CORE IDEA 2: GENETICS AND INHERITANCE
TOPIC 2.5: GENE EXPRESSION AND REGULATION (PART 2)
(TRANSLATION & TRANSLATIONAL + POST-TRANSLATIONAL CONTROL)

Learning Outcomes:

(c) Describe how the information on DNA is used to synthesize polypeptides in prokaryotes and eukaryotes (description of the processes of transcription, formation of mRNA from pre-mRNA and translation is required)

(j) explain how differential (i.e. spatial and temporal) gene expression in eukaryotes can be regulated at different levels:

(iv) translational level (half-life of RNA and initiation of translation)
(v) post-translational level (biochemical modification and protein degradation)

Use the knowledge gained in this section in new situations or to solve related problems.

Textbooks and References

Campbell, Urry, Cain, Wasserman, Minorsky, Reece (2018) **Biology – A Global Approach (11th Edition)(Global Edition) Chapter 17: Expression of Genes pg. 397 – 406, Chapter 18: Control of Gene Expression pg. 427 - 429** (Pearson Publication) ISBN-10 1-292-17043-3

Note: This textbook is available in our library. You may wish to borrow them to supplement your reading when necessary.

Contents

Learning Outcome 2(c) modified:

Describe how the information on DNA is used to synthesise polypeptides in prokaryotes and eukaryotes. (Description of the processes of **translation**)

1 The Genetic Code: A Blueprint for Life

- The genetic code is a set of rules that determines how the information in DNA and RNA is translated into proteins, which are the building blocks of all living organisms. It's like a language that cells use to understand the instructions in their genetic material.

Retrivel Practice: Recall that from Topic 2.1 Structure and Function of Nucleic Acids

DNA and RNA: The Carriers of Genetic Information

- DNA (Deoxyribonucleic Acid): Contains the instructions for building proteins. It's made up of four nucleotides: adenine (A), thymine (T), cytosine (C), and guanine (G).
- RNA (Ribonucleic Acid): Acts as the messenger that carries instructions from DNA to the ribosomes of the cell. In RNA, thymine (T) is replaced by uracil (U).

1.1 Codons: The Language of the Genetic Code

- A codon is a **sequence of three nucleotides** in **mRNA** that specifies a particular amino acid or a stop signal for translation. For example, the mRNA sequence **AUG codes for the amino acid methionine** and also serves as the **start codon to initiate translation**.
- There are a total of **64 possible codons**. (Fig. 1.1.1)

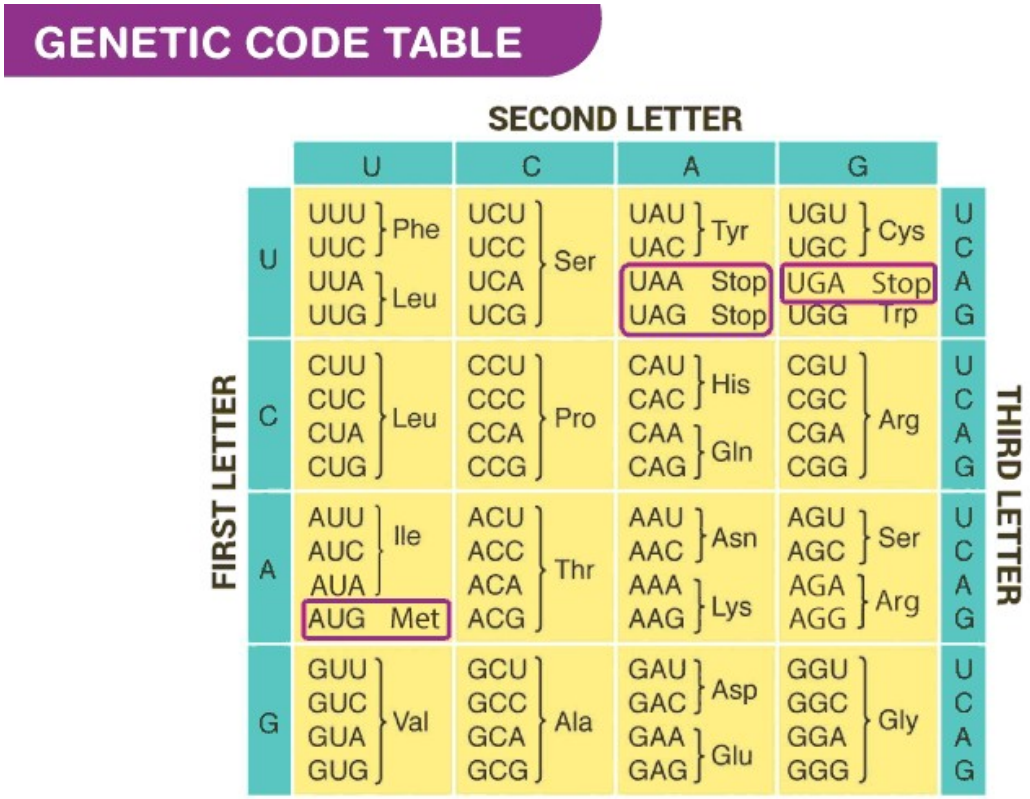
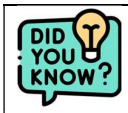


Fig. 1.1.1: Genetic code table



The Logic Behind 64 Possible Codons

- The logic behind having 64 possible codons in the genetic code is based on the structure of DNA and RNA molecules, and the way the genetic information is encoded.

Number of Nucleotides:

- There are **four different nucleotides (A, U, C, G)** in RNA.

Combination Logic:

- If we consider combinations of these nucleotides in sets of one, two, and three:
  - **Single Nucleotide (1-letter combinations):**
    - **Only 4 possible codons** (A, U, C, G). This is not enough to code for 20 amino acids.
  - **Two Nucleotides (2-letter combinations):**
    - The number of **possible combinations is 4 × 4 = 16**. Still **not enough for 20 amino acids**.
  - **Three Nucleotides (3-letter combinations):**
    - The number of **possible combinations is 4 × 4 × 4 = 64**. This is **more than enough to code for 20 amino acids**.

- Using three nucleotides per codon gives us 64 possible combinations, which are more than sufficient to code for all 20 amino acids as well as start and stop signals in the genetic code.

- Here's how these 64 codons are used:
  - **Amino Acid Coding:**
    - There are 20 different amino acids, and **each one is coded by one or more codons**. This **redundancy** helps protect against mutations.
  - **Start Codon**:
    - One codon **(AUG**) acts as the **start codon**, signaling the beginning of protein synthesis. It also **codes for the amino acid methionine**.
  - **Stop Codons:**
    - **Three codons (UAA, UAG, UGA)** act as stop codons, **signaling the end of protein synthesis**.

1.2 Amino Acids: The Building Blocks of Proteins

- There are 20 different amino acids that can be combined to form proteins.

- Each codon specifies one of these amino acids. For example, the codon GGU codes for glycine, and the codon UUU codes for phenylalanine.

1.3 How the Genetic Code Works

Retrivel Practice: Recall from Topic 2.4 Gene Expression and Regulation (I)

1.3.1 Transcription: Copying the Code from DNA to RNA

- Transcription is the process where a segment of DNA is copied into mRNA. This mRNA strand carries the genetic information from the DNA in the nucleus to the cytoplasm, where proteins are made.

1.3.2 Translation: Reading the Code to Build Proteins

- Translation occurs at the ribosome, where the mRNA is read in sets of three nucleotides (codons).

- Each codon matches complementarily with the anticodon of a tRNA molecule that carries a specific amino acid.

- The ribosome moves along the mRNA, reading each codon and adding the corresponding amino acid to the growing polypeptide chain.

1.4 Main features of the Genetic Code

- **Triplet Code:**
  - The genetic code is a **triplet code**, meaning that **each amino acid is encoded by a sequence of three nucleotides** called a codon. For example, the codon AUG codes for the amino acid methionine.

- **Degeneracy/Redundancy:**
  - The genetic code is **redundant**, which means that **most amino acids are encoded by more than one codon**. For instance, the amino acid leucine is encoded by six different codons: UUA, UUG, CUU, CUC, CUA, and CUG.

- **Unambiguous:**
  - **Each codon specifies only one amino acid**. This means that the codon AUG always codes for methionine and nothing else.

- **Universal:**
  - The **genetic code is nearly universal**, meaning that it is the **same in almost all organisms**, from bacteria to humans.

  - This universality suggests that all life shares a **common evolutionary origin**.

- **Start and Stop Signals:**
  - **Start Codon**: The codon **AUG** not only **codes for methionine** but also serves as the **start signal for translation**.

  - **Stop Codons**: There are **three stop codons (UAA, UAG, UGA)** that **do not code for any amino acid** and **signal the end of translation**, causing the ribosome to release the newly formed polypeptide.

- **Non-Overlapping and Continuous:**
  - The genetic code is **read in a non-overlapping manner**. **Each nucleotide is part of only one codon** and codons are read one after another without skipping any nucleotides.

  - For example, in the sequence AUGGCU, the codons are AUG and GCU, read sequentially without overlap.

- **Fixed Polarity**
  - **Codons are always read in the same 5' to 3'** direction on the mRNA. Reading in the reverse direction would specify different amino acids.

Checkpoint 1
Choose true or false and justify your choice.

Q	Statement	Choice	Justification
1	Each codon in the genetic code specifies more than one amino acid.	T / F	
2	There are more codons than amino acids because some amino acids are encoded by more than one codon.	T / F	
3	The genetic code is read in a continuous, non-overlapping manner from the start codon to the stop codon.	T / F	

Checkpoint 2
Use the Genetic Code Table in Fig. 1.1.1 to complete the following questions:

1) Given the mRNA sequence 5'-AUG GCU UUU AAA UGA-3', determine the sequence of amino acids produced during translation.

