Chapter 18

Regulation of Gene Expression
Control of Prokaryotic (Bacterial) Genes
Bacterial metabolism

- Bacteria need to respond quickly to changes in their environment
  - if they have enough of a product, need to stop production
    - why? waste of energy to produce more
    - how? stop production of enzymes for synthesis
  - if they find new food/energy source, need to utilize it quickly
    - why? metabolism, growth, reproduction
    - how? start production of enzymes for digestion
Feedback inhibition

- product acts as an allosteric inhibitor of 1st enzyme in tryptophan pathway

- **but this is wasteful production of enzymes**

Oh, I remember this from our Metabolism Unit!
Different way to Regulate Metabolism

- Gene regulation
  - instead of blocking enzyme function, block transcription of genes for all enzymes in tryptophan pathway
  - saves energy by not wasting it on unnecessary protein synthesis

Now, that's a good idea from a lowly bacterium!
Gene regulation in bacteria

- Cells vary amount of specific enzymes by regulating gene transcription
  - turn genes on or turn genes off
    - turn genes OFF example
      if bacterium has enough tryptophan then it doesn’t need to make enzymes used to build tryptophan
    - turn genes ON example
      if bacterium encounters new sugar (energy source), like lactose, then it needs to start making enzymes used to digest lactose
Bacteria group genes together

- **Operon**
  - genes grouped together with related functions
    - example: all enzymes in a metabolic pathway
  - **promoter** = RNA polymerase binding site
    - single promoter controls transcription of all genes in operon
    - transcribed as one unit & a single mRNA is made
  - **operator** = DNA binding site of repressor protein
So how can these genes be turned off?

- **Repressor protein**
  - binds to DNA at operator site
  - blocking RNA polymerase
  - blocks transcription
**Operon model**

Operon: operator, promoter & genes they control serve as a model for gene regulation.

**Repressor protein** turns off gene by blocking RNA polymerase binding site.

**repressor** = repressor protein

**AP Biology**
Repressible operon: tryptophan

Synthesis pathway model
When excess tryptophan is present, it binds to \textit{tryp repressor protein} & triggers repressor to bind to DNA
- blocks (represses) transcription

conformational change in repressor protein!
Tryptophan operon

What happens when tryptophan is present?

Don’t need to make tryptophan-building enzymes

Tryptophan is allosteric regulator of repressor protein

(b) Tryptophan present, repressor active, operon off

Tryptophan is allosteric regulator of repressor protein
Inducible operon: lactose

Digestive pathway model
When lactose is present, binds to **lac repressor protein** & triggers repressor to release DNA

- induces transcription

**conformational change in repressor protein!**
Lactose operon

What happens when lactose is present?

Need to make lactose-digesting enzymes

Lactose is allosteric regulator of repressor protein
Jacob & Monod: *lac* Operon

- Francois Jacob & Jacques Monod
  - first to describe operon system
  - coined the phrase “operon”
Operon summary

- **Repressible operon**
  - usually functions in **anabolic** pathways
    - synthesizing end products
  - when end product is present in excess, cell allocates resources to other uses

- **Inducible operon**
  - usually functions in **catabolic** pathways,
    - digesting nutrients to simpler molecules
  - produce enzymes only when nutrient is available
    - cell avoids making proteins that have nothing to do, cell allocates resources to other uses
Regulation of Gene Expression In Eukaryotes

The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.
The BIG Questions…

- How are genes turned on & off in eukaryotes?
- How do cells with the same genes differentiate to perform completely different, specialized functions?
Evolution of gene regulation

- Prokaryotes
  - single-celled
  - evolved to grow & divide rapidly
  - must respond quickly to changes in external environment
    - exploit transient resources

- Gene regulation
  - turn genes on & off rapidly
    - flexibility & reversibility
  - adjust levels of enzymes for synthesis & digestion
Evolution of gene regulation

- **Eukaryotes**
  - multicellular
  - evolved to maintain constant internal conditions while facing changing external conditions
    - homeostasis
  - regulate body as a whole
    - growth & development
      - long term processes
    - specialization
      - turn on & off large number of genes
    - must coordinate the body as a whole rather than serve the needs of individual cells
Points of genetic control

- The control of gene expression can occur at any step in the pathway from gene to functional protein:
  1. packing/unpacking DNA
  2. transcription
  3. mRNA processing
  4. mRNA transport
  5. translation
  6. protein processing
  7. protein degradation
Evolution of new traits can develop as mutations occur that turn on or turn off genes. **Turn those genes off!**

1. **Pre-transcription**
   - DNA packing - coils up DNA, like a chromosome
   - DNA methylation – methyl groups block transcription factors

2. **Post-transcription**
   - RNA Interference- breakdown by siRNA triggers degradation of mRNA

3. **Translation**
   - Regulatory proteins block start of translation

**Turns Genes On!**
- Histone acetylation

Histone acetylation is a turn-on for me!
1. DNA packing

How do you fit all that DNA into nucleus?

