

Organisation and Control of Prokaryotic and Eukaryotic Genome – Summary

ORGANISATION

A. Prokaryotes:

- Single DS circular DNA
 - Associated with small amount of proteins
- Located within nucleoid region
- Smaller DNA rings – Plasmids
- Protein coding genes usually arranged in an operon.
- Genes closely packed – very few non-coding gaps

B. Eukaryotes:

NON-CODING REGIONS:

- Introns
 - Within genes
 - Alternate RNA splicing – codes for more than one polypeptide
- Transposons
 - Between genes
 - Short inverted repeats that flank coding DNA
 - E.g. GAA Gene AAG
 - Move from one location on genome to another
 - DNA intermediate; cut-and-paste mechanism
- Retrotransposons
- Simple Sequencing Genes (a.k.a. SATELLITE DNA)
 - Between genes
 - Most mammals – near centromeres
 - In *Drosophila* – in both centromeres and telomeres.
 - Microsatellites

- 1-3 bp, 15-100X
 - relatively stable, highly polymorphic DNA markers in linkage mapping.
- Minisatellites
 - 20-100 bp, thousands of times
 - used in DNA fingerprinting
- Pseudogenes
 - Genes that have lost function
 - Due to random mutations
 - Evolutionary significance

C. Telomeres

- Multiple repetitions of short **non-coding** nucleotide sequence
 - In humans, TTAGGG repeated 100-1000X
- Function:
 - Protects genes from erosion via successive rounds of DNA replication
 - **Telomeres serve as buffers (sacrificial protection)**
 - **To ensure that critical proteins will still be synthesised in the daughter cells despite the shortened chromosomes.**
 - **Cells will also undergo apoptosis after a limited number of cell division/mitosis (≈ 50) \rightarrow i.e. when a critical length of the telomere is reached.**
 - **This limits the extent of accumulated mutations and prevents the development of cancer.**
 - Prevents fusion of ends with the ends of other chromosomes (chromosomal mutation)
 - **Which can disrupt regulatory control of genes on the adjoined chromosomes**
 - Maintains integrity of chromosomal ends
 - Broken chromosomes that lack telomeres recognised as defective by cellular DNA repair machinery, which remedies situation by putting broken ends together, restoring the telomeres.
 - Inappropriate repair:
 - chromosome fusion OR
 - attracts enzymes that degrade the chromosome entirely.

D. Centromeres

- Appear as constrictions in eukaryotic chromosomes.
- Ensure proper segregation of chromosomes:
 - Hold sister chromatids together
 - Mitosis: up to beginning of anaphase
 - Meiosis: up to anaphase 2
- Elaboration of kinetochore (composed of DNA and proteins)
 - Kinetochore – site at which chromosomes attach to the spindle fibres (both during mitosis and meiosis)
 - Motor proteins in kinetochore – assist movement of sister chromatids to opposite poles (during anaphase, after the centromere holding 2 sister chromatids divides)

F. Gene Amplification

- Process of increasing number of copies of a gene
- 2 mechanisms:
 - DNA replication
 1. Single strand broken
 2. Broken strand replicated – double stranded break occurs
 3. Leads to chromosomal rearrangement, including gene amplification
 - Recombination and segregation
 - Misalignment and recombination between sister chromatids
 - One chromatid with a duplicated segment
 - Another with a deleted segment
 - Further rounds of misalignment causes linear amplification of duplicated segment.
- Significance varies with organism
 - In developing South American clawed frog, simultaneous transcription of amplified rRNA genes allows ribosome synthesis to be completed in 60 – 80 days instead of 1000.
 - Association with drug resistance in malignant tumours in mammals.
 - Generation of multiple copies of oncogenes in mammals.

CONTROL

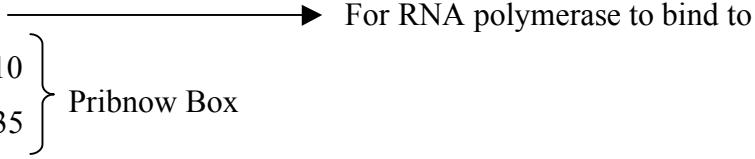
4 levels:

