

2. Gene basics + genotype-phenotype connection



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beSocratic 1 review

An introduction to using beSocratic in DEVO Fall 2021

Background: The typical beSocratic activity consists of a number of "pages". You are presented with background information, tasks to complete, questions to answer, and explanations to construct.

Activities are not graded, you earn points when you complete them (seriously). Responses will be reviewed at the beginning of the next class period.

Your answers should be mechanistically "plausible", that is, they make sense and work rather than that they are "correct". In biology, correct answers generally have to be memorized.

When all tasks on a page have been completed the **Next** button will appear.

The number of pages in the activity and where you are appear at the bottom of each page.

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We sometimes use multiple choice questions: you may be limited to a single choice, sometimes multiple choices are allowed.

As with graphs and drawings, you will often be asked to explain or expand on your choices.

How can we encourage you to engage in in-class discussions ↓

(suggestions welcome)

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Q: Which best describes you?

- I never ask questions in class
- I ask questions when something is unclear
- I ask question of classmates rather than instructors
- I find people who ask questions annoying, they slow down the class
- I love people who ask good questions, the responses often clarify my own thinking
- I find it helpful when the reasons wrong answers are wrong are clearly explained
- where am I?

Which topics would you like to learn more about in this class? →

suggestions welcome

What areas of cell-molecular biology would you like to see reviewed? →

topics to review

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You may be familiar with the common term "model". For us a model is a mechanistic (often molecular) or cellular level) description that explains a behavior.

A model is made of parts and assumptions about how those parts interact and behave. Consider this Rube Goldberg machine (→) that wipes one's lips after a sip of soup!



A good model does not contain extraneous bits - all parts are needed. This is often referred to as [Occam's razor](#). At the same time, a good model "works", it provides a mechanism for how a behavior occurs.

The model's parts act in ways consistent with scientific principles. Remove or alter a part/step and the machine no longer functions as expected. For our purposes what is critical is that the model is plausible, not necessarily correct.

Scientific models are judged based on their simplicity, their predictive accuracy, and the range of behaviors they can explain. As an example, a scientifically plausible model for the movement of a rock (or a planet) need only consider the forces acting on it, as well as its momentum, but not its history (unlike biological systems).

Q: What factors combine to complicate the building of biological models? →

Think about the histories of organisms and their molecule level details

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A reflection on teaching

groupQ: What, exactly, do you think you should learn (or know) about genes and why?

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A review / practice thinking about... genes

(things every MCDB major should know)

groupQ: How did (or could) we come to know that genetic information is stored a chemically stable molecule?

Griffith (1928) - transformation (S-R *Strept. pneumoniae*)

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A review / practice thinking about... genes

groupQ: How (exactly) did (or could) we come to know that genetic information is stored in DNA?

Avery et al (1944) - transforming principle - nucleic acid

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A review / practice thinking about... genes

groupQ: How can new genes arise de novo?
+ why is having a plausible mechanism important?

A socratic hint:
Does RNA synthesis data suggest possible a mechanism?

[Schlotter \(2015\) Genes from scratch – the evolutionary fate of de novo genes](#) - Trends in Genetics.

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Q: Give RNA vaccines and mutating viruses, any questions you have trouble answering (as a nascent molecular biologist) for family and friends?

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A reflection on teaching

groupQ: What does knowing the “parts of a gene” (or the features of protein structure) enable you to do?

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Types of mutations (alleles)

- Null (complete loss of function)
 - Gain of function
 - Hypomorphic (amorphic)
 - Hypermorphic
- Q:** How do they work (mechanistically)?
- Antimorphic
 - Neomorphic
 - Null (loss of function)
 - Gain of function
 - Neutral
 - others?

groupQ: Which is most likely to produce a dominant allele + why?

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groupQ: How might a mutation (in the coding region) that does not alter the encoded polypeptide's sequence produce a phenotype?

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The complex link between genotype & phenotype

The "failure to realize the importance of these two points, namely, that **a single factor (gene) may have several effects** (proteins with multiple roles), and that **a single character (trait) may depend on many factors**, has led to much confusion".

– T.H. Morgan et al (1915)

- one gene may influence multiple processes
 - expressed at different times & places in response to different signals → different outcomes
- "downstream" effects influenced by feedback (and stress) responses
 - context effects (other genes expressed)
 - epigenetic effects (DNA/histone modification)
- genetic background effects → modify outcomes
- stochastic events (mutation / monoallelic gene expression / noise)

[Conceptual simplicity and mechanistic complexity: the implications of un-intelligent design - a bioliteracy blog post](#)

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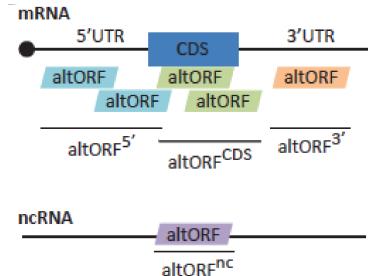
Q: Why is the early idea of “one gene one enzyme (one protein)” not accurate?

