

DEVO (MCDB 4650) Activity #1 - Class 2 - What is a Gene? 2020

Genes are a key feature of all organisms, they store genetic information.

This information is created through the processes of mutation and selection.

In cells, genes are regions of double-stranded DNA molecules. A typical DNA molecule (a chromosome) contains many genes.

An obvious question then is how do we, and the cell, recognize a gene within a DNA molecule? Explain how a gene is recognized by the cell and experimentally (→)

a biofundamentalist activity

In the box ↓ draw a schematic of the key structural features of typical eukaryotic gene.

How does a cell "find" a gene? how does a molecular biologist (you) find and define a gene?

Simple linear structure (ds-DNA) with nearby promoter(s), and distant enhancers, exons/introns - regulated by sequence specific DNA binding proteins.

A similar diagram can be made on the other strand, in the opposite orientation.

The positions of genes within a DNA molecule is recognized by the binding sites of transcription factor. Experimentally, single cell RNA sequencing can reveal the presence of transcripts whose sequences can be mapped to the genomic (DNA) sequence.

It is common to refer to "gene expression". What does it mean that a gene is expressed? (↓)

what exact is "expressed"?

How can you tell (experimental evidence) that a gene is expressed? (↓)

What is produced when a gene is expressed?

What needs to occur for a gene to be expressed? (↓)

what is required, what changes when a gene "turns on"?

Gene expression = recruitment of DNA-dependent, RNA polymerase recruitment and RNA synthesis. Depending on the gene, this RNA can be processed (introns removed) and translated to generate a polypeptide.

Alternatively, in some genes the RNA synthesized may not be translated, but rather processed in various ways and then interact with mRNAs, regulating their translation of stability.

Look for the presence of RNAs
Transcription factors must bind, recruit and activate RNA polymerase

Epigenetic factors or processes can enhance or repress gene expression.

Which is NOT an epigenetic factor or process (↓) (check all that apply).

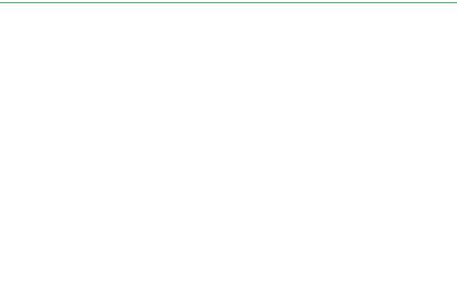
- a change in DNA sequence
- a modification of a DNA base
- a modification of a histone
- a change in chromatin folding
- the binding of a transcription factor
- no idea how to answer

Illustrate (↓) and explain (in the box below) a plausible model for how how epigenetic effects could allow or enhance the expression of a particular gene.

explain your model please

The only one that is not epigenetic is changing the DNA sequence - that is a mutation (and not directly, physiologically reversible).

Epigenetic modifications will influence the accessibility of DNA, as well as the affinity of transcription factor binding, and the recruitment and activation of RNA polymerase.

<p>How are epigenetic effects different from genetic effects? They are (check all that apply) (↓)</p> <ul style="list-style-type: none"> <input type="radio"/> irreversible <input type="radio"/> reversible <input type="radio"/> regulateable <input type="radio"/> random <input type="radio"/> non-random <input type="radio"/> no idea how to answer 	<p>Which do think is the most important, biologically, and why? speculation is welcome</p>	<p>They are reversible, regulatable, and non-random. Their regulatable nature can be used to control the expression of specific genes in specific cell types and under specific conditions.</p>
<p>Explain what "accessible" means in terms of chromatin and genes, both structurally and functionally? Use a drawing (→) to illustrate your explanation (↓).</p> <p>who needs access to what?</p>	 <p>Any ideas?</p> <p>(→)</p>	<p>The transcription factors involved in regulating specific genes must be able to collide with and bind to specific DNA sequences If the DNA is wrapped with proteins (and folded on itself - there can be no space for proteins, so no binding. You can ask whether proteins of specific site can localized to regions of DNA, or you could determine whether smaller molecules could react with DNA, or whether various transposable elements insert in the region.</p>
<p>Which of the following terms (as applied to mutations) have you been introduced to and feel that you understand (↓)?</p> <ul style="list-style-type: none"> <input type="radio"/> hypomorphic <input type="radio"/> null <input type="radio"/> hypermorphic <input type="radio"/> antimorphic <input type="radio"/> neomorphic <input type="radio"/> loss of function <input type="radio"/> amorphic <input type="radio"/> most are new to me! 	<p>Which is most likely to act in a dominant manner, and why – provide a plausible mechanism?</p> <p>B I U x; x²</p> <p>How would it work?</p>	<p>It is difficult to predict the behavior of an allele, but one simple model would involve an antimorphic mutation - such an allele produces a gene product (polypeptide) that interacts with the wild type gene product and reduces or inhibits its activity. In the way an antimorphic allele/gene product can mimic a null (no wild type gene product present) and is most likely (but not absolutely certain) to produce a phenotype!</p>

Using CRISPR CAS9, you generate a deletion in the region of the gene encoding the normal translation start. Under what conditions might such a mutation not produce a "loss of function" mutation? Diagram (↓) your mechanism & explain (↓) your reasoning.

how can you get around the effect of the mutation

In many genes there are "alternative" sites of transcription or "downstream" sites of translation initiation. By disrupting the known transcription/ translation start sites, alternative sites may become active - and their activity may partially (or totally) rescue the effect of the mutation.



Genes can overlap. What is it about the structure of DNA that makes that possible? (→)

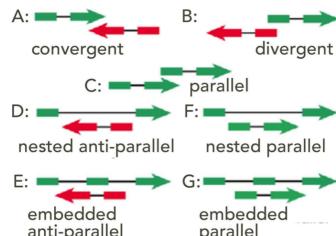
When eukaryotic genes are diagrammed typically thick lines indicate exons, thin lines indicate introns, and arrows indicate the direction of transcription (RNA synthesis).

Here (→) are some possible arrangements of over-lapping genes. Circle those arrangements where you might expect that expression of one gene would strongly influence the expression of the other.

Describe the model of gene expression regulation you based your predictions on (in the box ↓)

describe the model you use for your predictions

how can gene overlap and still function?



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



The two strands in a double-stranded DNA molecule are antiparallel.

Although you can justify many choices, my own favorite would be B, since binding of transcription factors and RNA polymerase to one strand, and the movement of RNA polymerase on one gene could disrupt the binding of transcription factors / polymerase etc on the other.

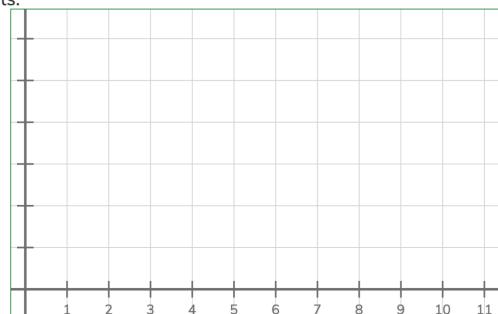
Overlapping genes often influence each other's expression. Let us see whether we can make predictions of such effects.



Consider this pair of genes (↑), in which one gene (top strand) is initially expressed, while the other (bottom strand) is not.

Assume that the originally unexpressed gene is turned on at time =4; present a model (a graph) of the behavior of the originally expressed gene as a function of time.

