

3. Signals, thresholds, and community responses

Tuesday, 31 August 2021

remember: TURN ON ZOOM RECORDING!

question/confusions from last time?

2. Gene basics (reviewed): genotype-phenotype connection

Which parts of the gene (-) have you been asked to learn (memorize and recognize?)

enter your list here...

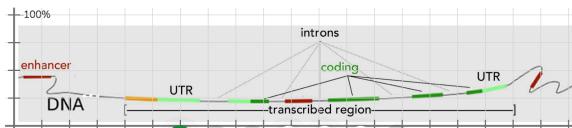
Have you completed both parts of the MCDB cell & molecule (or equivalent) course sequence?

- yes
- no
- yes, but I am not sure that I completely understand it

In past courses you have been introduced to the various "parts" of a eukaryotic gene. Now let's see if we can use them to make a model!

You are asked to predict the likelihood that a 4bp deletion within a gene will lead to a "loss of gene function" (LoF) effect. Below is a schematic of a gene.

Draw your estimate (y-axis) of the probability that a 4bp deletion will produce a LoF phenotype, the x-axis is the position of the mutation along the gene.



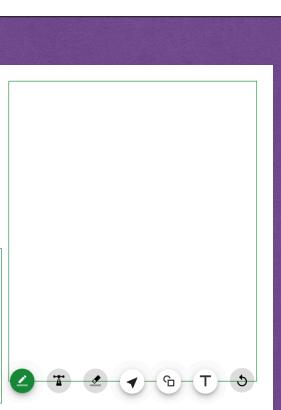
Describe the ideas that you used to make your predictions (the box can scroll)

or is prediction impossible, and if so why?

In molecular biology we often construct and use chimeric polypeptides; for example, appending moieties such as green fluorescent protein or a transcriptional repressor or activator domain to a transcription factor.

What features of polypeptide and protein structure make this possible and why is it important evolutionarily? Draw a cartoon that illustrates your thinking (→) and explain your logic (↓)

What ideas did you consider in constructing your explanation?



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Which of these types of mutation would feel comfortable explaining to someone else? →

- null mutation
- gain of function mutation
- loss of function mutation
- neomorphic mutation
- hypermorphic mutation
- hypomorphic mutation
- antimorphic mutation
- most are new to me

Which are the most likely to produce a dominant phenotype and how would that work? ↓

explain your model of the mechanism involved (why is the mutated form of the gene "dominant" for a specific phenotype?)

explain your thinking

Will a mutation that is dominant for one phenotype necessarily be dominant for all phenotypes associated with a gene? →

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How are epigenetic effects different from genetic effects →

- irreversible
- reversible
- regulatable
- random (stochastic)
- non-stochastic (directed)
- no idea how to answer

Why are epigenetic effects reversible while mutations are (generally) not? ↓

explain your thinking

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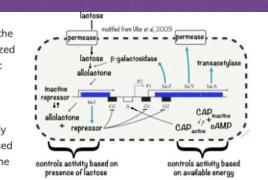
Lactose enters an *E. coli* cell through a channel encoded by the LacY gene. Production of allolactone (from lactose) is catalyzed by the product of the LacZ gene. Stable expression of the lac operon depends upon inhibition of the lac repressor by allolactone. Both LacY & LacZ are parts of the lac operon.

Given that LacI, which encodes the repressor, is constitutively expressed, how is it that LacZ and LacY can ever be expressed (since they are needed for i) lactose to enter the cell and ii) the synthesis of the inhibitor of the lac repressor? (1)

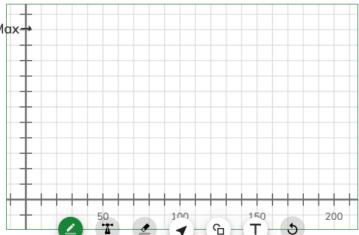
what is your model of the onset of lac operon expression, how does lactose concentration influence it?

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Assume that you have a clonal culture of wild type *E. coli*. At time = 25 lactose is added to the culture media (at a level sufficient to produce maximum LacZ expression. Draw (1), as a function of time, the behavior of three different cells in terms of lac operon expression (select different colors for each cell).



Explain the assumptions behind your graphs and why you might expect different cells to behave differently (1).

what assumptions did you base your graph on...

