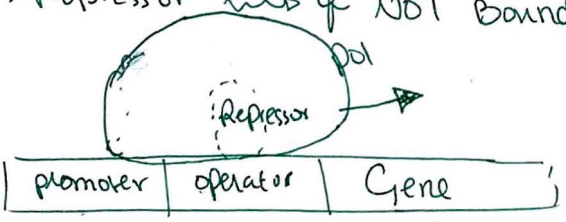
Genes
- Inducible → Regulation of expression involves these
  - ↳ expressed temporarily, ↑ or ↓, complicated structure.
- Constitutive → "housekeeping"
  - ↳ expressed permanently, fixed state, simple structure

Regulation of expression
1) Transcription
2) RNA processi
3) RNA transpor
4) Translation
5) post-translatio
↳ (phosphoryla

- Type A Response:
  - Inducing signal → ↑ expression (continued).
  - → in Response to: Intracellular conc. of Nutrient. (commonly observed in prokaryotes)
- Type B Response:
  - Regulatory signal → ↑ expression (Transient)
  - → during development of organism.
- Type C Response:
  - termination of signal → persists (↑ expression)
  - → during development of tissue / organ.
- Functioning unit of Genomic DNA, w/ single promoter, common operator => Operon (Cluster of Genes).
  - * primarily in prokaryotes.
- operon = promoter + Regulator + operator + structural genes.
  - Regulator: b w/ inducers & corepressors @ cytoplasm. → Control operator.
  - operator: Repressor binds to it.
  - structural genes: Co-regulated by operon.

* Operon Classification:
1. Catabolic (Inducible)
   - → Lac / Ara operon
2. Anabolic (Repressible)
   - → Trp operon.
3. Others

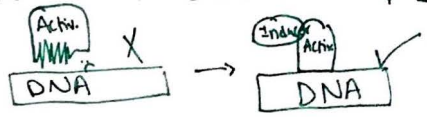
operator ⟺ Repressor ⇝ if NOT Bound → Promoter ⟺ Pol



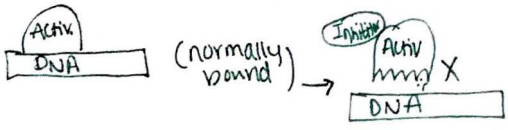
Inducer → binds Repressor → changes shape & unbinds. (un-negative control)

(-)ve control → Repressor

(+)ve control → Activator
- Normally can't bind ⇝ Inducer binds to it & it changes shape & can bind.



((+)ve Inducible Operons) ⇝ Activate transcription
+ Activator binds to Both DNA & RNA pol.



((+)ve Repressible Operons) ⇝ Stop transcription.

Lactose Genes.

promoter near LacZ

Lac Operon (3 structural genes):
- LacZ → β-Galactosidase.
- + LacY → β-Galactosidase permease. (transport of lactose across membrane).
- + LacA → β-Galactosidase acetyltransferase. (protects other intracellular galactosides from β-galactosidase).

Inducer: Allolactose.

Repressor: Glucose.

* Transcription of Lac operon → 1 polycistronic 3 genes

* LacI → diff. gene → Lac Repressor (LacI protein).

° NO Lactose: Repressor ⟺ operator. (sigma ✗ RNA pol.)

° Negative Regulation of Gene Expression: [Structural Genes Initially in a suppressed state]

• Lactose: Inducer ⇝ Releases LacI protein from O.
↳ Allolactose (stronger).

BOTH: Negative Allosteric effectors for Repressor (prevent Negative Control).

* Monod & Jacob Screen Results.
→ (1) LacZ Mutants.
→ (2) LacY Mutants.
→ (3) LacI Mutants: prod. β-galactosidase & permease even in absence of Lactose. (or Presence)
→ Constitutive Mutants.

* Reg. Site = Promoter + Operator + CAP ("Catabolism activator protein")
↕ Requires cAMP ← ⊖ Glc inhibits Adenylyl Cyclase.
CAP Protein

* Glc Regulates through:
(1) ↓ cAMP (inhibit Adenylyl Cyclase)
(2) dephosphorylation & inactivation of Gal. Permease in E. Coli Membrane.
⇒ Lac I still bound.

* HIGH Glc & HIGH Lac ⇝ infrequent, Negligible transcription.

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* Bacteria: Transcription product = polycistronic mRNA (operon inc. Reg. elements)

* mRNA of Most Inducible proteins ($t_{1/2}$ = <2 Mins.) high turnover

* primary function of transcriptional control:
→ Adjust Cell's Need of proteins according to environmental conditions

* polycistronic mRNA → different PP chain
* cistron (DNA) → 1 PP. (1 hereditary unit).