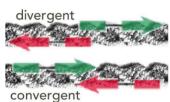
Explain (↓) the logic of your thinking.



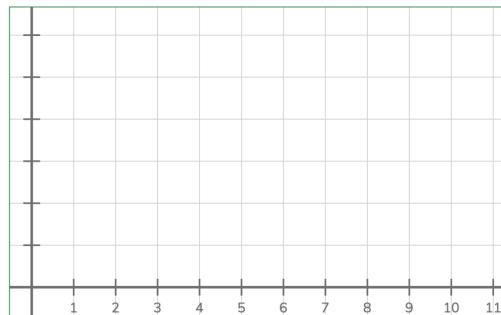
What assumptions is your prediction based on?

We would expect that activation of the gene on the anti-parallel strand will lead to a reduction of the synthesis rate of the gene on the other strand.

Given the balance between synthesis and degradation, we expect a decrease in the polypeptide encoded by the gene.



Now assume that instead of divergent, the genes are oriented in a convergent manner - would this change your response?

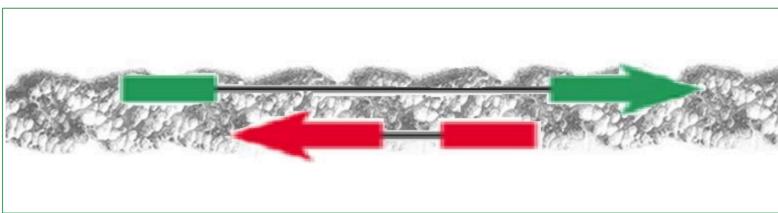


Explain the logic of your answer below



What assumptions is your prediction based on?

Here (↓) are two overlapping genes, one the 5'→3' and the other on the 3'→5' strands of a DNA molecule. Indicate 5' end of each gene and with a box indicate where you think their promoter regions are likely to be located.



Devise a model in which a single mutation (mark with an * in your drawing above)



would change the polypeptide encoded by one gene while inhibiting the expression of the other gene. Explain your reasoning in the box below (↓).

What processes or molecular interactions does your mutation need to influence?

We might expect less of an effect. It is even possible that the polymerases will pause, and allow each to pass by each other.

Could happen....

There are a number of ways to experimentally manipulate gene expression. Pick the method that you feel most confident in your understanding of their mode of action and limitations? (→)
we will consider explanations in class.

Explain (↓) which of these methods do you think is the most specific in its effects and why.

what determines its specificity?

- shRNA, RNAi, etc.
- morpholinos
- CRISPR-CAS9
- antibody injection
- not confident about any

What types of non-specific effects might it produce and explain why (↓)

where does non-specificity come from

Promoters / TF binding sites upstream of the first exon (the square end of the arrow icon).

A mutation in the bottom gene could disrupt promoter, as well as a splice junction sequence.

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Cut DNA (CRISPR CAS9) or destabilize RNA (shRNA, etc) - an antibody could bind to an inhibit normal function of gene product.

non-specific effects associated with off-target effects (primarily).

The end!

DEVO (MCDB 4650) Activity #2 Class 3 – Stochastic gene expression

Molecular numbers & half-lives:

An important feature of biological molecules is a property known as half-life. 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 and repair processes.

Consider a population of molecules. The molecules' half-life is defined as the time it takes for 50% of the molecules present at time=0 to be degraded. Degradation removes molecule from the system (and can also create molecules with new functions - fragments of the original molecule).

As in the case of radioactive decay, the half-life of any particular molecule cannot be predicted accurately - why is that (\rightarrow)

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.

Q1: What factors will influence the predictability of a cellular system? (\downarrow)

Describe explicitly the various processes involved in your model.

- because it involves the intrinsic instability of the molecule factors
- it is predictable, it is based on the molecule's chemical properties
- because it depends on random collision between molecules
- not sure, I would just be guessing

The third choice - degradation depends upon stochastic (random) collisions and subsequent degradative (catalytic) events.

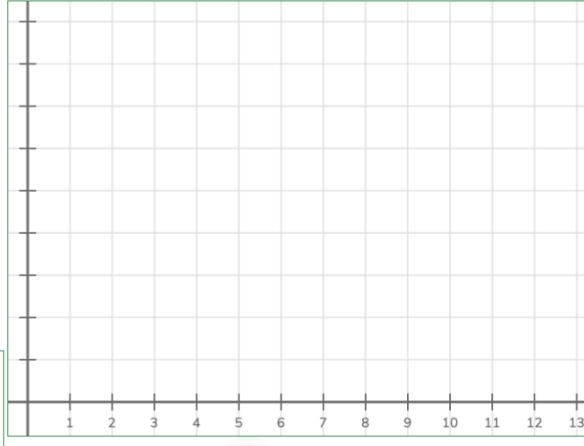
The more molecules involved in a particular processes (e.g. transcription factor proteins and the regulation of a particular gene), the more predictable the response will be. But within cells, the numbers of molecules are necessarily limited, and there are many "similar" interactions - for example, a transcription factor will be colliding DNA and binding with various affinities.

The *CRYBB* gene encodes the Crybb protein.

Consider a cell type in which the concentration of Crybb [Crybb] is constant.

In response to an extracellular signal at time = 2, expression of *CRYBB* is inhibited. Draw out the relationship between [Crybb] (y-axis) as a function of time (x-axis). Why does [Crybb] change?

what assumptions are you making?



When the concentration of molecule is constant, we can assume that its synthesis and degradation rates are equal. If synthesis is inhibited, the effects of degradation will become apparent - the level will drop, and the rate will reflect the half-life of the molecule.

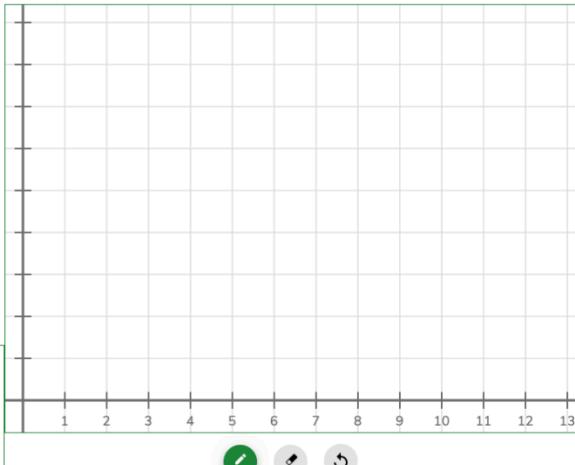
It is worth remembering that half-life is not an inherent quality of molecule, but a reflection of degradative processes, so it is regulatable.

Other factors can influence the levels of a protein in a cell.

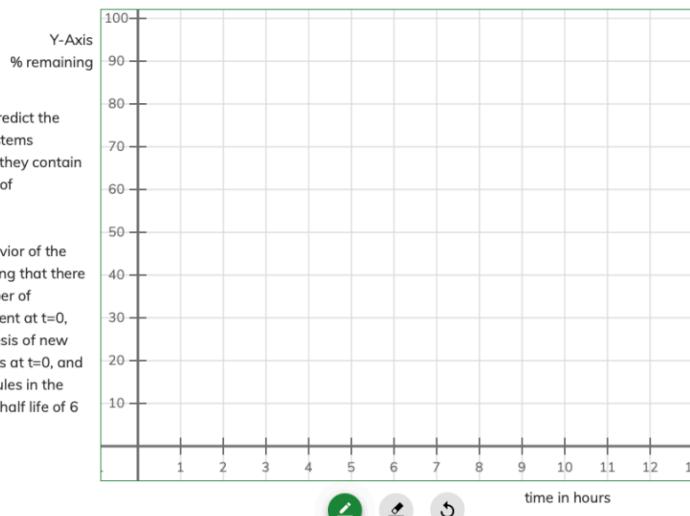
How would your graph change (or would it?) if, at time = 4 a signal occurred that inhibited the degradation of Crybb protein (assume that, as previously, the inhibitor of *CRYBB* expression was added at t=2).

