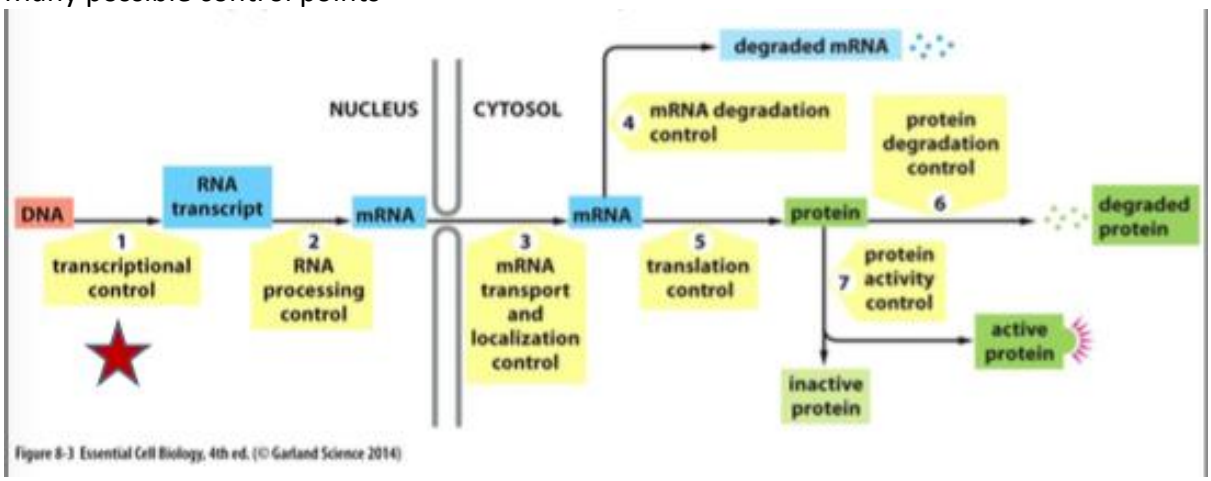
Table of Contents

Gene Expression- Overview

Differentiating cells

- Achieved through changes in gene expression
- All cells contain the same whole genome
- A typical differentiated cell only expresses ~50% of its total gene

Overview

- Many possible control points
- 

Transcriptional control

- main mechanism of gene expression regulation
- regulation by proteins (regulatory proteins) binding directly to DNA at promoters, regulatory DNA sequences (aka regulatory sites)- can be distant from the gene
- Different effects on expression: activate (on) repress (off) enhance (low to high)

