Control of Prokaryotic and Eukaryotic Genomes II

1. Introduction

If you extract the genome from two different cells of an organism and analyse them, you will find that they are identical. They contain the same number and type of genes. We know that the expressed proteins determine the specific function of the individual different cells. By extrapolation, this means that if all the genes were expressed into functional proteins in each cell, there should be no difference in how these two cells should look or function. How then do both cells have distinct characteristics and specific functions? We will look at the concept of gene regulation to derive the answer in part 2 of our lecture series.

In order to understand the gene regulation mechanisms in both the eukaryotic and prokaryotic genomes (with the focus on eukaryotic genome control as outlined in LO2j), a clear understanding of the mechanisms of gene expression in both must be first learnt. Thus, a snapshot of the overarching structure of this lecture series is as such:

1. Brief overview of gene expression in Prokaryotes and Eukaryotes
2. The various stages of gene expression in Eukaryotes and the associated gene regulation mechanisms involved (covered in greater detail).
3. The various stages of gene expression in Prokaryotes and the associated gene regulation mechanisms involved (covered in less detail).
4. A summary table comparing the control of Prokaryotic and Eukaryotic Genomes (to consolidate understanding and as a side-by-side reference)

2. Learning Outcomes

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

i. chromatin level (histone modification and DNA methylation)
ii. transcriptional level (control elements, such as promoters, silencers and enhancers, and proteins, such as transcription factors and repressors)
iii. post-transcriptional level (processing of pre-mRNA in terms of splicing, polyadenylation and 5' capping)
iv. translational level (half-life of RNA and initiation of translation) post-translational level (biochemical modification and protein degradation)

3. References

Campbell, N.A. and Reece, J.B. (2008). Biology, 8th edition. Pearson.

4. Organisation of Lecture Content

5. 4 Gene Regulation

Notes to self

Gene expression refers to the t**ranscription of a gene to synthesize a functional RNA and/or protein product.**

Gene regulation in all organisms including prokaryotes and eukaryotes is crucial. This means that prokaryotes and eukaryotes must regulate which genes are expressed and how high/ low the frequency of their expression must be at any given time.

The control of gene expression/ gene regulation thus refers to the **regulation of the amount and timing of appearance of the functional gene product**.

Question: Why is it important to control gene expression?

- Control of gene expression is important due to several reasons.
    - Some genes are expressed in **all the cells all the time.** These genes are said to be **constitutively expressed**.
        - These are essential genes that code for proteins responsible for metabolic functions (e.g. respiration) common to all cells.
    - A cell must be **responsive to changes in the environment**. They therefore must have the ability to **continuously turn genes on and off in response to circumstances and demands**.
        - E.g. The lac operon, in which some enzymes involved in lactose metabolism are expressed by *E Coli* only in the presence of lactose and absence of glucose.
    - Cellular differentiation: Different cell types need to synthesize **different sets of proteins** in order to be **specialized (**having **specific structures and functions**).
        - Although all cells within a multicellular organism have the same genome (as they were derived from a single zygote), they have specific functions. This is because they **express different genes at a specific time during development (temporal) or within a specific tissue (spatial),** resulting in **different proteins** produced.
        - This **spatial and temporal regulation** is called **differential gene expression.**

Notes to self

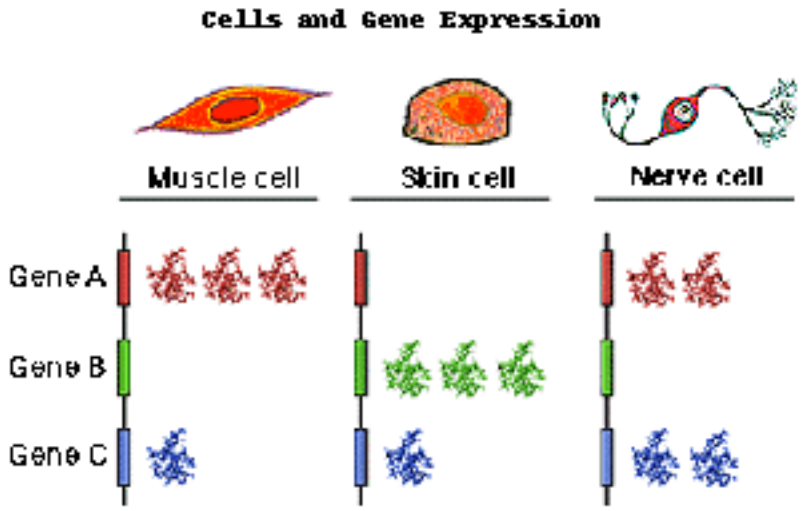


Figure 1. Differential Gene Expression in individual cells of an organism.
At any given point in time, the type of genes and quantity of gene products expressed in each cell type differs. Note: Gene C is likely an essential or "housekeeping gene" as it is expressed in all cell types shown.

- Regulation of gene expression can occur at the following levels:
  - **Genome**
  - **Transcriptional**
  - **Post- transcriptional** (RNA processing applies to Eukaryotes only)
  - **Translational** (Eukaryotes only)
  - **Post- translational** (Eukaryotes only)

- Each of these key stages represents a **potential control point** where the expression of a particular gene can be regulated i.e. increased or decreased expression. Hence, **not every stage needs to be activated** during regulation.

- Figure 2 shows a schematic diagram of the main levels of gene expression and potential regulation points, using a eukaryotic cell as an example.

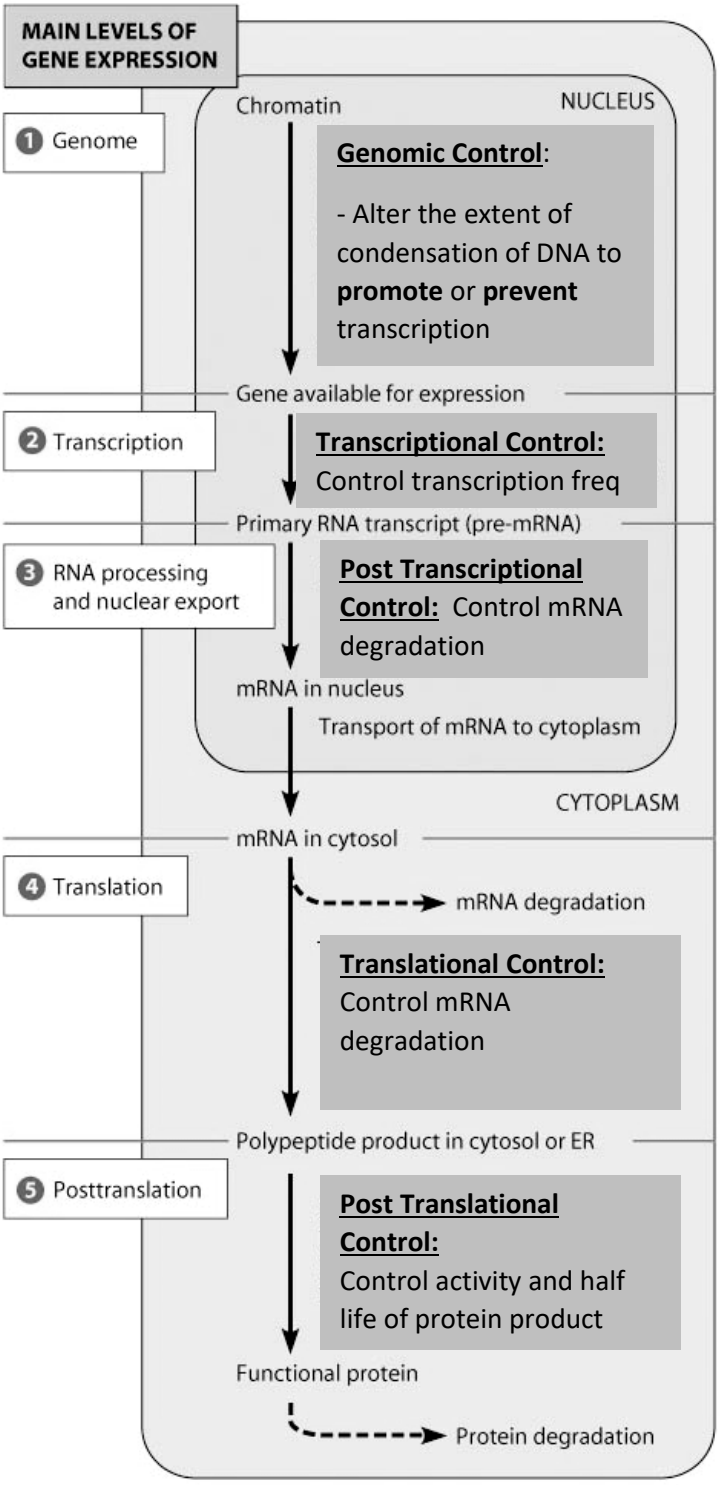


Figure 2.

Five key stages at which eukaryotic gene expression can be regulated

6. Brief Overview of Transcription and Translation in Prokaryotes and Eukaryotes

Transcription	Eukaryotes	Prokaryotes
Initiation	<u>Promoter recognized by general transcription factors</u> 1) General / basal transcription factors recognise and bind to the TATA box of the promoter. 2) Recruitment of RNA polymerase. 3) Transcription initiation complex (TIC) is formed.	<u>Promoter recognized by sigma factor</u> 1) Sigma factor binds to core RNA polymerase to form RNA polymerase holoenzyme. 2) RNA polymerase holoenzyme scans along the DNA, and its sigma factor recognizes and binds to the promoter elements at both the -10 and -35 sequences.
Elongation and termination	RNA polymerase unwinds and separates the DNA double helix and transcribes the template strand from the start point of transcription to the termination sequence.	
Post transcriptional modification	**Eukaryotes**	**Prokaryotes**
Process	1) Addition of 5'cap to pre-mRNA 2) Splicing - the process where introns (non-coding regions within a gene) are excised and exons (coding regions within a gene) are joined together. 3) Addition of poly A tail at 3'end of pre-mRNA A mature mRNA is formed.	None
Translation	**Eukaryotes**	**Prokaryotes**
Initiation	1) Small ribosomal subunit, eukaryotic translation initiation factors and initiator-tRNA form a complex. 2) Complex from 1) scans the mRNA to locate the start codon, AUG. 3) Binding of the large ribosomal subunit completes the translation initiation complex.	1) Translation initiation factors bind to the small ribosomal subunit and facilitate the binding of the small ribosomal subunit to Shine-Dalgarno sequence on the mRNA. 2) Initiator tRNA then binds to start codon, AUG, on mRNA 3) Large ribosomal subunit binds.
Elongation and termination	The ribosome continues to translate the remaining codons on the mRNA until the ribosome reaches a stop codon on mRNA (UAA, UGA, or UAG). Termination occurs when one of the stop codons enters the Aminoacyl-tRNA (A) site. These codons are not recognized by any tRNAs. Instead, they are recognized by release factors which trigger the eventual hydrolysis of the bond between the polypeptide and the tRNA.	