Explain your reasoning

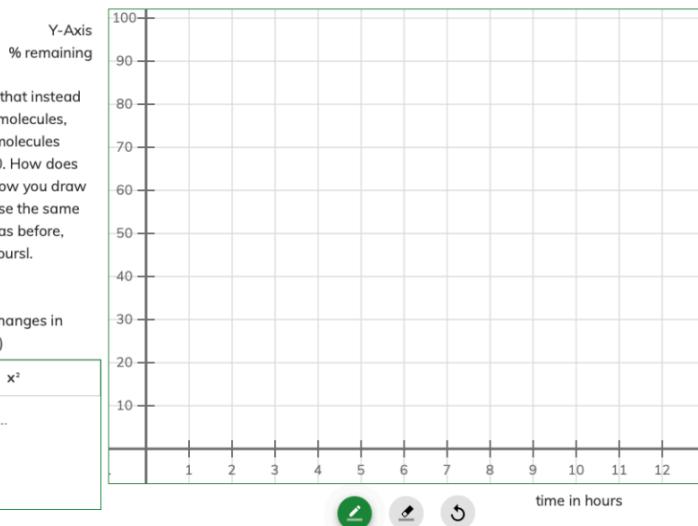
what is changing?



We might expect that the level of protein would increase, until a new steady state level was reached, when synthesis and degradation rates are equal (balanced).



We would expect a smooth decrease, the level dropping to 50% at the time determined the half life.

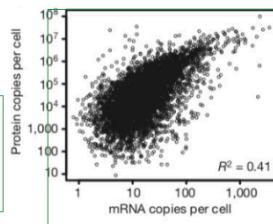


We would see random steps down, since the degradation of any one molecule reflects a 5% decrease in concentration.

In their data, Schwanhauser et al found that the levels of specific mRNAs and proteins were correlated.

Q: Draw your estimation of the correlation line (→) and explain (↓) what exactly does a "correlation" mean to you?

What kinds of conclusions or predictions does it enable you to make in general and for a specific protein?



What is the variation in protein numbers when there are ~8 mRNA molecules per cell? (↓)

- 10 to 50
- between <10 and $>10^7$
- no variation, #RNA = # protein
- no idea

Circle the two most dramatic examples (↑) in which the correlation between RNA and protein numbers breakdown and propose a model (↓) by which such a lack of correlation might arise.

Identify the key aspect of your model

The line would be straight running from the bottom left to the top right .

The variation (second) between <10 to $>10^7$

It could be that the degradative (or synthetic) processes are regulated – turned off or turned on.

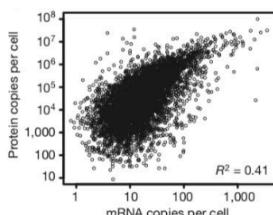
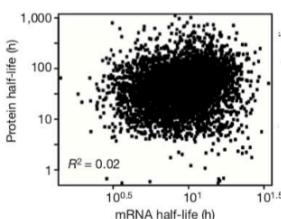
A (possibly) surprising observation was the lack of a significant correlation between mRNA and protein half lives (\rightarrow).

Explain (\downarrow) how you can tell, just by looking at these plots, whether or not there is a correlation between the two plotted variables.

is there a trick?

How might the lack of correlation between mRNA and protein half-lives influence your ability to predict the behavior of a particular system (for example in terms of the regulation of gene expression) (\downarrow)?

what would be effected



No obvious relationship between half-lives - at any particular mRNA half-life, the protein half-life can vary dramatically

The smaller the number of mRNAs/proteins in a cell, the noisier the responses to various stimuli are likely to be.

Questions to answer:

1) What factors determine the steady state concentration of a protein and the mRNA that it is in a cell?

can these be regulated?

2) Speculate on why a particular mRNA or protein would have a short versus a long half-life?

would impacts does changing half-life have?

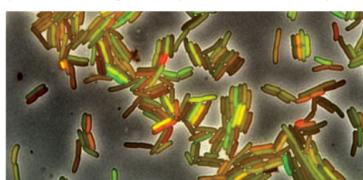
Rates of synthesis and degradation.

The ability to change rapidly will depend on the number of molecules present.

Up to now, we have been talking primarily about numbers of molecules and changes in half-life, but how might stochastic effects influence gene expression?

Consider the classic experiment by Elowitz et al, in which a strain of the bacteria *E. coli* has been engineered to express a red fluorescent protein and a green fluorescent protein. In both genes, expression was driven by the same promoter. Fluorescence microscopy can be used to determine whether a particular cell is expressing one or the other (red or green), both (yellow) or neither.

Assume that you isolate one of each "type" of cell and then grow up a new culture of each - what would you expect to observe?



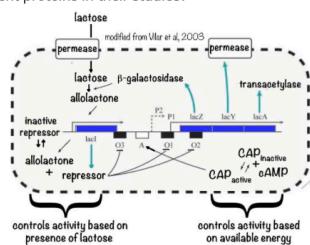
- all the cells will have the same phenotype as the original "parental" cell
- all four phenotypes will be present
- impossible to predict
- no idea

explain your thinking, please

Because the "decision" to express one or the other fluorescent protein is stochastic, and has no impact on future events - a particular cell will be expected to produce all various patterns of expression.

Elowitz et al used a lac promoter to control the expression of fluorescent proteins. In this system gene expression is inhibited by the constitutively expressed lac repressor, a protein present in small numbers in the cell. Does this information help you explain (↓) the stochastic expression of fluorescent proteins in their studies?

yes or no, explain



In the lac system, expression of the operon depends upon the inhibition of the repressor by allolactone. The reaction that forms allolactone is catalyzed by the LacZ gene. Lactose enter the cell through a channel encoded by the LacY gene. Expression of both LacZ and LacY is controlled by the lac repressor.

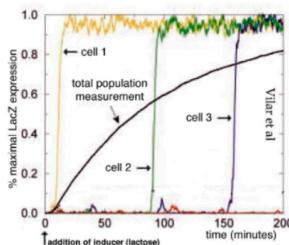
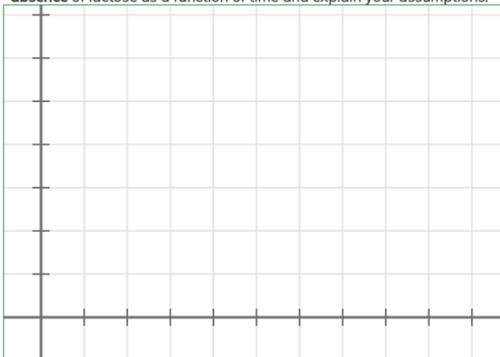
Given that the lac repressor is always expressed, how is that the lac operon can ever turn on? (→)

how can lac expression be turned on?

The small number of repressor mean that the operon will be expressed periodically, and stochastically.