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Molecular numbers & half-lives: An important feature of biological molecules is a property commonly known as "half-life", but perhaps more accurately as degradation rate.

Unlike the case with radioactive atoms, a biomolecule's half-life is not a constant of the universe, but can vary depending on context - it can be regulated by signaling systems and active degradative processes.

Consider a population of molecules. The molecules' half-life is the time it takes for 50% of the molecules present at time=0 to be degraded.

Degradation removes a molecule (it can create molecules with new functions).

As in the case of isotope decay, the half-life of any particular biomolecule cannot be predicted accurately - **why is that (→)**

That said, if the population of molecules is large enough, we can accurately predict the **average** time between a molecule's synthesis and its degradation.

Q: While the half-life of an isotope is a constant, the half-life of a biomolecule can be variable; how is that possible? (1)

Describe explicitly the various processes you have in mind in your model.

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Schwanhäusser et al (2011) found a correlation between RNA levels of and the polypeptides they encoded (top panel), but little if any correlation between RNA and polypeptide half-lives (bottom panel).

Q: How can you tell, just by looking at these plots (→), whether or not there is a correlation between the two plotted variables (↓)?

is there a trick?

Q: Why are we using the term polypeptide rather than protein? (↓)

is there a good reason?

Q: How is it that an RNA and the polypeptide it encodes can have different half-lives?

what determines half-life?

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A key biological question is how a single genome can produce multiple different cellular behaviors.

The answer involves the factors that control which genes are expressed in a particular cell. To reveal "noise" in gene expression **Elowitz et al. (2002)** inserted genes encoding green and red fluorescent proteins into *E. coli* cells.

The expression of these genes were driven by identical promoters derived from the lac gene.

When clones of such cells were examined by fluorescence microscopy four distinct phenotypes were observed: cells were either red, green, yellow (the combination of red & green), or dark.

As discussed in class, the lac promoter is inhibited by the binding of lac repressor proteins. Normally, there are ~10 lac repressor proteins per cell.

Q: Predict (and explain your thinking) what will happen to the frequency of these fluorescent phenotypes if the number of copies of the lac repressor protein was increased 10-fold (↓)

explain your thinking

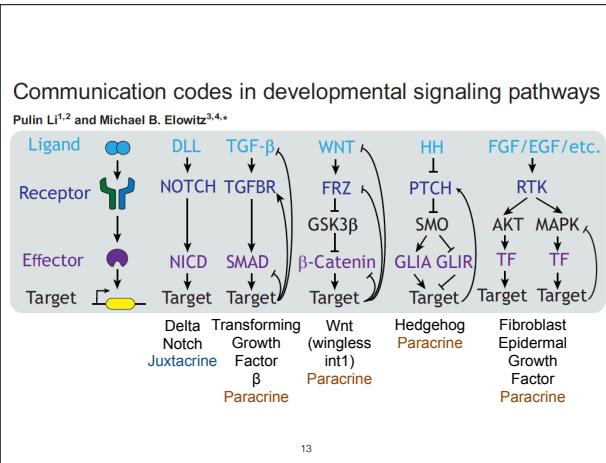
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Signal responses and threshold: general considerations

- – ligand (secreted or membrane-bound) (agonist)
- + ─ antagonists (various mechanisms)
- – receptor (membrane-bound or cytoplasmic)
- ─ activate or inhibit
- ↓ or ─ adaptor / effector
- ↓ – activate or inhibit or mixed

Impacts on the system

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Signal responses and threshold: general considerations

- [signal molecule]
- [receptor]
- [antagonists] (against ligand and receptors)
- Ligand-based response (e.g. kinase activation - phosphorylated target, stabilized / destabilized protein)
- Opposing (homeostatic) responses (e.g. target dephosphorylation, complex disassembly)
- Downstream interactions (affinities, transport reactions, nuclear retention, turnover)
- Downstream effects (gene expression)
 - Feedback (influence stability/expression of system components).

Any others?

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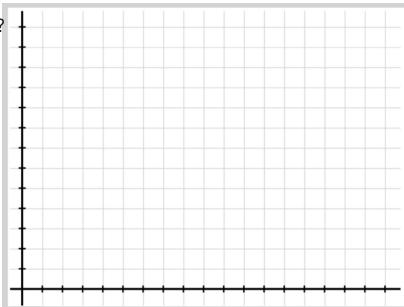
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Signal-response in (most) biological systems

- What does a typical signal response curve look like?