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* Coinducer (Actions):
(1) Repressor → Inac. Repressor (dissociates)
(2) Inac. Inducer → Active Inducer (binds)

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* Co Repressor Actions:
(1) Inac. Repressor → Ac. Repressor (Associates)
(2) Active Inducer → Inac. (dissociates).

Activator proteins:
(1) AraC pr. → Ara operon
(2) CAP pr. → Lac operon

Ara Operon = 3 genes for Arabinose Metab. + promoter + Initiator sequence ⇕ AraC (activator)

↝ {Arabinose (pentose) Metab in E. Coli}

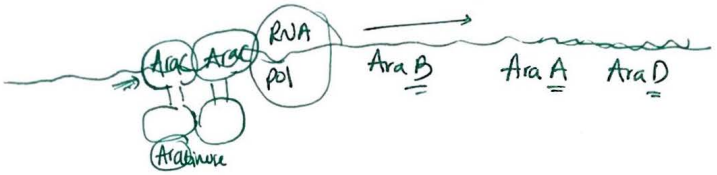
ONLY @:

* HIGH Arabinose → HIGH expression

(NO Arabinose → No expression).

↳ Arabinose ⟺ AraC protein ⟺ Initiator

→ helps RNA polymerase bind @ promoter → transcription.



Trp. Operon = 5 genes for Trp synthesis + promoter + operator.

* Controlled by:

(1) * @ LOW Trp → HIGH Expression.

(there all Trp repressors, can't bind operator on their own).

↳ Trp: Corepressor ; Trp ⟺ Repressor ⟺ opelator.

* Trp Repression isn't always complete ⇒ We have Attenuation.

(2) Attenuation: "pause structure", "hairpin-loop".

↳ high Trp → * Ribosomes pass over Trp codons quickly → terminator loop → premature abortion.

↳ low Trp → Ribosomes stall @ Trp codons → Antiterminator loop → transcription continues.

Eukaryotic Control of Gene Expression.

- Eukaryotic Genome = 1000x prokaryotic (More genetic info, greater specialisation, enormous Amt that doesn't code (Exons))
- Additional Genetic info → Regulation
  @: (1) differentiation of tissues.
  (2) Biologic processes
  (3) Response to complex environmental challenges.
- Control @: (1) Transcription.
  (2) processing of mRNA.
  (3) Transport & localisation of mRNA.
  (4) Translation
  (5) degradation of mRNA
  (6) Protein Activator Control.

DNA Remodelling: Histone exchange.
Using: ATP-dependent Chromatin Remodelling complex
requires: ATP.
- 1 ATP → H2A-H2B dimer exchange.
- 2 ATP → exchange of entire Nucleosome core

CHROMATIN
2 forms.

Euchromatin ⟺ Heterochromatin.

Euchromatin	Heterochromatin
less coiled	highly condensed
less Methylation	Methylated.
✓ transcriptionally active	✗ transcriptionally inactive.

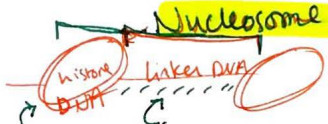
(Switch : ON & OFF Genes)

DNA: highly organised.
- Consequence:
  1) Better control of expression
  2) Controlled Access of TF

Histone octamer → 30 nm DNA fibre
= 8 subunits
= H2A + H2A + H2B + H2B + H3 + H3 + H4 + H4
DNA linker →

N-tail terminals: H2B, H3

Nucleosome ~ 200 Nucleotides.



* Wrapped ↝ 250 ms.
Unwrapped ↝ 10-50 ms ← Sequence specific DNA-binding protein binds to? unwrapped.

Chromosome Length: 10,000x shorter than DNA Length.

* Mechanisms:

1 Chromatin Remodelling.
* each cell contains the same complement of genes (Exception: Ab-producing cells ~~genes~~)
↝ Differential expression.
* DNA w/ β-Globin gene cluster → "Active" chromatin: Reticulocytes.
→ "Inactive" " : Muscle cells.

Processes:

A) Histone Acetylation & DeAcetylation:
* Lysine Residues @ Amino terminals of Histone Molecules (H3)?
* Reduces (+)ve charge → ↓ Binding affinity w/ (-)ve DNA.

Acetylation ↝ Readier Access ↝ ↑ Transcription
De-Acetylation ↝ ↓ Transcription

* Catalysed by: HAT (Histone Acetyl Transferases)
HD (HDAC ↝ Histone Deacetylases)

* Acetylation: HAT + coactivator → Euchromatin.
DeAcetylation: HDAC + corepressor → Heterochromatin.