2) Determine the mRNA sequence that would code for the amino acid sequence Proline-Valine-Serine.

2 TRANSLATION

- In eukaryotes, during translation, the mature mRNA is "read" according to the genetic code, to produce the primary structure of protein. The translation of mRNA into polypeptides required many protein molecules and RNAs e.g. tRNA and rRNA.

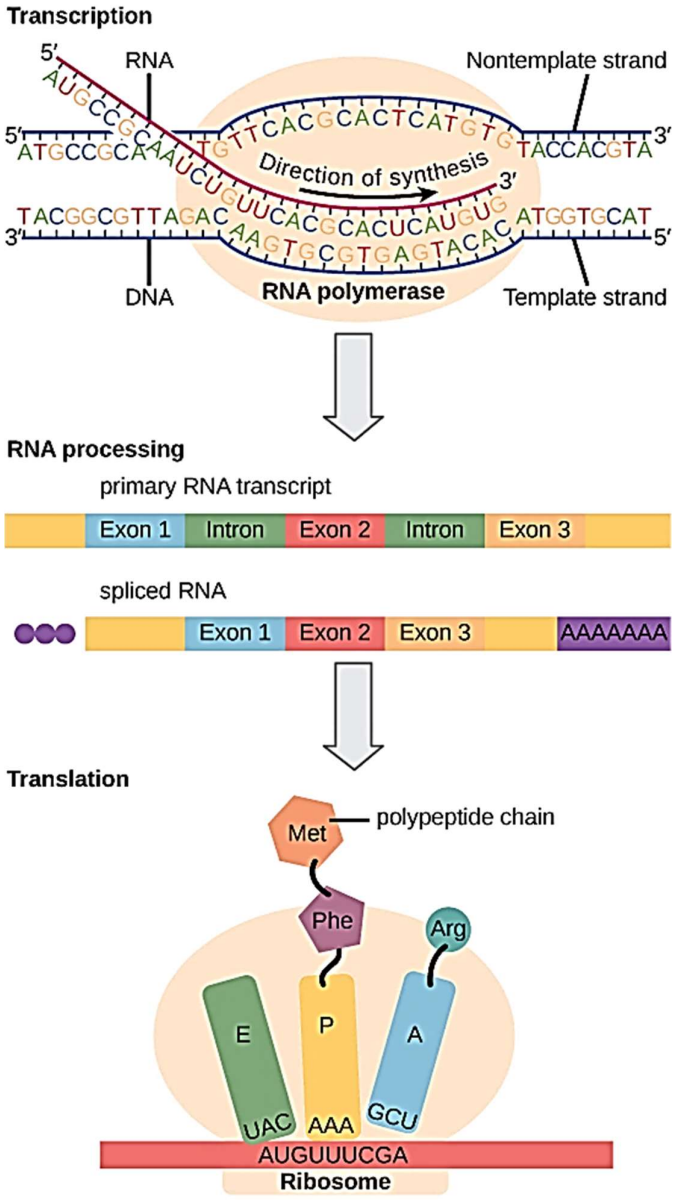


Fig. 2.1: Process of Translation and the components involved

2.1 Amino acid activation

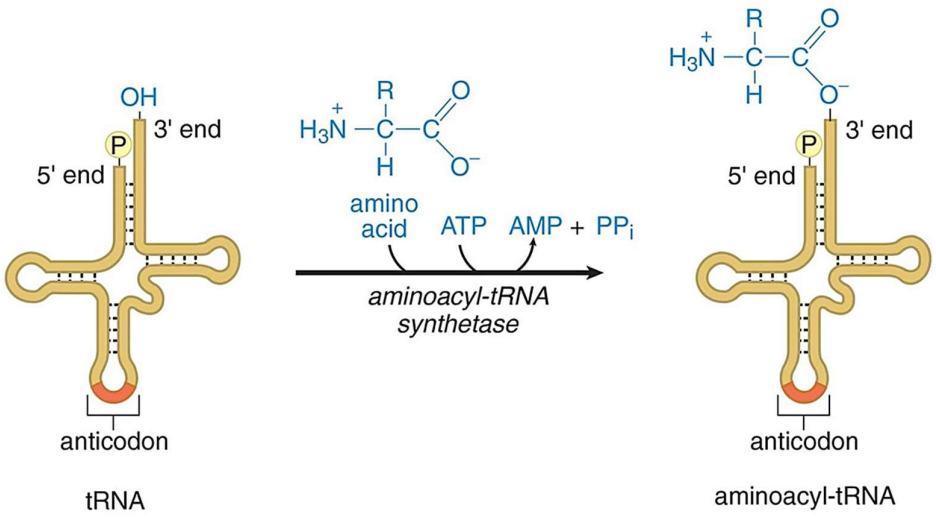


Fig. 2.1.1: Amino acid activation

- Before translation can occur, tRNA needs to be attached to the correct amino acid via the process of **amino acid activation**. The activation reaction is catalysed by **specific <u>aminoacyl-tRNA synthetases</u>**. Amino acid activation is **NOT part of translation process** but essential for translation to occur.

- There is at least 1 aminoacyl-tRNA synthetase for each amino acid. Each aminoacyl-tRNA synthetase is highly specific for a given amino acid.

- The first step is the formation of an aminoacyl-AMP from an amino acid and ATP. ATP is hydrolysed to release energy to result in the formation of an aminoacyl-AMP.

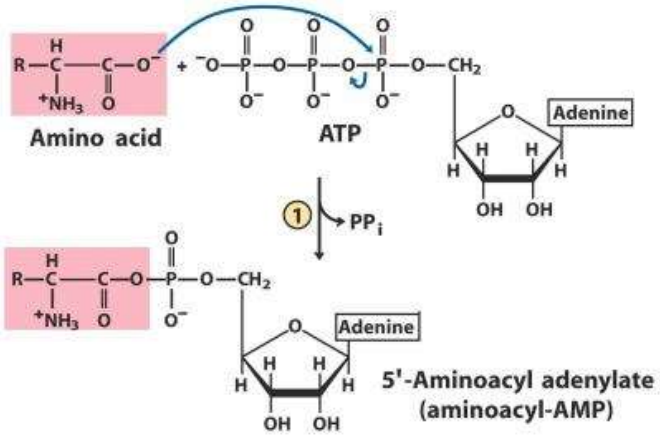


Fig. 2.1.2: Formation of aminoacyl-AMP

- Aminoacyl-AMP is tightly bound to the active site of the enzyme by non-covalent interactions (e.g. hydrogen bonds, hydrophobic interactions, etc).

- tRNA then binds to the active site. The bond between AMP and amino acid is broken to allow the transfer of the aminoacyl group of aminoacyl-AMP to a particular tRNA molecule to form **aminoacyl-tRNA**.

- The amino acid is attached via an ester linkage to the 3' end of the tRNA. This results in the formation of an activated amino acid. The activated amino acid is also known as charged tRNA, thus this process is also knowed as tRNA charging.

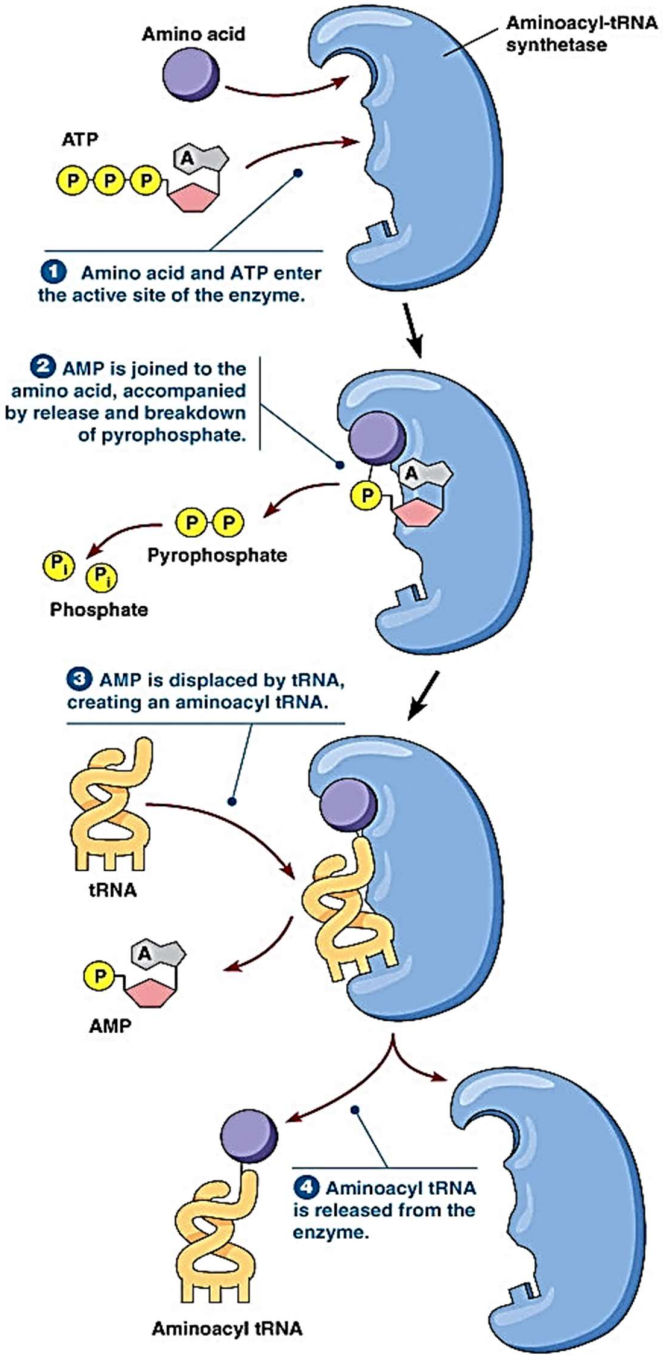


Fig. 2.1.3: Amino acid activation

Retrivel practice: Recall from Topic 1.1 Organelles and Cellular Structures

2.2 Structure and Function of Ribosomes

- Each ribosomal subunit is made up of ribosomal proteins and rRNA.