- Linear or sigmoidal?
- Stable or adaptive?

- **groupQ:** Under what conditions would the response not be stable (constant over time in the presence of the agonist)?

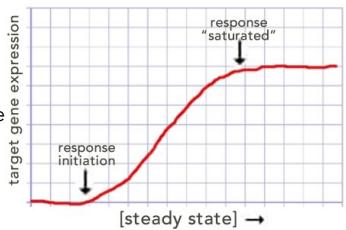


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Signal-response in (most) biological systems: sigmoidal

- **groupQ:** Why is the response generally not linear?
- **Q:** Why is there no apparent response at the low levels of signal?
- **Q:** Why does the response saturate?



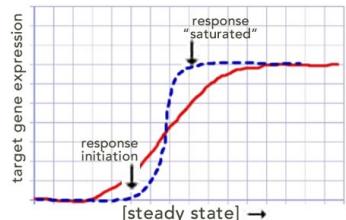
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Threshold responses

- **Q:** What is a threshold response?

- **groupQ:** How might the cell produce a threshold response?



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Signal-response in biological systems can be

- Adaptive
 - Reversible (back to pre-signal state)
- "Developmental"
 - Response leads to change in state, change in patterns of gene expression and cell behavior.
 - Post-signal state different from pre-signal state

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Cooperation between cells: the basis of developmental processes.

Q: Why do unicellular organisms cooperate?

- Regulate behaviors that make sense only within a dense culture
 - Synthesis and secretion of ("expensive") enzymes
 - For isolated cells, both enzymes and products diffuse away
 - DNA uptake machinery (useful DNA has to be present)
 - Secretion of molecules involved in biofilm formation (mechanical coordination)
 - Secretion of antibiotics (systems that protect from antibiotics)
 - Persister phenotypes
 - Programmed cell death (nutritional support for neighbors)

Evolutionary adaptations are based on costs vs benefits

The ecology and evolution of social behavior in microbes

Corina E. Tarnita*

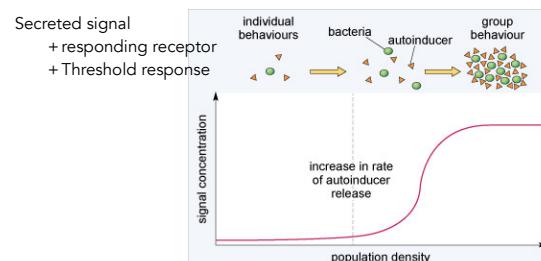
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Q: How might do unicellular organisms monitor population density (cell/volume) ?

Quorum sensing



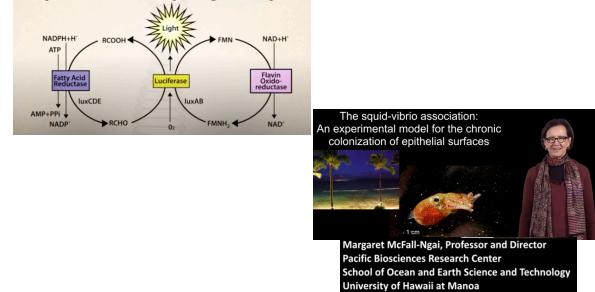
<https://www.open.edu/openlearn/science-maths-technology/general-principles-cellular-communication/content-section-1.1>

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Bioluminescence genes (*lux*) encode luciferase enzyme & proteins for recycling aldehyde