7. Eukaryotic Gene Expression and Regulation

Notes to self

A. At the Genome Level

A1 Chromatin Modification

- Organization of DNA into chromatin helps to:
  (a) Pack DNA into a compact form that fits inside the nucleus of a cell.
  (b) Regulate gene expression - physical state of DNA in / near a gene determines if the gene is **accessible** for transcription.

- Chromatin modification functions as a coarse control of gene expression by **making regions of DNA more or less accessible for transcription through the enzymatic addition or removal of chemical groups** from chromatin.

- Some sections within a chromosome are more compact than others. Chromatin is a dynamic structure that alternates between two states:

  (a) **Heterochromatin**
  - **Highly compacted** form of DNA where DNA winds **more tightly** around histones.
  - Genes are **not usually expressed**/ are **silenced** as **RNA polymerase and transcription factors** are **unable to access and bind to the promoter**.
  - Hence, **prevents** the formation of the **transcription initiation complex**.

  (b) **Euchromatin**
  - **Less compacted** form of DNA where DNA winds **less tightly** around histones.
  - Genes are **usually expressed** as RNA polymerase and transcription factors **are able to access and bind to the promoter**.
  - Hence, **allowing** the formation of the **transcription initiation complex**.

- Three key processes of chromatin modification include:
  - Chromatin modifying complex
  - DNA methylation
  - Histone acetylation / deacetylation

A1.1 Chromatin remodelling complex

Notes to self

- **REGULATION**

  Chromatin remodeling complex are protein complexes that **alter structure of nucleosomes** temporarily:

  (a) Results in **DNA being less tightly bound to histones**
  - Allows RNA polymerase and transcription factors involved in gene expression to access the promoter to **initiate transcription**.

  (b) Can also result in **DNA being more tightly coiled around histones**
  - Prevents RNA polymerase and transcription factors involved in gene expression from accessing the promoter, thereby **blocking transcription**.

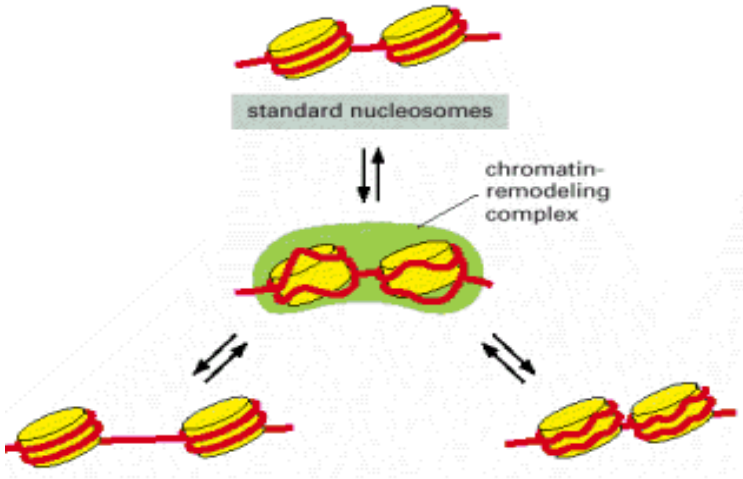


Figure 3: Chromatin remodeling complex and their effects on nucleosomes

A1.2 DNA Methylation

Notes to self

- DNA methylation refers to the **chemical attachment of methyl groups (-CH_3) to selected cytosine (C) nucleotides located in the sequence CG**.
- This process is catalysed by **DNA methyltransferases**.
- DNA methylation is associated extensively within heterochromatin. In some species, it is essential for the **long-term and usually permanent inactivation of genes** during cellular differentiation.

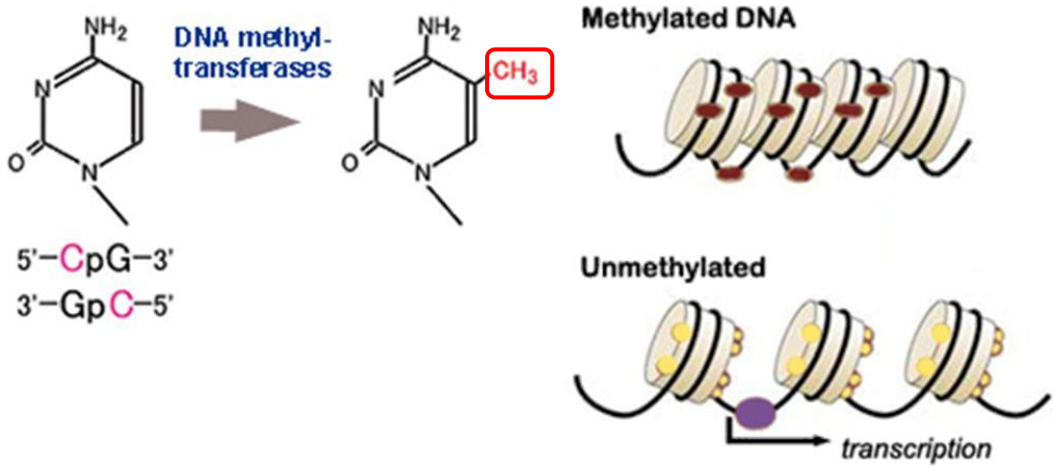


Figure 4: DNA methylation on selected cytosine residues result in inactive highly compacted DNA

- **REGULATION**

DNA methylation usually **prevents transcription** by:

(a) **Blocking binding of transcription factors** and hence, preventing assembly of transcription initiation complex at promoter.

(b) **Recruiting DNA-binding proteins** (i.e. transcriptional **repressors**, **histone deacetylases** and repressive chromatin remodeling complexes) to the methylated DNA to condense chromatin
- Results in **gene silencing/ no gene expression**.

A1.3 Acetylation/Deacetylation of Histones

Notes to self

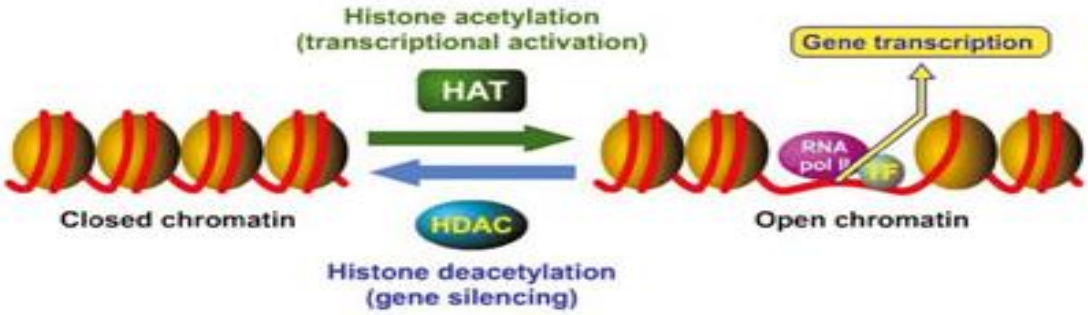
- **Histone <u>acetylation</u>** and **<u>deacetylation</u>** allows **<u>chromatin to decondense and condense</u>**, respectively, thus alternating between loose and tightly condensed states. Gene expression can then be regulated.

- **<u>REGULATION</u>**
  Acetylation of histones is catalysed by **<u>histone acetyltransferase (HAT)</u>**:

  – **<u>Addition of acetyl groups</u>** (-$COCH_3$) to lysine residues **<u>removes positive charges</u>** on histones.
  – This decreases the electrostatic interactions between negatively charged DNA and positively charged histones.
  – Tight binding between DNA and histones is loosened, making the **<u>promoter region more accessible to RNA polymerase and general transcription factors</u>**.
  – Acetylation works together with chromatin remodeling complex, **<u>allowing formation of the transcription initiation complex resulting in transcription</u>**.

- **<u>REGULATION</u>**
  Deacetylation of histones is catalysed by **<u>histone deacetylase (HDAC)</u>**:

  – **<u>Removal of the acetyl groups</u>**.
  – This restores a tighter electrostatic interaction between DNA and histones, **<u>inhibiting transcription</u>**.



<u>Figure 5 Histone acetylation and deacetylation.</u>
<u>HAT</u> <u>= **H**istone **A**cetyl**T**ransferase,</u> **<u>HDAC</u>** <u>= **H**istone **DeAC**etylase.</u>

A2 Gene Amplification *Notes to self*

- Gene amplification refers to the replication of a specific gene multiple times to create **more copies of that gene**.

- **REGULATION**
  Gene amplification results in an i**ncrease in the number of copies of a particular gene of interest** instead of increasing its frequency of transcription.

  All other genes remain in normal number except for the gene of interest which exist in **high copy numnber**.

  During transcription and translation, **increased copies of mRNA** and **increased copies of the required protein** will be obtained.

  E.g. During development, the actin gene is amplified to give rise to muscle development in chicken.

B. At the Transcription Level

- Control elements are non-coding DNA segments that **transcription factors bind** to in order to regulate transcription.

- Control elements can be divided into two categories:

(a) Proximal control elements **(e.g. promoters)**
  - Non-coding DNA that usually lie directly **upstream of the transcription start site**.
  - They are bound by **general transcription factors.**

(b) Distal control elements **(e.g. enhancers and silencers)**
  - Non-coding DNA that can be **located thousands of nucleotides upstream or downstream of the gene.**
  - They are bound by other types of transcription factors known as **specific transcription factors.**

- Transcription factors are **gene regulatory proteins** required for transcription. They **bind to control elements** as well as **other transcription factors/ proteins**.

- Regulation at the transcriptional level is the **most important and most universally used control point** of gene expression.