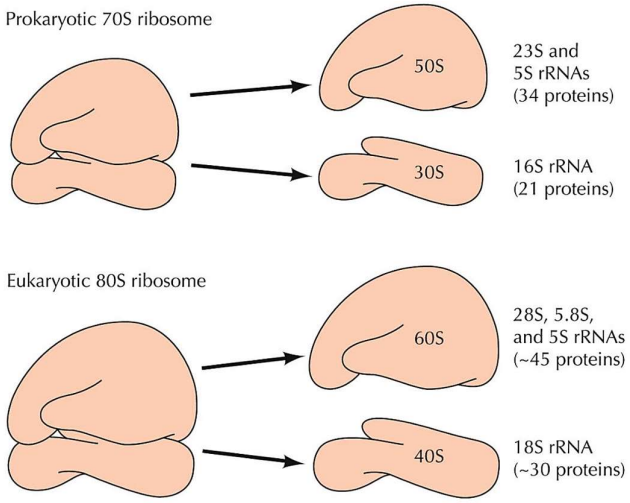


Fig. 2.2.1: Structure of ribosomes

- The large subunit of the ribosome contains three adjacent tRNA binding sites:
  - **Aminoacyl-tRNA binding site (A site)** for binding a charged tRNA molecule i.e. tRNA molecule attached to the next amino acid in the protein
  - **Peptidyl-tRNA binding site (P site)** for binding a central tRNA molecule containing the growing polypeptide chain
  - **Exit site (E site)** for the discharge of a uncharged tRNA i.e. tRNA molecule without the amino acid

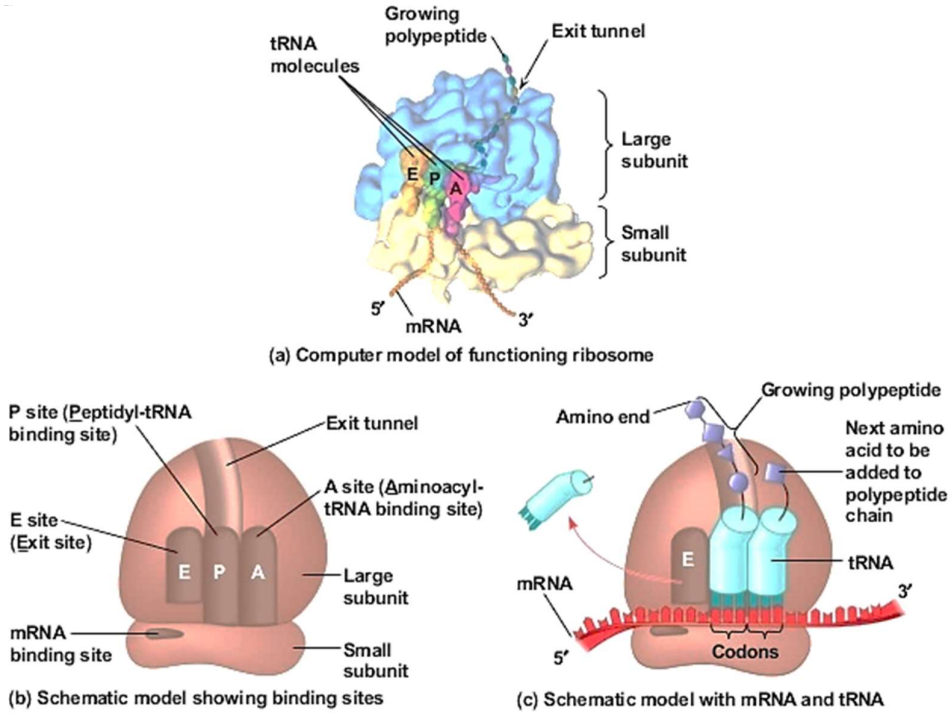


Fig. 2.2.2: Schematic Diagram showing the binding sites in a ribosome

2.3 Steps in Translation

- Translation involves the **uncoding** the **mRNA sequence** into a **sequence of amino acids** in a polypeptide chain and occurs in the **cytosol** of cells.

- Translation involves **3 steps**; Initiation, Elongation, Termination. All three stages require additional proteins to facilitate the translation process. **Energy** is also required and is provided by the **hydrolysis** of **guanosine triphosphate** (GTP).

Step 1: Initiation

1. A **small** ribosomal subunit **binds to the mRNA** at the **5' end**. Initiation factors will bind followed by the binding of a **specific initiator tRNA** carrying the amino acid **methionine** ($tRNA^{met}$).

    - ❖ In **eukaryotes**, the small ribosomal subunit binds to the **5' 7 methylguanosine cap** of the **mature mRNA**

    - ❖ In prokaryotes, small ribosomal subunit to Shine-Dalgarno sequence so that the start codon can be correctly positioned

    - Initiator tRNA with anticodon, 3'-UAC-5' carries N-formyl methionine in prokaryotes
    - Initiator tRNA with anticodon, 3'-UAC-5' carries **<u>methionine</u>** in **eukaryotes**

2. Small subunit **translocates downstream, from 5' to 3'** along the mRNA until it reads a **start** codon, **AUG**.

3. **Anti-codon** of initiator tRNA with methionine forms **hydrogen** bonds with **start** codon via complementary base pairing.

4. **Large** ribosomal subunit is recruited to complete the **translation initiation complex** with the aid of proteins known as **translation initiation factors**. A **GTP** molecule is hydrolysed to provide the **energy** for the formation of the translation initiation complex.

5. Initiator tRNA is situated in the **peptidyl-tRNA** (**P**) site of the ribosome large subunit. The **aminoacyl-tRNA (A) site is empty**.

6. Synthesis of polypeptide will be from the **initial methionine** at the **N-terminus** towards the final amino acid at the **C-terminus**, i.e. **N → C**.

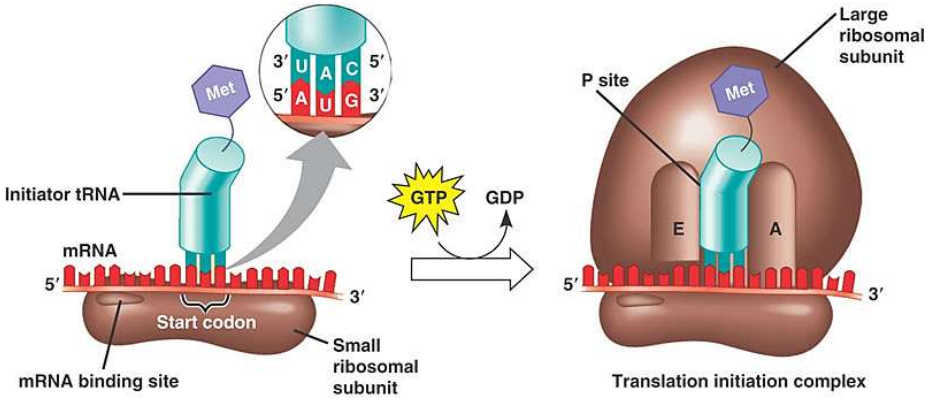


Fig. 2.3.1: Formation of translation initiation complex in eukaryotes.

Step 2: Elongation of the polypeptide chain (in both eukaryotes and prokaryotes)

7. The **anticodon** of an **incoming aminoacyl tRNA** (charged tRNA) **base pairs** with the complementary mRNA codon in the **A** site. **2 molecules of GTP** are hydrolysed to provide the energy for the **codon recognition**.

8. **rRNA** acting as the **peptidyl transferase** which is found in the **large subunit** catalyses the formation of a **peptide bond** between the **new amino acid** in the **A site** and the **carboxyl end** of the **growing polypeptide** in the **P site**.

9. The polypeptide is now attached to the **tRNA** in the **A site**. Ribosome **translocates** along the mRNA towards the **3'** end, **moving the tRNA** in the **A site** to the **P site**.

10. The **uncharged tRNA** in the P site is moved to the **exit (E) site** where it is **released**.

11. The mRNA moves along with its bound tRNAs and bring the **next codon** to be **translated** into the **A site**. **A molecule of GTP** is hydrolysed in this **translocation process**. The cycle then repeats.

