

Different expression of protein-encoding genes in prokaryotes and eukaryotes:

- Prokaryotes have functionally similar genes regulated under one operon, one transcription start site but many translation start site on mRNA
- Eukaryotes have promotor for individual genes, mRNA undergoes processing.

• Lac operon in E.Coli

- CAP - Promotor - Operator - LacZ - LacY - LacA
- CAP: Catabolite activation protein binding site
- Promotor: allows $\sigma 70$ binding, assembly point of transcriptosome
- Operator: binding point of repressor
- LacZ: β -galactosidase (Catabolism of lactose)
- LacY: Permease (lactose transporter)
- LacA: Transacetylase (removal of toxic waste)

• Prokaryotic transcription regulation: Lac operon

- At low glucose levels, cAMP concentration increases (Glucose \leftarrow Adenylyl cyclase \rightarrow cAMP)
- cAMP binds to CAP, bind to enhancer region, upregulate Lac Operon transcription
- At high lactose level, lactose bind to lac repressor, sequestering from it binding with operator, upregulate lac
- Vice versa

• Eukaryotic transcription regulation:

- Assembly of initiation complex
- Regulation via proximal promotor region
- Regulation via distal enhancer region (with mediator)
- Unique expression of different regulators/general TFs in different tissues.

• Levels of regulations:

- Basal transcription level: influenced by repressor/activator
- Tissue specific regulation: presence of unique TFs in certain tissues
- mRNA processing regulation: Regulate 5'cap, 3'poly-A, alt splicing create different transcripts

• Transcription factors

- Transcription factors usually have two domains: Ligand binding domain and DNA binding domain
- E.g. Glucocorticoid receptor: responsive to growth hormone, GH bind to LBD, form dimer, DBD bind to DNA.

Epigenetics: Inheritable changes in phenotype caused by reversible changes on chromatin but not DNA sequence itself

Methylation:

- Addition of a methyl group to the CpG sequence
- Extensively in eukaryotes but lost in yeast and worms.
- Unmethylated DNA indicates foreign gene in bacteria
- Functions:
 - Methylation repress promotor activity, repress transcription, controls X-chromosome inactivation
 - Blocks splicing sites \rightarrow alt splicing, inhibit transposable element relocation
 - Involved in imprinting, inherit phenotypes

CpG islands: region of high CpG sequence, found in 5' end of the gene in promotor region(~1-2kb)

Methylation inhibition mechanism:

- CpG with attached methyl group - physical impedement for protein binding
- However, Methyl-CpG binding protein bind exclusively to methylated CpG, can action/recruit proteins

Maintenance methylation and De Novo methylation

- Maintenance methylation:
 - enzyme involved: ubiquitously expressed DNA methyltransferase (DNMT1)
 - After replication, daughter strands are hemimethylated, DNMT1 binding methylated other strand
- De Novo methylation
 - Enzymes involved: DNMT3a and 3b.
 - Create new methylation patterns, developmentally regulated
 - E.g. methylation erasure after fertilisation, rewriting methylation pattern in early development.

Imprinting: exception to erasure and rewriting during fertilisation.

- During gametogenesis, a gene is turned on/off at a locus depending on the sex of the organism
- Some genes are expressed only in eggs, others only in sperms, this ensures offspring only have one set of active allele.
- During gametogenesis/meiosis, imprinting are erased and pattern is established based on the sex of the gamete producing individual.
- Imprinting methylation occurs at imprinting control region (ICR) upstream of the gene.
- E.g. Igf2/H19 genes in human
 - During female gametogenesis, egg generated by the female removes all methylation at this allele, CTCF bind to imprinting control region, prevent enhancers to mediate and promote Igf expression.
 - In male gemetogenesis, the Igf/H19 gene in the sperm is methylated, inhibit H19 transcription, promote Igf expression.
 - IGF allele have a father origin-dependent expression pattern, H19 have a mother origin dependent expression pattern. Imprinting genes have a parent-of-origin-dependent expression pattern.
 - In offsprings, one allele express H19, other express Igf, both at moderate amounts.
 - Defect: Beckwith-Weidemann syndrome(Igf +/+), abnormal large size, low blood sugar
 - Defect: Silver-Russel syndrome(H19+/+), stunted growth, big head

Eu/Pro protein expression genes regulated as

Lac structure, regulation

Eukaryotic regulation levels, methods

TF structure

Epigenetics def

DNA Methylation mechanism function

Two types of methylation

Imprinting