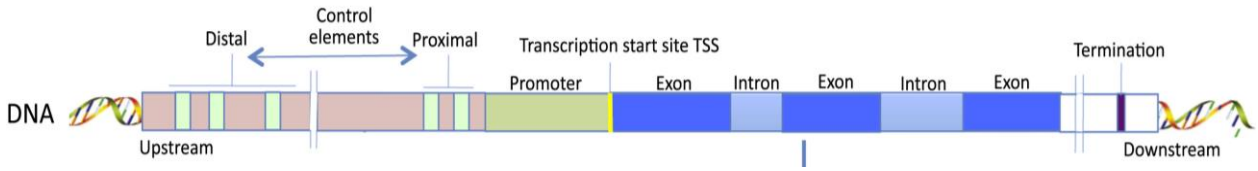


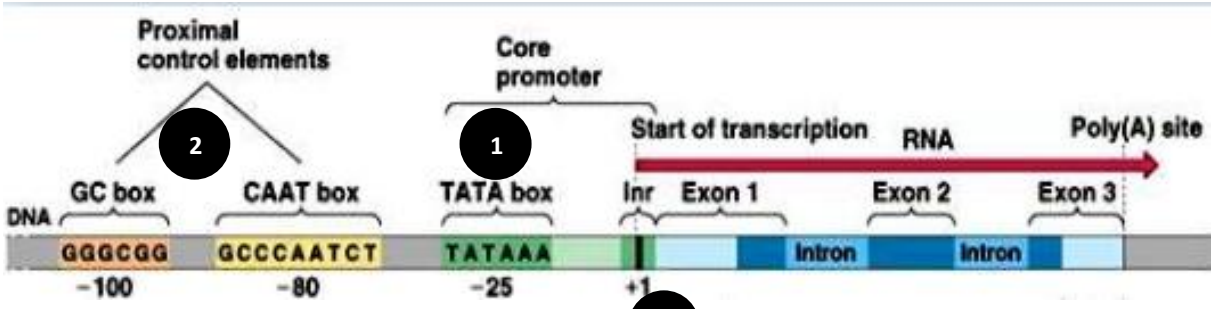
Figure 6 Eukaryotic control elements (proximal and distal)

B1 Promoter

- The **promoter** is located **just upstream of the transcription start site** of a gene.
- The promoter functions as **recognition site** for the **binding of transcription factors** and **RNA polymerase.**
- It is also therefore the **site of transcription initiation**.

Critical Elements within the Eukaryotic Promoter:

- Within the promoter, there are **critical elements/short sequences** that improve the efficiency of the promoter.
- They function to help **recruit RNA polymerase and general transcription factors** to the promoter.



1

TATA box

- Located at a relatively fixed position (at -25 site)
- Has a consensus sequence of 5'-TATAA-3'

REGULATION

- Important in determining **precise location of transcription start site**.
- Its deletion will result in transcription starting at a variety of locations,
- resulting in a truncated/ non-functional protein.

(NOTE: It is similar in function to Pribnow box (-10 element) in prokaryotic promoters)

2

CAAT box

- CCAT box commonly found at -75 site;
- May not be always present.
- Has a consensus sequence of 5'-GGCCAATCT-3'

GC box

- GC box commonly found at -90 site.
- May not be always present.
- GC can be found in multiple copies.
- Has a consensus sequence of 5'-GGGCGG-3'

REGULATION

- **Improves efficiency** of promoter, helps **recruit general transcription factors and RNA polymerase**
- Similarity of critical elements not crucial to regulation of gene expression

Figure 7 Critical elements in Eukaryotic Promoter

B2 Enhancers and Silencers

Notes to self

- Enhancers and Silencers are non-coding DNA sequences that help to alter the frequency of transcription:

  (a) **Enhancers,** when bound by **specific transcription factors** known as **activators**, **increase frequency of transcription** by promoting assembly of transcription initiation complex.

  (b) **Silencers**, when bound by **specific transcription factors** known as **repressors, decrease frequency of transcription** by inhibiting assembly of transcription initiation complex.

B2.1 Increasing Transcription Frequency: Enhancers + Activator

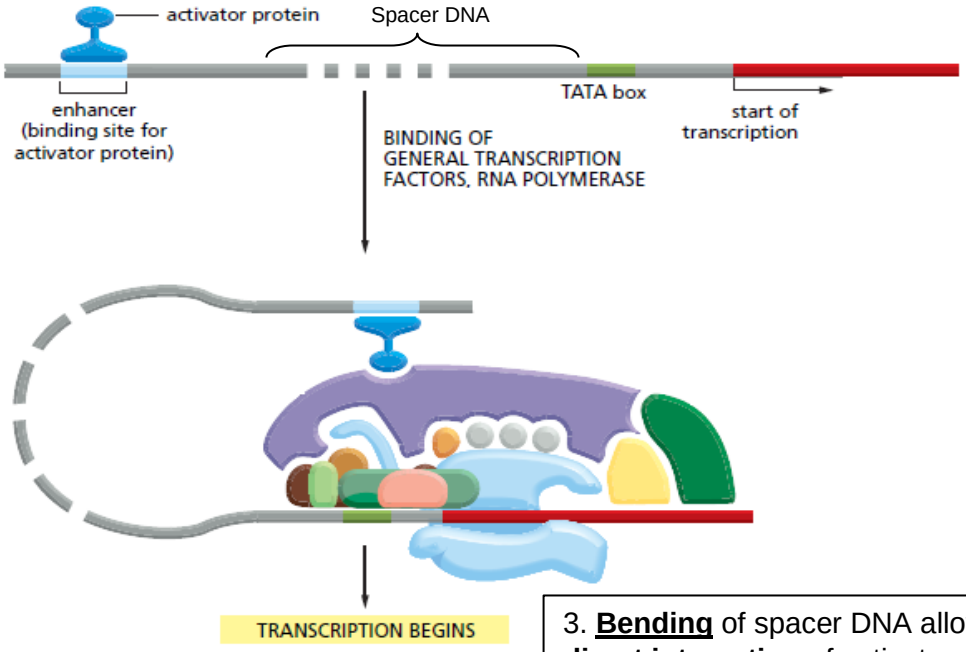
- The binding of general transcription factors and RNA polymerase to the promoter only results in a **low (basal) level of transcription**.

- **REGULATION**
- **Specific transcription factors** called **activators** recognise and bind to **enhancer sequences.**
- This results in **gene activation**, where the **frequency of transcription** of the genes they control is **increased**.
- Enhancers are **positive regulatory elements** involved in the **upregulation** of transcription as they **promote the assembly of the transcription initiation complex** via their interaction with **activators.**

- Activators bind to enhancers to increase frequency of transcription by 2 ways:
  1. Promote assembly of transcription initiation complex (TIC)
  2. Increase accessibility to promoter DNA

Mechanism 1: Promote assembly of TIC

1. Activator proteins bind to enhancer sequences in DNA.



2. **Spacer DNA** (regions of untranscribed DNA between genes) bend.

3. **Bending** of spacer DNA allows **direct interaction** of activators with RNA polymerase and general transcription factors at the promoter, promoting the assembly of TIC.

Figure 8 Activator proteins bind to enhancer region, causing DNA bending to facilitate TIC formation

Mechanism 2: Increasing accessibility to promoter DNA

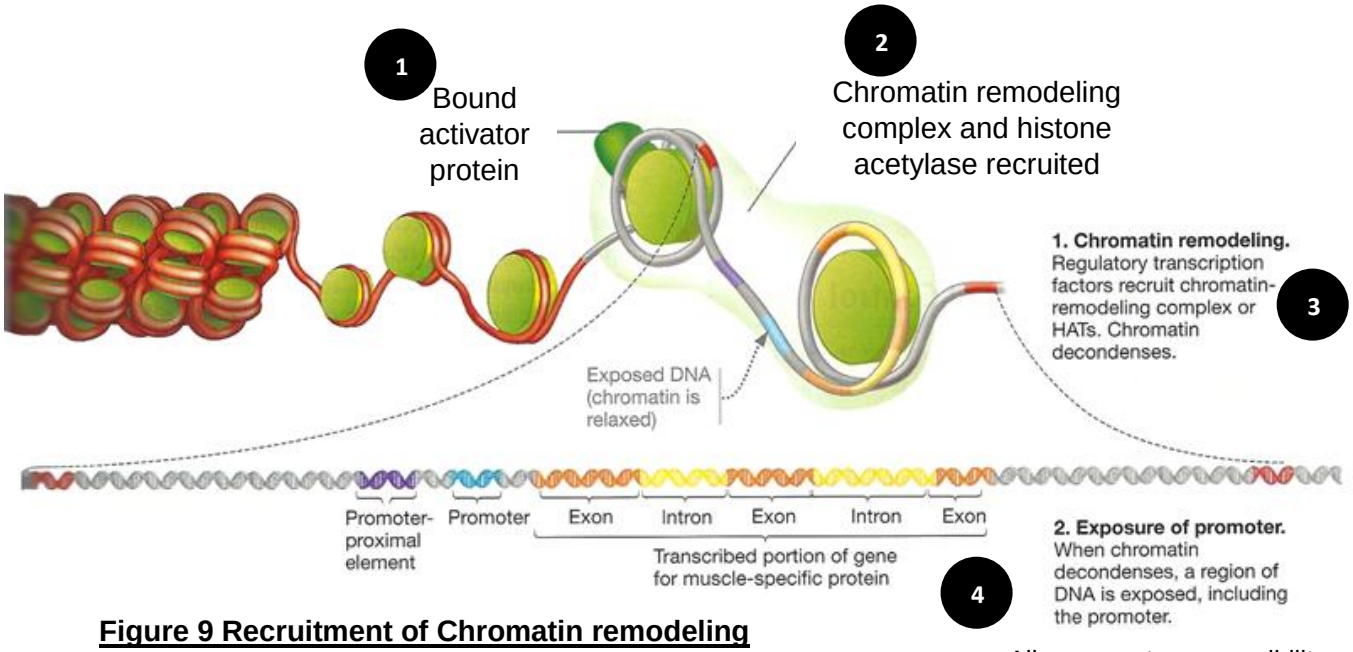


Figure 9 Recruitment of Chromatin remodeling complex and histone acetylases to decondense DNA as a result of activator binding to enhancer region

Allows greater accessibility of general transcription factors and RNA polymerase

B2.2 Decreasing Transcription Frequency : Silencers + Repressor

Notes to self

- **REGULATION**
- **Specific transcription factors** called **repressors** recognise and bind to **silencer sequences.**
- This results in **gene silencing/ repression,** where the f**requency of transcription** of the genes they control is **decreased.**
- Silencers are **negative regulatory elements** involved in the **downregulation** of transcription as they **prevent the assembly of the transcription initiation complex** via their interaction with **repressors.**

- Repressors bind to silencers to decrease frequency of transcription by these ways. Repressor proteins may:
  1. **Recruit histone deacetylases** (or other chromatin modifying enzymes), causing the DNA to bind more tightly to the histones, thus restricting access to general transcription factors and RNA polymerase.
  2. **Interact** with the **proteins at the TIC** and **prevent the correct assembly** of a functional TIC.
  3. **Interfere with the correct binding of transcription factors and activators** to DNA by **binding at or near to the promoters or enhancers** respectively.
  4. **Bind to the activator proteins** to prevent them from carrying out their function.