- some genes encode “non-coding” RNAs
- many proteins contain more than one subunit (polypeptide)
- some polypeptides are part of more than one protein (molecular complex).
 - e.g. β -catenin
 - adhesion + cytoskeletal organization
 - wnt inter-cellular signaling + gene regulation
- a region of DNA may encode multiple gene products

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groupQ: How do we know (what methods) reveal how many gene products are encoded by a single stretch of DNA?



modified from Samandi et al., 2017. Deep transcriptome annotation enables the discovery and functional characterization of cryptic small proteins.

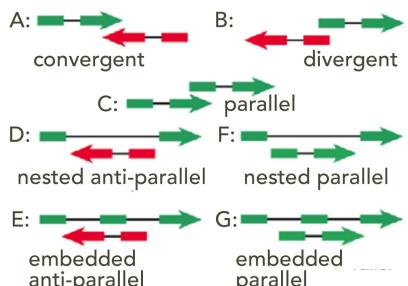
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Genes (regulatory sequences) can overlap.

Mutations in one gene can have unexpected effects on others

Predictions? →

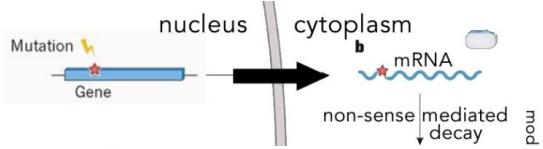


modified from Soldà et al. 2008. Non-random retention of protein-coding overlapping genes in Metazoa. BMC genomics 9: 174.

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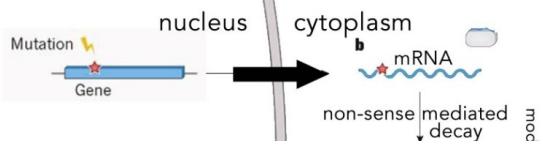
Unexpected complexities: non-sense mediated decay (NMD)



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Unexpected effects: non-sense mediated decay (NMD)



groupQ: How does (could) the NMD system "know" where the normal "end" of an mRNA should be?

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Factors controlling irreversible outcomes:

cell differentiation

groupQ: How can changes in gene expression (in response to signals and such) become effectively irreversible?

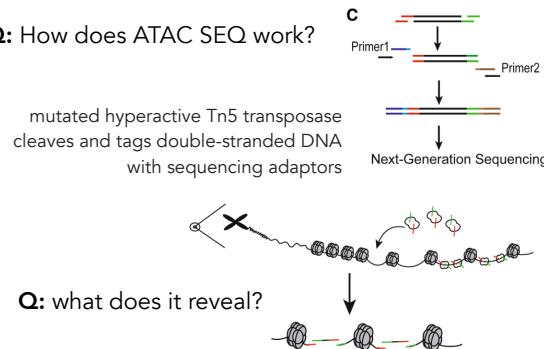
- Impacts on "background" patterns of gene expression that influence response - it is the system that responds (not specific genes).
- Changes in DNA modification/chromatin structure that influences transcription factor binding

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Visualizing chromatin accessibility - ATAC SEQ

Q: How does ATAC SEQ work?



Q: What can happen (beside the elimination for the gene product) when a mutation eliminates a gene product?

- Effects due directly to the absence of activity dependent on gene product
- secondary (stress / folding / interaction) effects on interacting molecules
 - possible toxic interactions (e.g. mitochondrial function)
- Influenced of genetic background effects

genotype-phenotype connection is complex

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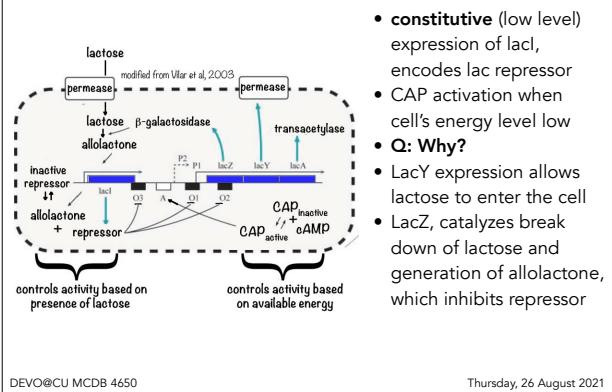
- During development, expression of a mutant adult globin gene replaces a normal fetal globin gene.
- A possible treatment - turn on fetal gene
- **groupQ:** What type(s) of mutation would this be?

