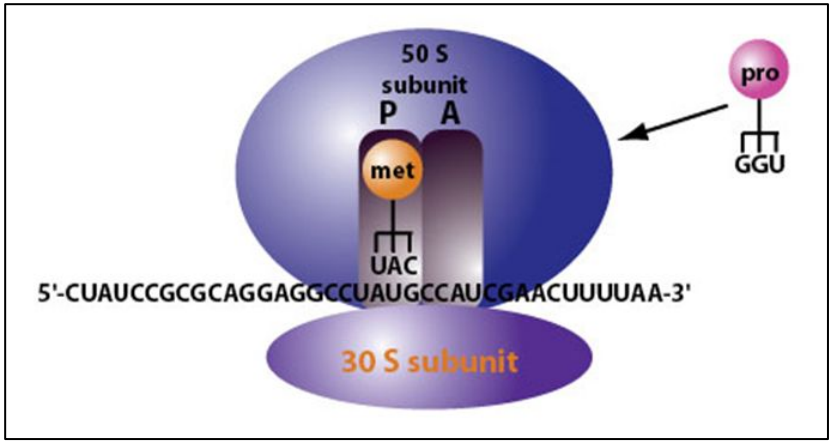
In prokaryotes:

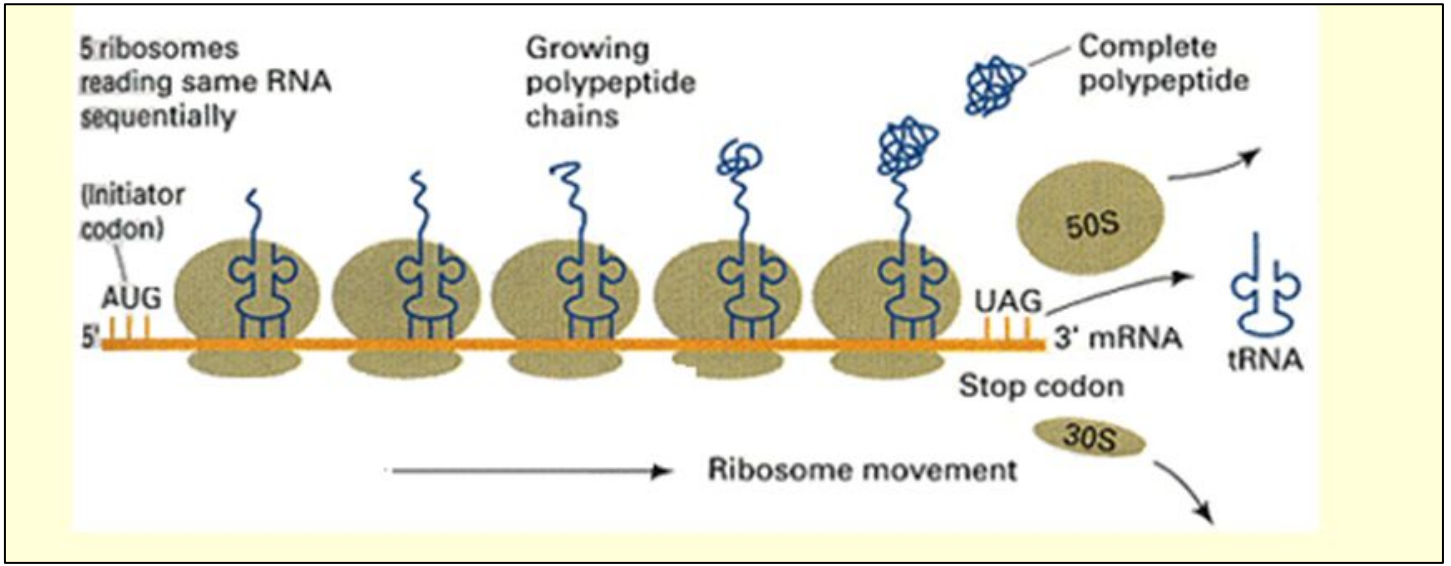
- **2 RF's:**
  - **RF 1:** Recognizes UAA and UAG
  - **RF 2:** Recognizes UGA and UAA