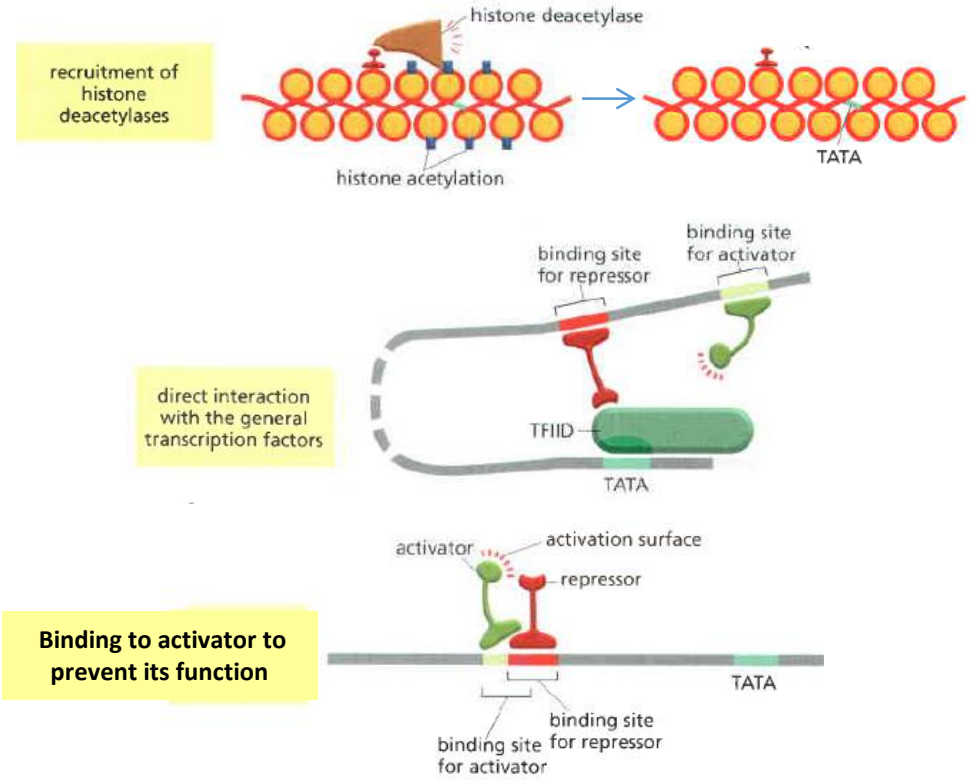


Figure 10.

Mechanisms of repressor protein and silencer interaction to decrease frequency of transcription.

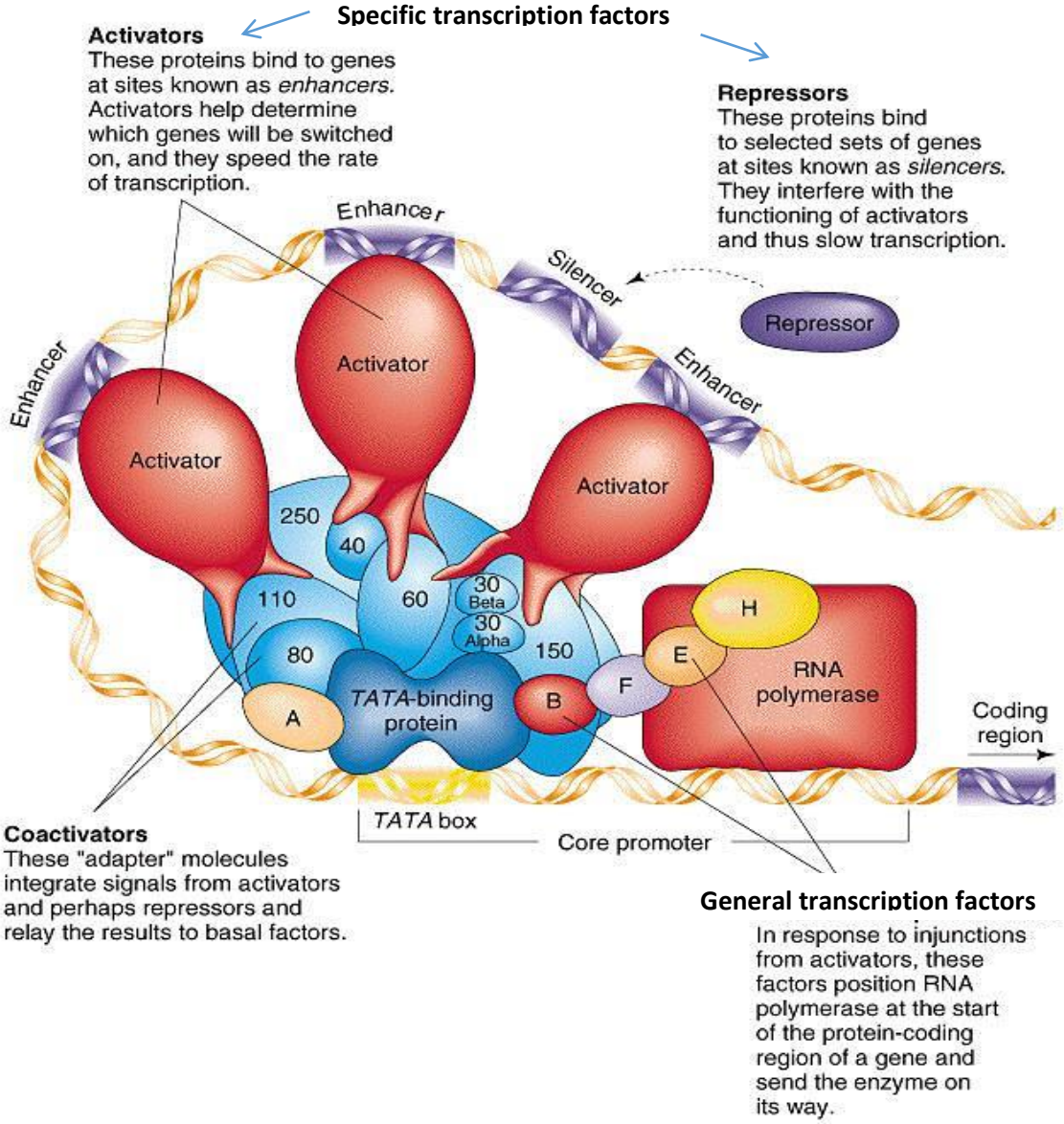


Figure 11 Summarises the function of proteins involved in initiating transcription (basal transcription factors) and regulating the frequency of transcription (activators, repressors).

C. At the Post - Transcription Level

Notes to self

- RNA processing takes place after transcription. Majority of post-transcriptional modifications occur on mRNA.

- RNA processing occurs only in **eukaryotes**, in **nucleus** of the cell. **In prokaryotic cells which lack nuclei, transcription and translation occurs simultaneously. Thus in prokaryotes, mRNA undergoes little or no modification following synthesis** by RNA polymerase.

- RNA transcript formed **immediately after transcription** is known as **pre-mRNA**. It will be modified to become a **mature mRNA** to enable its export out of the nucleus for translation.

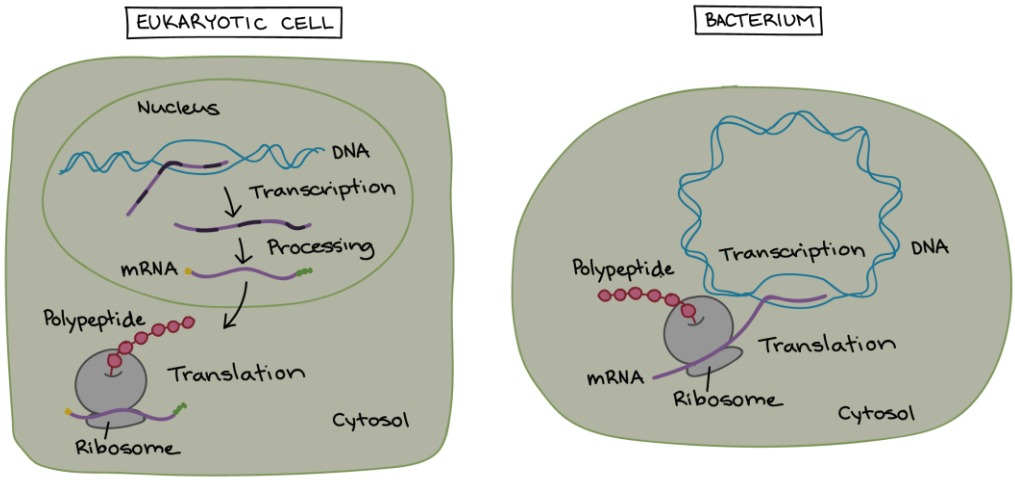


Figure 12 Comparison between eukaryotic and prokaryotic (bacterium) cells. Eukaryotes have membrane-bound nucleus, while prokaryotes lack a nucleus (only nucleoid region present). RNA processing occurs only in eukaryotes

- **REGULATION**
- RNA processing is crucial to regulate gene expression.

  (1) Translation of completely processed pre-mRNAs ensures that functional proteins are produced. Otherwise, incompletely processed pre-mRNAs containing introns produce defective proteins that might interfere with functioning of the cell.

  (2) By altering mRNA stability, it affects the amount of protein that can be made.

- There are three key processes in RNA processing. They are carried out in the following order:
  1. **Capping at the 5’ end**
  2. **Splicing of pre-mRNA**
  3. **Polyadenylation (Adding a poly A tail to the 3’ end)**

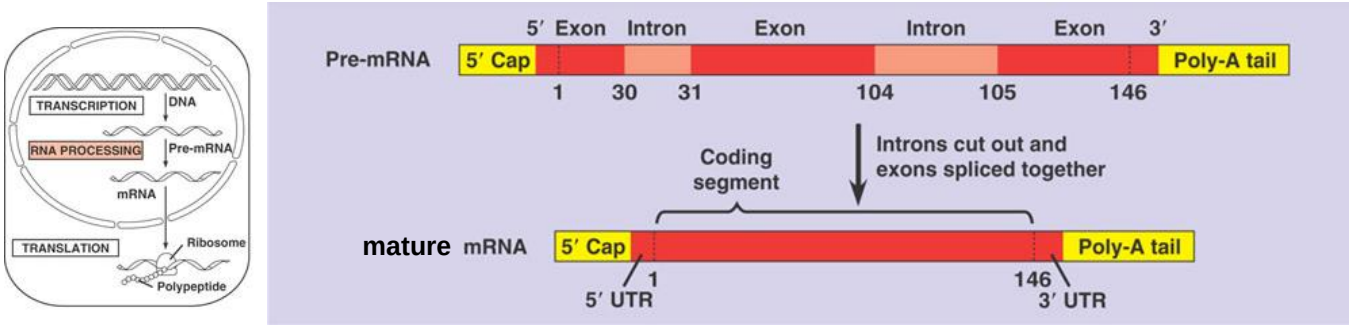


Figure 13. Key processes that occur during post-transcriptional modification

C1 Addition of the 5’ 7-methylguanosine cap

- The **5’ end** of the **pre-mRNA** is **capped with a 7-methylguanosine residue / modified guanine nucleotide**.
- **REGULATION**
- Importance of 5’ cap:
  - (i) **Processing (mRNA splicing and polyadenylation):**
    - **5’ cap** helps the cell to recognise mRNA (amongst all other RNA molecules in the cell). This ensures subsequent steps such as splicing occurs on the correct RNA molecule.
  - (ii) **Export out of nucleus**
    - 5' cap is recognised by certain proteins, which are required for the mRNA to exit from nucleus via nuclear pores.
  - (iii) **Half-life / stability**
    - 5' cap stabilises mRNA by protecting the growing pre-mRNA from rapid degradation by cellular ribonucleases.
  - (iv) **Translation**
    - 5’ cap helps promote translation initiation. The cap is recognised by eukaryotic initiation factors. Subsequent binding of initiation factors to the cap helps recruitment of mRNA to small ribosomal subunit.