* Ubiquitination:
H2A → inactivation.
H2B → activation.

DNA Methylation

→ Symmetrical CG sequences.
→ Predominantly @ Cytosine. (→ 5-Methylcytosine).
→ Inactivates gene expression.

*CpG Island:
DNA region w/ Many CG
C can become methylated.
↳ Mammals: 70-80% of CpG Methy

* Lysine Methylation & Acetylation all competing rxns.

Acetyl-Lys ← Lysine → Mono methyl-Lys → Dimethyl-Lys → Trimethyl-Lys.
"Methylated 3 times"

* Serine phosphorylation (→ phosphoserine).

B) Modification of DNA.

Gene: Tyrosine Aminotransferase.
→ Acute demethylation of deoxycytidine at a specific region in Response to: Glucocorticoids
⇒ ↑ rate of transcription.

C) DNA binding proteins.

Serial binding of transcription factors ⇒ directly disrupts Nucleosome ⇒ prevents re-formation] ↑ Trans Ctiph

* DNA Methylation is inherited by daughter cells.
⇒ Maintenance Methylase (Maintains Methylation @ same sites after DNA Replication)

* Effects of DNA Methylation:
1) inactivation of single gene expression.
2) " " genes from part of chromosome.
3) " " genes of whole chromosome (X)

in Short: Most imp histone Modifications:
1) Acetylation.
2) Methylation.
3) Phosphorylation.
4) Ubiquitination.

Histone Modification sites:

H2A → Mainly Acetylation
H2B → " Acetylation → one Phosphorylation & one methylation
H3 → " Methylation
H4 → " Acetylation

Modification	State	effect/Meaning @ H3
Methylation		gene silencing / inactivation / heterochromatin formation.
M	K A	Expression
P	K A	Expression
M		Silencing of Hox gene, X chromosome inactivation

(شوفي السلايد أحلى/أسهل للحفظ).

Hox genes → genes that control all other genes.

- HOX A @ chromosome 7 —control→ HOXA – 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13.
  1–13 except 8 & 12
- HOX B @ chromosome 17 → HOXB – 1, 2, 3, 4, 5, 6, 7, 8, 9, 13.
  1–9 + 13
- HOX C @ chromosome 12 → HOXC – 4, 5, 6, 8, 9, 10, 11, 12, 13.
  4, 5, 6, 8–13
- HOX D @ chromosome 2 → HOXD – 1, 3, 4, 8, 9, 10, 11, 12, 13.
  1, 3, 4, 8–13

للحفظ بالبنفسجي

(Conductor زي ال تشغلهم)
فيه أول وفيه آخر.

Cells from Nerves/Muscles → diff Morphologies, same DNA.

Promoters:
- Basal : 40 bp upstream → in all protein coding genes.
- Upstream : 200 bp upstream → structure & Binding factors vary

* Repeated Initiation → ~~Rede~~ Amplification.
* Repetitive DNA sequences for: rRNA genes & tRNA genes (Hundreds)
  - ↳ during early development of Metazoans they were Needed for Amplification (e.g. eggshells).
* Gene amplification example:
  Ca. Cells → ↑ DHF reductase genes (Resistance to Methotrexate)

* Eukaryotic Reg is More complex than prokaryotic:
  1. larger genome — P: 4.6 Mb, 2000 protein; E: 23 X 50-250 Mb, 40,000 genes.
  2. diff cell types
  3. Absence of genomes
  4. Chromatin structure.
  5. uncoupled transcription & translation.

* Enhancers → increase activity of promoters.
  - ↳ binding site for Reg. proteins.
  - ↳ distant, upstream or downstream, promiscuous (any, More than one),

  (promoters initiate lower levels of transcription)

* Silencers → Repress.
* Tissue Specific Gene Expression is Mediated by? Enhancers, Enhancer-like Elements.

* Immunoglobulins → Light Chain (e.g. ~~IgG~~ (25 kDa)) → V, J, C
  - V: 300
  - J: 5
  - C: 10

  @ same Region of same chromosome ⇒ Recombination (Gene Rearrangement)

* Heavy Chain (e.g. IgM (50 kDa)) → (1) Alternative polyadenylation).

Alternative RNA processing:
1) Alternative promoters.
2) intron-exon splice sites.
3) polyadenylation sites

mRNA
- (m): 2700 bases, remains attached to Membrane
- different carboxyl terminal
- (s): 2400 bases, secreted.

