Outline Nov. 8

- Review the lactose (*lac*) operon
  - Predicting phenotypes of partial diploids

Examples of other operons:

- arabinose (*ara*) operon
- arginine (*arg*) operon
  - A repressible operon
- tryptophan (*trp*) operon
  - Two kinds of regulation
    - Repression
    - Attenuation

What are the similarities and differences in the control of these operons?

Types of Gene Regulation

- Gene regulation can occur at various steps
  - The amount of product depends on
    - rate of mRNA synthesis (transcription),
    - mRNA degradation,
    - protein synthesis (translation) etc.
- Prokaryotes commonly control transcription

Types of Gene Regulation

- Constitutive genes are always expressed
  - Tend to be vital for basic cell functions (often called housekeeping genes)
- Inducible genes are normally off, but can be turned on when substrate is present
  - Common for catabolic genes (i.e. for the utilization of particular resources)
- Repressible genes are normally on, but can be turned off when the end product is abundant
  - Common for anabolic (biosynthesis) genes

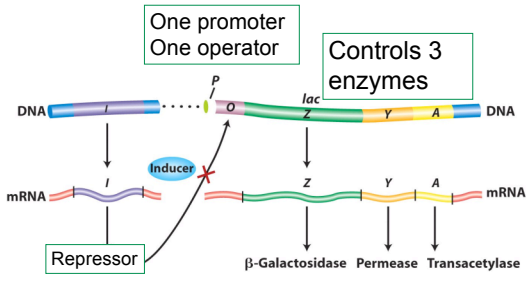
Terminology

- Repressors and Activators are proteins that bind to DNA and control transcription.
  - Those genes are said to be repressible or inducible
- Inhibitors and Inducers: small "effector" molecules that bind to repressors or activators

Operons

- In Prokaryotes, functionally related genes are regulated as a unit, called an operon.
- Operons consist of:
  - Several structural genes
  - ONE promoter and one terminator
  - A control site (operator)
  - A separate regulator gene (codes for protein that binds to operator)

Organization of the lac operon



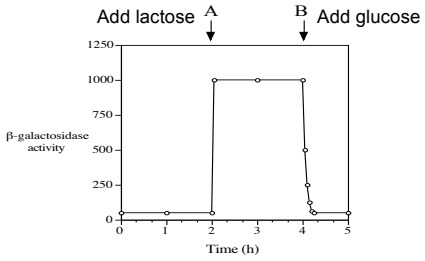
Overview of the lac Operon

- Gene is normally off
  - There is no transcription because a repressor binds to the control site
- When lactose is present, it inactivates the repressor, allowing transcription to begin.
  - Produces both lacZ (to break down lactose) and lacY (to let it into the cell)
- When lactose is used up, the repressor is again free to bind to DNA, and halt transcription.
- Glucose must be absent. If glucose is present, transcription doesn't start.

Glucose must be absent

- "Catabolite repression"
  - A separate regulatory mechanism controls the binding of RNA polymerase to the promoter.
  - If glucose is present, there is little cAMP, so the activator complex (CAP-cAMP) can not bind to the promoter region.

Cells respond quickly to available sugars



Some practice

- What is the phenotype:

lacI+ lacO+ lacZ- lacY+ / F' lacI- lacO+ lacZ+ lacY-

  - Will there be B-galactosidase activity?
    - With lactose? Without lactose?
  - Will there be permease activity?
    - With lactose? Without lactose?
  - Are the genes inducible (is there a difference with and without lactose)?

More practice

lacI+ lacO+ lacZ- lacY+ / F' lacI- lacO+ lacZ+ lacY-

- Make a table:

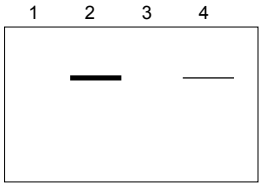
	-lactose	+ lactose	Interpretation
B-gal			
Permease			

Even More practice

  - Will there be B-galactosidase activity?
    - With lactose? Without lactose?
  - Will there be permease activity?
    - With lactose? Without lactose?

Which is more effective in regulating lac: repressor or CAP?

