Gene regulation I
Biochemistry 302

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What constitutes “gene regulation”

• Extracellular signals
  – Chemical (e.g. hormones, growth factors)
  – Environmental stress (e.g. temperature)

• Intracellular effects
  – Change in cell phenotype
  – Change in gene expression
    • Cell cycle/replication
    • Transcription
    • Translation
Purpose of gene regulation in simple organisms vs metazoans

- Control of cell growth and division
  - Bacteria – environment
  - Lower eukaryotes (yeast) – environment
  - Higher eukaryotes – constant environment, respond to chemical signals

- Control of cell differentiation and development (metazoans)
  - Mediated by precise genetic programs
  - Generally irreversible (e.g. muscle differentiation)
  - Endpoint may be death (e.g. rbc’s, B-cells, skin cells)

The rate of synthesis and overall abundance of specific mRNA transcripts may differ by over four orders of magnitude from cell type to cell type in metazoans.
Paradigms of transcriptional regulation come from study of prokaryotic transcription factors

- **Role of transcription factors**
  - Regulators of RNA Pol binding affinity
  - Regulators of RNA Pol “isomerization”

- **Regulation of transcription factors by “ligands”**
  - DNA-binding
  - Protein-protein interaction

- **Historical view (Jacob and Monod)**

\[
RNAP + P \xrightarrow{K_B} RNAP:Pc \xrightarrow{k_2} RNAP:Po \xrightarrow{\text{elongating complex}}
\]

- Affinity
- Rate
Sugar utilization in prokaryotes: a paradigm of transcriptional regulation

- *E. coli* prefer to eat glucose but can adapt to environmental changes in nutrients
  - Lactose, arabinose, maltose
  - Metabolism of some sugars requires expression of specialized genes

- Jacob and Monod studying *E. coli* mutants displaying altered lactose metabolism, 1960s
  - Predicted the existence of an unstable mRNA template to account for rapid changes in β-galactosidase activity
  - Predicted the existence of a repressor that regulated the level of mRNA by binding to a specific operator sequence on DNA
  - Proposed a unifying hypothesis of gene regulation in which control of transcription initiation is the key feature
Structure of the lactose operon (structural genes + regulatory elements)

\[\text{lacZ}: \beta\text{-galactosidase (cleaves Lac} \rightarrow \text{Glc, Gal)}\]

\[\text{lacY}: \beta\text{-gal permease (transport protein)}\]

\[\text{lacA}: \text{thiogalactoside transacetylase (converts non-hydrolyzables)}\]

\[\text{lacI (or lacR): lac repressor (regulates the lacZYA cluster)}\]

CRP site: cAMP receptor protein (mediates regulation by glucose)
Operon model based entirely on indirect evidence from genetic analysis

lactose or allolactose in *E. coli*

**Fig. 26-18**

RNA polymerase

Primary control via repression!

**(...but it has largely stood the test of time.**
lac operon is up-regulated by inducer-repressor interaction and activator binding

**cAMP receptor protein (CRP) or catabolite activator protein (CAP)**

Isopropyl β-thiogalactoside (IPTG) used in the lab

Trans-activation not predicted by Jacob and Monod, also thought that the lac repressor was RNA.
Mapping of protein binding sites in the lac regulatory region via DNase I footprinting

Note how operator elements are imperfect palindromes (inverted repeats)
*lac* repressor: an allosteric gene regulatory protein

- First regulatory protein shown to bind DNA
  - Purified on the basis of IPTG affinity (Gilbert and Muller-Hill, 1966)
  - Tetramer of four identical subunits (~38 kDa)
  - Low intracellular concentration (~10⁻⁸ M)
- Binds specifically to operator \((K_a = 10^{13} \text{ M}^{-1})\) and non-specifically to DNA \((10^6 \text{ M}^{-1})\).
  - In response to inducer binding, repressor affinity changes from ‘specific’ to ‘non-specific’ mode.
  - Three binding sites (centered at +11, −82, and +432) but only +11 and −82 are true operators elements w/ repressor forming a DNA loop between them.
Crystal structure of the lac repressor (1996) provides insight into mechanism

- Tetramerization helix: joins two dimeric units
- Core domain: binds inducers e.g. IPTG, allolactose
- Headpiece: DNA-binding domain comprised of hinge & helix-turn-helix motif


93 bp loop

+11

−82

T Helix

N-terminal subdomain of core

C-terminal subdomain of core

HTH

Hinge
Molecular basis of inhibition of RNAP by lac repressor

-35 promoter site
-10 promoter site
CRP/DNA complex
-60

Lewis, M. et al. (1996)
Science 271:1247
Hinge helix region: an allosteric switch

- **no IPTG**
  - Packed $\alpha$-helical “hinge”
  - $\alpha$-helices stabilized by DNA (operator) and core contacts

- **w/ IPTG**
  - Rotation and translation of N-term subdomains
  - Destabilization of the hinge helices
  - Unpacking of hinge helix $\rightarrow$ headpiece “unfolding”
  - Loss of specific DNA contacts: concerted folding and binding in reverse
  - Repressor now has same low affinity for operator and non-operator sequences
IPTG drives *lac* repressor DNA-binding helices apart

Conceptual model of allosteric changes that disrupt DNA-binding

Rotation and translation
Other side of the coin: the biosynthetic \textit{trp} operon

• Amino acid biosynthesis consumes energy
  – Advantageous to turn off synthesis of biosynthetic enzymes when end product (amino acid) is available.
  – Regulatory goal is to repress gene activity.

• \textit{E. coli trp} operon (in contrast to \textit{lac})
  – \textit{trp} repressor is \underline{activated} by ligand binding.
  – Additional regulation by premature termination of transcription (“\underline{attenuation}” – regulatory dimmer switch involves ribosome positioning on 5’ mRNA)
    • Discovered by Charles Yanofsky, common to many biosynthetic operons including Trp, Leu, and His
    • Regulation by changes in RNA secondary structure
    • Extends the possible range of transcription rates (moderate to high trp levels)
Schematic of the *E. coli* trp operon (regulation by “activated” repression)

Fig. 26-33
Most stable secondary structure of the \textit{trpL} (\textit{trp} leader region)

Ribosome stalling due to lack of \textit{trp}-tRNA occurs when \textit{trp} levels are low favoring 2-3 base-pairing while disfavoring 3-4 base-pairing and premature transcriptional termination.
Attenuation – cooperation between translation and transcriptional machinery