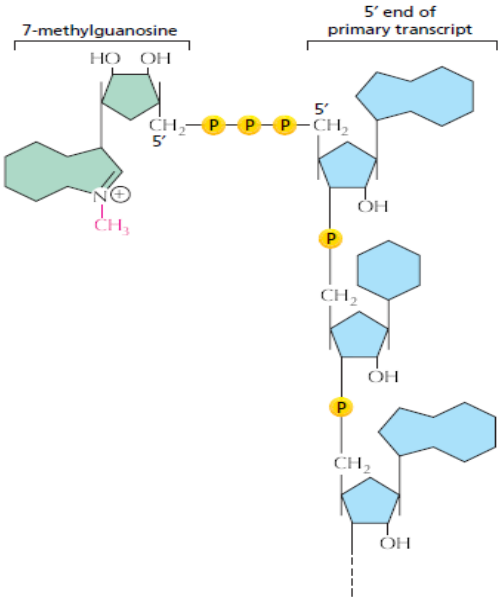


Figure 13. 7-methylguanosine cap attached to 5’ end of primary transcript.

[Note: 5’ cap is added as mRNA is being synthesised]

C2 Splicing of pre-mRNA

- Splicing is a process during which introns are **excised** and exons are **joined together/ spliced together** by **spliceosome.**
- Points of excision are very precise and are determined by sequence of nucleotide at intron-exon boundaries, known as **splice sites**.

- **Mechanism of RNA Splicing:**

I. **Splice sites** located at the ends of introns are recognised and bound by **small nuclear ribonucleoproteins (snRNPs). These snRNPs** contain **small nuclear RNA (snRNA)** which sequence is **complementary to the splice sites**.

II. The snRNPs **assemble to form a spliceosome**. The spliceosome **cuts out the introns and exons flanking the introns** are **spliced (joined)** together. A segment of intron is removed as a loop of RNA known as a **lariat**.

III. The spliceosome then dissociates, and mature mRNA is released.

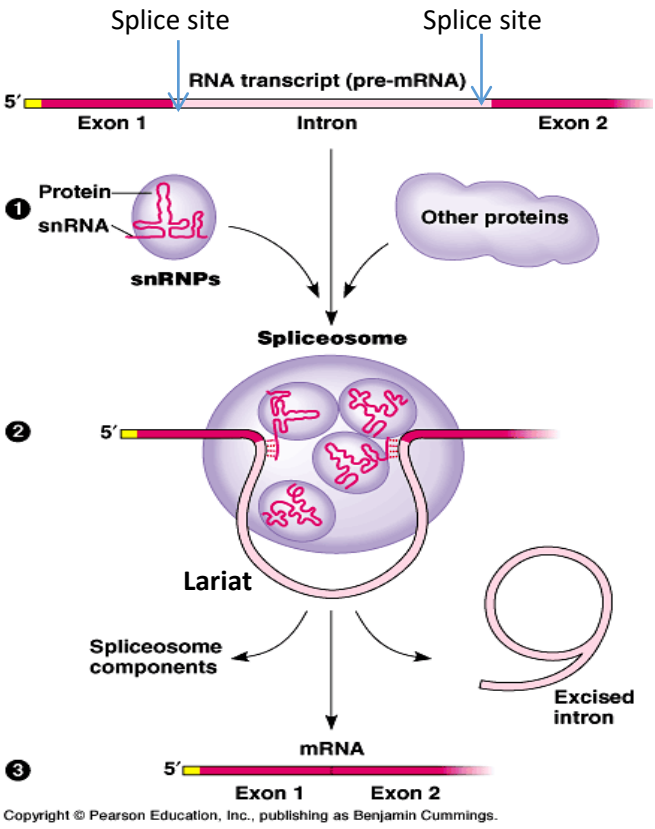


Figure 14. Mechanism of RNA splicing.

- **REGULATION**
- Importance of **alternative splicing** [Refer to 'Organization of Prok and Euk Genomes Part I]
  - Alternative RNA splicing ocurs when **different snRNPs (with different snRNA sequences) recognise different splice sites** and therefore **cut out and splice together different combinations** of introns and exons respectively.
  - The **advantage** of alternative splicing is that **from 1 gene, different mature mRNA may be generated, resulting in different polypeptides and finally different functional proteins formed**. Thus having different polypeptides at any one time, different metabolic acitivites can be easily controlled.

C3 Polyadenylation – addition of a poly-A tail to the 3' end of mRNA

- At the **3' end of the pre-mRNA**, a **poly-A tail** consisting of about **50 to 250 adenine nucleotides** is synthesized.
- This reaction is catalyzed by the **enzyme poly-A polymerase**.

- **REGULATION**
- Importance of poly-A tail at 3' end of mRNA:
  - Enhances half-life/ stability of mRNA transcript by slowing down its degradation by ribonucleases in nucleus and cytoplasm
  - Serves as a signal to direct the export of mature mRNA from nucleus to cytoplasm.
  - Necessary with 5' cap for translation, by recruiting initiation factors to form translation initiation complex.

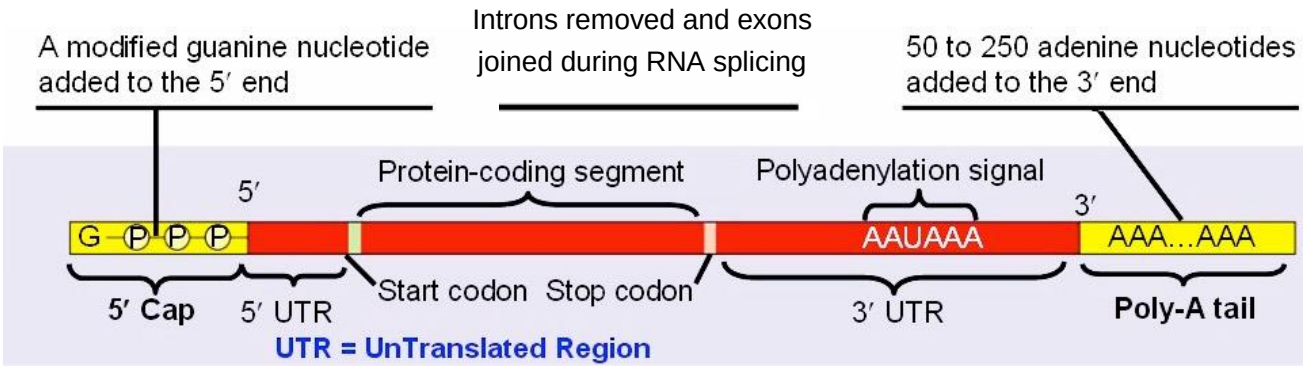


Figure 15 : Summary of post-transcriptional modification in eukaryotes

D. At the Translational Level

D1 mRNA stability / Half-life

- The stability / half-life of an mRNA molecule in eukaryotes is influenced by the **length of poly-A tail.**

- **REGULATION**
- The **more stable** the mRNA, the **longer its half-life**, thus the **longer** it can be used as a **template** for translation into the desired proteins. If the protein is not required in large amounts, the mRNA will have a short half-life.
- During mRNA degradation, ribonucleases remove the poly-A tail steadily in the 3' to 5' direction. This occurs in the cytoplasm. Once a critical length of poly-A tail is reached, the removal of the 5' cap will be triggered by a different ribonuclease.

D2 Binding of small ribosomal subunit

- During translation initiation, small ribosomal subunit binds to 5' cap of mRNA.

- **REGULATION**
  - Translation initiation can be blocked by a **translational repressor protein**.
  - The translational repressor protein binds to:
    - 5'cap and/or its vicinity i.e. 5' untranslated region (UTR).
    - 3' untranslated region to interfere with the interaction between 3' poly-A tail, initiation factors and 5' cap which is needed for translation.

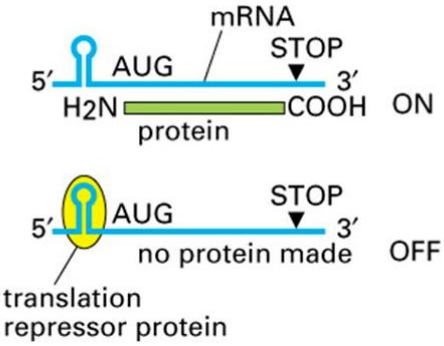


Figure 16: translational repressor protein

D3 Initiation Factors

- Initiation factors are proteins required to begin protein synthesis.
- They function to enable proper positioning of the small ribosomal subunit together with the initiator tRNA on the mRNA template, followed by the recruitment of the large ribosomal subunit.

  - **REGULATION**
  - Phosphorylation or dephosphorylation of initiation factors will determine their availability.

E. At the Post- Translational Level

- There are several mechanisms that regulate the activity of proteins by altering the protein's structure after translation. These include:
  - Proteolytic cleavage and activation
  - Chemical modification
  - Protein Degradation

E1 Proteolytic Cleavage and Activation *Notes to self*

- Many newly synthesized polypeptides cannot immediately serve as functional proteins.

  - **REGULATION**
  - **Removal of the inhibitory portions** of the polypeptide chain by **proteases** may activate proteins which are synthesised as **inactive precursors.**
  - **Eg.** Insulin is synthesised as an inactive precursor known as pro-insulin, which contains 59 additional amino acids that need to be removed for insulin to become active.

E2 Chemical Modification

- The activity of proteins may be regulated by the addition of chemical groups to the polypeptide chain.