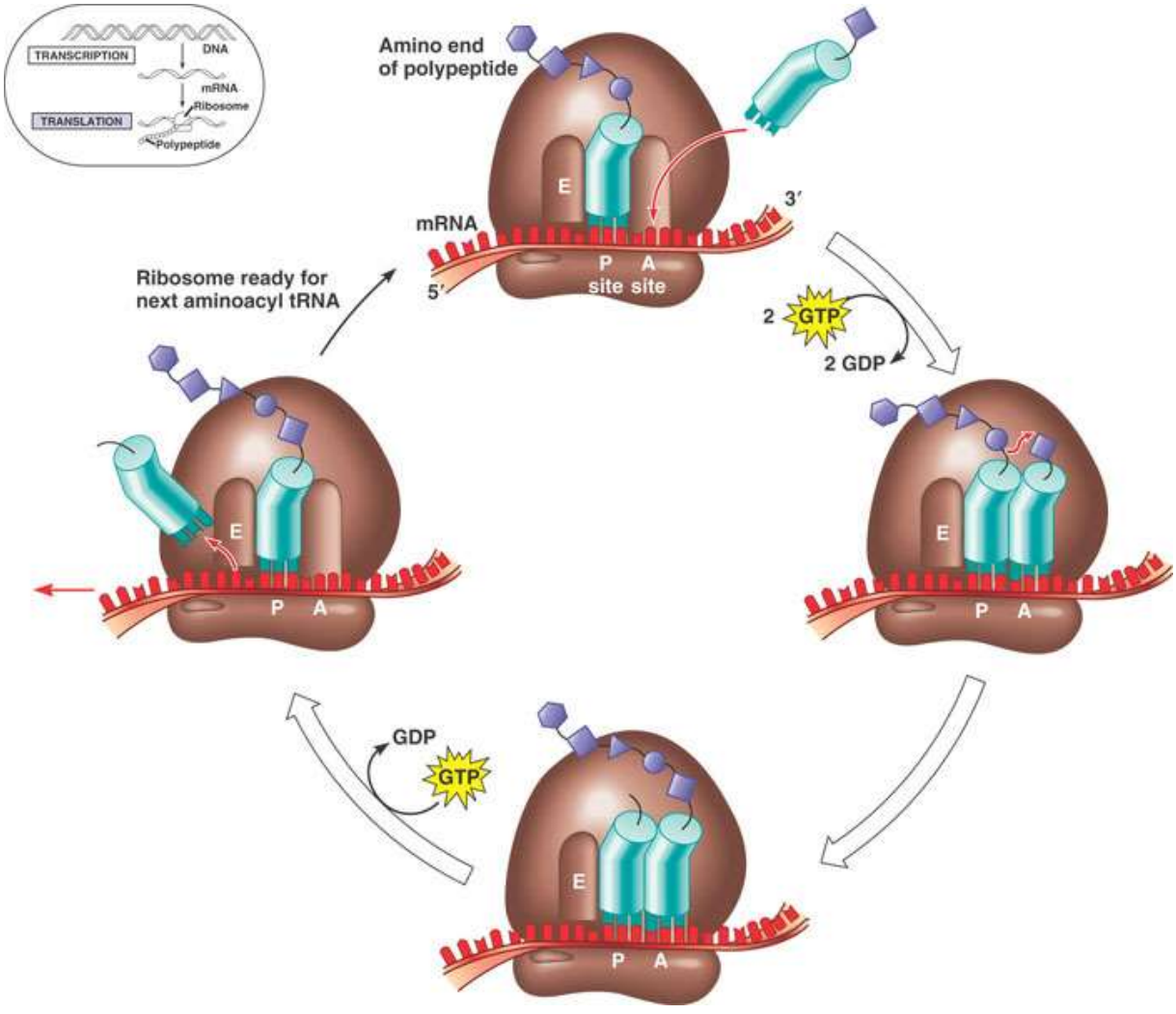


Fig. 2.3.2: Elongation during translation

Step 3: Termination (in both prokaryotes and eukaryotes)

12. The cycle repeats until a **stop** codon (UAA, UAG, UGA) in the mRNA reaches the A site.

13. A protein known as **release factor** binds directly to the **stop codon** in the **A site** and result in the addition of a **water** molecule to the polypeptide chain.

14. This results in **hydrolysis** of the **ester bond** between the **polypeptide chain** and **adenosine** at the **3'-end of tRNA** in the **P site**. This will release the polypeptide through the **exit tunnel** of the ribosome's large subunit.

15. The two ribosomal **subunits** and the **other components** of the assembly **dissociate**.

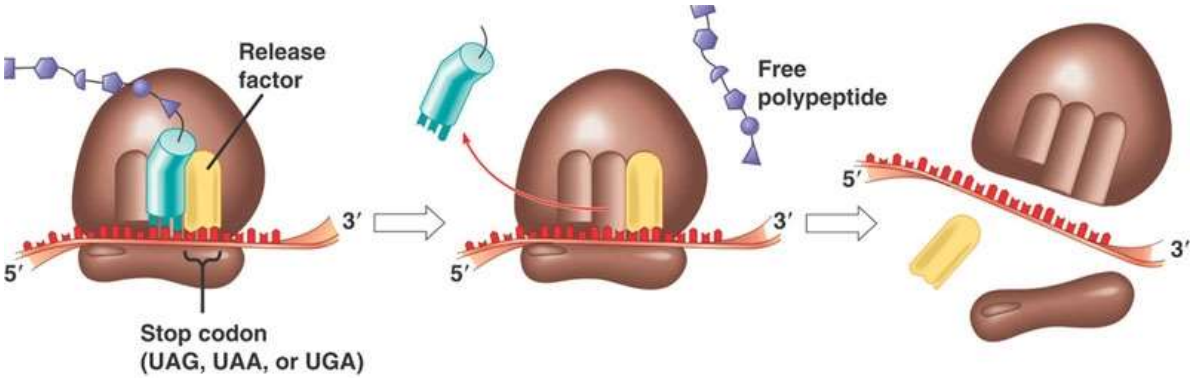
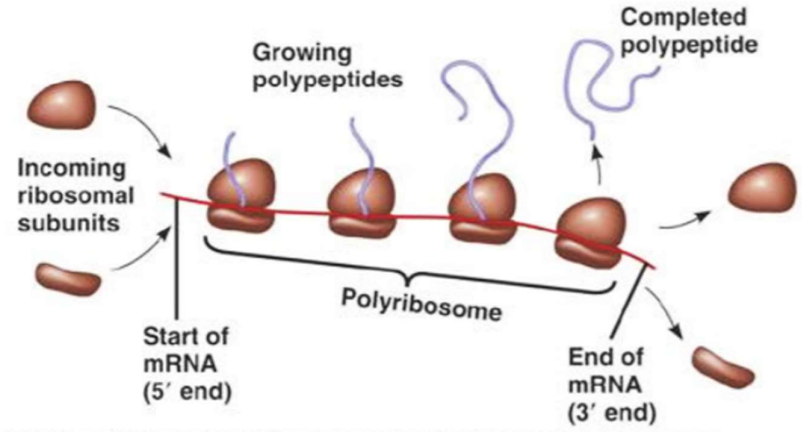


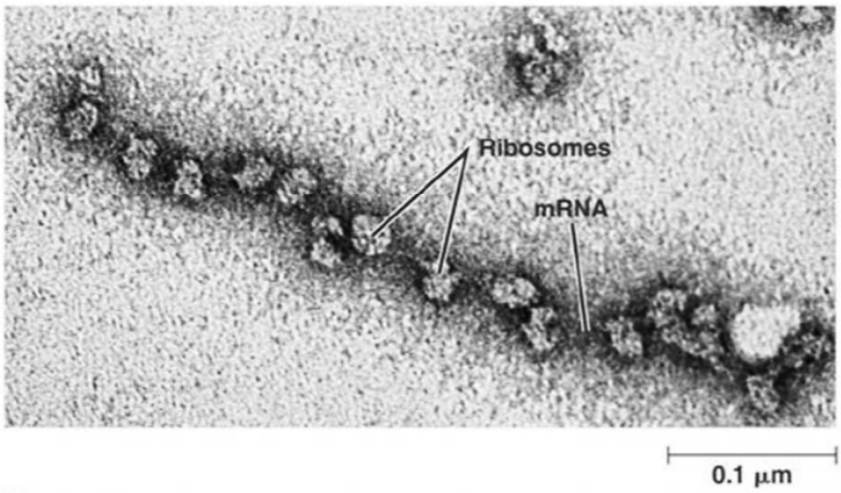
Fig. 2.3.3: Termination in translation

2.4 Polyribosomes

- Several ribosomes can associate into clusters known as **polyribosomes** in both prokaryotic and eukaryotic cells.
- Polyribosomes translate the **same** mRNA, producing **multiple** copies of the **same** polypeptide.



(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).

Fig. 2.4.1: Polyribosomes

Checkpoint 3

Fill in the blanks provided on translation process.

1 State the

(a) product of transcription

(i) carries genetic information from DNA .....

(ii) carries an amino acid .....

(iii) is a component of ribosomes .....

(b) product of translation .....

(c) enzyme needed in translation .....

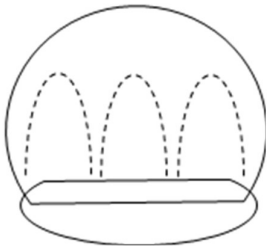
(d) location of translation .....

2 Using the genetic dictionary which contain the genetic code, list the amino acids coded by the following mRNA.

5' AUGCUCACUUUUUUCGGG 3'

.....

3 In the outline of the ribosome provided, label the exit site (**E**), the amino acyl site (**A**) and the peptidyl site (**P**).



Learning Outcome (j):

Explain how differential (i.e. spatial and temporal) gene expression in eukaryotes can be regulated at different levels:

iv. Translational level (half-life of RNA and initiation of translation)

3 Regulation at Translational Level

- Regulation at translational level refers to regulation of gene expression **determining level of protein synthesis**.