Here is the behavior of the lac operon measured in single cells as a function of time, and in the presence of extracellular lactose. (→)

In the graph, make a drawing of the expression of the lac operon in the absence of lactose as a function of time and explain your assumptions.



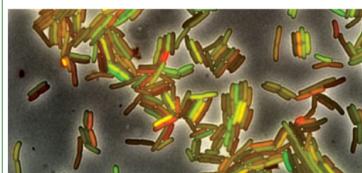
what assumptions did you base your graph on...

The operon will turn on (briefly) and then off, based on whether repressors are bound or not.

So let us (briefly) consider one more complication.

What might happen if instead of two different fluorescent proteins, Elowitz et al had used two different transcription factors? (↓).

would the behavior(s) observed be the same or different, and if different, how....



If it were transcription factors, their stochastic expression would be expected to lead to changes in gene expression, that could change the system in irreversible ways.

You are asked to explain how the activities in this exercise are relevant to embryonic development, are they relevant or irrelevant? (and explain/explain).

- obviously relevant
- apparently irrelevant, at least to me
- unclear, but hopeful they it will make sense as we proceed

be honest....

Done

Developmental systems (as we will see) often depend upon responses of cells to signals.

The expression of multiple receptors and response elements stochastically enables cells to respond dynamically to a range of possible signaling inputs, rather than only very specific responses.

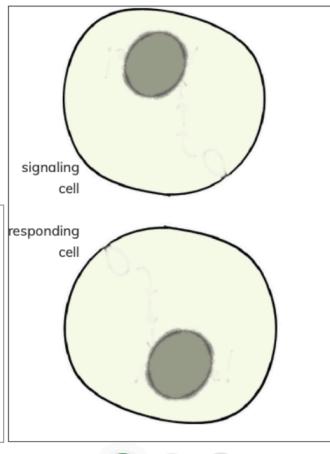
DEVO (MCDB 4650) Activity #3 – Class 4 Signaling gradients

Signaling, gradients & cellular responses: Cells influence each other in a number of ways.

Generate a schematic drawing of the important elements of a signaling system (\rightarrow)

Describe the various types of cellular effects that might occur when ligand molecules binds to receptors (\downarrow).

what can happen when a signaling molecule binds to its receptor?



The drawing should indicate that one cell is generating and releasing (or at least expressing on its surface) a signaling molecule, while the other is expressing on its surface a receptor protein for that molecule.

The binding of signaling molecule to receptor leads to an allosteric change in receptor structure, and so function, such that changes occur within the cell expressing the receptor. These changes can change protein function, most simply, changes to the localization of activity of transcription factors, leading to changes in gene expression and cell behavior.

The release of signaling molecule can be used to control and coordinate the behavior of neighboring cells. In a gradient, the concentration of the signaling molecule often changes over time (temporally) or over distance (spatially). These changes are influenced by a number of variables.

In a single isolated cell, located at the origin, indicate (\downarrow) the concentration of signaling molecule as a function of distance from the cell. Assume the system is a steady state.



What factors might influence the shape of the distribution (\downarrow)?

when does a behavior (in general) makes sense only at high density?



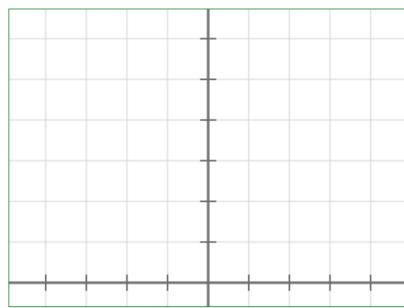
The concentration will peak at the origin and reflect the rate of signal synthesis and expression (secretion).

The shape of the curve (which decays away from the origin in both directions) will be influenced by signal molecule size and properties (diffusion rate, binding to various molecules in the environment) as well as its degradation (half-life) determined by various processes.

Assume that there are an increasing number of cells in the neighborhood of your cell. How will the concentration of the signaling molecule change over time?

Redraw (\downarrow) the [signal] concentration assuming that there are many nearby signal secreting cells.

Often the behavior of cells expressing receptors for the signaling molecule changes as a function of signal molecule concentration, why (\downarrow)?

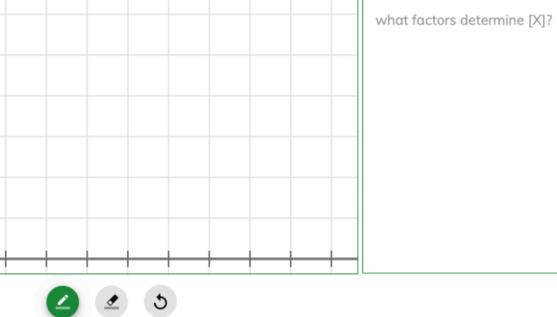
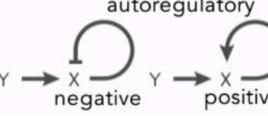
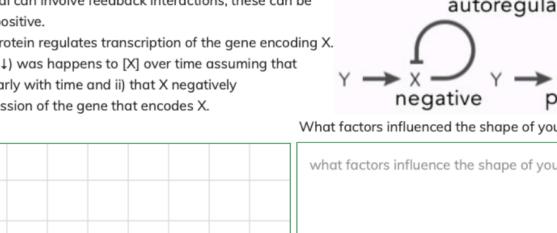


what controls whether a cell responds to signal or not?



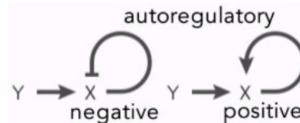
the more cells secreting the signal, the higher and broader the [signal] will be around the origin.

"Threshold" effects will depend upon binding affinities and processes such as cooperativity and the presence of feedback interactions, interactions that you may or may not have been introduced to in earlier courses.

<p>Taking part in a cooperative system to produce an expensive behavior is a social activity. All social system can be "invaded" by cheaters. An individual cell could "cheat" the community if (pick all that apply→)?</p> <p>How the percentage of cheaters in a community negatively impact the evolutionary "well being" of the community? (↓)</p> <p>what does "evolutionary well being" consist of?</p>	<input type="radio"/> it expressed the receptor but not ligand <input type="radio"/> it made ligand but not receptor <input type="radio"/> it benefited from the expensive activity, but not contribute to it <input type="radio"/> cheating is not possible <input type="radio"/> no idea	<p>Could be Express the receptor (only then can it respond)</p> <p>Or</p> <p>Benefit from the activity (e.g. the secretion of a degradative enzyme that enables it to import nutrients.</p>
<p>What benefits do cheaters gain and how might an organism/population protect itself against cheaters? →</p> <p>any plausible solutions?</p>		<p>Organisms need to identify (in some way) those organisms that fail to cooperate. Alternatively, they can make cheating difficult by linking cooperative behavior with an essential function, a function if lost leads to death.</p>
<p>Signaling systems often control gene expression networks. Such networks are often complex and incompletely characterized. In network diagrams a pointy arrow ("→") indicates a positive effect while a bar arrow ("→ ") indicates a negative effect.</p>		<p>As the signal increases, the level of Y increases (with a delay associated with the transcription of the gene, and the translation of the mRNA that encodes Y. As Y accumulates it will lead to expression of the gene encoding X.</p>
<p>Consider a simple regulatory interaction in which an increase in [signaling molecule] leads to a to an increase in the intracellular concentration of the transcription factor Y. Y acts directly and positively on the expression of gene X. Assume that the [signal] increases with time (x-axis). How does the level of the X gene product change over time (↓)? What factor(s) contribute to the level of the X gene product over time (↓)?</p>	<p>what factors determine [X]?</p>	<p>Both Y and X will accumulate until they reach steady state levels at which point synthesis and degradation rates are equal.</p>
		