In eukaryotes:

- **Single eRF** is present, which recognises all the 3 stop codons

After polypeptide is released, Ribosome falls off from the mRNA and dissociates into individual sub units


50 S
subunit
P
A
met
UAC
pro
GGU
5'-CUAUCCGCGCAGGAGGCCUAUGCCAUCGAACUUUUAA-3'
30 S subunit


5 ribosomes
reading same RNA
sequentially
Growing
polypeptide
chains
Complete
polypeptide
(Initiator
codon)
AUG
5'
50S
UAG
3' mRNA
tRNA
Stop codon
30S
Ribosome movement

Post translational modifications

- **PTM** refers to the covalent and generally enzymatic modification of proteins following protein biosynthesis.
- PTMs are important components in cell signaling, as for example when prohormones are converted to hormones.
- These modifications include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis and influence almost all aspects of normal cell biology and pathogenesis.
- Therefore, identifying and understanding PTMs is critical in the study of cell biology and disease treatment and prevention.

1. Phosphorylation

- **Protein phosphorylation** is a reversible post-translational modification of proteins in which an amino acid residue is phosphorylated by a protein kinase by the addition of a covalently bound phosphate group.
- Reversible protein phosphorylation, principally on serine, threonine or tyrosine residues
- Phosphorylation alters the structural conformation of a protein, causing it to become activated, deactivated, or modifying its function
- Phosphorylation plays critical roles in the regulation of many cellular processes, including cell cycle, growth, apoptosis and signal transduction pathways.

2. Glycosylation

- The addition of a carbohydrate moiety to a protein molecule is referred to as protein glycosylation.
- It is a common post translational modification for protein molecules involved in cell membrane formation..
- Protein glycosylation helps in proper folding of proteins, stability and in cell to cell adhesion commonly needed by cells of the immune system.
- The major sites of protein glycosylation in the body are ER, Golgi body, nucleus and the cell fluid.
- **Types of glycosylation:** Glycopeptide bonds can be categorized into specific groups based on the nature of the sugar–peptide bond and the oligosaccharide attached, including N-, O- and C-linked glycosylation, glypiation and phosphoglycosylation

Types of Glycosylation	
N-linked	Glycan binds to the amino group of asparagine in the ER
O-linked	Monosaccharides bind to the hydroxyl group of serine or threonine in the ER, Golgi, cytosol and nucleus
Glypiation	Glycan core links a phospholipid and a protein
C-linked	Mannose binds to the indole ring of tryptophan
Phosphoglycosylation	Glycan binds to serine via phosphodiester bond

3. Methylation

- **Protein methylation** is a type of post-translational modification featuring the addition of methyl groups to proteins.
- Many eukaryotic proteins are post-translationally modified on their N-terminus. A common form of N-terminal modification is N-terminal methylation (Nt-methylation) by N-terminal methyltransferases (NTMTs).
- Methylation is mediated by methyltransferases, and S-adenosyl methionine (SAM) is the primary methyl group donor.
- Methylation is a well-known mechanism of **epigenetic regulation**, as histone methylation and demethylation influences the availability of DNA for transcription.

4. Ubiquitination

- Ubiquitination is the addition of ubiquitin molecules to lysine residues of a protein.
- Following ubiquitination, most proteins are targeted to the 26S proteosome for degradation.
- Ubiquitin is an 8-kDa polypeptide consisting of 76 amino acids that is appended to the NH2 of lysine in target proteins via the C-terminal glycine of ubiquitin.
- Following an initial monoubiquitination event, the formation of a ubiquitin polymer may occur
- important in regulation of various aspects of receptor signaling and trafficking

