Control of Prokaryotic and Eukaryotic Genome

Genomic level
Eukaryotic only

- Chromatin remodelling complex
    - Made up of proteins that alter the structure of nucleosomes
    - Regulation:
        - Alters the structure of **nucleosomes** temporarily ⇒ DNA being less/more tightly bound to **histones** ⇒ Chromosomes condense/decondense ⇒ Allow/Prevent RNA polymerase and transcription factors access to the promoter to initiate transcription ⇒ Promotes/Inhibit transcription
- DNA methylation (Prevents transcription)
    - Regulation:
        - Addition of **methyl** group to selected **cytosine** nucleotides found in sequence CG ⇒ Recruits **DNA-binding proteins** to the methylated DNA to condense chromatin + Blocks binding of transcription factors and RNA polymerase to the promoter ⇒ Prevents transcription
- Histone acetylation (Promotes transcription)
    - Addition of **acetyl** groups to lysine residues by **histone acetyltransferase**
    - Regulation:
        - Removes positive charges on histones ⇒ Decreases the electrostatic interactions between negatively-charged DNA and histones ⇒ Loosens binding between DNA and histones ⇒ Makes the region more accessible to RNA polymerase and general transcription factors ⇒ Promotes transcription
- Histone deacetylation (Prevents transcription)
    - Removal of **acetyl** groups from lysine residues by **histone deacetylase**
    - Regulation:
        - Restores **positive charges** on histones ⇒ Increases the electrostatic interactions between negatively charged DNA and histones ⇒ Restores the tight interaction between DNA and histones ⇒ Makes the region less accessible to RNA polymerase and general transcription factors ⇒ Inhibits transcription
- Gene amplification
    - Increase **copy number** of a specific gene
    - Replicating the specific gene multiple times ⇒ Create more copies of the gene ⇒ More copies of mRNA ⇒ More copies of the required protein
    - To meet the high demand of the protein during certain stages of development
    - Mechanisms
        1. Rolling circle mechanism
            - Genomic chromosomes give rise to an **extrachromosomal circular**

DNA carrying the gene of interest
    - **Many copies of this circular DNA are then synthesised through the **rolling circle mechanism**
        1. One strand of dsDNA nicked
        2. DNA polymerase adds nucleotides to free 3' OH end to synthesise complementary DNA strand using intact strands as a template
        3. 5' end of nicked strand displaced as synthesis proceeds
        4. Displaced strand used as template and replicated discontinuously
2. Multiple rounds of DNA replication
    - Origin of replication in the DNA at the specific region of the chromosome initiates replication repeatedly and terminates randomly
    - Produces replication bubbles nested within larger replication bubbles, hence producing more copies of the gene of interest in the chromosome

Transcription
<u>Eukaryotic</u>

- Promoter strength
    - Critical elements
        - **TATA box** at -25 site determines precise location of transcription start site
        - **CAAT and GC boxes** improve efficiency of promoter by helping to recruit general transcription factors and RNA polymerase
    - Similarity of critical elements to consensus sequences are not crucial in controlling gene expression (rely more on enhancers/silencers)
- General Transcription Factors
    - Complementary in shape and charge to the TATA/CAAT/GC box/promoter
    - Binds to promoter region and recruits RNA polymerase to form transcription initiation complex to initiate transcription of the genes
- Presence of regulatory proteins
    - Activators → Enhancer
        - Increase efficiency of transcription by <u>promoting assembly of transcription initiation complex</u> when bound to **<u>enhancer region</u>** by:
            - Bending of spacer DNA to allow direct interaction of activators with RNA polymerase/general transcription factors at promoter
            - Recruiting histone acetylase/chromatin remodelling complex to increase accessibility of promoter DNA to RNA polymerase/general transcription factors
    - Repressors → Silencer
        - Reduce efficiency of transcription by <u>inhibiting assembly of transcription initiation complex</u> when bound to **<u>silencer region</u>** by:
            - Interfering with action of activator
                - Competitive DNA binding

        - Masking activation surface
        - Direct interaction with general transcription factors
    - Changing chromatin structure by recruiting histone deacetylase/chromatin remodelling complex

