TRANSCRIPTION IN EUKARYOTES

General process:

Initiation, elongation and termination

Initiation

Enzyme involved: RNA polymerase II

proteins involved: General Transcription factors

- **TBP (Tata binding protein)**
- **Other general transcription factors**

So what does TBP do?

- TBP binds to promoter at the TATA box
- Recruits other GTF which helps with binding of RNA polymerase to bind to the promoter
- Forming preinitiation complex

What does RNA polymerase do?

- Unwinds and unzips the DNA strands

Why does RNA polymerase need to do that?

- To expose the nitrogenous base so that it can act as a template for transcription
- For complementary base pairing for free nucleotides

Elongation

Enzyme involved: rna polymerase

What does rna polymerase do?

- Reads the DNA template from 3' to 5' direction
    - The rna synthesised from 5' to 3'
    - Free nucleotides are paired with their complementary bases via hydrogen bonds
- Catalyses formation of phosphodiester bonds between a 3' hydroxyl end to incoming 5' phosphate group

Termination

Enzyme involved: RNA polymerase

Products formed: Pre-mRna

Whats the terminator sequence called:
polyadenylation sequence signal

What does RNA polymerase do?

- transcribes a polyadenylation code AAUAAA past its signal sequence
- the growing chain is then Cleaved downstream 10-30 nts, RNA dissociates from DNA

What happens after transcription is done?

- RNA transcript is released
- RNA polymerase dissociates from DNA

Pre-mRNA modification (only in eukaryotes)

Why is it important?

- to produce mature mRna
- helps with translation

Note: can occur simultaneously with transcription

Addition of 5' caps (ALWAYS THE FIRST)

when does it occur?

- during synthesis of pre-mRNA

Enzymes involved: capping enzymes

What does it do?

- Capping enzymes catalyse the covalent attachment of 7-methylguanosine to the 5' end of the mRNA transcript, forming a 5'-5' triphosphate linkage.

Why does it do it?

- protects mRNA from degradation by 5' exonucleases, conferring stability to the capped mRNA
- serves as a binding site for translation initiation factors and ribosome to initiate translation.
- facilitates the export of mature mRNA from the nucleus to the cytoplasm.

addition of 3’ poly(A) tails

process: Polyadenylation

Enzyme involved: poly-(A)-polymerase

What does the enzyme do?

- The enzyme catalyses the addition of 50-240 adenine nuecleotides to the 3’ end

Why does the enzyme do that?

- to facilitate the transport of mRNA from nucleus to cytoplasm
- prevent degradation by the 3’ exonucleases
- facilitate binding of ribosomes to 5’ end of mRNA and initiate translation

Splicing

PROTEINS involved: splicesome

What does this protein do?

- splice out the introns from the mRNA strand

How does the protein do that?

- recognises the 5’ splice site, branch point, 3’ splice set
- excise the introns and ligate the exons

Why does it do that?

- introns allow alternative splicing to occur so that multiple different mRNA can be produced from a single gene
- to prevent addition of nucleic acids not needed which can affect the protein structure due to unfavourable R group interactions

TRANSLATION IN EUKARYOTES

general process: initiation, elongation, termination

Things to note

- Sequences of bases in mRNA provides coded information that is read by ribosomes in 3 consecutive nucleotides known as codons

- Characteristic of codons
    - Codons are universal as they are common in most organisms
    - Punctuated: consists of codons that signal when the sequence stops and begins
        - Start codon: AUG
        - Stop codon: UGA, UAG, UAA
    - Codons are non-overlapping as the same nucleotide are not used for more than 1 codon
    - Unambiguous as no codons code for more than one amino acid
    - Degenerate as One amino acid can be produced by more than one codons as codons have more than one tRNA and some tRNA can tolerate mismatch at the 3rd base of a codons

<u>Initiation</u>

Enzymes involved:

What does the enzyme do?

Plasmid are not part of the chromosomes

TRANSCRIPTION IN PROKARYOTES

TRANSLATION IN PROKARYOTES

ORGANISATION AND CONTROL IN EUKARYOTES

Differential expression: the process where cells become different from one another due to the unique sequences of genes that are expressed

Why is this important?

- So that they can produce different cell types in a single organism to carry out their specific functions from the same genome
    - Eg) liver cells, nerve cells
- Enable multicellular organisms to proceed through development stages
    - As organism progress through developmental stages, certain genes are activated or repressed at certain stages but not others.

Why do we need to regulate transcription and translation?

- To save energy and resources especially when the gene is not needed

<u>DNA Packing</u>

1st stage:

Proteins involved: histone proteins and Linker DNA

Products formed: 10nm nucleosome

Structure formed: beads-on-the-string

What do histones protein do?

- DNA winds around histone octamer containing 8 histones proteins
- Consist of high proportion of positively-charged amino acids, allowing them to bind tightly to the negatively-charged phosphate groups along DNA backbone via ionic and hydrogen bonds

What does linker DNA do?