<p>Response to a signal can involve feedback interactions, these can be either negative or positive.</p> <p>In our case, the X protein regulates transcription of the gene encoding X. Predict (and graph ↓) what happens to [X] over time assuming that i) [Y] increases linearly with time and ii) that X negatively regulates the expression of the gene that encodes X.</p>		<p>As the level of Y increases, it will lead to the expression of X. As X increases, it will increase until its concentration is high enough to inhibit the expression of X. It will then, presumably, decline due degradation processes. As it declines, it will pass a concentration at which it no longer inhibits the expression of the X gene, at which point X will increase again (since Y continues to be present).</p>
	<p>What factors influenced the shape of your graph? (↓)</p> <p>what factors influence the shape of your curve?</p>	<p>This pattern will continue over time, as long as Y is present.</p>

Now consider the behavior of the positive auto-regulatory circuit.

Assume that that i) the level of active Y increases linearly with time but ii) that X positively regulates the expression of the gene that encodes X. What factors did you consider in drawing your graph? (↓)



what influence the shape of your curve?



As before, the increase in Y will lead to an increase in X, which as the concentration of X increases, will lead to a further increase in X until such time that the rate of synthesis of X will be matched by the rate of its degradation.

One unrealistic aspect of the scenario is the assumption that Y continues to increase; while this might be the case in the short term, eventually the level of Y will plateau as the rate of its synthesis or activation will be balanced by the rate of its degradation (or inactivation).

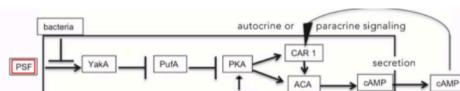
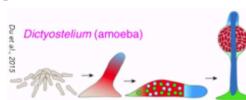
In many quorum sensing systems, there is a threshold concentration of signaling molecule - below which there is no cellular response, and above which the response is full on.

In the slime mold community behavior is based on two factors, the secreted quorum sensing protein PSF and signals from the presence of bacteria, which the slime mold eats.

Above a threshold concentration, PSF activates the protein kinase YakA, below the threshold YakA is inactive.

Make a model that might explain why YakA is not active below the PSF threshold (→) and what happens to PKA activity when YakA is active? (explain↓)

explain your model, and what happens to PKA activity



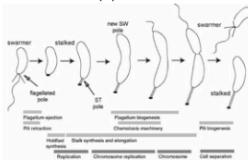
The question of thresholds is tricky, but one possible mechanism would be that increasing signal only leads to Yak activation above a threshold if the binding of the regulator of Yak is weak, or depends upon multiple sites occupied at the same time, a situation that only occurs when the concentration of signal is high enough - it would be influenced by factors beyond simple activator concentration

One YakA is active, PKA will be active, leading to synthesis of cAMP and the aggregation (and eventual slug formation, migration, and differentiation.

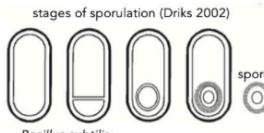
DEVO (MCDB 4650) Activity 4 Class 5 - Differentiation & Aggregation -

When we think about development, we typically think of multicellular organisms, although there certainly are developmental processes in unicellular organisms.

A few of the most obvious are sporulation (such as in *B. subtilis* →) and the formation (↓) of swimmer and adherent cells in *Caulobacter*.



These behaviors involve molecular systems that respond to environmental factors and initiate changes in gene expression and protein activities that lead to specific changes in cell structure & behavior.



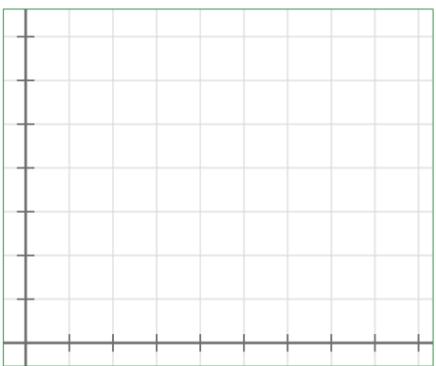
Predators can be a powerful driver of multicellularity. Borass et al found that when the unicellular predator *Ochromonas vallescia* was introduced into cultures of the unicellular alga *Chorella vulgaris* multicellular forms of *Chorella* appeared after ~5-10 days. Initially larger, these multicellular *Chorella* stabilized at ~8-cells, and became the dominant form of *Chorella* in the system, and were stable in the absence of the predator.

Borass et al argued that this was an evolutionary rather than a signaling event; what details (above) support their reasoning? how might you confirm it?

(→)

An signaling change would be expected to revert when the stimulus was removed. An evolutionary change might well be expected to be stable, and would be expected to be associated with changes in the genome - genetic changes that alter the expression of specific genes.

In relation to the Borass *et al* study, predict (and graph) how the number of cells in the *Chorella* colony will change over time (multiple generations) ↓.



What factors do you think will limit *Chorella* colony size? And how might a multicellular *Chorella* circumvent those limitations?

what physical factors limit colony size?

By getting larger chorella makes predation more difficult. Sticking together is a relatively easy way to increase size (in a single step).

BUT the initial change just might make be failure to separate - so that cell clusters increase in size, and adopt an irregular number of cells/colony (presumably colonies fragment mechanically). So over time, we might expect first no change in size, and then an increase ... later, there may be the appearance of evolutionary adaptations than limit size (and increase colony fragmentation).

How would the predator respond? indicate the effect of changing *Chorella* colony size (previous graph) on predator size (↓) and explain your reasoning, also consider the question, why might changing colony size be easier than changing predator size? (↓).



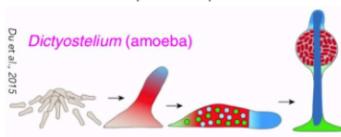
what physical factors limit colony size?

The structure of the predator is likely to be more difficult to change (not simple).

It might, in fact, not change at all - what might change would be the prey the predator prefers.... All depends upon the environment.

Another example of multicellularity, albeit transient, involves social evolution.

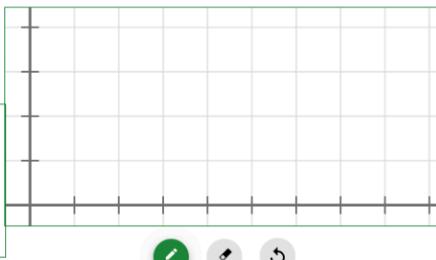
The cellular slime mold *Dictyostelium discoideum* is "normally" unicellular - crawling around in soil, eating bacteria, growing and reproducing. When conditions become hostile, however, it changes its strategy. A single cell's ability to move from place to place is limited. The solution ([adopted by a number of different species](#)) is to become transiently multicellular, cell cooperate (and sacrifice themselves) to generate a structure that lifts related cells out of the soil and allows for the dispersal of spores.



What are the units of concentration? Explain the factors that determined the shape of your graph (↓).

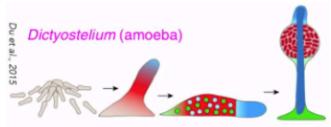
what factors control [PSF]?