Prokaryotic

- Operon
    - Genes coding for proteins involved in same biochemical pathway clustered together on one operon, controlled by a **single promoter** and transcribed into a single **polycistronic mRNA**
        - Polycistronic mRNA has independent start and stop codons ⇒ Translated independently
    - Allows the bacteria to coordinately regulate a group of genes that encode gene products with related functions ⇒ More efficient gene control
    - Regulation:
        - **Repressor**: Binds to operator, preventing RNA polymerase from binding to promoter, thereby preventing gene transcription
        - **Inducer**: Binds to and inactivates repressors, increasing transcription frequency (e.g. allolactose)
        - **Corepressor**: Binds to and activates repressors, preventing gene transcription
        - **Activators**: Binds to binding sites in the promoter, increasing affinity of RNA polymerase to the promoter, increasing transcription frequency (e.g. CAP)
- Promoter
    - Strength determined by how similar the **critical elements** [-10 (**Pribnow box**) and -35 sequences] are to the **consensus sequence**
    - Strength of promoter determines the frequency of transcription
- Sigma Factor
    - RNA polymerase holoenzyme = Core polymerase + Sigma factor
    - Recognises and binds to critical elements at promoter
    - Regulation:
        - Different sigma factors recognise different promoters
        - Availability of sigma factors determines which promoters the RNA polymerase holoenzyme can bind to and hence which set of genes are transcribed

Post-Transcription
Eukaryotic only

1. 5’ 7-methylguanosine cap of mRNA
    - Importance:
        - Helps cell to recognise mRNA ⇒ Ensures subsequent steps occur on the

correct RNA molecule
        - Signals proteins to export mRNA out of the nucleus via nuclear pores
        - Stabilises mRNA by protecting growing pre-mRNA from rapid degradation by cellular RNases
        - Recognised by **eukaryotic initiation factors** which recruits small ribosomal subunit to mRNA, thus promoting translation initiation
2. Alternative splicing
    - Introns excised and exons are joined together by spliceosomes
    - Spliceosomes made up of proteins and snRNA
    - Importance:
        - Allows 1 gene to generate different mature RNA by splicing together different combinations of exons and hence, different polypeptides which can control different metabolic activities
3. 3' poly-A tail of mRNA
    - Importance:
        - Enhances half-life of mRNA by slowing down its degradation by **RNases** in the nucleus and cytoplasm
        - Serves as a signal to direct the export of mature mRNA from nucleus to cytoplasm
        - Works with 5' cap to regulate mRNA translational efficiency

Translation

<u>Eukaryotic</u>

- mRNA stability
    - Influenced by length of poly-A tail
    - More stable ⇒ ↑ half life ⇒ Can be used as template for translation for longer period ⇒ ↑ protein product
    - Regulation:
        - mRNA degradation
            - **3' poly-A tail** steadily removed by **RNase**
            - Once critical length reached, removal of 5' cap is triggered
- Binding of small ribosomal subunit
    - Small ribosomal subunit binds to 5' cap of mRNA
    - Regulation:
        - **Translational repressor protein** can bind to 5' cap or 3' UTR ⇒ Interferes with the interaction between 3' poly-A tail, 5' cap and small ribosomal subunit ⇒ Blocks binding of small ribosomal subunit ⇒ Blocks translation
        - **Phosphorylation/Dephosphorylation** of eukaryotic initiation factors determines their availability for translation

<u>Prokaryotic</u>

- mRNA stability

    - Regulation:
        - Prokaryotic mRNAs have relatively **short half-life** which allows for rapid degradation by RNases ⇒ Allows cell to control gene expression by rapidly adjusting rate of synthesis of proteins
        - Synthesis of **antisense RNA** complementary to mRNA ⇒ Complementary base pairs with mRNA to form dsRNA ⇒ Blocks translation and targets the dsRNA for degradation by RNases
- Binding of small ribosomal subunit
    - Regulation:
        - Prevent binding of small ribosomal subunit, hence blocking translation initiation by:
            - Binding of translational repressor protein at/near Shine-Dalgarno sequence
            - Binding of anti-sense RNA complementary to mRNA at/near Shine-Dalgarno sequence
        - Regulating availability of Initiation factors

Post-Translation
Eukaryotic only

- Covalent modification
    - Regulation:
        - Further processing of polypeptides to form functional proteins by cleavage and/or covalent modification
            - e.g. glycosylation, disulfide bond formation, attachment of prosthetic groups
- Phosphorylation and dephosphorylation
    - Regulation:
        - Regulates cellular activity through phosphorylation by **kinase** or dephosphorylation by **phosphotase**
- Protein degradation
    - Determines how long the protein will remain in the cell to control protein activity/prevent aberrant activity
    - Proteins not needed can be hydrolysed to amino acids to be used for synthesis of new proteins
    - 1 polypeptide can be modified to form many different proteins ⇒ Smaller genome required
    - Regulation:
        - Addition of **ubiquitin** by ubiquitin ligase to the protein to tag for degradation
        - Proteasome recognises ubiquitin-tagged protein and cleaves the protein into smaller peptides that can be further degraded in cytoplasm