  - **REGULATION**
  - Types of chemical modification include:
    - **Glycosylation** - addition of one or more sugar monomers/ carbohydrate chain to form **glycoproteins**.
    - **Phosphorylation** – addition of a phosphate group, which may either **activate or inactivate** the protein (e.g. Eukaryotic initiation factors).

E3 Protein Degradation

  - **REGULATION**
  - The **degradation of proteins** is important in determining **how long** a particular protein is allowed to remain in the cell and carry out its function.
  - **Misfolded proteins** or **proteins that are no longer required** undergo a process known as **ubiquitination**.
  - Ubiquitination is a process by which **proteins to be degraded are tagged with ubiquitin molecules**. The addition of ubiquitin tags is catalysed by enzyme **ubiquitin ligase.**
  - These ubiquitin-tagged molecules are recognised and subsequently degraded by **proteasomes** into smaller peptides which can be further degraded by cytoplasmic enzymes.

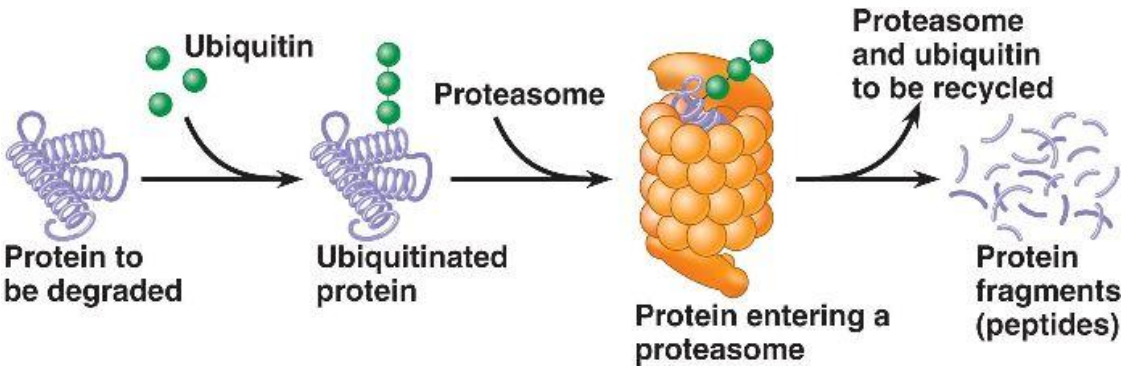
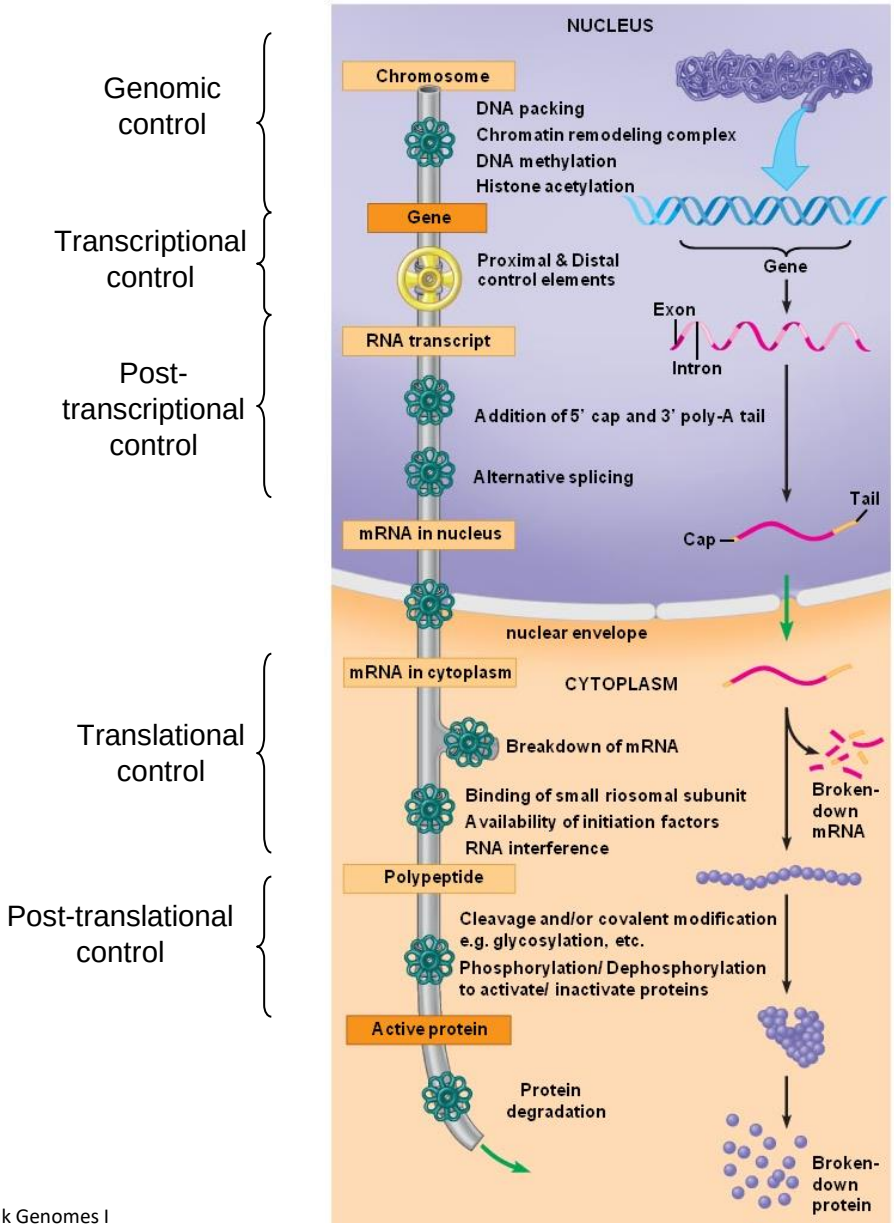


Figure 17. Degradation of ubiquitin-tagged protein into perptides/ amino acids (to be recycled for use)

Summary for Control of Eukaryotic Gene Expression



8. Prokaryotic Gene Expression and Regulation

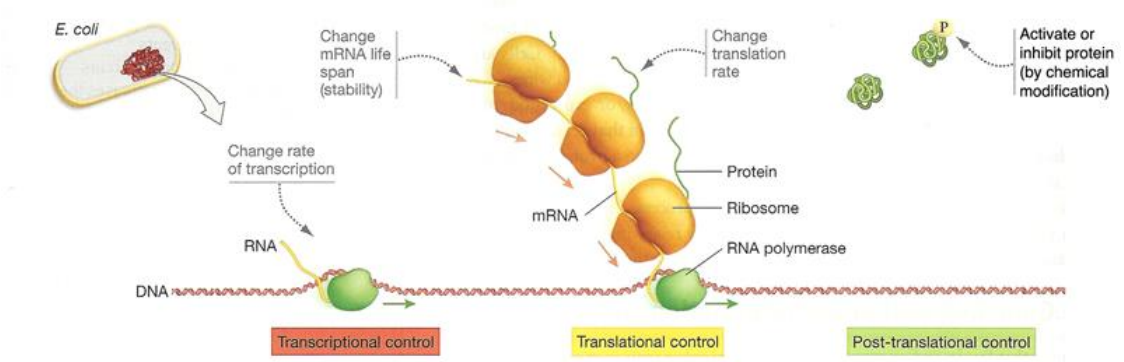


Figure 18. Levels of prokaryotic gene expression – Transcriptional, Translational, Post Translational

A. At the Transcriptional Level

- **Transcriptional control** regulates **when and how often** a gene is transcribed.
- For prokaryotes, **transcriptional control** is the **most important level** of regulation, followed by **translational control.**

A1 Promoter

- The **promoter** is a DNA sequence to which **RNA polymerase** and **general transcription factors** bind to **initiate transcription**.
- Understanding the structure of the promoter enables us to appreciate how the **strength of the promoter** would affect the **frequency of transcription**.

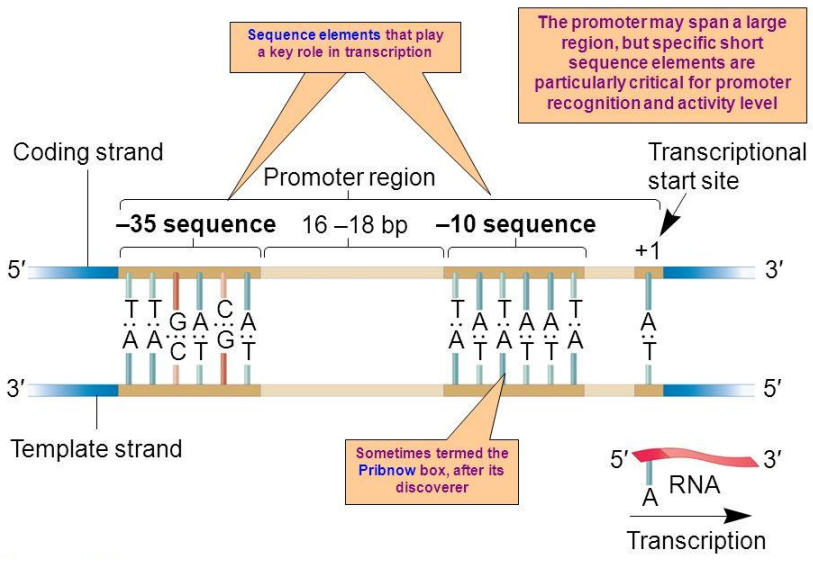


Figure 19. General structure of prokaryotic promoter.
By convention, nucleotide sequences referred to are on the non-template DNA strand. The promoter includes the **transcription start site (+1).** Preceding and just adjacent to it on the left is termed '**upstream**' and is denoted (-1). The direction of transcription after +1 site is termed "**downstream**'.