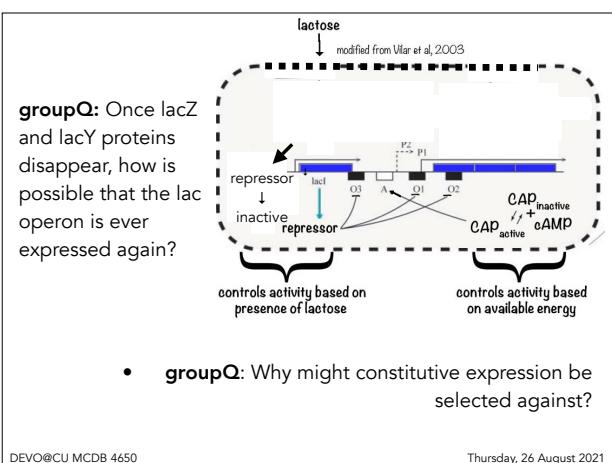
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Noise in biological systems

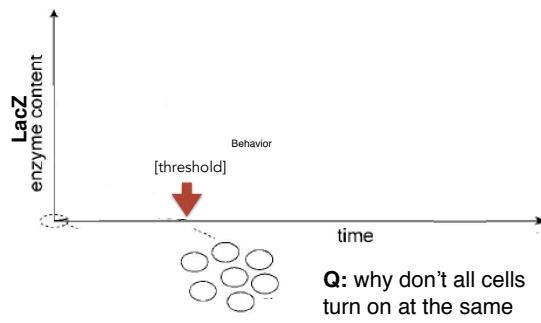
A classic system, the lac operon





Behavior of the lac system as a function of lac concentration

adapted from Vilars et al 2003

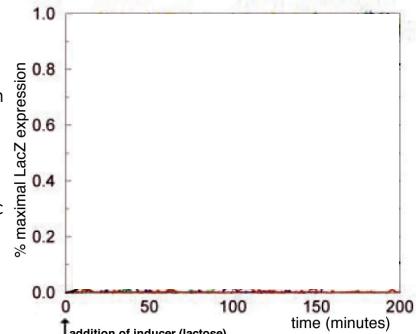


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Behavior of cells with [lactose] > threshold

- Rate of lactose entry into cell
- rates of formation & removal of [allolactone]
- affinity of allolactone for lac repressor
- [repressor]

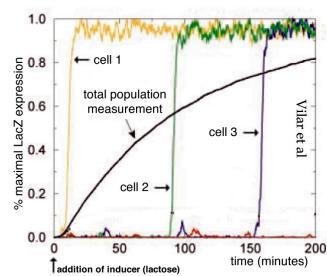


Q: How (and why) does the behavior change then [lactose] < threshold or absent?

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- how does behavior of system change if there are 1000 (or 2) lac repressor molecules instead of 10?



other factors to consider

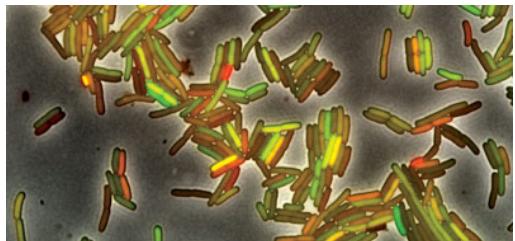
- bursting behavior during RNA / polypeptides synthesis
- half-lives of lacZ/lacY RNAs and proteins

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Further evidence for the noisiness of the lac operon

– supported by more modern single cell studies



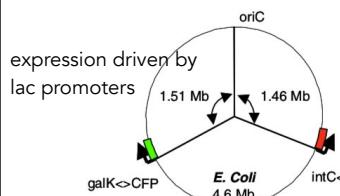
Elowitz et al.

bioliteracy post: [Biology education in the light of single cell/molecule studies](#)

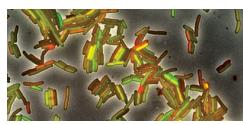
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basic set up of Elowitz et al study.



A map of the *E. coli* chromosome with the origin of replication (*oriC*), *cfp* and *yfp* loci indicated. Locations were chosen to avoid systematic effects associated with gene copy number while remaining sensitive to stochastic differences. The >> symbol denotes replacement by homologous recombination.



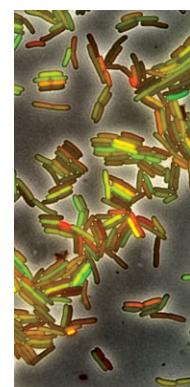
Observed phenotypes:
both genes on, both off,
or one or the other on

groupQ: Why did they place the insertions where they did, any ideas?

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Q: How would system be altered if instead of *cfp* and *yfp*, the inserted genes encoded transcription factors.