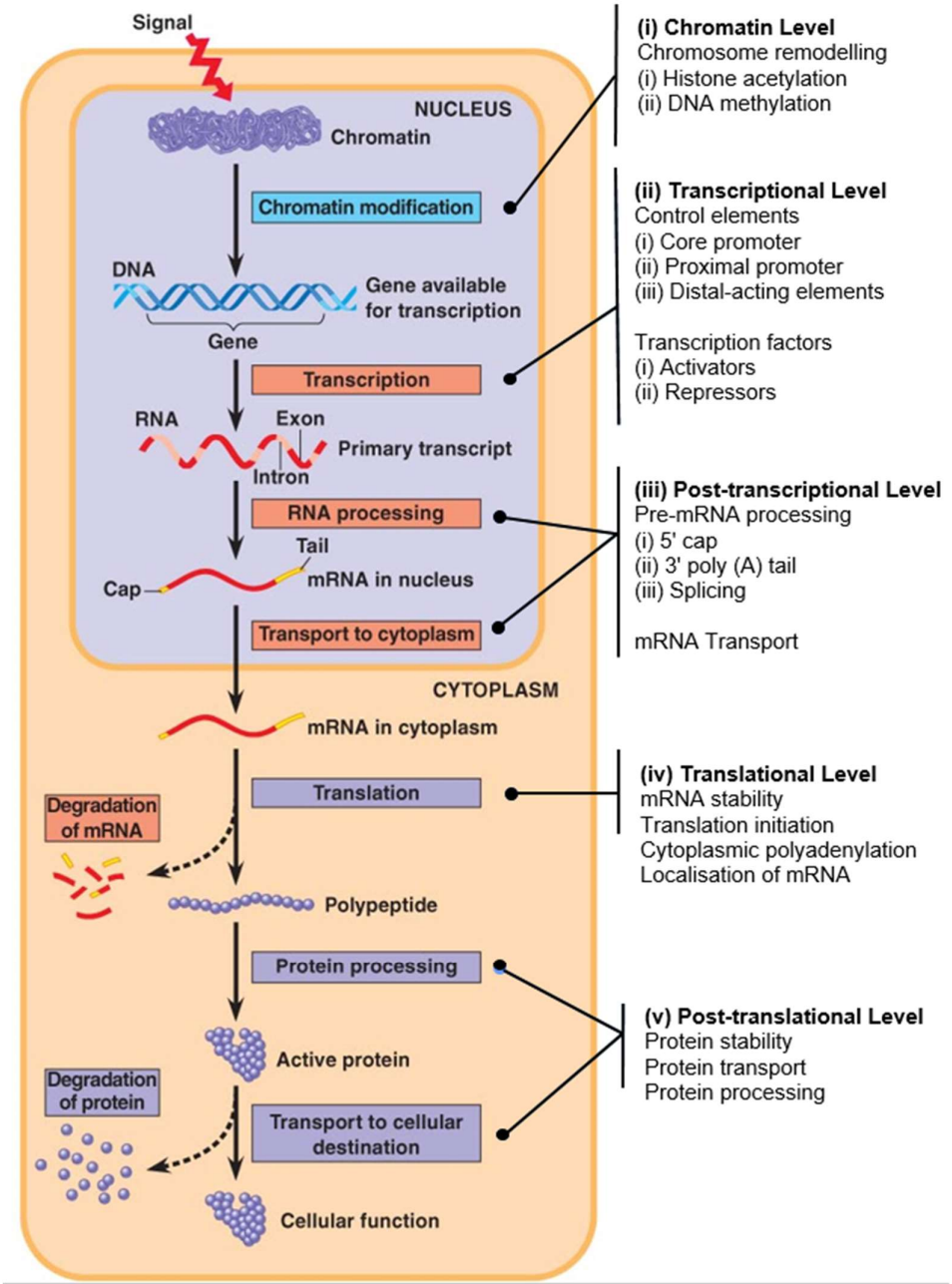


Fig. 3.1: Regulation of eukaryotic gene expression at various levels

3.1 Half-life of mRNA (mRNA Stability)

- The half-life of an mRNA molecule is the **duration it remains intact in the cell before being degraded**. This stability can significantly affect how much protein is produced from a given mRNA.
    - **Short-lived mRNAs** are **rapidly degraded**, **limiting protein production**.
    - **Long-lived mRNAs persist for extended periods**, allowing **more protein to be synthesized**.

3.1.1 Length of poly (A) tail

- mRNA stability depends on the **length of poly(A) tail.** The **longer the poly-A tail of mature mRNA**, the longer the mRNA can be used as a template for translation.

- After export from the nucleus, mRNA is protected from degradation by its **5' cap and poly(A) tail**. However, the poly(A) tail is gradually shortened by **exonucleases** in 3' to 5' direction, once it reaches the cytoplasm. Once the poly(A) tail is shortened to a critical length the mRNA undergoes degradation.

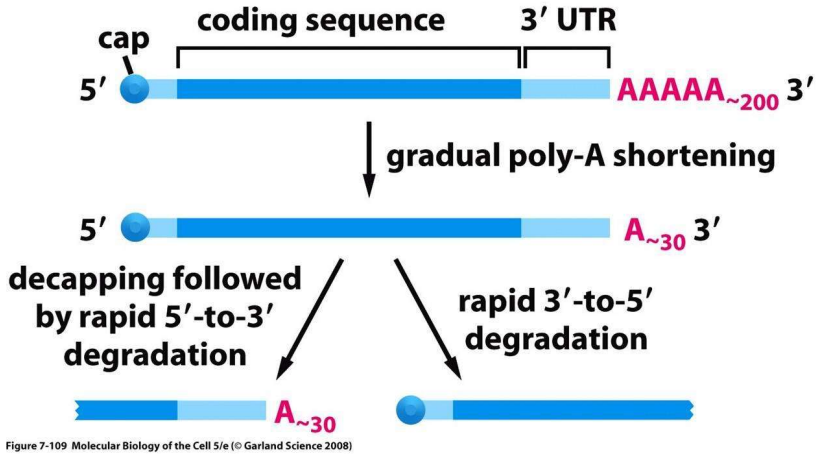


Fig. 3.1.1: mRNA degradation begins with enzymatic shortening of the poly(A) tail.

3.1.2 RNA-binding Proteins (RBPs)

- These proteins can bind to specific sequences in the mRNA, protecting it from degradation or marking it for destruction. For example, some RBPs bind to the 3' untranslated region (UTR) and stabilize the mRNA, extending its half-life.

3.1.3 AU-rich Elements (AREs)

- Sequences in the 3' UTR of mRNAs that attract proteins promoting rapid degradation. mRNAs with AREs tend to have shorter half-lives.

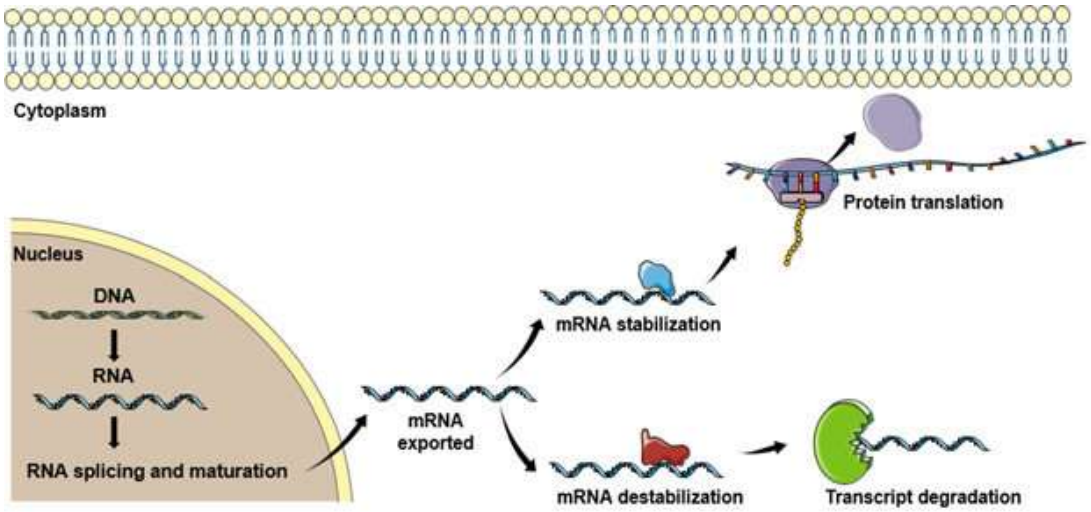


Fig. 3.1.2: Stability of mRNAs regulated by RNA-binding proteins.

3.1.4 MicroRNAs (miRNAs)

- Small non-coding RNAs that can bind to complementary sequences in the mRNA, leading to mRNA degradation or repression of translation. This interaction typically occurs in the 3' UTR of the mRNA.

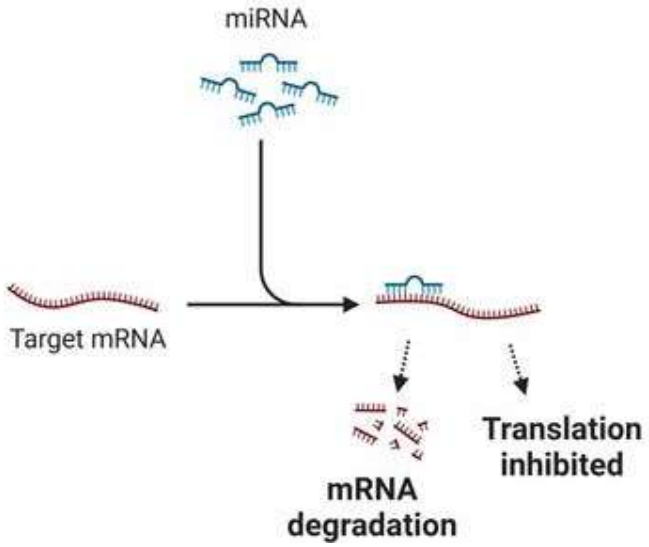


Fig. 3.1.3: Inhibition of translation by miRNA.

- Example: In red blood cells, mRNAs for haemoglobin are very stable and are translated repeatedly as the organism requires large amount of haemoglobin. However, for the mRNA for cyclin, which regulate the cell cycle, has a short half-life. This ensures that cyclins are only present when needed for cell division and are quickly degraded afterward.

3.2 Control of Translation Initiation

- The initiation of translation involves the assembly of the ribosome on the mRNA, starting at the start codon (AUG). This step is crucial for determining when and where translation begins.

