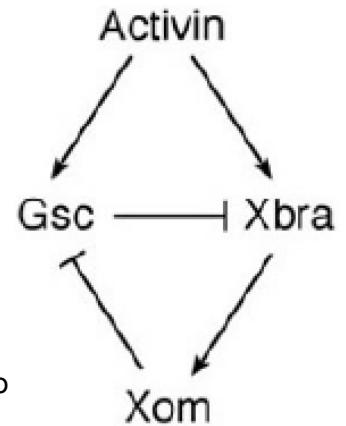


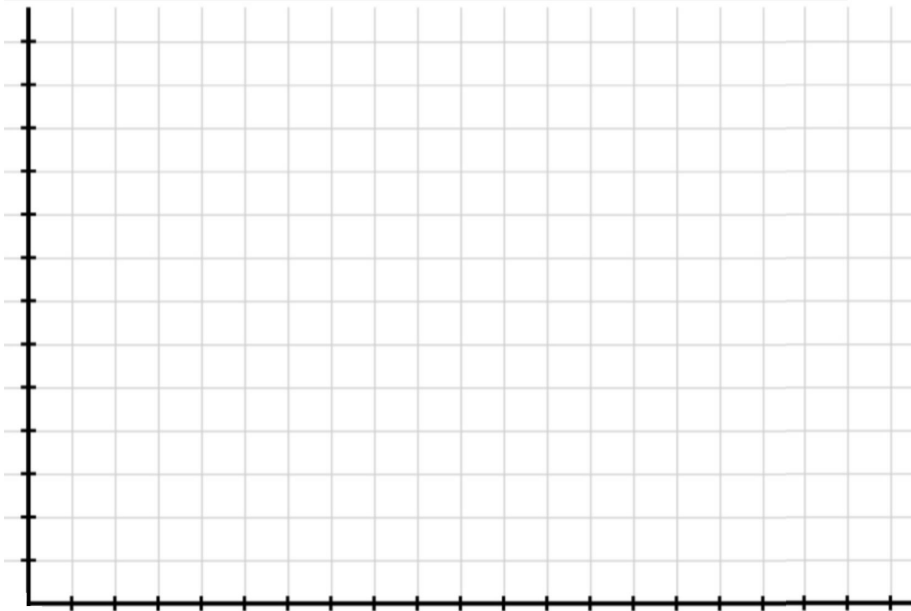
**MCDB 4650 MidTerm #1 exam Feb 2020 name:** \_\_\_\_\_

My suggestion - look through the test first before starting to work on the answers (it helps calm one down).

**Q1 (25 points total):** In the Saka & Smith model, the signaling molecule Activin positively regulates the expression of the genes encoding the transcription factors Goosecoid (Gsc) and XBrachyury (Xbra). YET, at a particular level of activin signaling, only one or the other gene (Gsc or Xbra) is expressed.



**A:** Consider a concentration of activin at which Gsc, but not Xbra is stably expressed. Draw out (below) as a function of time, the levels of the two RNAs (or proteins) (Y-axis) as a function of time (X-axis). Activin is added at time = 0.



**B:** Explain below (↓) how it is that Gsc comes to be the activin target gene that is expressed.

Early after activin addition on both Gsc and Xbra RNAs will rise slightly. As Gsc protein appears and its concentration increases, it will inhibit further Xbra expression (which will begin to decrease). While a small amount of Xbra will appear, leading to small amount of Xom expression, it is unlikely to influence Gsc expression. Gsc RNA will increase until it reaches steady state (where its degradation = synthesis).

**C:** What level of Xom RNA/polypeptide will be present at steady state (when Gsc is expressed)?

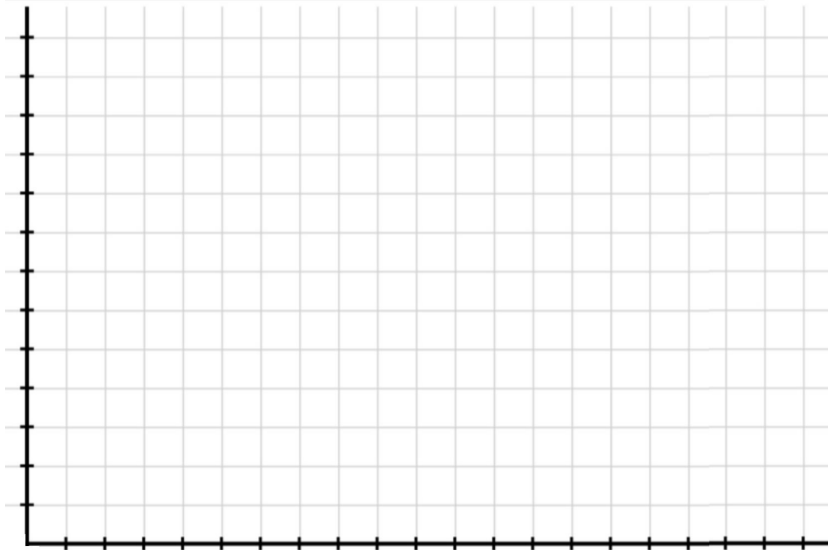
- detectable
- undetectable
- impossible to predict
- no idea how to answer

explain your answer: ↓

At steady state, there will be a high level of Gsc protein, which will inhibit Xbra and so Xom expression. I suspect that little or no Xom (or Xbra) RNA will accumulate (be detectable) at steady state.