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Other sources of molecular level noise:

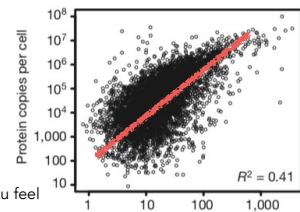
turnover of RNAs and proteins

- Radioisotope decay is determined by isotope half-life, an intrinsic property of the atom (lawful, but unpredictable for any particular atom).
- Biological turnover is an active (regulated) process.
- Controlled by sequence information, molecular conformation, and enzymes that carry out the breakdown (endo/exo- nucleases, proteinases).
- Involves stochastic collisions with other molecules
- **Q:** what is the difference between stochastic and random

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- correlation between concentration of RNA & its encoded polypeptide



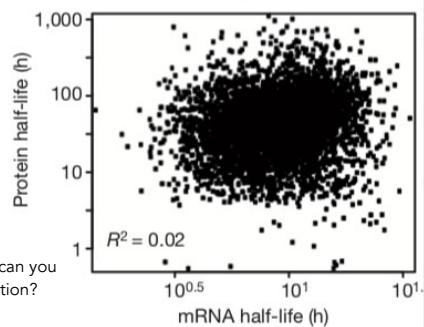
Q: For any particular RNA would you feel comfortable predicting the polypeptide's concentration? Explain

Published: 18 May 2011
Global quantification of mammalian gene expression control
Rüdiger Schwerdtfeger, Dorothee Busse, Na Li, Gunnar Dittmar, Johannes Schuchhardt,
Jana Wolf, Wei Chen & Matthias Selbach
Nature 473, 337–342 (2011) | Cite this article

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Lack of correlation
between RNA half-life
& polypeptide half-life.



Q: What conclusion(s) can you draw from this observation?

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Q: what, if anything, is the value of stochastic decision making?

It allows for multiple phenotypes within a particular environment.
– diversity in responses.

- As example, lac operon leads to a small number of cells that are lac on under energy-limiting conditions
- Cells can respond, without constant synthesis of enzymes/ transporter that are otherwise unneeded.
 - Population can contain multiple cell types (in a single environment)
 - Bacterial (cancer) population
 - Actively dividing cells
 - Persister (non-dividing cells)
 - Sacrificial cells (programmed cell death).

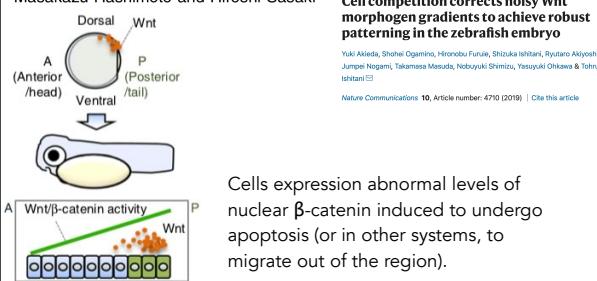
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Q: How do organisms deal with molecular/ cellular noise

Cell competition controls differentiation in mouse embryos and stem cells

Masakazu Hashimoto and Hiroshi Sasaki

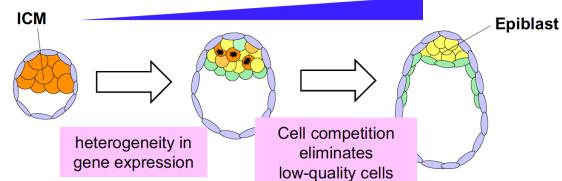


Cells express abnormal levels of nuclear β -catenin induced to undergo apoptosis (or in other systems, to migrate out of the region).

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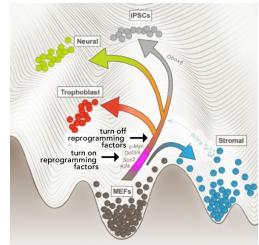
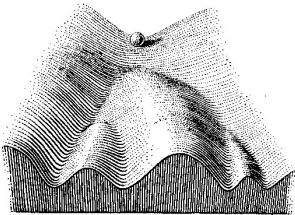
TEAD activity \Rightarrow pluripotency factors



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Due to molecular level noise: developmental processes are noisier than thought (revealed by single cell RNA SEQ)



Instead of a cell rolling (and dividing and differentiating) smoothly down a landscape (Waddington)

They display molecular (brownian + directed) motion.

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2. Gene basics + genotype-phenotype connection

Portfolio questions:

- 2.1 How do cells with the same genotype develop different phenotypes?
- 2.2 How can details of gene and protein structure help you make plausible predictions about the effects of specific mutations? What are the limits of such predictions?
- 2.3 What are the sources of noise in the lac operon (and gene expression in general); how are they central for the lac operon's normal function?
- 2.4 What roles does noise play in biological systems, how is noise manifest and controlled?

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