[2] Alternative Splicing & processing.

↝ 7 unique Tropomyosin in 7 diff tissues.

* Class Switching ↝ one gene switched off.
Closely related gene on.

* During intrauterine life, first Hb to be produced is: Embryonic Hb.*

⇒ 2 Zeta + 2 Epsilon —(by 6th Month of Intrauterine Life)→ $\alpha_2\gamma_2$ = HbF —(after Birth)→ HbA_1 (97%), HbA_2 (3%)

also @ Immunoglobulins (IgM or IgG)

1° Immune Response ↓ 2° immune Response.

in short:

IgM or IgG → Class Switching

Light chain variation → Gene rearrangement/Recombination.

Heavy chain variation → alternative adenylation

differentiate:

In Mammalian cells: Most mRNA $t_{1/2}$ = Measured in hours.

in prokaryotes: <2 Mins

Some mRNA $t_{1/2}$ = 10 - 30 mins (turnover rapidly).

* (mRNA stability is subject to Regulation)

effective in:

↳ Enzyme & its Normal substrate, or Receptor & its Normal ligand *

↳ Influenced by: Hormones.

↳ Ends of mRNA involved in its Stability

↳ e.g. Ferritin → low Iron → Stem loop @ 5' end stabilised by 90 kDa Protein (block progression of ribosome @ translation)

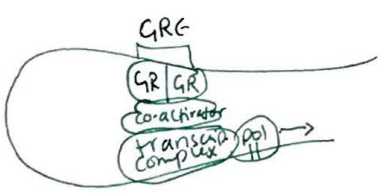
↳ high Iron → hairpin is lost → ↑ ferritin synthesis.

* DNA-Binding proteins:

↳ Steroids ⟺ Nuclear hormone Receptors ↝ bind DNA.
e.g. oestrogen. (TF)

conformational change

Recruitment of coactivators → catalysis of Acetylation @ Lys @ Histones → ↑ Transcription.

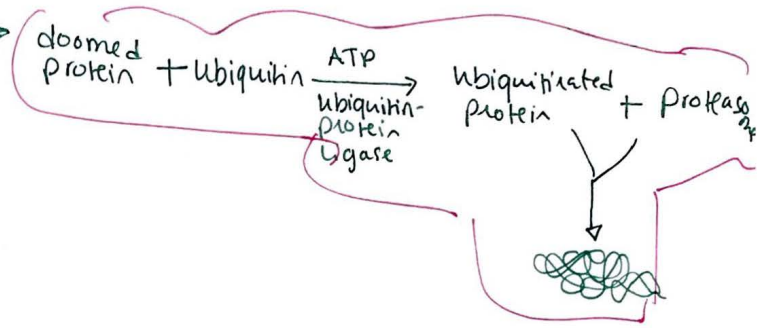


* RNA Editing.

e.g. Apo-B100 @ VLDL ← @liver. unedited mRNA: CAA (glutamine) —edited (deamination, NH_4^+) @intestines→ UAA (Stop! codon) → Apo-B48 @ CM

* protein Stability.

e.g. Ubiquitin Control proteolysis ↝ Cyclin Control of Cell cycle.



- Stability of protein depends on?

N-terminal AA.

Arg, Lys → $t_{1/2}$ = 3 Mins — Not super stable

Met, Ala → $t_{1/2}$ = 720 hrs — very stable.

+ Slide 43

Summary: Eukaryotic Gene Regulation.

* Chromatin Remodelling (hetero ⇌ eu)
1. Acetylation & Deacetylation ↳ Lys (@Histone)
2. Methylation. ↳ Cyt (@DNA)
3. Phosphorylation ↳ Ser
4. Ubiquitylation
5. DNA Binding Proteins ↳ (TF) Steroids → Nuclear Hormone Receptors

DNA-Related.
- Transcription ↳ promoters. (Basal/upstream)
- Gene Amplification ↳ rRNA & tRNA ↳ DHF reductase in Methotrexate immunity.
- Enhancers & Repressors. ↳ Produce diff. cell types.
- Class Switching ↳ Embryonic Hb → HbF ↳ IgM or IgG
- Gene Rearrangement. ↳ IgG Light chain.

RNA-related.
1. Alternative RNA processing.
   - A. Polyadenylation. ↳ IgM Heavy chains.
   - B. Splicing ↳ Tropomyosin
2. mRNA stability. ↳ ferritin
3. RNA editing ↳ Apo-B100 or B48

protein Stability
- ↳ Ubiquitin-dependent proteolysis
- ↳ Cyclins Control of cell cycle.