5. Acetylation

- Protein acetylation is one of the major post-translational modifications (PTMs) in eukaryotes
- acetyl group from acetyl coenzyme A (Ac-CoA) is transferred to a specific site on a polypeptide chain under the **catalysis of acetyltransferase**
- Acetylation occurs mainly on lysine
- N-acetylation occurs in almost all eukaryotic proteins through both irreversible and reversible mechanisms.
- N-terminal acetylation requires the **cleavage of the N-terminal methionine by methionine aminopeptidase (MAP)** before replacing the amino acid with an acetyl group from acetyl-CoA by N-acetyltransferase (NAT) enzymes.
- **This type of acetylation is co-translational,** in that N-terminus is acetylated on growing polypeptide chains that are still attached to the ribosome.
- While 80 to 90% of eukaryotic proteins are acetylated in this manner
- Lysine acetylation on histone N-termini is a common method of regulating gene transcription.
- Histone acetylation is a reversible event that reduces chromosomal condensation to promote transcription
- Histone deacetylase (HDAC) enzymes reverse the effects of acetylation by reducing the level of lysine acetylation and increasing chromosomal condensation.

6. Lipidation

- Attachment of lipid molecules to a protein or part of a protein attached to the cell membrane.
- Lipidation is a method to target proteins to membranes in organelles (endoplasmic reticulum [ER], Golgi apparatus, mitochondria), vesicles (endosomes, lysosomes) and the plasma membrane.
- This type of modification gives proteins distinct membrane affinities
- Lipidation increase the hydrophobicity of a protein and thus its affinity for membranes.

7. Proteolysis

- Proteolysis involves the breakdown of proteins into smaller polypeptides or amino acids through the hydrolysis of peptide bonds by a **protease.**
- Proteolytic processing is a ubiquitous and irreversible post-translational modification
- Consequently, the functional sequence of a protein can very rarely be predicted from its transcript, as proteolysis products form new (neo-) N and C termini.
- These proteolytic processing events, can result in activation, inactivation, completely altered protein function and they regulate a vast array of biological processes

Protein targeting

- **Protein targeting** or **protein sorting** is the biological mechanism by which proteins are transported to the appropriate destinations in the cell or outside of it.
- Proteins can be targeted to the inner space of an organelle, different intracellular membranes, plasma membrane, or to exterior of the cell via secretion.
- This delivery process is carried out based on information contained in the protein itself.
- Correct sorting is crucial for the cell; errors can lead to diseases.

Targeting signals

- Targeting signals are the pieces of information that enable the cellular transport machinery to correctly position a protein inside or outside the cell.
- This information is contained in the polypeptide chain or in the folded protein.
- The continuous stretch of amino acid residues in the chain that enables targeting are called **signal peptides or targeting peptides.**

- There are **two types of targeting peptides,** the presequences and the internal targeting peptides
  - **Presequence:** often found at the N-terminal extension and is composed of between 6-136 basic and hydrophobic amino acids
  - **internal targeting peptides:** known as signal patches, are composed by amino acid residues that are discontinuous in the primary sequence but become functional when folding brings them together on the protein surface composed of parts which are separate in the primary sequence.

Protein translocation:

- Since the translation of mRNA into protein by a ribosome takes place within the cytosol, proteins destined for secretion or a specific organelle must be translocated.
- This process can occur during translation, known as **co-translational translocation**, or after translation is complete, known as **post-translational translocation.**

Co-translational translocation:

- Most proteins that are secretory, membrane-bound, or reside in the endoplasmic reticulum (ER), golgi or endosomes use the co-translational translocation pathway.
- This process begins with the N-terminal signal peptide of the protein being recognized by a signal recognition particle (SRP) *while the protein is still being synthesized on the ribosome*.