Notes to self

- Bacterial promoters contain **two** important **critical elements/short sequences**.
- Sequences at the
  (a) -10 site are known as ***-10 sequence* / Pribnow box**
  (b) -35 site are known as ***-35 sequence***

- Comparing the **most commonly occurring bases within the critical elements, a consensus sequence is derived.**
- Many promoters have -10 and -35 sequences that are similar to the consensus sequence. The **consensus sequence** at:
  (a) -10 site is 5'-TATAAT-3'
  (b) -35 site is 5'-TTGACA-3'

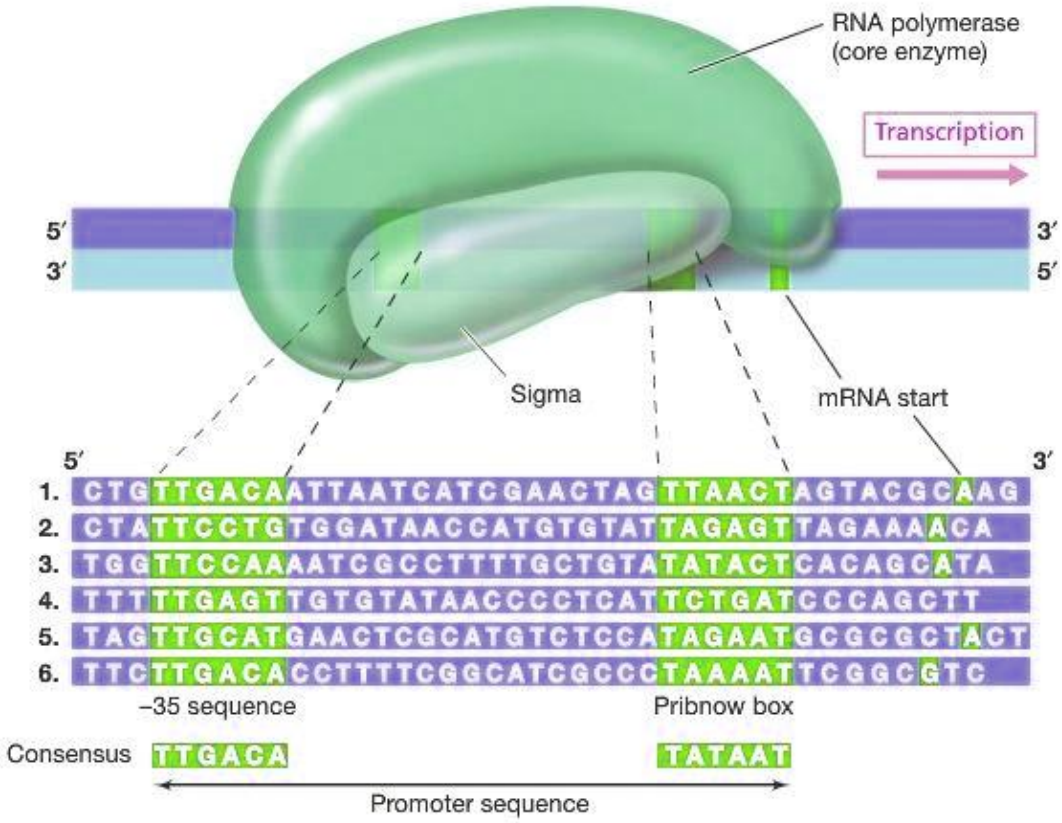


Figure 20. General structure of prokaryotic promoter showing (a) consensus sequence and (b) RNA polymerase holoenzyme binding.

(a) consensus sequence is derived by **comparing** the critical element/ short sequence of 6 different prokaryotic promoters (in this case illustrated in fig) on the non-template DNA strand. (b) RNA polymerase holoenzyme comprises the **core polymerase and sigma factor**. **Sigma factor** recognises and **binds to both the critical elements** at the promoter.

- The bacterial **RNA polymerase holoenzyme** is made up of
  - A **core polymerase** and
  - A **sigma factor**.
    - The **sigma factor** on the holoenzyme **recognizes and binds** to **both** the **critical elements at the promoter**. (Figure 20b).

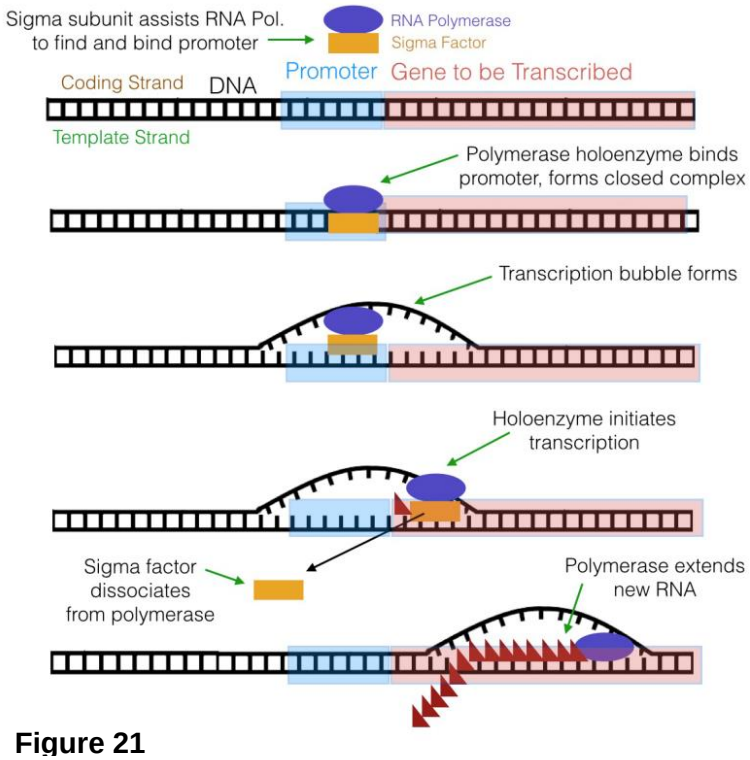
- The **shape of the RNA polymerase and sigma factor is complementary** to the **nucleotide sequence** that they bind to.

- **REGULATION**

- The **more the critical elements/ short sequences** in a given promoter **resemble the consensus sequence**, the **stronger** the promoter.
- Strong promoters allow **RNA polymerase** to bind with **greater binding efficiency**, thus **increasing the frequency of transcription**
  NOTE: avoid the term "rate of transcription'

A2 Sigma factor

- The **sigma factor** is a **protein subunit of RNA polymerase.**
- It is the **specific part** of RNA polymerase that **enables RNA polymerase to recognise and bind** to the **promoter** to initiate transcription.
- There are **different sigma factors, each recognises and binds to a different promoter.**



1. RNA polymerase holoenzyme comprises a core polymerase and sigma factor. Assembled RNA polymerase scans along DNA.

2. The sigma factor of RNA polymerase holoenzyme recognises and binds to the critical elements at both -10 and -35 positions.

3. RNA polymerase holoenzyme unzips part of the double stranded DNA for transcription. Only one of the strands is used as a transcription template.

4. After a short distance, sigma factor dissociates from polymerase. RNA synthesis continues.

Figure 21

- **REGULATION**
- Since **each sigma factor** recognises and binds to a **unique promoter**, the **availability of different sigma factors** can be controlled, thus **determining the genes that can be transcribe**.
- Changing the sigma factors allows **different** sets of genes or operons to be transcribed by the **same RNA polymerase core enzyme**.
- The sigma factor and RNA polymerase core enzyme recruited to the promoter on the DNA forms the **transcription initiation complex**.

Notes to self

A3 **Operon** (Covered under Genetics of Bacteria)

- The operon consists of
  - An operator
  - A common promoter
  - Structural genes with related functions

- The operon **enables the expression of the related structural genes** to be **controlled by a single promoter**, thus enable transcription to form a **polycistronic mRNA**.
- Associated with the operon is a **regulatory gene**, located upstream of the operon, which **encodes either an active or inactive repressor**.

- **REGULATION**
- Operons allow bacteria to **regulate** a group of structural genes that encode f**unctionally related gene products** in a **coordinate manner**.
- These means that the transcription of these genes can be **turned on or off together**/ the **frequency of transcription** of these genes can be **increase or decreased simultaneously**.
- By having coordinate control of the genes, the control of gene expression becomes **more efficient**.

- Small molecules that help the operon regulate gene expression include:
  - **Repressor**
    - When bound to operator, repressor **physically blocks** binding of RNA polymerase to promoter.
    - Hence the transcription of the genes within the operon is **prevented**.
  - **Inducer** (e.g. allolactose)
    - Can regulate transcription frequency.

B. At the Post- Transcriptional level

Notes to self

- **None.**
- RNA splicing and processing do not occur.
- **Transcription and Translation occur simultaneously in prokaryotes,** i.e. bacterial mRNA is used as a template for translation as it is being produced.

C. At the Translational level

- **Translational control** regulates the **number of polypeptides** synthesised.
- This is achieved via the following means:
  - mRNA stability/ Half-life
  - Binding of small ribosomal subunit
  - Initiation factors

C1 mRNA stability / Half-life

- The stability / half-life of an mRNA molecule remaining in the cytoplasm will determine the number of polypeptides that can be translated from it.
- The **more stable** the mRNA, the **longer its half-life**, thus the **longer** it can be used as a **template** for translation into the desired proteins.

- **REGULATION**
- Unlike eukaryotes, prokaryotic mRNAs have **relatively short half-lives.**
- This means that they are subject to **rapid degradation**
  - This is advantageous to bacterial cells as they can control gene expression by rapidly adjusting the synthesis of proteins in response to environmental changes.
  - mRNAs are degraded by **ribonucleases (RNases)** into ribonucleotides minutes after they are synthesised.

- **REGULATION**
- Certain changes in conditions induce bacteria to **quickly synthesise an antisense RNA**, which is **complementary** to a particular **mRNA**.
- Anti-sense RNA binds to mRNA via c**omplementary base pairing** to form a **duplex**, **reducing the half-life of mRNA**.
- Anti-sense mRNA thus either:
  - **blocks** the translation machinery from assembling and thus **prevents translation**.
  - Target RNA for **degradation.**

(NOTE: details of this mechanism is not required)

Notes to self

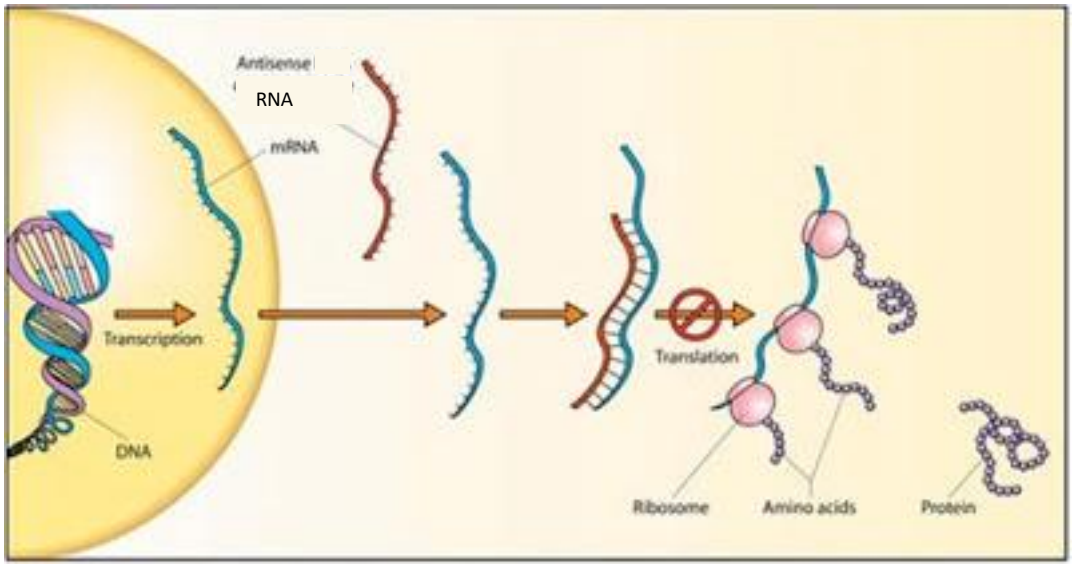


Figure 22: Antisense RNA forms double-stranded duplex and reduces half-life of mRNA, preventing translation of the mRNA.