**D:** Now, assume that the system has changed so that the binding affinities of the Xbra protein for the Xom promoter, and the Xom protein for the Gsc promoter are 10-fold higher than the binding affinity of Gsc for the Xbra promoter, but that the steady state level for all three proteins is similar. Make and justify a prediction for how this might influence the expression of Gsc, Xbra, and Xom (Y-axis) over time (X-axis) (→) at a particular activin concentration and explain your logic (↓).

We might expect that the result will be that it will take less time for Xbra protein to accumulate to a level necessary to activate Xom expression, and for Xom protein to accumulate so that Gsc expression will end up inhibited, leading to high Xbra expression at steady state. There could be some confusion about the wording of the question, so answers are graded very leniently.



**E:** Now consider how the system might be expected to change if the Gsc RNA/polypeptide were 10 times the size of the Xbra and Xom RNAs/polypeptides. At steady state, which will be expressed, Gsc or Xbra? and explain your choice.

Assuming that protein length does not influence binding affinity or turn-over rate, the RNA/polypeptide lengths would delay the accumulation of Gsc compared to Xbra and Xom proteins, so we would expect that at steady state we would expect to see Xbra / Xom rather than Gsc (high Xbra/Xom low Gsc).

**Q2 (20 points):** In the Elowitz et al experiment, genes encoding green and red fluorescent proteins were inserted at sites equidistant from the bacterial origin of replication (*oriC*). The same *lac*-based promoter was used to drive the expression of each gene.

The authors noted four types of cells in the population, those fluorescing green, those fluorescing red, those fluorescing both green and red (yellow), and those not fluorescing at all.

**A:** You watch the cells over a long period of time. What would you expect to see happen over time? It is important to know that the fluorescent proteins have been engineered to have short half-lives.

- no change in the color of individual cells
  - cells switch between all states
  - fluorescence disappears from all cells
  - all cells express both green and red proteins.
  - no idea
- Explain your answer: ↓

We are assuming that the expression of the two fluorescent protein encoding genes is stochastic (there is no feedback occurring that would stabilize the expression of one gene or the other). Also, since the proteins are unstable (short half live), the cells will switch randomly between states.

**B:** How would your answer change if the fluorescent proteins had long half-lives?

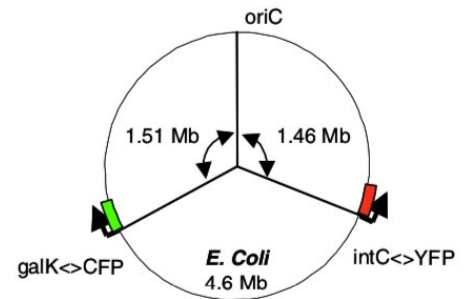
If the half life were long enough, we would expect that over time all cells would contain both fluorescent proteins. Even as the genes turned on and off stochastically.

**C:** How would your answer change if the promoter used was (unlike the *lac* promoter in the absence of lactose) strongly active and not regulated by the *lac* repressor?

Again, since the two gene do not interact with one another in a regulatory manner, we would expect both genes to be active all of the time, so that all cell would be found to express both proteins all of the time.

**D:** Now instead of genes expressing simple fluorescent proteins you used transcription factor-fluorescent protein chimeras containing different transcription factor domains. How would behavior of the cells be likely to change compared to the cells in the original experiment.

As the two genes turned on and off, the transcription factor chimeras would accumulate, and begin to influence the expression of genes their binding sites in their promoter regions. These "downstream" effects are unpredictable (since we do not know which genes are turned on or off), depending upon the pattern of gene activation (it is possible that there is no significant effect, if the downstream gene regulated do not greatly influence the expression of other genes or cell behavior).



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.

**Q3 (15 points):** The responses of genetically identical cells exposed to the same signaling molecule may differ from one another, different genes may be expressed.

**A:** What factors will influence cellular differences in gene expression?

When we say genetically identification, we mean no genomic differences, but at the level of gene expression, there may be stochastic changes influence which genes are expression (which protein binding sites are accessible, which DNA sequence have been modified, etc). These differences may influence the cell's responses to signaling factors (do they express the receptor and necessary downstream proteins?)