- The synthesis pauses while the ribosome-protein complex is transferred to an SRP receptor on the **ER in eukaryotes**, and the **plasma membrane in prokaryotes**. There, the nascent protein is inserted into the **translocon**, a membrane-bound protein
- Within the ER, the protein is first covered by a chaperone protein to protect it from the high concentration of other proteins in the ER, giving it time to fold correctly.
- Once folded, the protein is modified as needed (for example, by glycosylation), then transported to the Golgi for further processing and goes to its target organelles

Post-translational translocation:

- Some proteins are translated in the cytosol and later transported to the ER/plasma membrane by a post-translational system.
- **In prokaryotes** this requires certain cofactors such as SecA and SecB. This pathway is poorly understood **in eukaryotes,** but is facilitated by Sec62 and Sec63, two membrane-bound proteins.
- In addition, proteins targeted to other destinations, such as mitochondria, chloroplasts, or peroxisomes, use specialized post-translational pathways. Also, proteins targeted for the nucleus are translocated post-translation through the nuclear envelope via nuclear pores.

Differences between prokaryotic and eukaryotic protein synthesis

Eukaryotic Protein Synthesis vs Prokaryotic Protein Synthesis

Eukaryotic Protein Synthesis	Prokaryotic Protein Synthesis
Eukaryotic mRNA molecules are monocistronic, containing the coding sequence only for one polypeptide.	In prokaryotes, mRNA molecules are polycistronic containing the coding sequence of several genes of a particular metabolic pathway.
In eukaryotes, protein synthesis occurs in the cytoplasm.	In prokaryotes, protein synthesis begins even before the transcription of mRNA molecule is completed. This is called coupled transcription - translation.
In eukaryotes, most of the gene have introns or non coding sequences along with exons or coding sequences. The exons are joined together and introns are removed during mRNA processing.	Prokaryotes do not have introns (Except Archaebacteria). Therefore mRNA processing is not required.
The primary mRNA transcript in eukaryotes undergoes processing and splicing to change into a functional mRNA.	In prokaryotes splicing of mRNA transcript does not occur.
In eukaryotes, mRNA molecules are modified by the addition of a 5'G cap formed of methylated guanosine triphosphate.	No such cap is formed at 5'end of bacterial mRNA.
A poly A tail formed of about 200 adenine nucleotides is added at the 3'end of mRNA in Eukaryotes.	No poly A tail is added to bacterial mRNA.

In eukaryotes, 5'cap initiates translation by binding the mRNA to small ribosomal subunit usually at the first codon AUG.	In bacteria, translation begins at an AUG codon preceded by a special nucleotide sequence.
The first amino acid methionine entering the ribosome is not formylated.	The first amino acid methionine is formylated into N formyl methionine.
The pre imitation complex formation is initiated by nine initiated factors.	Only two initiating factors are involved.
In eukaryotes, the number of initiating factors is much more than prokaryotes. About ten IFs have been identified in reticulocytes an RBC. These are eIF1, eIF2, *eIF3*, *eIF4* , eIF5, eIF6 ,eIF4B, eIF4C,eIF4D, eIF4F	Three initiating factors found in prokaryotes. PIF-1 , PIF-2 , PIF-3
In eukaryotes small subunit of ribosome (40 S) gets dissociated with the initiator amino acyl tRNA (Met-tRNA Met) without the help of mRNA. The complex joins mRNA later on.	In prokaryotes, 30 S subunit first complexes with mRNA (30S-mRNA) when then joins with f Met tRNA f-

Atleast 4 elongation factors	3 elongation factors
1 release factor	2 release factors
mRNA is very stable and degradation occurs very slowly	Degradation of mRNA occurs continuously and while translation is in process

Protein splicing

- It is a post translational process that results in the excision of an internal protein region and ligation of its flanking sequences.
- **Intein (int**ernal prot**ein):** the central protein region, which is subjected to excision
- **N or C Extein (ext**ernal prot**ein):** corresponding flanking sequences
- It is an **autocatalytic process and is independent of any cofactors or enzymes.**