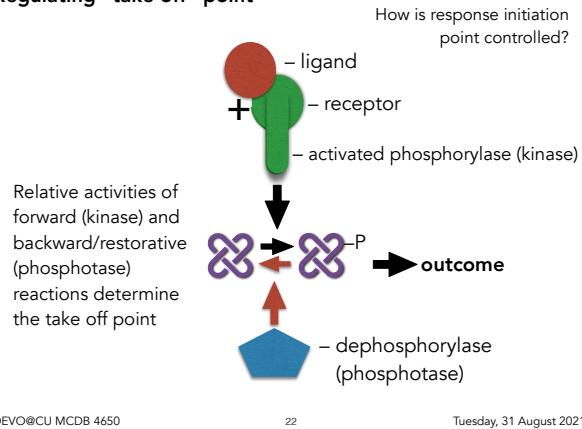


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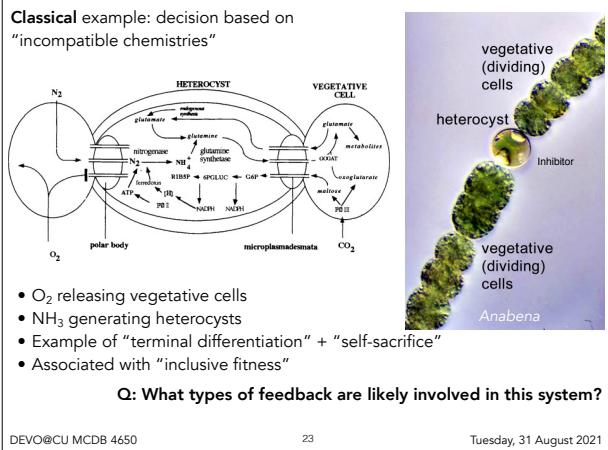
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Regulating "take off" point

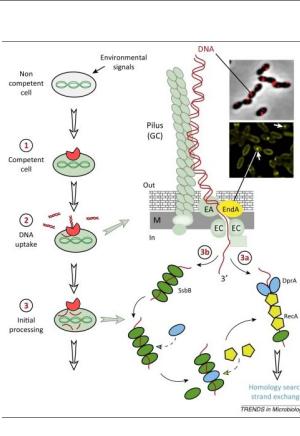


Classical example: decision based on "incompatible chemistries"



decisions in bacterial cells

Q: What factors will determine whether DNA should be imported from the environment? & what should the cell do with it.



"Decisions" by bacterial cells: programmed cell death

Q: What is difference between apoptosis and necrosis?

Q: What factors will determine whether a bacterial cell should commit "suicide" (apoptosis)?

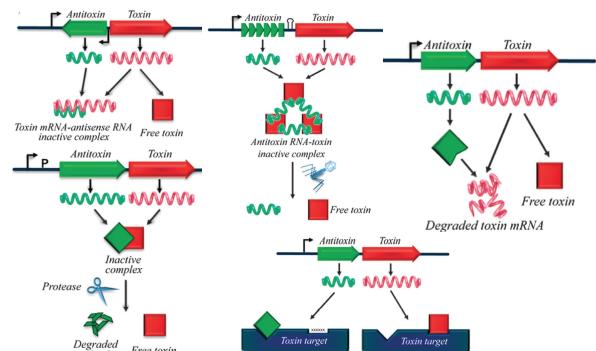
During infection by a lytic or lysogenic phage, killing cell kills the phage, protects the community.

In dense (starving) condition, kill cell can release nutrients that insure survival of relatives.

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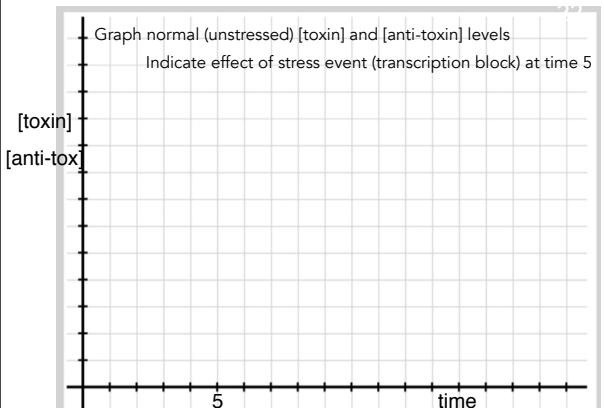
addiction (toxin-anti-toxin) modules



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addiction module stable toxin, unstable anti-toxin (levels)



Altruistic cell death

Q: How is such “self-sacrifice” possible, evolutionarily?

Q: Why would programmed cell death be regulated by quorum sensing?

Q: Are there alternatives to programmed cell death in stressed (crowded) conditions?

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Social cheaters

Q: What is a social cheater, how can selection influence their prevalence?

Q: Are anti-vax folk social cheaters?

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Portfolio questions:

- 3.1 Why are dose-response curves typically sigmoidal, not linear? Start by defining both.
- 3.2 How might “threshold” behavior be generated?
- 3.3 How does quorum sensing work and why is it used?

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