- Transcriptional
- Post-transcriptional
- Translational
- Post-translational

Stage	Prokaryotes	Eukaryotes
Transcription	<ul style="list-style-type: none"> • Promoter <ul style="list-style-type: none"> ○ Has consensus sequences <ul style="list-style-type: none"> ▪ TATAAT at -10 ▪ TTGACA at -35 • Sigma Factor • Operon 	<ul style="list-style-type: none"> • Promoter <ul style="list-style-type: none"> ○ TATA box at roughly -25 • Transcription Factors <ul style="list-style-type: none"> ○ ** required to recognise the TATA box and to recruit RNA polymerase • Control Elements <ul style="list-style-type: none"> ○ 4 types: <ul style="list-style-type: none"> ▪ Promoters <ul style="list-style-type: none"> • High level of transcription: binding of transcription factors to control elements beyond the promoter on DNA. ▪ Promoter-proximal elements (PPE) ▪ Enhancers <ul style="list-style-type: none"> • are distal control elements • looping mechanism ▪ Silencers <ul style="list-style-type: none"> • Competitive DNA binding • Masking activation surface • Direct interaction with the general transcription factors
Post-Transcription	<ul style="list-style-type: none"> • mRNA is immediately ready for translation 	<ul style="list-style-type: none"> • 5' 7-methylguanosine (7-MG) capping • 3' PolyA tail • RNA Splicing
Translation	<ul style="list-style-type: none"> • mRNA degradation <ul style="list-style-type: none"> ○ degraded by nucleases after only a few minutes • initiation of translation <ul style="list-style-type: none"> ○ anti-sense RNA 	<ul style="list-style-type: none"> • mRNA degradation <ul style="list-style-type: none"> ○ half-lives from minutes to months • initiation of translation <ul style="list-style-type: none"> ○ initiation factors
Post-Translation	<ul style="list-style-type: none"> • Covalent Modification (In Golgi Apparatus) • Phosphorylation/Dephosphorylation • Feedback control 	
	Cytoplasm	Golgi Apparatus

A. Prokaryotes

Transcription

- Promoter
 - Two short sequence elements within the promoter
 - Located approximately at -35 and -10
 - Consensus sequences  For RNA polymerase to bind to
 - TATAAT at -10
 - TTGACA at -35
- Sigma Factor
 - Subunit of RNA polymerase
 - Recognizes promoter elements (at both -10 and -35)
 - Disassociates from polymerase once transcription begins
 - Different sigma factor for different genes
 - **Specificity ensures only certain genes are transcribed only when correct sigma factor becomes available
- Operon
 - Repressible trp operon
 - Inducible lac operon

Post-Transcription

- mRNA is immediately ready for translation

Translation

- mRNA degradation
 - short lifespan
 - degraded by nucleases after only a few minutes
- initiation of translation
 - anti-sense RNA
 - binds to mRNA to down-regulate its translation

Post-Translation

- Covalent Modification
 - Occurs in cytoplasm

- Phosphorylation/Dephosphorylation
- Feedback control

B. Eukaryotes

Transcription

- Promoter \longrightarrow For RNA polymerase to bind to
 - TATA box at roughly -25
- Transcription Factors
 - ** TRANSCRIPTION FACTORS required to recognise the TATA box and to recruit RNA polymerase
 - vs. prokaryotic sigma factor
 - specific in binding (to proteins, other transcription factors and control elements)
- Control Elements
 - a.k.a. cis-acting elements
 - non-coding DNA sequences
 - bound by transcription factors to help regulate the transcription of a gene
 - 4 types:
 - Promoters
 - basal level of transcription: transcription factors interacting with RNA polymerase with promoter.
 - High level of transcription: binding of transcription factors to control elements beyond the promoter on DNA.
 - Promoter-proximal elements (PPE)
 - lies within 100-200 bp upstream from start site of transcription and TATA box
 - CAAT box and GC box found within -50 and -100 region
 - Enhancers
 - (compared to PPE) are distal control elements
 - positive regulatory elements; upregulation of transcription
 - thousands of nucleotides upstream, downstream of transcription start or even within an intron
 - bound by transcription factors known as activators.
 - looping mechanism

- direct interaction of the activators with the RNA polymerase or transcription factors at the TAT site upregulates/stimulates transcription of a gene.
- Silencers
 - DNA elements
 - Inhibit gene expression
 - Bound by transcription factors known as repressors
 - Mechanism:
 - Competitive DNA binding
 - Masking activation surface
 - Direct interaction with the general transcription factors

Post-Transcription (still pre-mRNA)

- 5' 7-methylguanosine (7-MG) capping
 - prevents degradation by cellular nucleases
 - recognition of resultant mRNA by initiation factors (promoting ribosome binding to initiate translation)
- 3' PolyA tail
 - DNA 10 – 35 nucleotides beyond AAUAAA sequence cleaved enzymatically.
 - PolyA polymerase then adds about 200 adenine nucleotides to 3' end.
 - Enhances stability of mRNA, impeding degradation by nucleases
 - Direct the transport of mRNA from nucleus to cytoplasm
- RNA Splicing
 - Spliceosome removes introns, joins exons
 - Various permutations of splicing

Translation

- mRNA degradation
 - half-lives from minutes to months
- initiation of translation
 - initiation factors
 - needed to enable ribosomes to attach to the mRNA for initiation of translation

Post-Translation

- Covalent Modification
 - Occurs at golgi apparatus
- Phosphorylation/Dephosphorylation
- Feedback control