3.2.1 Initiation Factors

- Proteins such as eIF2 play a critical role in the formation of the initiation complex. Phosphorylation of eIF2 can inhibit its activity, preventing the initiation of translation.

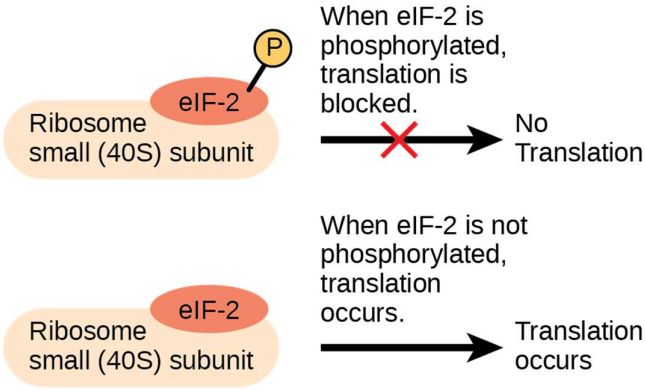


Fig. 3.2.1. eIF2 phosphorylation inhibits translation initiation.

3.2.2 Internal Ribosome

- Entry Sites (IRES): Some mRNAs have IRES elements that allow ribosomes to bind and initiate translation independently of the 5' cap structure, enabling translation under conditions where cap-dependent translation is inhibited.

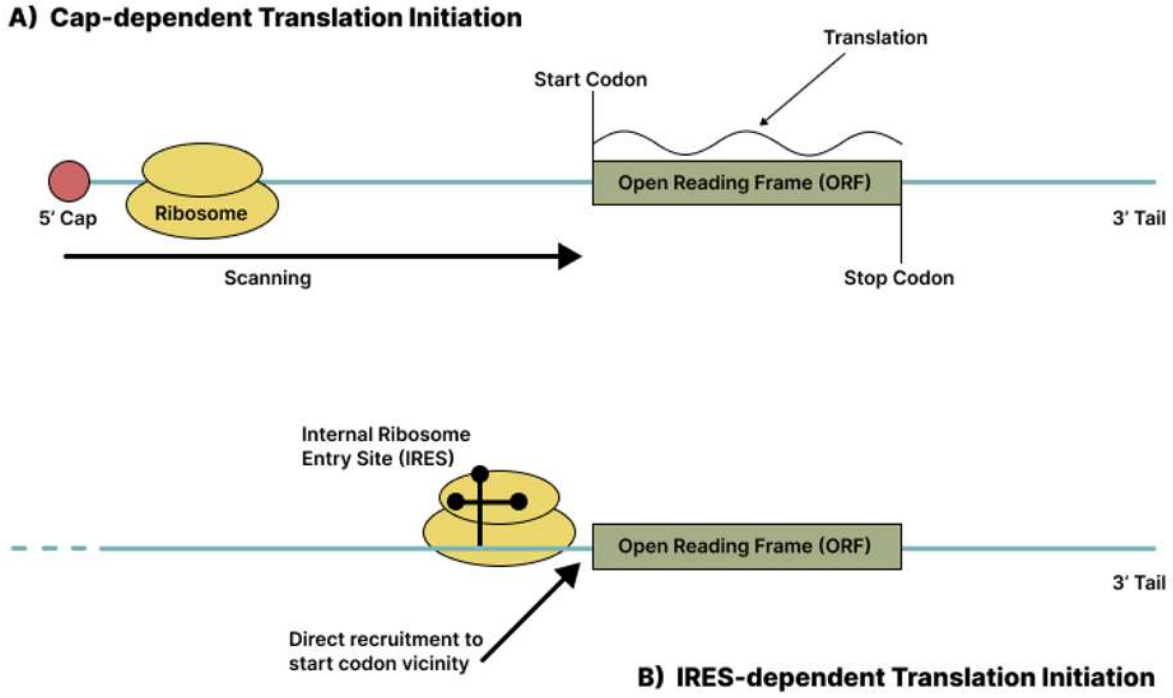


Fig. 3.2.2: Schematic diagram of (A) cap-dependent initiation of translation compared to (B) IRES-dependent initiation of translation.

3.2.3 Translational Repressors

- Proteins that bind to specific sequences or structures in the mRNA can prevent the recruitment of ribosomes. Examples include iron regulatory proteins (IRPs) that bind to iron-responsive elements (IREs) in the mRNA and inhibit translation under low iron conditions.

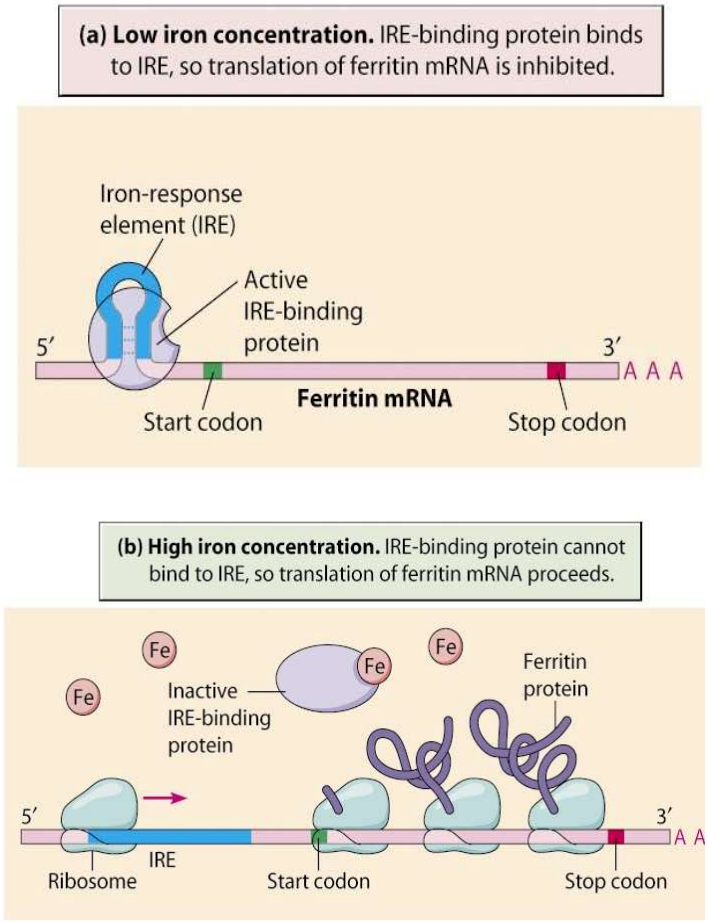


Fig. 3.2.3: Regulation of expression of ferritin by concentration of iron.

3.2.4 Secondary Structures in the 5' UTR

- The presence of secondary structures, such as hairpins or stem-loops, in the 5' UTR can impede ribosome scanning and reduce translation efficiency. The melting of these structures, often facilitated by RNA helicases, is necessary for efficient translation.

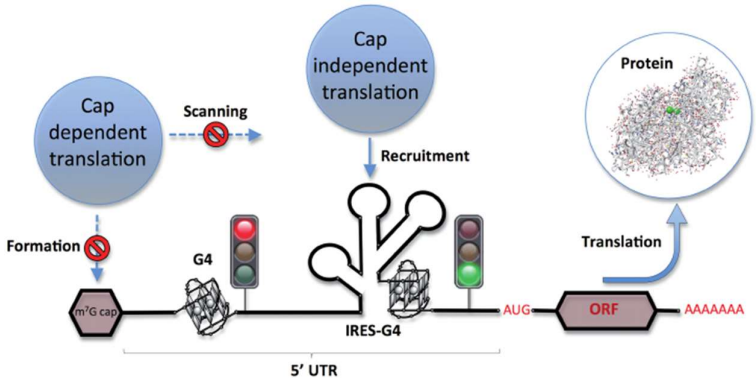


Fig. 3.2.4 Schematic illustration of the hairpin loop structure in 5'-UTR block cap-dependent translation and facilitates cap independent translation via IRES.

Learning Outcome (j):

Explain how differential (i.e. spatial and temporal) gene expression in eukaryotes can be regulated at different levels:

v. Post-translational level (biochemical modification and protein degradation)

4 Regulation at Post-translational Level

- Once a protein is synthesized, it can undergo various chemical modifications that influence its activity, stability, localization, or interactions with other molecules. These modifications are essential for the **functional diversity of proteins** and **protein's concentration** after translation.

4.1 Biochemical Modification

4.1.1 Phosphorylation

- Addition of a phosphate group to serine, threonine, or tyrosine residues by kinases. Phosphorylation can activate or deactivate enzymes and other proteins.