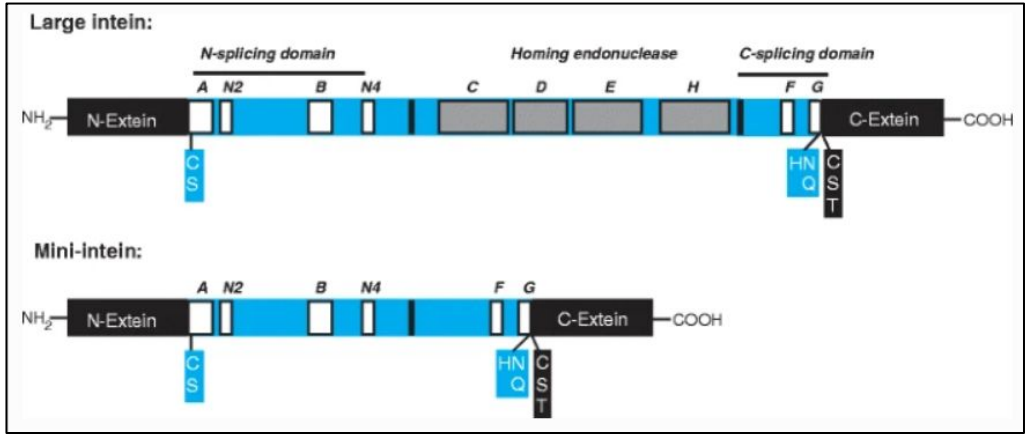
General organization of an intein:

- Inteins usually vary in size from 134-650 aa residues
- Divided into 2 large groups:
  a. Classical or large inteins
  b. Mini inteins
- **Classical or large inteins:** consists of two domains
  a. Hint: catalyses protein splicing
  b. Central endonuclease domain

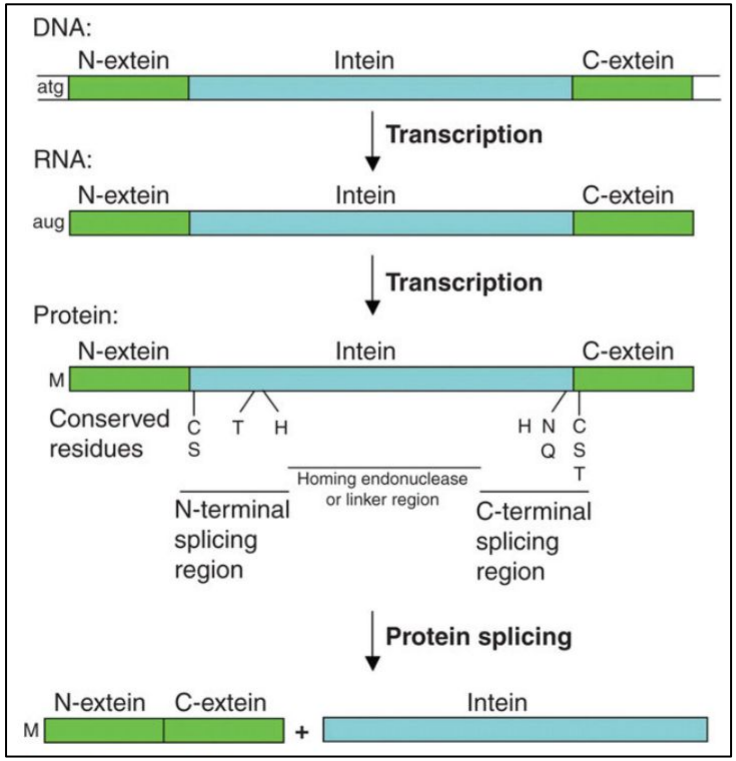
  And 10 conserved aa sequence motifs: A, N_2, B, N_4, C, D, E, H, F, G

- **Mini inteins:** central endonuclease domain is replaced by a linker sequence, which lacks catalytic activity and lacks central aa sequence motifs C, D, E and H

