BIS101 F2013 Lecture 13: Bacterial Gene Expression

Gene regulation possibilities

Why regulate gene expression?
* respond to changing environment
* save resources -- don't waste amino acides, nucletoides, ATP making stuff that isn't necessary
* development -- in eukaryotes different tissues, different time periods, need different sets of genes.

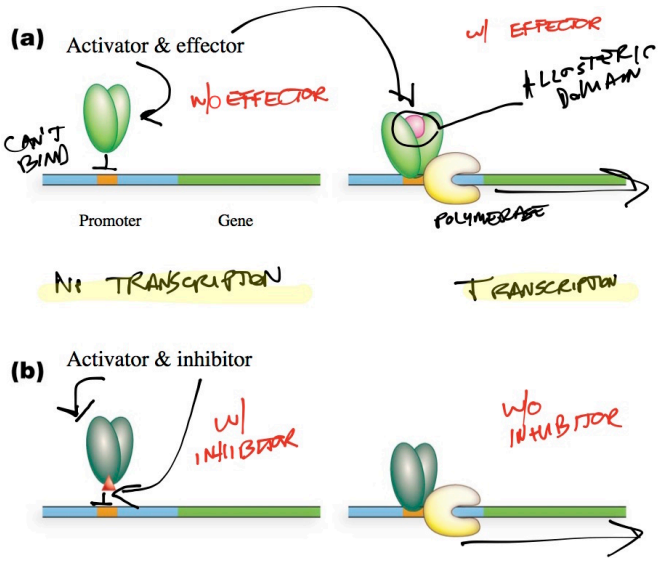
organisms have a number of methods to regulate gene expression. today we'll focus on some ways it's done in prokaryotes, that get across many of the main concepts.

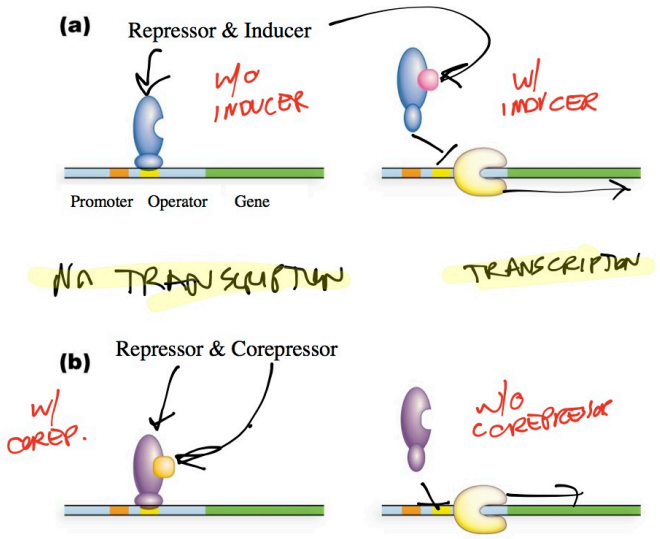
**for general knowledge of operon Ch. 5 not needed, but strongly recommended in order to do HW and information on test.""

constitutive? (always on) vs regulated:

transcriptional regulation can affect 1) initiation 2) amount

- **Activator**
    - binds to activator binding site on DNA (on or near promoter region, or in enhancer sites in eukaryotes)
    - facilitate binding of RNA polymerase and initiate transcription
    - can require binding of an **effector**
    - **allosteric domain** and conformation change
    - **inhibitor** can prevent from binding
- **Repressor**
    - binds to control sequence called an **operator** and prevents transcription
    - **corepressor** molecule can be required for binding
    - **inducer** molecule can prevent from binding





cis regulation vs. **trans** regulation cis means "on this side" ? -- a sequence that affects gene regulation of nearby, linked genes. trans means affects distant or unlinked genes.

Gene: What is a gene?

Fundamental physical and functional unit of heredity which carries information from one generation to the next

A segment of DNA, composed of a transcribed region and usually a regulatory sequence that makes possible transcription.