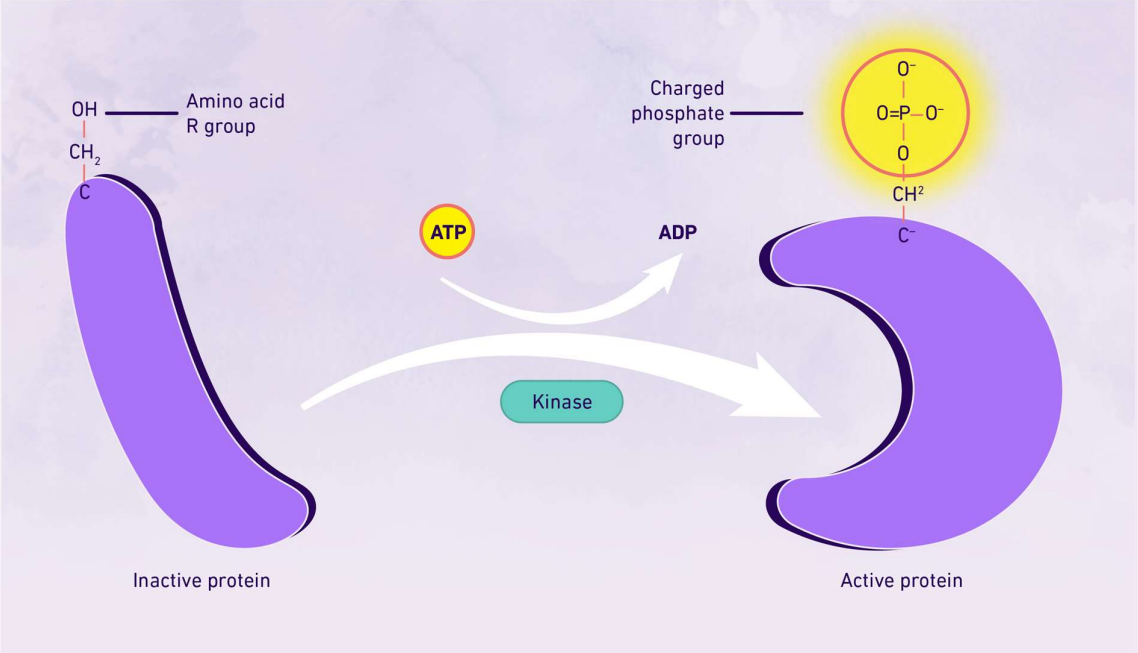


Fig. 4.1.1: Protein phosphorylation.

4.1.2 Glycosylation

- Addition of sugar molecules to asparagine, serine, or threonine residues. Glycosylation can affect protein folding, stability, and cell surface localization.

4.1.3 Ubiquitination

- Addition of ubiquitin to lysine residues, often marking the protein for degradation by the proteasome.

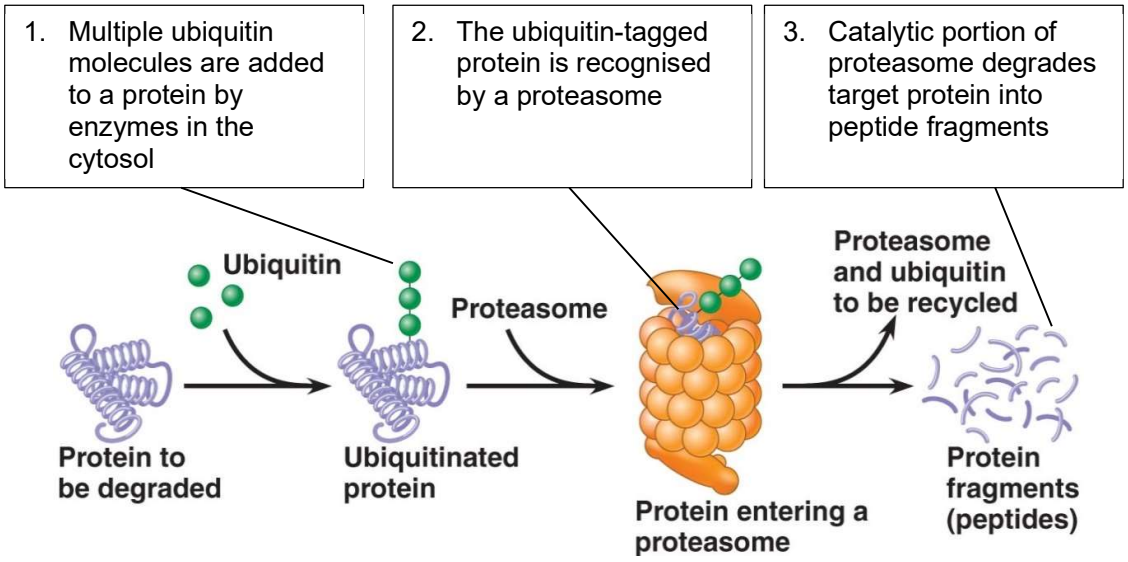


Fig. 4.1.2: Ubiquitinylated proteins are destined for degradation by the proteasome

4.1.4 Acetylation and Methylation

- Addition of acetyl or methyl groups to lysine residues, often affecting protein interactions and gene expression.

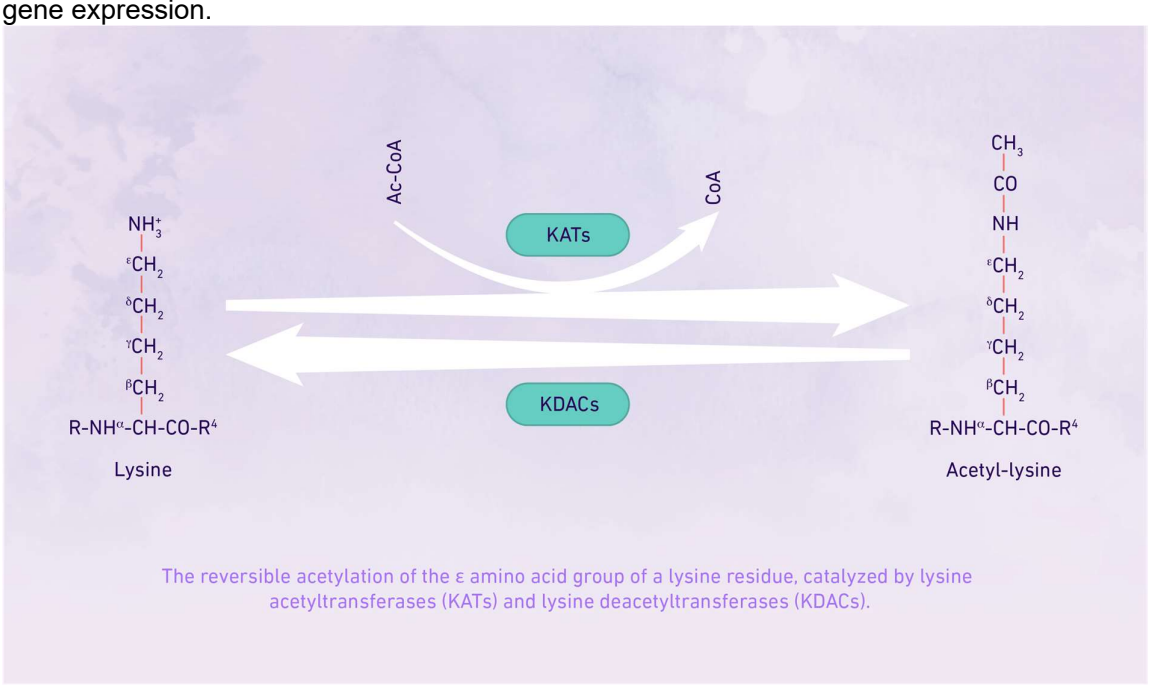


Fig. 4.1.3: Acetylation of a lysine side chain.

- Example: The tumor suppressor protein p53 is regulated by phosphorylation and acetylation, which affect its stability and ability to activate target genes involved in cell cycle arrest and apoptosis.

4.2 Protein Degradation

- Protein degradation is a selective process that removes damaged, misfolded, or unneeded proteins, maintaining cellular homeostasis.

4.2.1 Ubiquitin-Proteasome System (UPS)

Proteins destined for degradation are tagged with ubiquitin molecules. The ubiquitinated proteins are recognized and degraded by the proteasome, a large proteolytic complex.

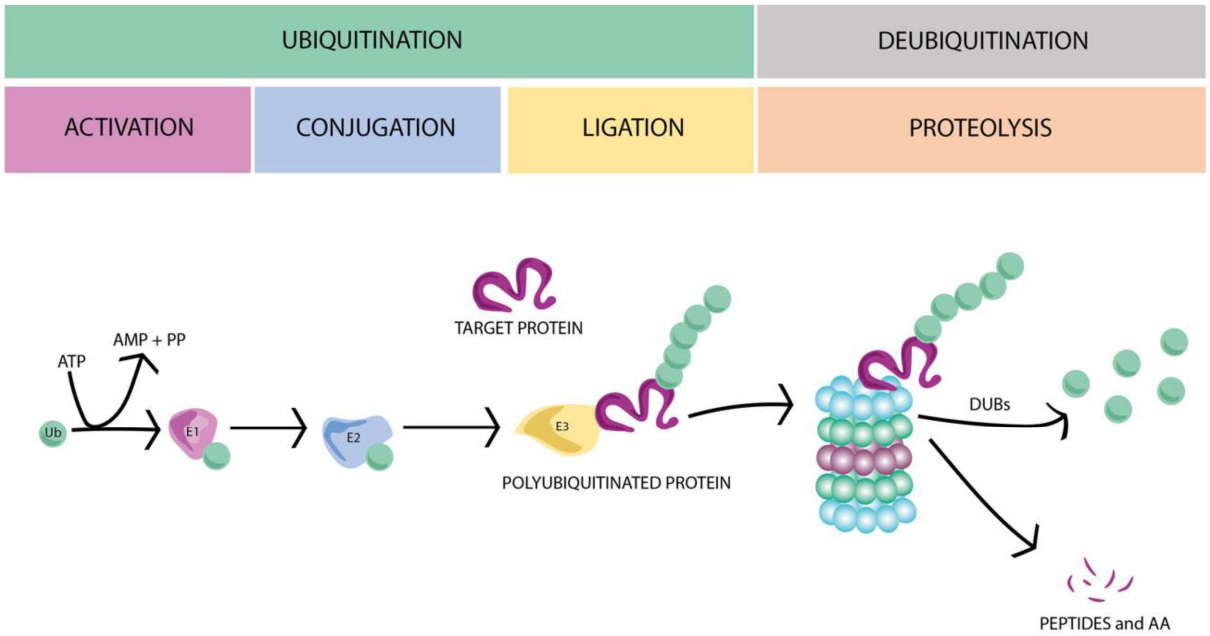


Fig. 4.2.1: The ubiquitin–proteasome system (UPS). Ubiquitin-activating enzyme (E1) activates the ubiquitin (Ub) through an ATP-dependent reaction. Then, the activated ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2) and to the target protein by an E3 ubiquitin ligase. Following several rounds of ubiquitination, the polyubiquitinated protein is recognized by the proteasome and degraded to small peptides and amino acids (AAs). To finish the UPS cycle, the polyubiquitin molecules are disassembled by deubiquitinating enzymes (DUBs) and recycled for new rounds of ubiquitination.