- DNA coiling & folding
  - double helix
  - nucleosomes
  - chromatin fiber
  - looped domains
  - chromosome

from DNA double helix to condensed chromosome
Nucleosomes

“Beads on a string”

- 1st level of DNA packing
- histone proteins
  - 8 protein molecules
  - positively charged amino acids
  - bind tightly to negatively charged DNA
DNA packing as gene control

- Degree of packing of DNA regulates transcription
  - tightly wrapped around histones
    - no transcription!
    - genes turned off
  - heterochromatin
darker DNA (H) = tightly packed
  - euchromatin
lighter DNA (E) = loosely packed
DNA methylation

- **Methylation of DNA** blocks transcription factors
  - no transcription!
  - → genes turned off
- attachment of methyl groups (–CH$_3$) to cytosine
  - C = cytosine
- nearly permanent inactivation of genes
  - ex. inactivated mammalian X chromosome = Barr body

![Methylation of DNA](image)
DNA Methylation

• DNA methylation can cause long-term inactivation of genes in **cellular differentiation**

• In **genomic imprinting**, methylation regulates expression of either the maternal or paternal alleles of certain genes at the start of development
Epigenetic Inheritance

- Although the chromatin modifications just discussed do not alter DNA sequence, they may be passed to future generations of cells.

- The inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called epigenetic inheritance.

http://www.youtube.com/watch?v=kp1bZEUgqVI

Oh gosh! My bad habits can be passed to my chicks and grandchicks!
Epigenetics Mechanisms

RNA Interference

Gene Expression

Histone Modifications

DNA Methylation
Histone acetylation

- **Acetylation of histones** unwinds DNA
  - loosely wrapped around histones
    - enables transcription!
    - genes turned on
  - attachment of acetyl groups (–COCH₃) to histones
    - conformational change in histone proteins
    - transcription factors have easier access to genes
Human transgenerational epigenetic phenomena?

Don't Count Dad Out

So if a pregnant mother's diet can affect the child's epigenetic outcome, can dad's diet do the same? Quite possibly, according to scientists who delved into the well-kept, historical records of annual harvests from a small Swedish community.

These records showed that food availability between the ages of nine and twelve for the paternal grandfather affected the lifespan of his grandchildren. But not in the way you might think.

Shortage of food for the grandfather was associated with extended lifespan of his grandchildren. Food abundance, on the other hand, was associated with a greatly shortened lifespan of the grandchildren. Early death was the result of either diabetes or heart disease. Could it be that during this critical period of development for the grandfather, epigenetic mechanisms are "capturing" nutritional information about the environment to pass on to the next generation?

http://en.sevenload.com/videos/tX02lnf-Nova-The-Ghost-In-Your-Genes-1-6

AP Biology  View NOVA special “A ghost in your genes”

Time magazine 2010

Utah Epigenetics website
Mechanism exist to “open up” chromatin.

Chromatin remodeling complexes alter primary structure of chromatin.

Histone modifying enzymes alter histone tail modifications.
Mechanism exist to “condense” chromatin

Histone modifying enzymes alter histone tail modifications

DNA methylases,

Recruitment of chromatin binding proteins Polycomb proteins Heterochromatin Protein

Tied up in knots. When not condensed, chromatin exists in a "beads on a string" conformation (left). But when treated with PRC1, the beads clump together (middle, right).
Mechanism exist to “open up” chromatin

Chromatin remodeling, histone modifications

Mechanism exist to “condense” chromatin

Histone modifications, DNA methylation, chromatin binding proteins

Can alter gene activity without change in DNA
Is it the existing chromatin state heritable?

Regulatory roles of chromatin

if yes: EPIGENETIC REGULATION

if no: CHROMATIN REGULATION
Epigenetic/chromatin phenomena

Chromatin-based restriction of genome accessibility during differentiation

Selective activation of genome after perception of stimulus (influence of environment/stress)

Mitotic maintenance of cell identity (or loss thereof in cancer)

Dosage compensation in the male versus female genome (X inactivation in mammals)

Memory, Behavior, Aging
RNA interference

- **Small interfering RNAs (siRNA)**
  - short segments of RNA (21-28 bases)
    - bind to mRNA
    - create sections of double-stranded mRNA
    - “death” tag for mRNA
      - triggers degradation of mRNA
  - cause **gene “silencing”**
    - post-transcriptional control
    - turns off gene! = no protein produced

siRNA
Control of translation

- Block initiation of translation stage
  - regulatory proteins attach to 5' end of mRNA
    - prevent attachment of ribosomal subunits & initiator tRNA
    - block translation of mRNA to protein
Turn your Question Genes on!