"Northern Blot" detects mRNA on a gel



1: no sugars
2: lactose
3: glucose
4: glucose+lactose

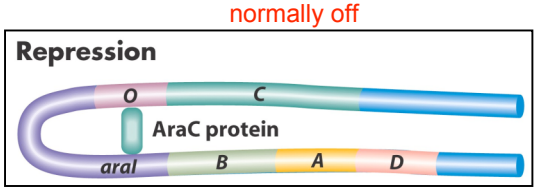
What effects do you predict for mutations in the operator?

- What is the phenotype for:
  lacI+ lacO^c lacZ- lacY+ / F' lacI+ lacO+ lacZ+ lacY-
  - What does the operator do?
  - What will be the consequence if it is non-functional?
  - Will the mutation be cis-dominant, trans-dominant, or recessive?

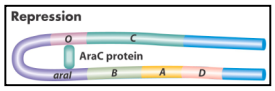
	-lactose	+ lactose	Interpretation
–B-gal			
–Permease			

Arabinose operon

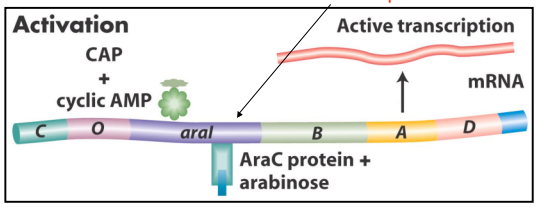
Transcription is normally off



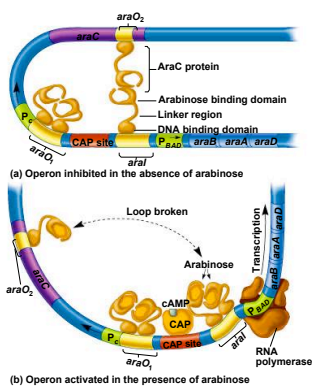
Arabinose operon



Arabinose binds to the repressor and "unlocks" transcription

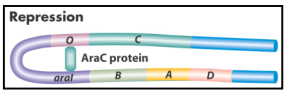


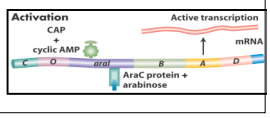
("Keep the operon turned off, until there is something to metabolize")



Where is the likely mutation?

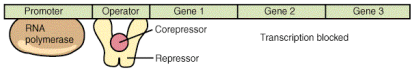
1. AraB is expressed when arabinose is added, but not araA.
2. Enzymes are never expressed, even when arabinose added to the medium.



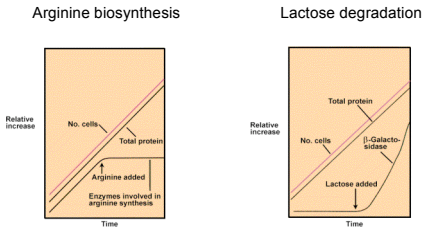


Arginine: a repressible operon

- Arginine is an essential amino acid. Transcription is normally on.
- When excess arginine is present, it binds to the repressor and changes its shape. Then the repressor binds to the operator and blocks arginine synthesis.
  - ("Don't synthesize arginine if plenty is already available")



Repressible and Inducible operons



Repression Induction

Brock Biology of Microorganisms, vol. 9, Chapter 7

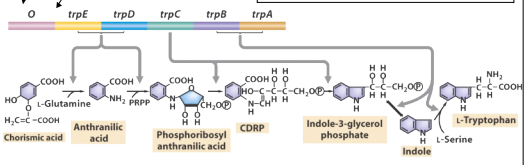
The *trp* operon

- *trp* is another example of a repressible operon
- Contains genes for the synthesis of tryptophan
- Normally on; If the end product (tryptophan) is abundant, the operon is turned off.