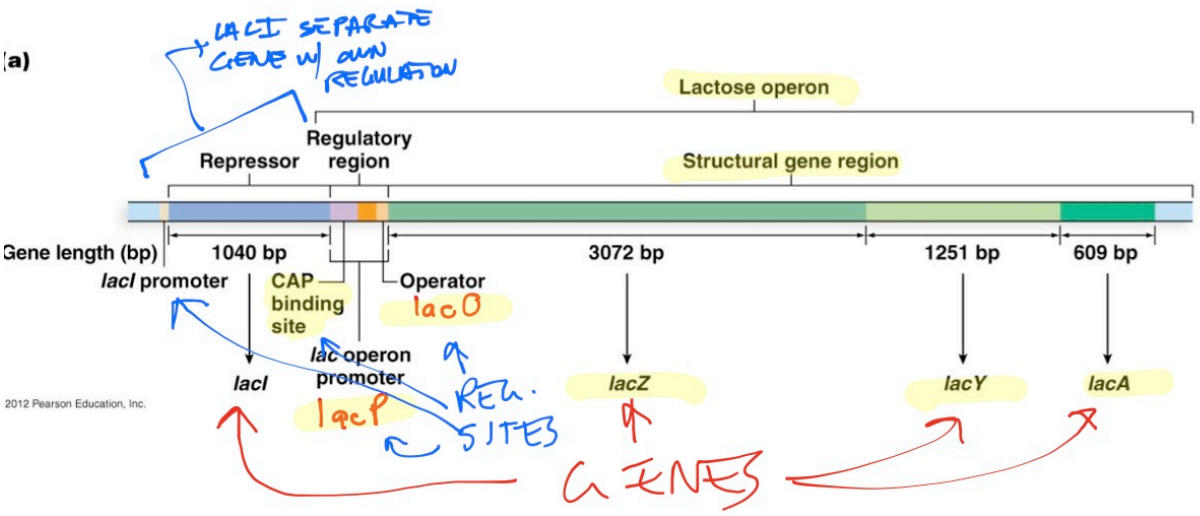
What is an operon ? a group of genes that are coregulated. Each codes for a separate product, but all share common transcription and regulation.

makes for efficient regulation of a system or pathway.

polycistronic transcription (vs. monocistronic): makes multiple proteins from same transcript; common in bacteria, rare in eukaryotes

lac operon

- *lac* operon



normally E. coli uses glucose for metabolism

lac operon is **inducible** -- normally not highly expressed, doesn't take up or use lactose in absence of glucose, lac+ (wild type) can induce expression of operon to use lactose

confusing naming

genes:

lacZ lacY lacA are protein-coding DNA

- permease **lacY** allows lactose in
- lactose -> galactose + glucose by enzyme beta-galactosidase (**lacZ**)
    - occasionally produces allolactose
- **lacA** transacetylase -> not well known but perhaps transfer acetyl group from nonmetabolizeable sugars so as to prevent reentry into cell?

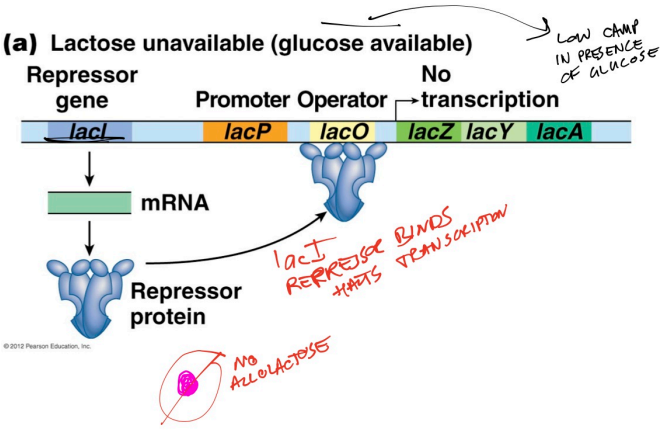
regulatory regions

lacP promotor

CAP binding site binds CAP-CAMP complex: catabolite activator protein (CAP) and cyclic adenosine monophosphate (cAMP) (signal transduction). bends DNA to open for RNA pol and ++ expression

glycolisis ? (metabolic pathway get energy from glucose) reduces availability of cAMP, keeping expression low