Of course, this scheme makes little sense if there are only a few cells around, so *D. discoideum* uses a quorum sensing system to monitor cell density. Cells secrete the prestarvation factor (PSF) protein. Draw a graph (↓) of the relationship between [PSF] (y-axis) and cell density (x-axis).



There is no reason to migrate if life is good. *D. discoideum* uses a second signaling system, the generation and secretion of cyclic AMP (cAMP) to monitor the availability of food. If the local population density of *D. discoideum* cells is sufficiently high (above a threshold), starving cells will secrete cAMP. As extracellular [cAMP] increases cells will migrate towards each other and aggregate into a slug that can then migrates, find a good location and differentiates.

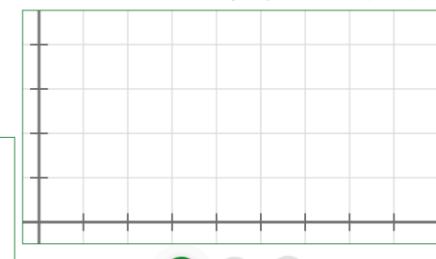
Approximately 20% of the cells in the slug will undergo what is known as terminal differentiation - they form the holdfast and stalk (and die). The remaining cells form spores that are lifted up out of the soil and released; they can drift in the air and colonize new sites. Which cells of the slug become stalk and which spores is a stochastic process involving different gene expression programs.



Why is it important, for *D. discoideum*, that the process of cell fate determination be random, what might you expect to happen if it weren't (↓)?

explain you thinking (what is key for "self-sacrificing" behavior to occur?)

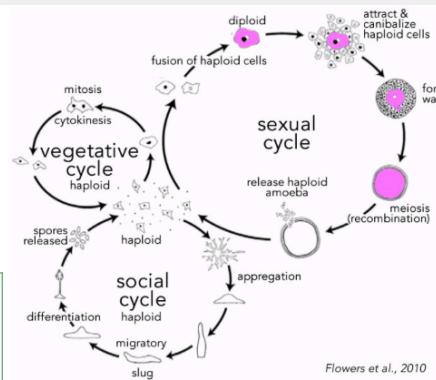
Assume that bacterial cell numbers are decreasing over time. Indicate your prediction of [cAMP] (↓).



D. discoideum also has a (weird and rare) sexual cycle (→) with distinct mating types. Two cells of different mating type fuse; haploid amoeba are attracted, induced to die and either consumed or used to form a wall around the group. The resulting giant diploid cell enters meiosis, forming recombinant haploid cells that are released.

As a check, what is the "purpose" of meiosis, why might it be rare in Dicty & why would it be beneficial (feel free to speculate ↓).

what is the value of meiosis under stressful conditions?



We would expect that [PSF] would reflect the concentration of cell – cells per volume. We would have to assume that the environment is rich (that is, full of food) and that [cells] is increasing with time.

Then [PSF] would increase in parallel to [cells].

We would expect that as the concentration of bacterial cells decreases (which will be a function of nutrients in the environment as well as the [dicyt cells], which eat them), there would be some threshold at which dicyt cells begin to synthesize and release cAMP.

The purpose of sexual reproduction is (it is thought) to generate new genetic variation - variation based on the reshuffling of maternal and paternal alleles through the meiotic process of chromosome alignment and crossing over (recombination).

New genetic variations in the population might be better able to deal with a hostile environment.

DEVO (MCDB 4650) Activity 5 Class 6 - Asymmetries -

Thinking about multicellularity, and the generation of distinct cell types gets us to think about cellular asymmetries, how they arise and what are their "down stream" effects. Asymmetries can be intrinsic or imposed.

Illustrate in a diagram (↓) intrinsic asymmetries within a "typical" eukaryotic cell and explain your thinking (→).

remember the nature of the cells involved



There is a generic internal asymmetry based on the common fact that the nucleus is asymmetrically located within the cell, with the microtubule center (if the cell has a microtubule center) in the geometric center of the cell. BUT most cells are within some environment that itself may be asymmetric.

Immediately after a eukaryotic cell divides, each of its daughters has an inherent asymmetry axis.

Illustrate in a diagram (↓) and explain the origin of this asymmetry? (→)

remember the nature of the cells involved

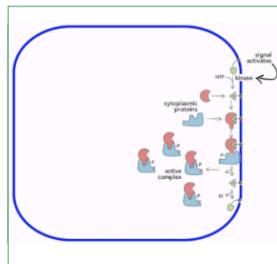


The spindle poles will define an axis - with the nuclei reforming (after cytokinesis) - so each daughter cell will have a spindle pole/centrosome - nuclear axis.

Here is a schematic of a cellular system that could generate an asymmetric cell.→

- The source of the asymmetry in this system is:→
- intrinsic to the cell
 - imposed on the cell
 - stochastic
 - no idea I can justify

Indicate how might this cell divide so as to generate either i) two similar or ii) two different cells? →



Explain your reasoning (→)

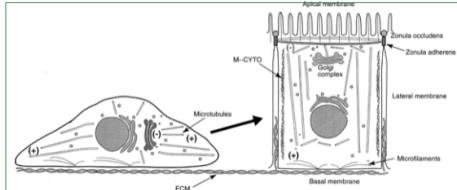
Why are cells similar or different?

Extrinsic factors (asymmetry of signaling) will lead to asymmetries in the cell.

A horizontal division plane will generate two similar cells, while a vertical division plane will generate two asymmetric cells (we might think).

What types of intrinsic asymmetry axes might be present in an isolated, crawling fibroblast versus a cell within an epithelial sheet. Indicate the axes present (→) and explain the basis of your thinking (1).

What cellular structures are responsible for these axes?



How might interactions with the substrate and/or neighboring cells influence cellular asymmetries (1)?

how might these influence the cell?

Think about the organization of cells within a colonial organism.

Illustrate (1) the various factors that could lead to the appearance of different cell types?

Explain your reasoning.↓

why would they become different?

In the cell on the left, there is an asymmetry arising from interactions with the substrate, creating front-back and top-bottom axes.

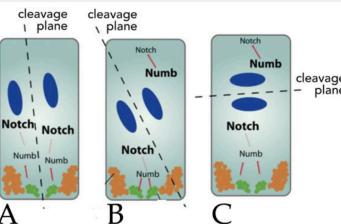
and in the cell on the right, there is an even more dramatic apical-basal (based on substrate attachment) and little or no front-back axis.

The most obvious differentiation event would be cells on the surface of a colonial organism versus those cells that are internal to the colony.