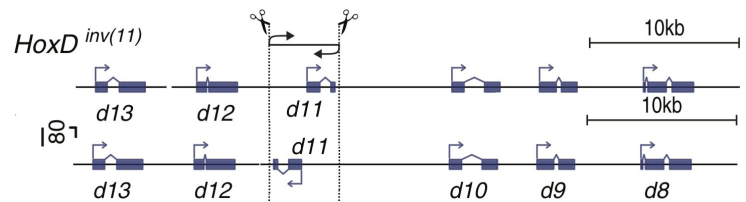
**B:** There are cases in which genes vary in their accessibility between cells of similar "types". The most dramatic case involves X inactivation in XX female mammals, but variations occur through what is known as monoallelic expression. What process is NOT responsible for X-inactivation and monoallelic expression?

- mutation
- the inaccessibility of gene regulatory sequences
- DNA methylation of transcription factor binding sites
- binding of various repressor proteins
- no idea how to answer

explain what is happening during monoallelic expression ↓

In a diploid organism, there are maternal and paternal versions of each gene (on the autosomes, that is, the non-sex determining chromosomes). These genes can be different (different allelic variants), and so either regulated differently or encode different gene products (polypeptides or non-coding RNAs). During monoallelic expression, the environment of the two genes comes to be different, either through differences in chromatin structure / folding or DNA/histone/etc modification, leading to the expression of one gene (one allele) and not the other. If the alleles are different, with different effects on the cells expressing them, then the cells may behavior differently (assuming the gene is normally expressed in that cell type).

**C.** Consider the HOX gene D11. Using CRISPR CAS9 you engineer the reversal of the gene, so that it is transcribed in a direction opposite from all of the other genes in the cluster. You find that the level of expression of the two neighboring genes, D12 and D10 are reduced. Provide a plausible (testable) model for how the inversion of D11 could influence the expression of D12 and D10.



It is possible that enhancer elements present in the region around d11 no longer function to enhance expression of d12 and d10; alternatively, the expression (RNA transcription) of d11 may influence chromatin organization or the binding of transcription factors (for example enabling repressive transcription factors to bind) that influence d10 and d12 expression. One could compare TF binding patterns between wt and flipped versions of the chromosome.

**Q4: (10 points): A:** You analyze a population of cells in which there is a high level of a particular mRNA but a low level of the corresponding polypeptide: provide a molecular scenario that could produce that outcome.

Two possibilities come to mind, either translation of the mRNA is inefficient (it rarely productively interacts with ribosomes, etc) and so polypeptides are rarely synthesized or the polypeptide may be rapidly degraded following synthesis.

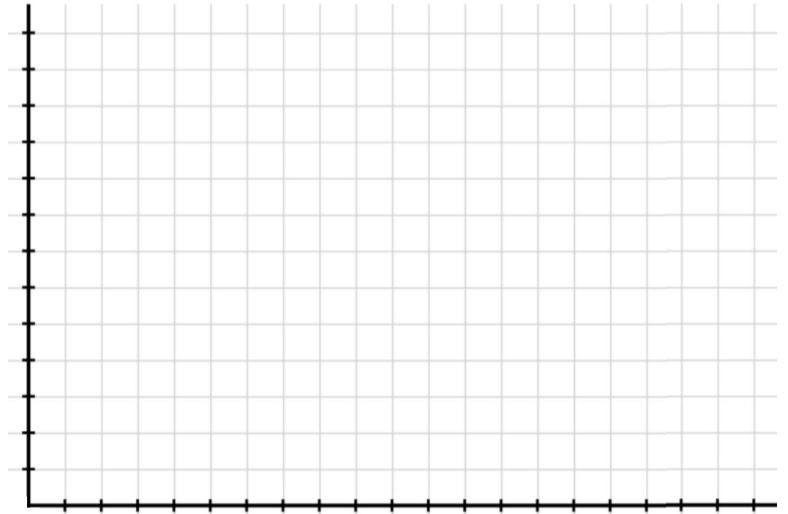
**B:** Explain (provide a model for) how regulation of half-life can influence the speed at which a cell can respond to changes in its environment.

Imagine that the response to a signaling molecule involves regulating the synthesis (activity) of both positively and negatively acting transcription factors. If the negatively acting transcription factors have long half lives, it will take a long time after the addition of the signal for them to disappear from the cell and this will impact the cell's response to the signal. BUT if the negatively acting transcription factors are unstable (rapidly turned over), as soon as expression is inhibited (by the signal) they will disappear, allowing for the full signaling response.

**Q5 (20 points):** A key feature of quorum sensing is the nature of the response to a changing (increasing) quorum signal.