- Connect nucleosome together giving rise to bead on string structure, shortening the length of DNA

2nd level:

Product formed: 30mm fibre

Structure formed: zigzag or solenoid structure

How is the structure formed?

- Nucleosome associate with each other, further shortening the molecule

3rd level:

Product formed: 300 nm fibre

Proteins involved: protein scaffold

How is the product formed?

- The 30 nm chromatin fibre formeds looped domains attached to a protein scaffold

Final level:

Products formed: 700 nm metaphase chromatid

How is this product formed?

- During mitosis/meiosis, the 300 nm fibre coil and fold,further compacting the chromosomes

REGULATION IN CHROMATIN LEVEL

- ➔ Tightening or loosening of ~~chromatins~~ **nucleosomes**(refer to dna packing) in order to increase or decrease rate of transcription
- ➔ Undergoes covalent modification by modifying such as acetylation methylation and phosphorylation

Histone modification

Enzyme involved: Histone acetyltransferase (HAT) and Histone deacetylase (HDAC)

Loosening of chromatin structure (histone acytelation)

Enzyme: HAT

What does HAT do?

- Catalyses the addition of acetyl group to lysine side chains
- Neutralises positive charge on lysine side chains and disrupts electrostatic attraction between histone protein and negatively charged DNA backbone (phosphate group)
- As the forces are disrupted, chromatin structure is loosened, allows GTF to AND RNA Polymerase to access prometer more readily, INCREASING RATE OF TRANSCRIPTION

Tightening of chromatin structure (histone deactyelasation)

Enzyme: HDAC

What does HDAC do?

- Catalyses the removal of acetyl group from the lysine side chains
- Restore electrostatic attraction between DNA and histones, tightening the chromatin structure, making it less accessible for GTF and RNA pol to bind to promoter, inhibiting transcription

DNA methylation

Enzyme involved: DNA methyltransferase (DNMT)

Role of enzyme: catalyses the addition of methyl group to carbon 5 position of cytosine, forming 5-methylcytosine

Methylation

- The adding of methyl groups to the cytosine in the CpG islands inhibits the activator from binding to the enhancer, inhibiting transcription
- This is because the methyl groups block the binding of the activator protein to an enhancer element

- Also, methylated CpG pg islands recruit other DNA binding proteins such as histone deacetylase and methyl CpG binding proteins

- HDAC removes acetyl groups from histone proteins, chromatin adopt a more closed conformation → restricting access to GTF and RNA pol to bind to promoter, inhibiting transcription

Regulation at transcriptional level

<u>GTF:</u>

- proteins that are required for the binding of RNA polymerase to the promoter for transcription to occur
- essential for transcription of all genes
- Initiates transcription at a low rate so that few transcripts are produced

<u>Specific transcription factors:</u>

- Selectively bind to control elements other than promoter
- Interact with components of the transcription apparatus
- Control RNA polymerase II via mediator

What does the mediator do?

- Phosphorylation of RNA polymerase II to regulate transcription
- Activators bind and stimulate mediator to phosphorylate RNA polymerase II
- Repressors does the opposite

<u>Combinatorial control</u>

What is involved in this process?

- Multiple DNA control elements and proteins that control transcription
- Some TF
- One or more activator or repressor

<u>Coordinate control</u>

Post-translational level

- Basically pre-mRNA modification

Translational level

Half-life of mRNA

- Determined by length of it poly-A-tail
    - Longer length, longer it can be used as a template to make proteins

How is the mRNA removed?

- Enzyme involved: ribonucleases

How does the enzyme remove mRNA

- Gradually shortens the tail until critical length triggers
    - Removal of 5' cap and exposed mRNA will rapidly degrade by its 5' end
    - Degradation from the 3' end

Formation of translation initiation complex

- Needs interaction of 5'cap and 3' poly-A-tail before small ribosomal subunit can be recruited for initiation of translation

How does initiation of translation be regulated?

- Repressors that bind to the 5' end of mRNA
- Repressors recognize the 3'-UTR sequence and decrease translation by interfering communication between 5' cap and 3' poly-A-tail
- Phosphorylation and dephosphorylation to activate TIF

Post-translation level

Biochemical modifications

Proteins and enzymes involved:

TYPE OF MODIFICATION	**DESCRIPTION**	**Enzymes**
Phosphorylation	Addition of phosphate groups	Kinases
Dephosphorylation	Removal of phosphate groups	phosphatases

Acetylation	**Addition and removal of acetyl groups**	
glycosylation		
proteolysis		

Protein degradation

Enzyme involved: proteasomes

Protein involved: ubiquitin

What is the role of protein and enzyme?

- Ubiquitin target signals recognized by large, protein-degrading enzymes such as proteasomes