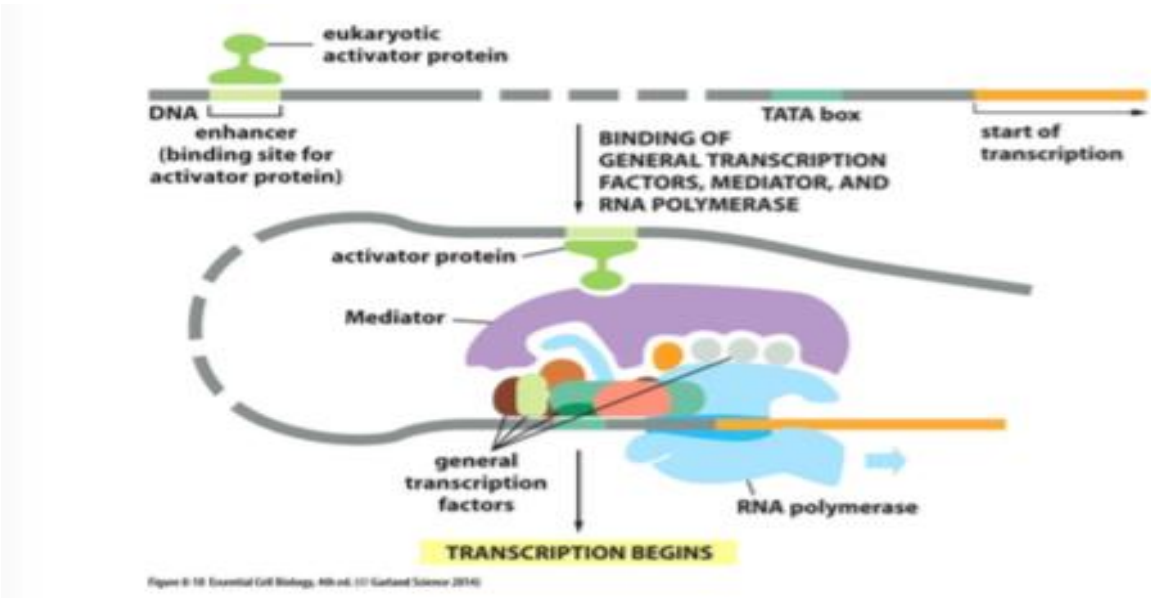
Prokaryotes

- One or two regulatory proteins, impact gene expression
- often simple switches (activators/repressors)

Eukaryotes

- combinatorial control of expression
- multiple regulatory proteins that influence the level of transcription at any gene at any time
- several thousand regulatory proteins in humans
- more complex, more utility, fine tuning

Combinatorial control Diagram



Regulatory proteins

- also called: transcriptional regulators
- bind to specific sequences, base components via the major groove of DNA helix
- recognise surface features of DNA at a sequence
- bind from outside (no unzipping)

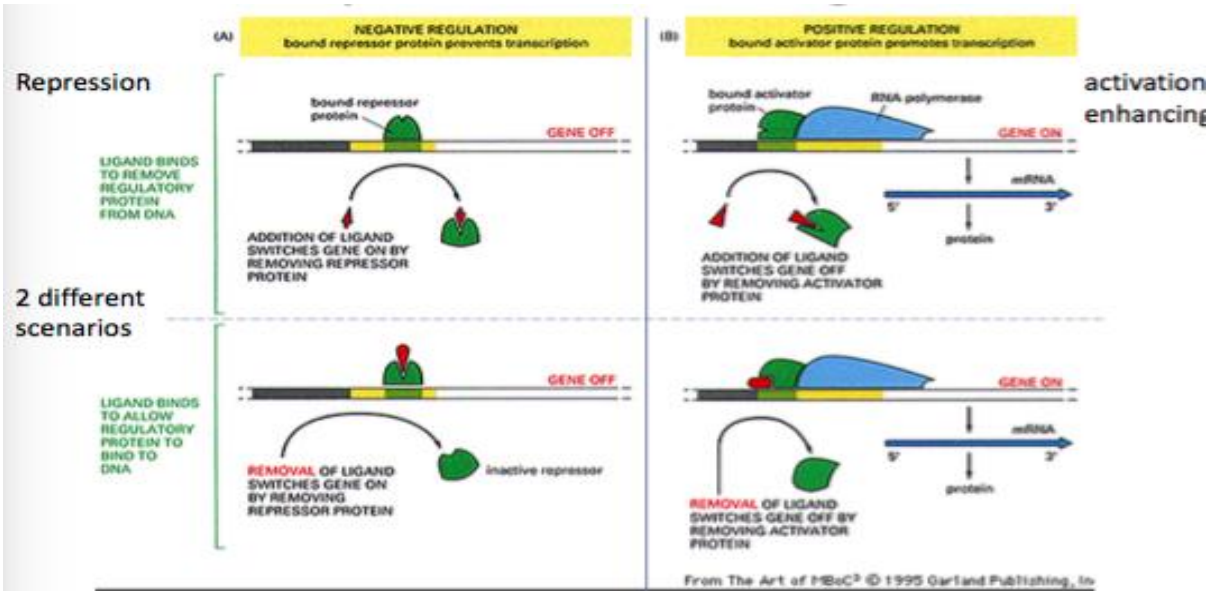
Double Helix

- usually represented as uniform 36 twist/bp
- 10 nucleotides/turn
- It is actually sequence dependent resulting it to be non-uniform
- TATA box of promoters changes the conformation of DNA at that location
- Allows lock and key interaction with regulatory proteins (no unzipping)

Specific binding

- DNA binding proteins have specific shapes
- Interact with 8-10bp long sequences in the double helix, have specific surface features
- Leucine zipper- strong and specific bond in nature

Regulatory protein influences



Transcriptional activators

- Switch genes on in their active form
- Work on promoters that function poorly unless the activator proteins bind to help transciprtion