Glucose, no lactose



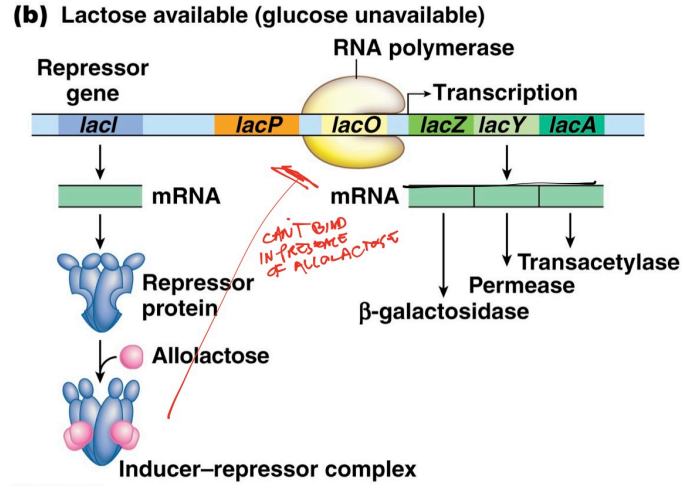
lacI repressor protein binds to lacO operator

lacO actually overlaps promoter and transcript; RNA pol can't bind

lacI operate cis or trans? (trans)

mutations in lacO cis or trans ? (cis)

Lactose, no glucose



RNA pol binds to lacP promoter (not operator as in figure)

cAMP abundant, CAP-cAMP open DNA -> lots of transcription

Problem ? need lactose to get started, but how's it get in with no permease?

repressor falls off occasionally!

Glucose and lactose?

cAMP very limited, so low expression of operon

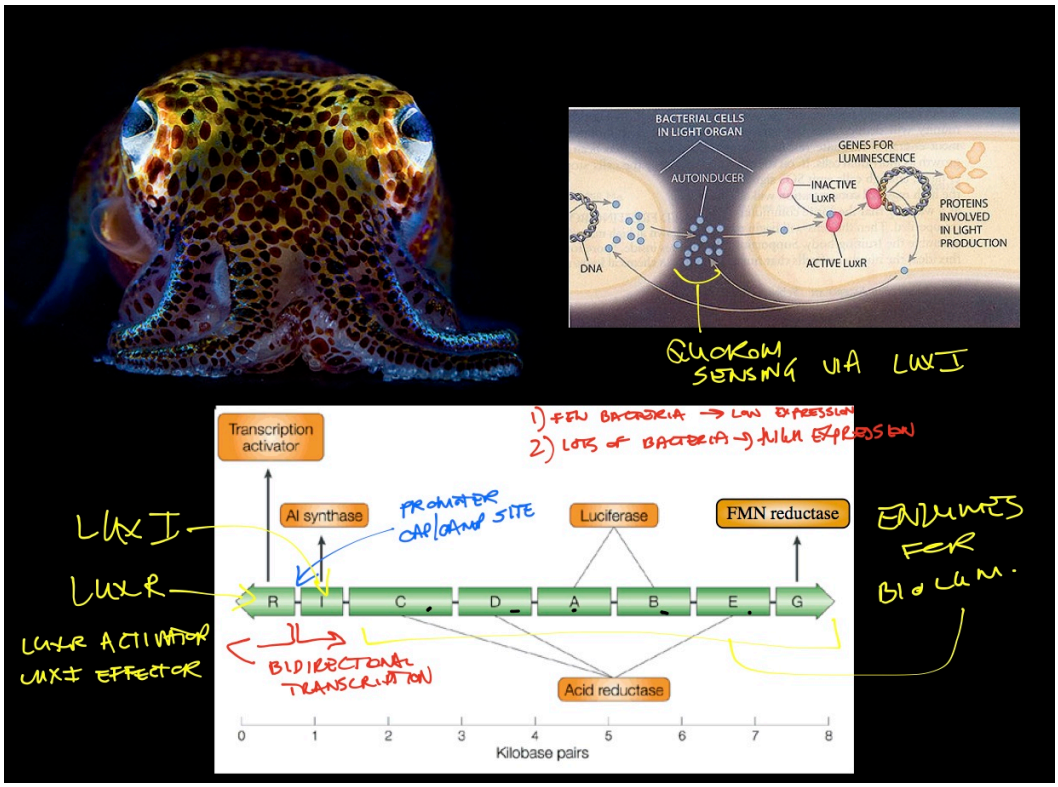
allolactose inducer may be present, no binding of repressor