C2 Binding of small ribosomal subunit

- In prokaryotes, a few nucleotides **upstream of each AUG start codon** on a **polycistronic mRNA**, is where a **Shine-Dalgarno sequence (5' – AGGAGG-3')** is found.
- This sequence is **recognised by the small ribosomal subunit**, which will **bind** to it so that the **start codon can be correctly positioned in the subunit** before the initiation tRNA and large ribosomal subunit can assemble for translation.

- **REGULATION**
- Translation initiation can be blocked by:
  - Binding of a **translational repressor protein** at or near to the **Shine Dalgarno sequence**
    - This prevents the binding of the small ribosomal subunit to the Shine-Dalgarno sequence, thus preventing proper ribosome assembly and prevents translation.
  - **Binding of anti-sense RNA complementary to the mRNA near/ at the Shine-Dalgarno sequence**.
    - This prevents the binding of the small ribosomal subunit to the Shine-Dalgarno sequence, thus preventing proper ribosome assembly and prevents translation.

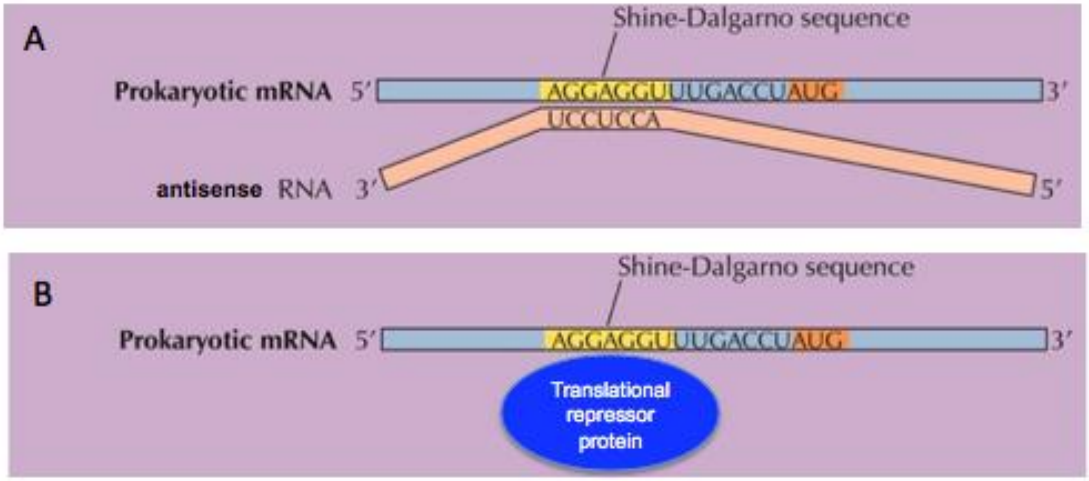


Figure 23: Binding of A) antisense RNA and B) translational repressor protein to Shine Dalgarno sequence upstream of start codon AUG

C3 Initiation Factors

- **Initiation factors** are **necessary for the proper positioning** of the **small ribosomal subunit** together with the i**nitiator tRNA on the mRNA**, and the subsequent **recruitment of the large ribosomal subunit** which form the **translation initiation complex**.

- **REGULATION**
- **Availability of initiation factors** controls initiation of translation.

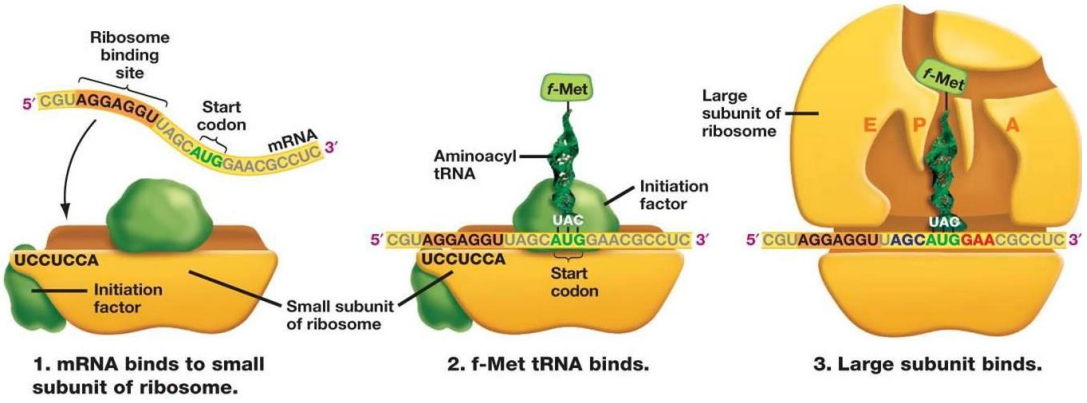
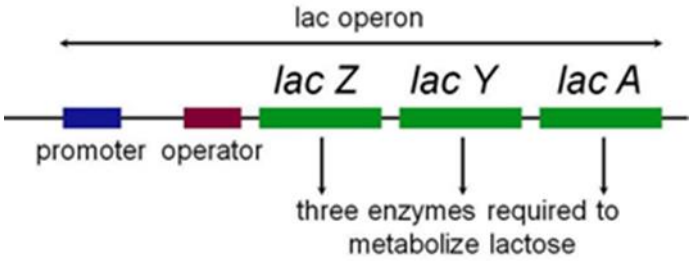


Figure 24: initiation factors required for translation initiation.

D. At the Post-Translational level

- **Controls proteins that are already present in the cell**.
- **Activate or inhibit** protein function
- **Regulation not significant,** although processes including **covalent modification, phosphorylation/ dephosphorylation and protein degradation** may occur to regulate protein activity.

Regulation of Gene Expression		
Feature	**Prokaryotic Gene Expression**	**Eukaryotic Gene Expression**
Genome Level	• Chromatin modification e.g. histone acetylation **cannot occur** as prokaryotic DNA is **not associated with histones.**	• Chromatin modification e.g. histone acetylation can occur, resulting in conversion between **euchromatin and heterochromatin**. ○ Eukaryotic DNA is associated with histones (and other proteins).
	• **DNA sequences** (promoters and operators) serve as **on/off switches.**	• **Degree of condensation** of chromatin serves as **major on/off switches** for gene expression**.**
Transcription Level	• Functionally related genes are organized in an **operon**, under the **control of a single promoter**. • These genes are transcribed together to give rise to a **polycistronic mRNA.**  	• Genes are not organized into operons. • Each gene has its own promoter and gives rise to a **monocistronic mRNA**.

	• **Few control elements**, which are usually located **close** to the promoter and genes under its control.
	  ○ E.g. operator is located close to the promoter of genes.
		• **Many control elements** that can be located **proximally or distally** upstream/ downstream of a gene.
		  ○ E.g. proximal and distal control elements

	• Only **one** RNA polymerase involved. All RNAs are synthesized by the same RNA polymerase.	• **Five** different RNA polymerases present. o Three main types of RNA (mRNA, tRNA and rRNA), synthesized by **three different RNA polymerase.**
	• General transcription factors **not required** as RNA polymerase can directly recognize and bind to **Pribnow box** of prokaryotic promoters. o Occurs with the aid of **sigma factors, which increases** RNA polymerase affinity for the **promoter**. o Prokaryotic RNA polymerase associates with the sigma factor to form RNA polymerase **holoenzyme**.	• General transcription factors required. o General transcription factors and RNA polymerase II assemble at the **TATA box** of eukaryotic promoters to form the **transcription initiation complex.**
Post-transcriptional Level	• Post-transcriptional modifications do not occur. Primary transcripts (pre mRNA) are the actual mRNA.	• Primary transcripts (pre-mRNA) undergo processing to produce mature mRNA: o Addition of 5' cap o RNA splicing o Addition of 3' poly (A) tail

Translational Level	• Translation is often coupled to transcription. Both processes occur **simultaneously**.	• Translation is **not coupled** to transcription. • mRNA must move from the nucleoplasm, across the nuclear envelope, via a nuclear pore to the cytoplasm for translation to occur. • RNA transcript is not free to associate with ribosomal subunits prior to completion of transcription.
	• Control at this includes mRNA stability and half-life (antisense RNA reduces RNA half-life and prevents translation initiation complex assembly), binding of small ribosomal subunit (by translational repressor protein or antisense RNA complementary to Shine Dalgarno sequence upstream to AUG) and availability of initiation factors.	• Control can occur at pre-translational level, when **regulatory proteins bind at the 5' UTR or the 3' poly (A) tail of mature mRNA**. This **prevents binding with the small ribosomal subunit** and the assembly of the translation initiation complex.
	• mRNAs are **polycistronic** and have **multiple start codons**, allowing for the direct synthesis of **several different polypeptides.**	• mRNAs are **monocistronic** and have only **one** start codon, allowing for the synthesis of only **one kind of polypeptide.**

Post-translational Level	• Post-translational modification **not significant**, though the following processes can still occur: ○ Covalent modification ○ Phosphorylation/ dephophorylation ○ Degradation	○ Post-translational modification can occur in the form of: ○ Chemical modification (glycosylation and phosphorylation) ○ Proteolytic Cleavage and Activation ○ Degradation (ubiquitination)

Keywords include:

Promoter	Consensus sequence	Translation initiation complex	Silencers
RNA polymerase	Frequency of transcription	mRNA	Specific transcription factors
General transcription factor	Shine Dalgarno sequence	Chromatin remodeling complex	TATA box
Transcription initiation complex	Translation initiation factors	DNA methylation	Activators
-10 sequence/ Pribnow box	AUG, start codon	Histone acetylase	Repressors
-35 sequence	UAG, UAA, UGA, Stop codon	Histone deacetylase	5’ cap
RNA polymerase holoenzyme	Small ribosomal subunit	Negatively charged DNA	Poly A tail
Sigma factor	Large ribosomal subunit	Enhancers	