<p>In a particular organism, such as the fruit fly <i>Drosophila melanogaster</i>, it is possible to accurately predict the embryonic axes based on the structure of the egg.</p> <p>It would be reasonable to assume that ... (→)</p> <p>Explain your reasoning (↓)</p> <div style="border: 1px solid green; height: 150px; width: 100%;"></div>	<ul style="list-style-type: none"> <input type="radio"/> the egg already one asymmetry axis <input type="radio"/> the egg already has two asymmetry axes <input type="radio"/> axis formation is based on later embryonic events <input type="radio"/> embryonic axis are determined by the site of sperm entry <input type="radio"/> no idea 	<p>Q1: the egg has pre-established asymmetry axes (both A-P and D-V).</p>
<p>In other types of organisms, such as the mouse and human, the unfertilized egg has a asymmetry axis associated with the meiosis II spindle and the position of the first polar body – please draw that axis (→)</p> <p>It is NOT possible, however, to accurately predict the embryonic axis based on the structure of the egg because (↓)</p> <ul style="list-style-type: none"> <input type="radio"/> one asymmetry axis is not enough <input type="radio"/> two asymmetry axes is not enough <input type="radio"/> axis formation is based on later embryonic events <input type="radio"/> axes are determined by the site of sperm entry <input type="radio"/> no idea <p>Explain your reasoning (↓)</p> <div style="border: 1px solid green; height: 150px; width: 100%;"></div>	 <p>The axis would go through the first polar body (line from lower left to upper right).</p> <p>In mammals, embryonic axes will be determined later within the inner cell mass, which forms as embryonic cells come to find themselves in different environments.</p>	
<p>Predict the type of fossil evidence that might enable you to deduce the presence of a multicellular stage in a dicytostelium-like organism (→)</p> <p>Explain you reason for why trace fossils provide evidence for the presence of at least one organismic axis (here is a link to consider) (↓)</p> <div style="border: 1px solid green; height: 150px; width: 100%;"></div> <div style="border: 1px solid green; height: 150px; width: 100%;"></div>	<p>We might expect to see linear tracts, indicating that the organism can move in a coordinated, directional way. We might then expect that the front end is different from the backend (anterior-posterior axis), and perhaps a dorsal-ventral axes as well.</p> <p>The anterior end might well be specialized to respond to the environment in order to influence the direction of movement.</p> <div style="text-align: center; margin-top: 10px;"> L S D </div>	

Which of these cleavage planes would be most likely to generate two similar daughter cells? (→)

- A
- B
- C
- all equally likely
- unpredictable
- no idea



Explain your choice (↓) and describe which is most likely to produce an unpredictable outcome. Include a description of what it means to be similar or different at the cellular level.

what would make the outcome unpredictable?

I would assume A because each daughter cell would inherit similar environment, as well as a similar set of intracellular components.

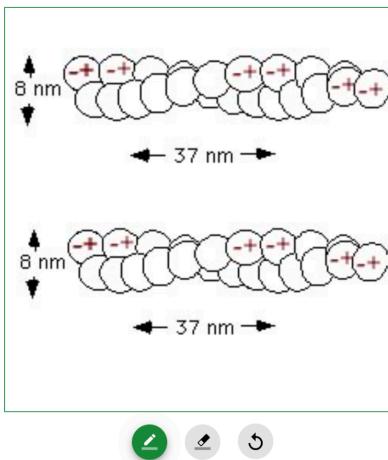
In contrast B and especially C generate immediately distinct cells, with different environments, as well as different cell-cell interactions. The result is likely to be differences in signaling systems, responses, and patterns of gene expression.

LEFT-RIGHT Asymmetry:

Consider an actin filament. Draw (→) the different ways a myosin motor might move along the filament to produce a straight (top) or a curvy movement (bottom).

Explain your drawing / thinking (↓).

how does a myosin move along a microfilament?



Just to check

Gene expression on the two strands of a DNA molecule may be influenced by DNA replication because

(choose one (↓) and explain your thinking (→))

- of replication induced changes in DNA sequence
- of replication induced changes in DNA modification
- replication will displace transcription factors
- replication



explain your thinking here.

Depending on your geometries (myosin-actin binding and step size - that is, how many actins between binding sites), one might expect that a motor that switched between back and forth between strands might move straight, but one that always bound to the same actin strand would circle around the microfilament.

end

The old strand would be methylated or otherwise modified, while the newly synthesized strand would not yet be.

Modification take time, and so will change as a function of time from the time of synthesis, reaching a plateau level (eventually).

DEVO (MCDB 4650) Class 8 - Signaling - Activity 7

Define what is meant by a chimeric protein? (→)

In their studies of myosin 1D and myosin1C, Lebreton et al made chimeras between parts of the two proteins. Why did they do that? (↓)

- What was of critical importance in the chimeras they used? They (→) pick all that apply
- retained enzymatic activity
 - were stable
 - were chiral
 - bound actin
 - inhibited myo1D activity
 - not sure

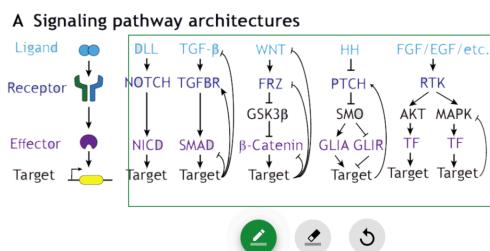
A1: parts of two (or more) distinct polypeptides, fused together - all retain their functionality

A2: to identify the region of myosin 1D molecule responsible for the behavior (directionality of movement).

Q3: they need to retain activity and are chiral, retainingly activity implies an ability to bind actin.

Multicellular animals rely on a restricted set of signaling systems. They consist of a signaling molecule (ligand), encoded for by a number of genes, receptors for that ligand, down stream effector(s), and targets.

Show are five of the most common signaling systems. Assume that the target is a gene. Circle the cases (→) in which you predict that target gene expression would vary over time, even if the concentration of ligand remained constant. Explain your reasoning. (↓)



We might expect that any signaling pathway with feedback interactions will be expected to change over time, since the level of feedback will be changing as the function of time (since they are not on initially).

In this diagram, only Notch does not appear to have feedback, but in fact that is not the case.

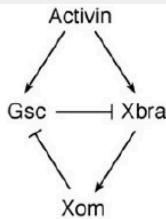
Essentially all networks involve various forms of feedback.

Activin (a TGF β family ligand) activates the expression the genes Gsc and Xbra in a concentration-dependent manner. Saka and Smith modeled this gene network here (→) and through a set of differential equations.

Typically, at any particular [activin] only one or the other gene is expressed.

Make and justify a prediction of which gene (Xbra or Gsc) is expressed at low versus high [activin]. What factors are considered in your model? (↓)

explain here

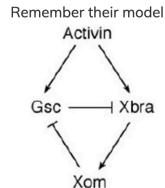
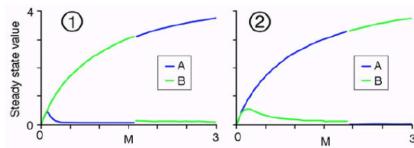


One possible model. Because the negative feedback on Gsc associated with Xbra expression (through Xom) involves the transcription and translation of two genes/ RNAs, we might reasonably expect that its effects could take longer than the effect of Gsc on Xbra expression. So when activin is added, perhaps Gsc protein accumulates to an effective concentration first (before Xbra/Xom) thereby inhibiting Xbra expression.

Of course the system would be effected by binding affinity of Gsc and Xbra/Xom for their DNA targets, as well as the various half-lives of the RNAs and polypeptides involved.

The Saka and Smith model gives different predictions (1 and 2) depending on various presumptions. At low [activin] or [M] (along the X axis) you may notice that at very low [M][activin] both *Xba* and *Gsc* are expressed at a low level.
Can you provide a plausible explanation for why this might occur?

how would that be possible?

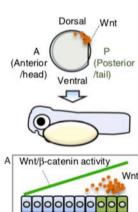


Research article
A mechanism for the sharp transition of morphogen gradient interpretation in *Xenopus*
Yasushi Saka^{1,2} and James C Smith¹
BMC Developmental Biology 2007.