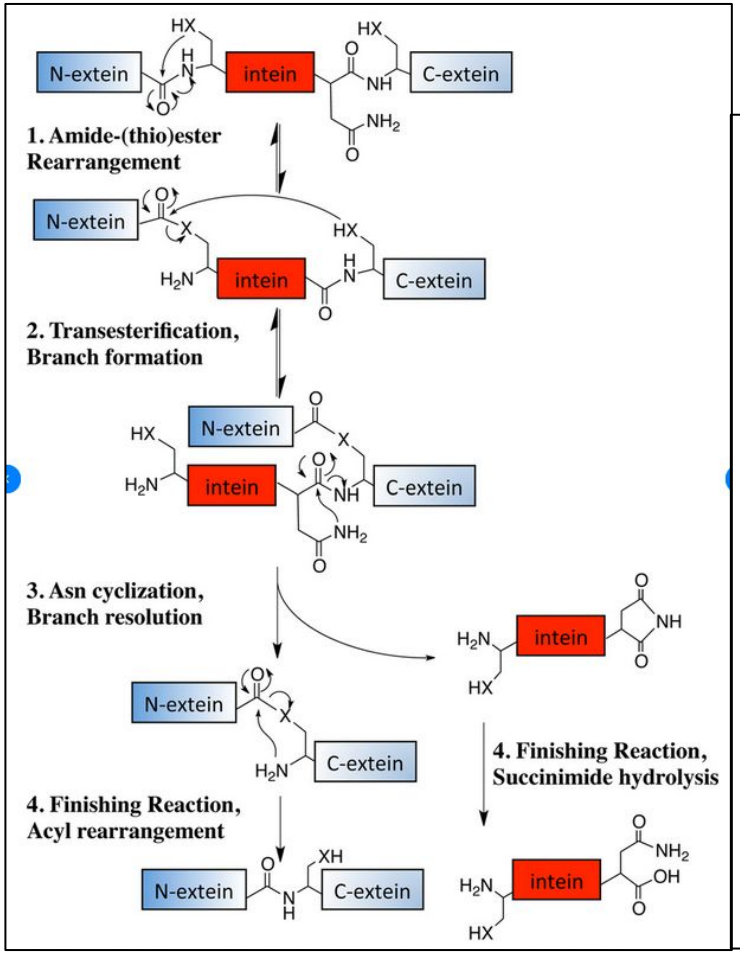
Structure of classical and mini inteins:



Mechanism of protein splicing



Basic mechanism of protein splicing.



X represents an oxygen or a sulfur atom.

X=S (Cys) and X=O (Thr, Ser)

Step1: autocatalytic N-X shift @ N terminal splicing site: yield ester or thioester bond

Step 2: transesterification, yields branched intermediate: N extein is transferred to the side chain of residue +1 of C extein, protein with 2 N terminus

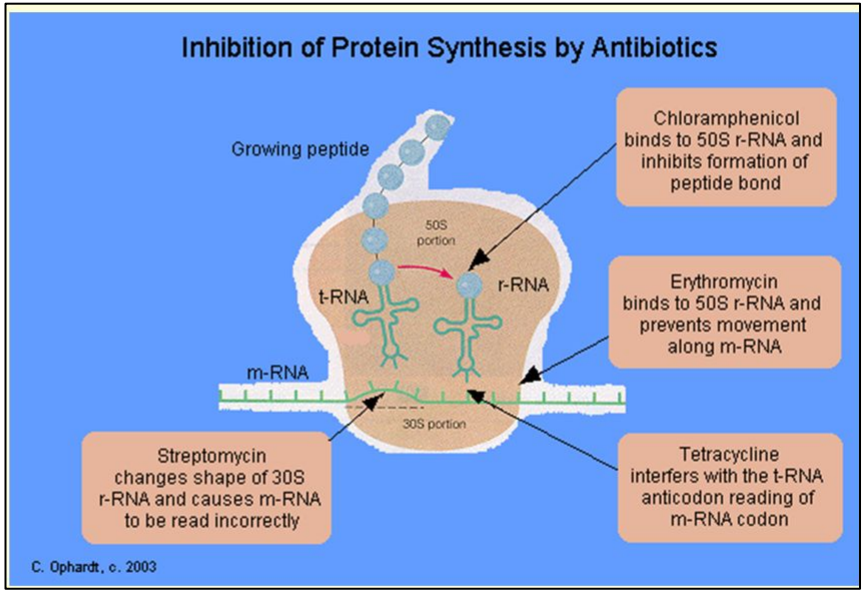
Step 3: C terminal Asn cyclization: cyclization of intein C terminal Asn to resolve branched intermediate and cleave peptide bond between intein and C-extein