**A:** Graph out (→) and describe (↓) the key features of a typical quorum sensing system reflected in the response of cells (Y-axis) as a function of the concentration of the quorum signal [S] (X-axis).

In a quorum sensing system, the cellular response will display a threshold pattern - below the threshold concentration there will be no response, above the threshold concentration the response will turn on fully. The graph will have a step like shape.



**B:** Quorum sensing systems are generally used to control behaviors that make sense at one (e.g, high) cell density but not at another (e.g, low density). Explain the role and reason why slug formation, migration, and differentiation in *Dictyostelium* are controlled by quorum sensing?

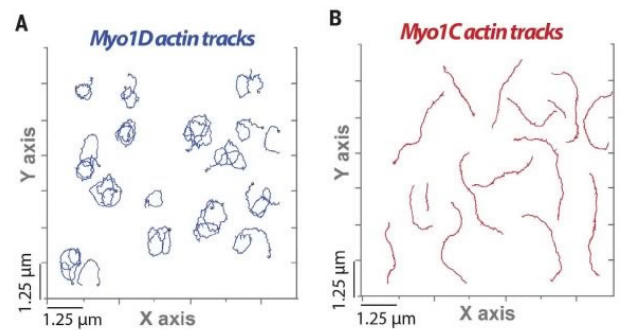
In *Dictyostelium*, the individual organisms cooperate to produce a behavior that no individual unicellular organism could produce, forming a structure that enables them escape (into the wind) one environment and travel to another. These behaviors depend on accumulating enough cooperating cells, under starvation conditions, to form a large enough "slug" and subsequent fruiting body (stalk and spores). Because the cells are starving, a single cell could not divide sufficiently to produce the necessary number of cells required to make an effective, that is, macroscopic structure.

**C.** How is a threshold response in a signaling system like quorum sensing, how is it different?

The two are basically very similar (although there are many different underlying molecular mechanisms), and characterized by a distinct threshold that leads to changes in gene expression, protein activity, and cellular behavior. Typically when we think of quorum sensing we are thinking about single on-off switch that measures the number of organisms per unit volume, whereas signaling often occurs in densely packed cells, but can lead to a number of different thresholds that lead to multiple distinct states. Quorum sensing is generally associated with a change in signal as a function of time (and cell density) whereas signaling is more often thought of a change in signal molecule as a function of distance from a source (although it is true that signaling molecule concentrations are also changing as a function of time).

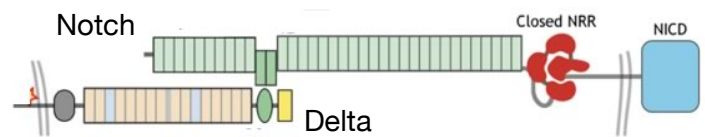
**Q6 (5 points):** A: Based on the myosin-1D paper we considered, myosin-1D appears to be necessary for left-right axis formation in the fruit fly *Drosophila*. In an *in vitro* system, myosin-1D protein attached to a surface leads to the curved movement of actin filaments, while the related protein myosin-1C leads to their straight movement (→). Provide a plausible explanation for how two structurally similar motor proteins, both of which move to the + end of actin filaments can produce such different behaviors?

to We can think of the nature of the step, does on the motor (head domain) follow a single strand (move in a curvy manor along the active filament) or does switch between strands (both of which have the same polarity) and so moved in a straighter path?



Myo1D (A) drove counterclockwise, circular motility of actin, where- as Myo1C (B) did not show any turning bias in these conditions. (Lebreton et al., 2018)

**Q7 (10 points):** In the delta-notch signaling pathway, signaling in the responding cell involves the cleavage of the notch regulatory region (NRR) by two proteases, releasing the notch intracellular domain (NICD), which moves to the nucleus.



**A:** Indicate the direction of the molecular movement initiated by the binding of Delta to Notch (↑) and describe plausible mutations that could lead to a failure of Notch signaling, while still producing full length Notch and Delta proteins.

The structure moves to the left (delta pulled into the cell, pulling notch along with it).

Mutations could influence 1) the expression of the Notch/Delta proteins on the cell surface, 2) the binding interaction between Notch and Delta, 3) the ability of the notch regulatory regions to open (e.g. a new disulfide bond or interaction that stabilizes the NRR), or 4) changes in the NICD that impact its movement into the nucleus or its interactions with other proteins.

**B:** Consider a pair of cells, both of which express Delta and Notch. Explain how the movement of the NICD into the nucleus could produce different behaviors (patterns of gene expression) in the two cells.

The answer is very much like that for question 3A - if the two cells are expressing different genes, and have different patterns of DNA accessibility and modification, or different levels of transcription factor activities, their responses to the activation of the NICD could well be quite different.