The trp Operon

Also a promoter and a special "leader" peptide, trpL

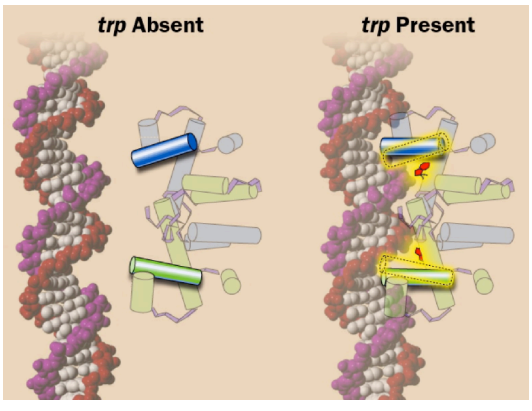
5 genes: E, D, C, B, A



Same order as enzymes for trp synthesis

Trp operon

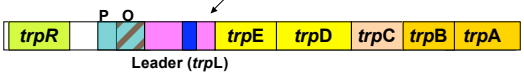
- Two regulation mechanisms, repression and attenuation
- Repressor (trpR) is activated by tryptophan
  - Changes shape so it can bind to the operator.
  - 70x reduction in synthesis
- As with lac and arabinose, the repressor protein is produced by another gene (trpR) far away



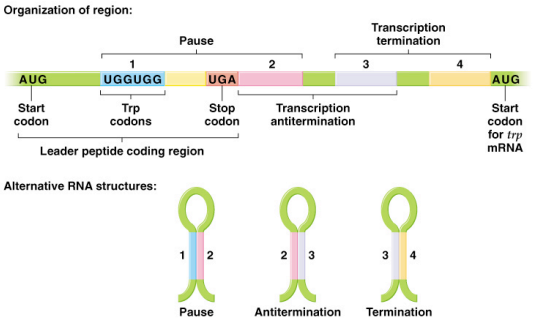
Attenuation

- Attenuation depends on an interaction between transcription and translation in a "leader sequence" at the beginning of the operon.
  - 10x reduction

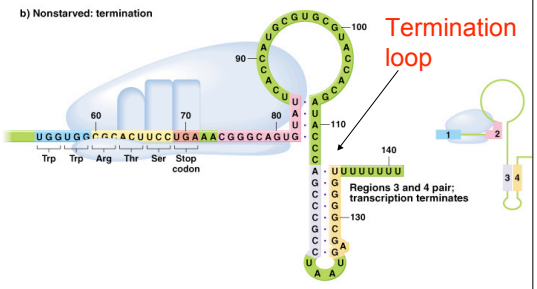
The leader has several trp codons



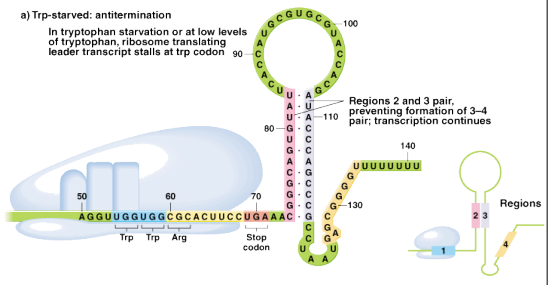
Four Regions in the Leader Sequence can pair



Trp common; forms termination loop



Trp low; transcription occurs



Attenuation is common in other operons that synthesize amino acids.

Here are some other leader sequences:

pheA: Met—Lys—His—Ile—Pro—**Phe—Phe—Phe**—Ala—**Phe—Phe—Phe**—Thr—Phe—Pro——

his: Met—Thr—Arg—Val—Gln—Phe—Lys—**His—His—His—His—His—His**—Pro—Asp——

leu: Met—Ser—His—Ile—Val—Arg—Phe—Thr—Gly—**Leu—Leu—Leu—Leu**—Asn—Ala—Phe—Ile—Val—Arg—Gly—Arg—Pro—Val—Gly—Ile—Gln—His——

thr: Met—Lys—Arg—Ile—Ser—Thr—Thr—Ile—**Thr—Thr—Thr—Ile—Thr—Ile—Thr—Thr**—Gly—Asn—Gly—Ala—Gly——

ilv: Met—Thr—Ala—**Leu—Leu**—Arg—**Val—Ile**—Ser—**Leu—Val—Val—Ile**—Ser—**Val—Val**—**Val—Ile—Ile—Ile**—Pro—Pro—Cys—Gly—Ala—Ala—Leu—Gly—Arg—Gly—Lys