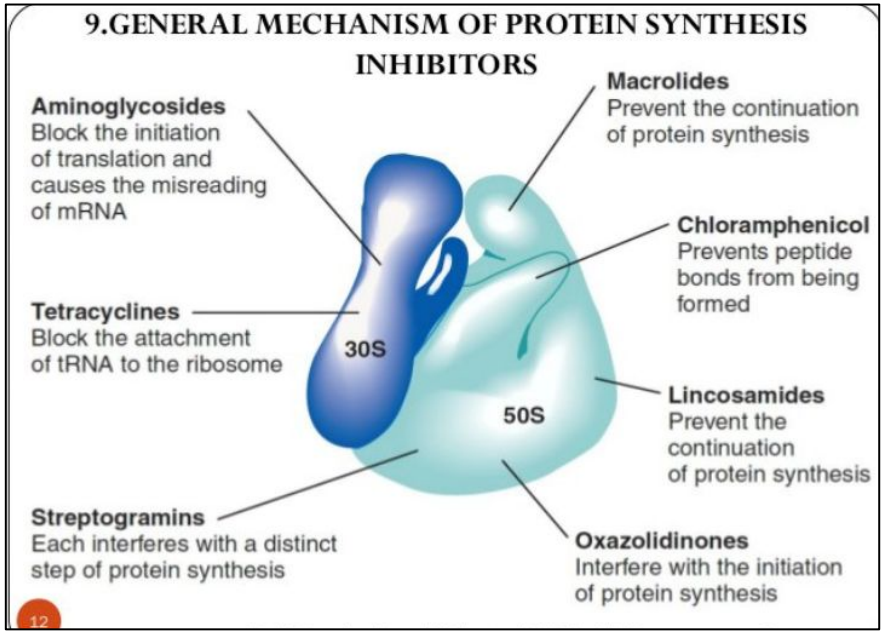
Step 4a: C terminal succinimide hydrolysis: yield free intein : succinimide occurring at C end of intein is hydrolysed to yield Asn or Iso Asn.

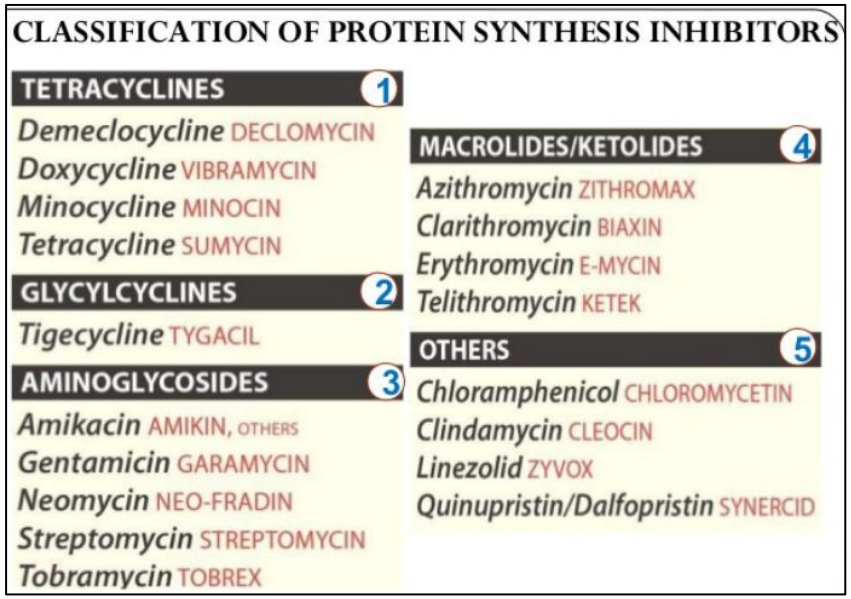
Step 4b: X-N shift: Spontaneous reaction, Since N-X shift ester bond is energetically disadvantageous, it is reversed to form **peptide bond between 2 exteins**