Mutants of lac operon

- Oc repressor can't bind: effect ? constitutive expression of lac operon (cis/trans?)
- I- bad repressor, can't bind.
- Z- or Y- can't make functional genes
- complementation ? because can make a merodiploid by using F plasmid

Go over F' I+P+OcZ-Y+A+/ I+P+O+Z+Y-A+ F' can't make galactosidase, bacteria can't make perm ease. neither functions F' also constitutively expressed b/c can't bind promoter. het functions normally -> constitutive expression of permease, inducible expression of galactosidase

Hawaiian bobtail squid



Operon worke dout by Joanne Engelbrecht here at UCD.

bioluminescescent underbelly to be camouflaged at night against starry/moonlight sky contains special organs to capture and cultivate Alivibrio fischeri bacteria

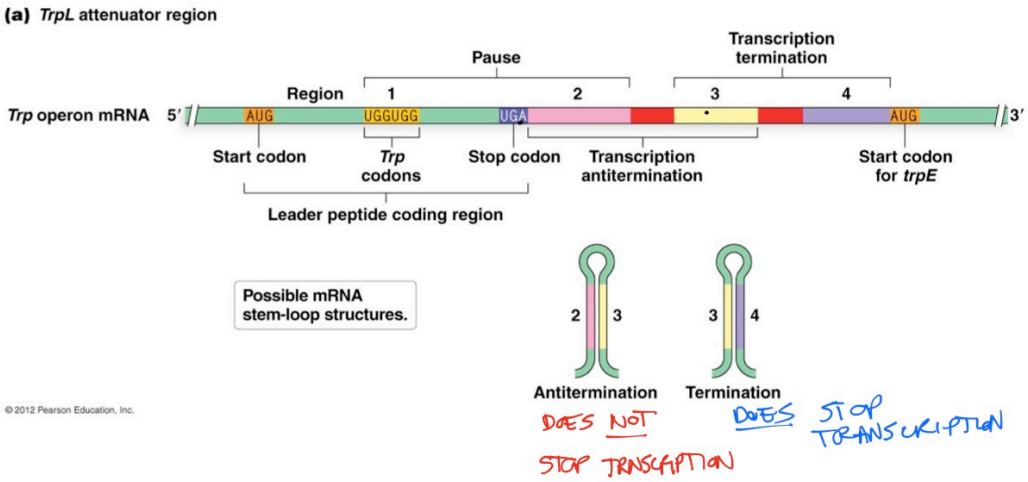
a. fischeri lux operon is expressed under glucose starvation (CAMP binding site) bidirectional transcription between I & R

also binding site for transcription activator luxR, which only binds when bound to its effector luxI

luxI protein secreted from cell. a single cell doesn't produce enough luxI to bioluminesce (free-living A. fischeri don't bioluminesce!)

when cells are concentrated, enough luxI produced -> feedback loop

Attenuation of tryp operon



(draw "some genes to make tryptophan" downstream)

makes amino acid tryptophan

has repressor-cosupressor model (tryptophan is corepressor, needed to bind & repress)

but instead of just on/off like lac, has dimmer switch in form of repeats in RNA

initial short polypeptide w/ repeated tryp codons

lots of tryp: ribosome stops at small stop codon and is on top of 1&2, loop 3-4 forms and terminates transcription of polymerase

little tryp: ribosome stalls in initial polypeptide b/c can't make it through tryp AAs. stalled ribosome let 2-3 mRNA loop form