Repressors

- Switch genes off in their active form
- E.Coli: affects ability to biosynthesis aa tryptophan (needs it to function)
- 5 gene encode enzymes required
- trabscribed as an operon with a single promoter
- when tryptophan is low this operon is transcribed
- tryptophan is high, tryptophan represson binds to tryptophan and then to the operator (DNA sequence recognised by regulatory protein)
- If a mammal eats a protein rich meal, stops cell synthesising its own until it is done, when E coli removes active repressor and produces again

Two Signal regulation

- More than one type of regulatory protein that influences expression, even in prokaryotes

Lac operon in E.coli, lactose digestion

- First gene in the operon LcZ produces an enzyme that breaks down lactose (galactose+ glucose)
- Controlled by cap activator + lac repressor
- If glucose available it uses that, if not lac operon switched on so other C sources (like lactose) be used
- Glucose and lactose: operon off
- Glucose no lactose: operon off

Lac Repressor

- No glucose, No lactose: No point in having operon switched on operon off, lac repressor binds to operator
- Lactose, no glucose: allolactose binds to lac repressor and changes shape so it can't bind to operator any more
- Two switches has increased the opportunity for control of gene expression

CAP Activator

- No glucose: it is activated
- Cell produces cyclic AMP (cAMP)
- cAMP binds to CAP which can bind to DNA
- Can't bind without cAMP and operon is switched off

Combinatorial Control

Prokaryotes

- Operon: cluster of genes transcribed as a single mRNA from a single promoter- can have very small genomes, low level of control (no finessing)
- Operator: regulatory sequence within operon promoter
- Two signal
- One promoter controlling expression in all

Eukaryotes

- Similar but more complex than prokaryotes, repression, enhancement possible in gene expression in control
- More control
- One controller per gene

Repressor/activator coordination

- When both bound they cancel each other out
- More complex situations (multiple regulatory proteins interact very many ways)
- Competitive DNA binding: overlapping repressor or activator sites
- Masking activation surface: proteins interact to mask one of the surfaces
- Direct interaction with general transcription factors

Combinatorial control

- Coordinated combined control mechanism
- PEPCK gene involved in glycolysis diagram (promoter and regulatory region)

Regulatory proteins

- Nuclear binding regulatory proteins come from the cell itself

- Co-ordination occurs in multiple layers
- Intracellular regulation: self-regulation

Intercellular regulation:

- coordinated cell signalling – e.g. hormones
- Also GTP binding proteins, protein phosphorylation, translocation to the nucleus

Cell Signalling

- Can induce fast or slow responses to extracellular signals
- Can have multiple effects
- Fast response (sec-min): extracellular signal interacts with surface proteins and alters cell behaviour
- Slow response to extracellular signals: altering gene expression of protein synthesis

Cell Signalling example

- Protein binding to extracellular recepto
- Stimulates cascade of sequential action
- Produces change in gene expression

Example 2

- Protein binding to intracellular receptor
- Stimulates cascade
- Produces change in gene expression
- Cortisol binding to dimer and going into nucleus which changes gene expression

Example 3

- Coordinated expression between tissue can be initiated by cell contact
- E.g.: extracellular matrix, a secreted substance outside the cell, used to glue layers of cells together like skin cells, can interact with other cells to form coordinated structures
- E.g. organ formation in embryogenesis

Control of expression in eukaryotes

- Transcription affected by nucleosome packaging affecting g.e.
- Repressors and activators do not have to bind close to the promoter can be several thousand bases away

RNA polymerases

- RNA polymerase I- most rRNA genes
- RNA polymerase II- all protein encoding genes, miRNA genes and genes for noncoding RNAS (those in spliceosomes)
- RNA polymerase III-tRNA genes, 5S rRNA genes, genes for many other small RNAs
- RNA polymerases are gene specific, require general transcription factors to initiate transcription
- Combined effects on transcription
- Concentration of transcription factors varies between cells and over time
- TF= Transcription factor I= number polymerase e.g. TFIIB

Eukaryotic key feature

- Regulatory proteins can bind away from the gene
- IN order for an activator protein to interact with a complex the DNA has to bend around

Distance of promoters

- Most are ~500bp from promoter
- Always a gap between it and promoter needs enough separation for the DNA to flex

Combinatorial control

- Multiple proteins binding to multiple sequence
- Presence or absence of any components affects gene expression
- Different in different stages and tissues