https://slidetodoc.com/intein-mediated-protein-splicing-jayanti-tokas-1-rubina/

Inhibitors of Translation





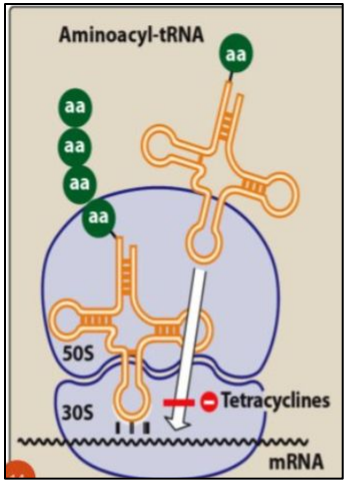

CLASSIFICATION OF PROTEIN SYNTHESIS INHIBITORS
TETRACYCLINES 1
Demeclocycline DECLOMYCIN
Doxycycline VIBRAMYCIN
Minocycline MINOCIN
Tetracycline SUMYCIN
GLYCYLCYCLINES 2
Tigecycline TYGACIL
AMINOGLYCOSIDES 3
Amikacin AMIKIN, OTHERS
Gentamicin GARAMYCIN
Neomycin NEO-FRADIN
Streptomycin STREPTOMYCIN
Tobramycin TOBREX
MACROLIDES/KETOLIDES 4
Azithromycin ZITHROMAX
Clarithromycin BIAXIN
Erythromycin E-MYCIN
Telithromycin KETEK
OTHERS 5
Chloramphenicol CHLOROMYCETIN
Clindamycin CLEOCIN
Linezolid ZYVOX
Quinupristin/Dalfopristin SYNERCID

1. Aminoglycosides:

- Molecules comprised of amino sugars.
- Includes streptomycin, gentamycin, kanamycin, etc.
- They transport through cell membrane, binds to ribosomes and inhibits protein synthesis
- **Bactericidal in action:** –Combination of membrane damage and inhibition of protein synthesis is bactericidal
- Aminoglycosides bind various sites on both ribosomal subunits

  –Freeze translation after initiation step, prevent polysome formation

  –Interfere with codon recognition, resulting in misreading
- **Gentamicin** bind to 30S ribosomal subunits and induce misreading of mRNA codons
- **Streptomycin** changes the shape of 30S rRNA and causes misreading of mRNA codons



2. Tetracyclines

- Tetracycline and its semi-synthetic derivatives (e.g., minocycline and doxycycline)

bind to 30S ribosomal subunits and

reversibly block the attachment of the charged tRNA to the A-site.

- They have bacteriostatic activity

3. Clindamycin

- Clindamycin binds to 50S ribosomal subunits and blocks peptide bond formation between amino acids located in the P- and A-sites
- It has excellent activity against gram-positive aerobes and anaerobes, as well as gram-negative anaerobes

4. Macrolides

- Macrolides bind 50S ribosomal subunits and block translocation and peptide movement through the exit site.
- They are bacteriostatic
- Eg: Erythromycin, Azithromycin, clarithromycin

5. Chloramphenicol

- binds to 50S ribosomal subunits and inhibit protein synthesis at the peptidyl transferase reaction i.e inhibits peptide bond formation

6. Linezolid

- Inhibits the formation of 70S Initiation complex by binding to a site on 50s Subunit near the interface with 30s subunit