4.2.2 Autophagy

Cellular components, including proteins, are enclosed in autophagosomes and delivered to lysosomes for degradation. This process is crucial for recycling cellular materials during starvation or stress.

4.2.3 Lysosomal Degradation

Some membrane proteins and extracellular proteins are internalized and delivered to lysosomes for degradation.

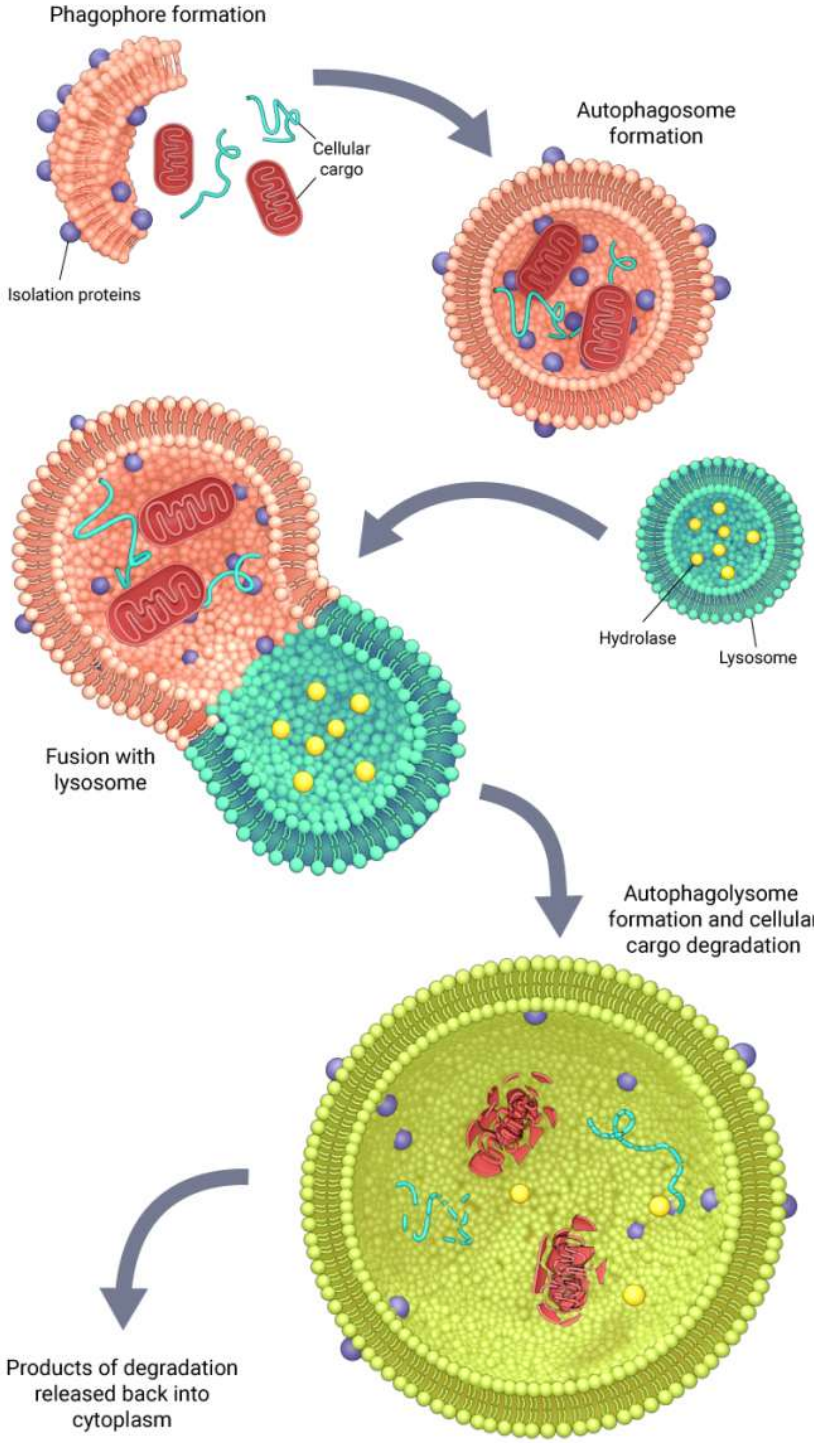


Fig. 4.2.2: Schematic depicting the mechanism of autophagy

CHECKPOINT 4

For each statement, indicate if it is true (**T**) or false (**F**).

1 Brain cells contain insulin gene. _____

2 Introns are non-coding sequences. _____

3 Introns are found in genes. _____

4 Introns consist of nucleotide base sequences. _____

5 No amino acids would be added to the polypeptide chain if a ribosome reads introns which are not removed during splicing. _____

6 The telomere codes for telomerase. _____

7 Centromere is a protein complex which spindle fibres attach to during nuclear division. _____

8 A dysregulation in gene expression may lead to cancerous growth. _____

9 Histidine modification can influence the rate of transcription in eukaryotes. _____

10 RNA polymerase binds to promoter sequence in eukaryotes but binds to operator sequences in prokaryotes. _____

11 A silencer is a gene. _____

12 Enhancers code for activators. _____

13 mRNAs are degraded in the cytoplasm as a form of control to limit the rate of translation. _____

14 The rate of translation initiation can be varied to regulate the amount of protein formed. _____

15 The number of ribosomes in a cell can be varied to regulate the amount of proteins synthesized in a cell. _____

16 All somatic cells in an organism contain the same set of genes regardless of their function. _____

17 Which of the following describes a form of post-transcriptional gene regulation?

A degradation of polyA tail

B methylation of DNA

C splicing of 5' cap

D mRNA editing _____

(i) Cis-acting Elements
1. Core promoter
2. Proximal promoter
3. Distal-acting elements

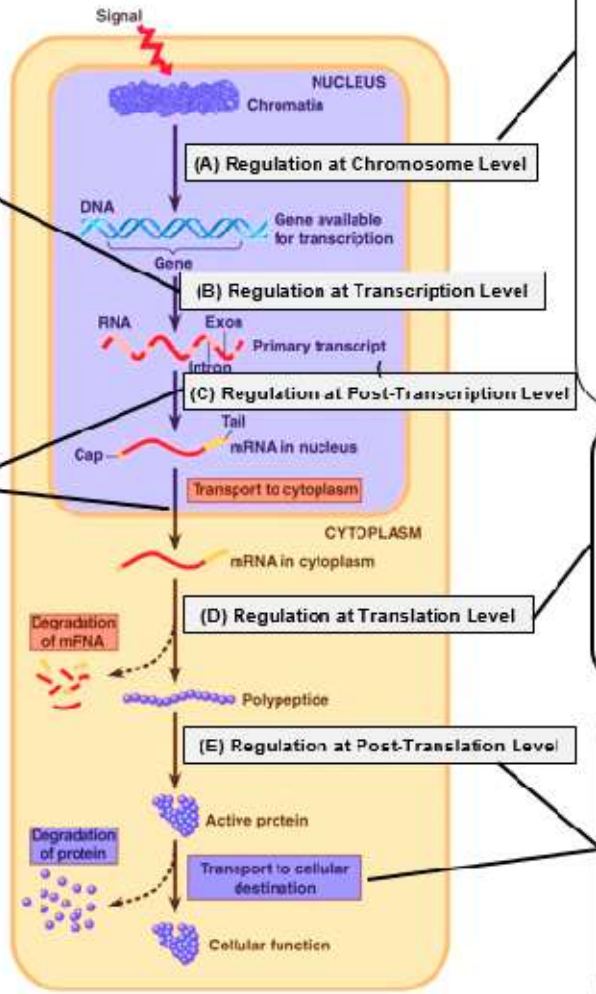
(ii) Trans-acting Elements
1. Basal TFs
2. Specific TFs (Activators, Repressors)

(iii) DNA Looping

(i) Pre-mRNA Processing
1. 5' cap involves the addition of a methylated G at the 5' end
2. 3' poly(A) tail is formed by adding 100 to 200 A to the 3' end
3. Splicing can be include all exons (constitutive) or some exons (alternative) carried out by ribozymes or spliceosomes

(ii) mRNA Transport

(iii) mRNA Editing



(i) Gene Amplification
Specific DNA segments are repeatedly replicated to produce multiple copies of a particular gene

(ii) Chromosome Remodelling

1. **Histone Acetylation**
Acetylation or deacetylation of histone tails by HATs or HDACs ➡ more relaxed or compact chromosome conformation ➡ ↑ing or ↓ing transcription.

2. **DNA Methylation**
Methylation of CpG islands by DNA methyltransferase ↓ transcription rates of genes nearby

1. mRNA Stability
Half-life of mRNA molecule can be lengthen or shortened by ↑ing or ↓ing the poly(A) tail

2. Gene Silencing
mRNA can be degraded or translation can be inhibited by siRNA or miRNA that is complexed with RISC.

1. Protein Stability
Proteins targeted for proteolytic degradation are ubiquitylated, then recognised and degraded by proteasome.

2. Protein Transport
Proteins with specific signals can be retrieved from the GA back into the rER

3. Biochemical Modification
Many proteins need to be modified before becoming functional e.g. preproinsulin → proinsulin → insulin