See previous panel:

The outcome and your explanation will depend on assumptions you make on protein binding affinities for DNA regulatory sites, RNA and polypeptide lengths, half-lives, such

A complexity, becoming apparent through the use of single cell resolution techniques, involves the stochastic nature of cellular responses. In the case of Alkieda et al. (2019) this involves cellular responses to Wnt signaling gradient (\rightarrow).



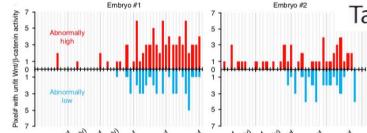
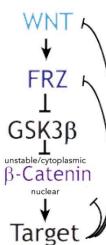
In the zebrafish (\leftarrow) a Wnt ligand gradient is involved in establishing the embryo's head-tail axis. The result is that posterior cells "should" have high levels of nuclear β -catenin and target gene expression while anterior cells should have low levels of both.

Yet, when examined the authors found a number of "eccentric" cells (\downarrow) that express the "wrong" level of β -catenin and target gene expression.

As development proceeds, the number of eccentrics decreases; the surrounding cells induce eccentrics to undergo cell death. Blocking the signaling system involved in inducing the death of eccentric cells leads to later stage developmental defects.

In what other processes might the elimination of eccentric cells be useful?

any spring to mind?

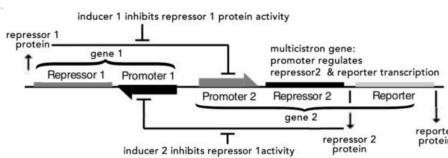


An obvious example would be cancer, in which a cell escapes regulatory control of growth and cell division. By becoming "different" from neighbors, it might trigger feedback signaling and induce its apoptotic death.

DEVO (MCDB 4650) Class 9 - Signaling - Activity 8

Signaling and HOX introduced

Given this circuit (\rightarrow), and assuming that all necessary positively acting transcription factors are present, what will be the level of the gene 1 and gene 2 expression? (\downarrow)



- one on and the other off
- both on at a low level
- both on at a high level
- impossible to predict
- no idea

At time = 10, inducer 1 is added at a concentration sufficient to inhibit the repressor 1 protein. Graph (\downarrow) the level of the repressor 2 protein as a function of time.

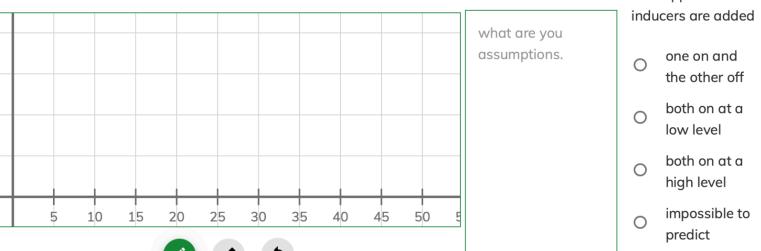
and explain the reasons for your answer to the question and your graph (\downarrow)



Now assume that you add induced 2 rather than inducer 1 at time = 10.

Graph (\downarrow) the level of the repressor 2 protein as a function of time.

and explain the reasons for your answer to the question and your graph (\downarrow)



This (\rightarrow) is the sequence logo for the hypothetical transcription factor Zok1. A T at position 1 and an A at position 5 are essential for binding. Binding is weaker if bases other than those indicated are present at the other positions.



Consider two accessible sequences TTCAACTT and TTGCACTT. At a high concentration of Zok1 protein; which site will be more likely to be occupied (\downarrow), i.e. have a Zok1 protein bound?

- TTCAACTT
- TTGCACTT
- Both will be bound equally
- neither will be occupied
- no idea how to answer

Now consider the situation at a low concentration of Zok1 protein; which site (\downarrow) will be more likely to be occupied?

Explain the reasoning behind your choice? (\downarrow)

- TTCAACTT
- TTGCACTT
- Both will be bound equally
- neither will be occupied
- no idea how to answer

here

Answer depends upon assumptions. One assumption might be that since positively-acting TFs are present, both genes will be one (stochastically) at a low level, a level too low to stably repress the other

Adding inducer 1 will lead to inhibition of repression of gene 2, which will lead to increased repressor 2 protein, which will stably repress gene 1. SO, gene 2 (repressor 2) will increase and plateau when expression and degradation reach steady state.

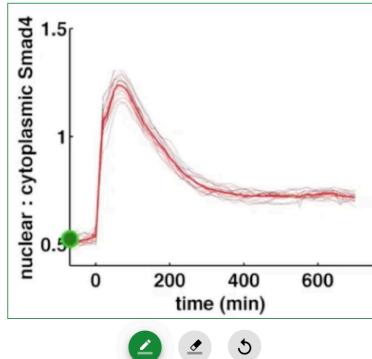
Once this state is reached, gene two will be expressed even if inducer 1 is removed.

Exactly as before, except that gene 1 will be expressed and gene 2 will be inhibited.

Q1: At a high concentration of Zok1, both sites will be occupied, but

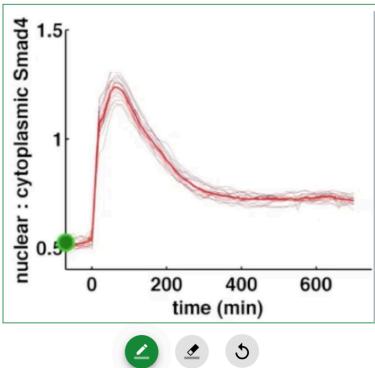
Q2: at the lower concentration, the TTCAACTT site will be occupied, because it contains a better match to the consensus.

Here is the response of nuclear SMAD (transcription activator) to a constant level of activin over time. Assume that the different genes have different binding affinities for the SMAD protein, and that SMAD binding activates transcription. Graph the response of a gene with a low affinity SMAD binding site (\rightarrow). and explain the logic of your response (\downarrow).



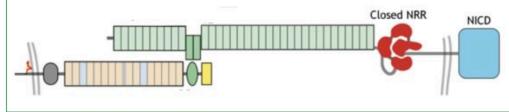
A gene with low affinity SMAD response sites will track along with the level of SMAD, dropping as nuclear SMAD concentration drops (and depending upon the site's binding affinity).

Compare the behavior of a gene with a low affinity SMAD binding site to a gene with a high affinity SMAD binding site (\rightarrow). and explain the difference between the two and what might happen with genes with intermediate affinity SMAD binding sites (\downarrow).



Since the SMAD level does not go to the pre-activin exposure level, a gene with a high affinity SMAD response site will increase expression that is likely to stay high (since nuclear SMAD concentration does not return to baseline level).

In Notch signaling, a Delta protein on one cell interacts with a Notch protein on a neighboring cell. The result is a change in both proteins. A conformational change in the Delta protein will lead to its endocytosis which will lead to the unfolding of the Notch regulatory region (NRR) of the Notch protein. In this diagram (\downarrow), indicate the direction of movement in the delta-notch complex



What processes, associated with Notch signaling, are dependent on the unfolding of the NRR? (\downarrow)

what are you assuming?

Predict what would happen to Delta and Notch signaling if there were a mutation in the Notch protein that blocked the unfolding of the NRR. (\downarrow)

explain your reasoning

- nothing
- no notch signaling
- block delta endocytosis
- no idea

The movement of Delta into the cell requires the cleavage of the NRR site in Notch. This requires that the NRR site unfolds in response to movement of Delta.

If the NRR site cannot unfold, there will be no Notch signaling (since NICD will not be released) and Delta will not be endocytosed - or so we predict.