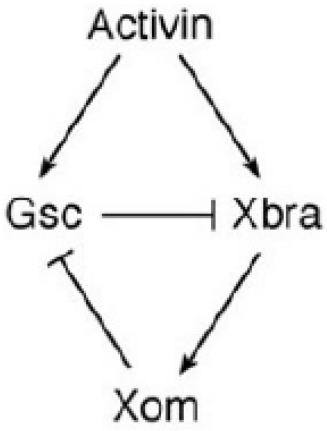
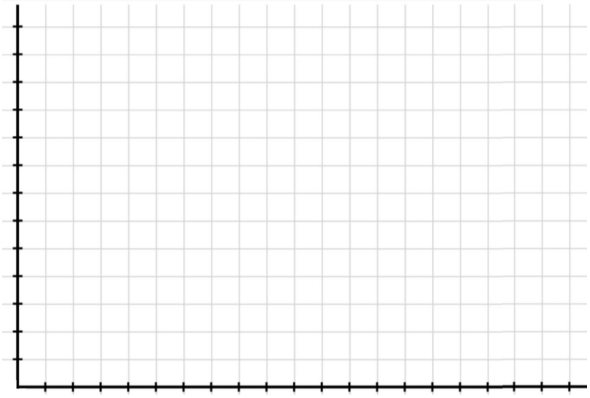
MCDB 4650 MidTerm #1 exam Feb 2020 **name:**_____

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.

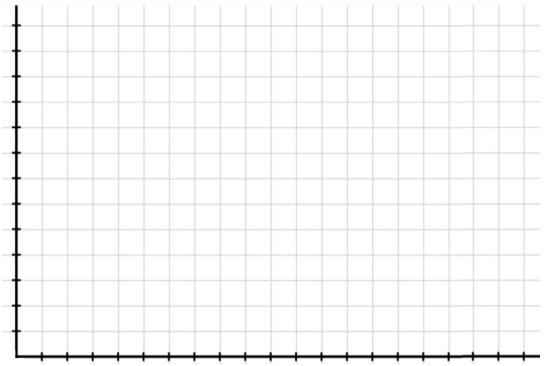


B: Explain below (↓) how it is that Gsc comes to be the activin target gene that is expressed.

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: ↓

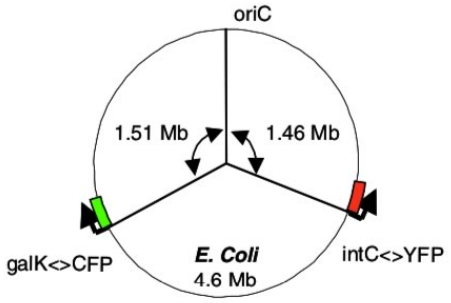
D: Now, assume that the system has changed so that the binding affinities of the Xbra protein for the Xom promoter, and the Xom protein for the Gsc promoter are 10-fold higher than the binding affinity of Gsc for the Xbra promoter, but that the steady state level for all three proteins is similar. Make 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 (↓).



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.

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 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 stochasric, differences. The <> symbol denotes replacement by homologous recombination.

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: ↓

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

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?

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.

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.

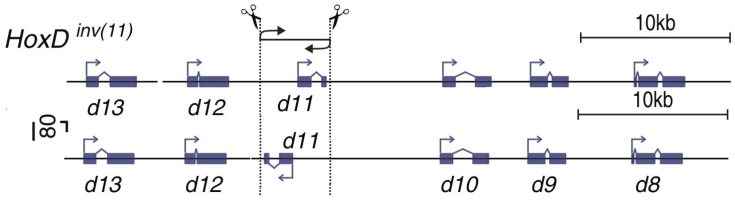
A: What factors will influence cellular differences in gene expression?

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 ↓

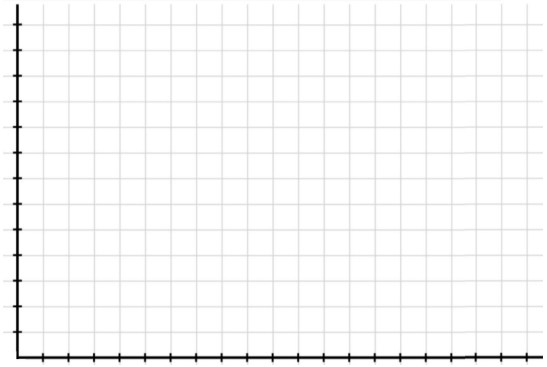
C. Consider the HOX gene D11. Using CRISPR CAS9 you engineer the reversal of the gene, so that it is transcribed in a direction opposite from all of the other genes in the cluster. You find that the level of expression of the two neighboring genes, D12 and D10 are reduced. Provide a plausible (testable)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



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.

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.

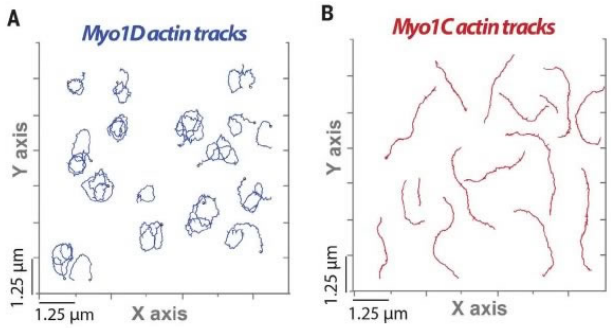
Q5 (20 points): A key feature of quorum sensing is the nature of the response to a changing (increasing) quorum signal.
A: Graph out (→) and describe (↓) the key features of a typical quorum sensing system reflected in the response of cells (Y-axis) as a function of the concentration of the quorum signal [S] (X-axis).



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?

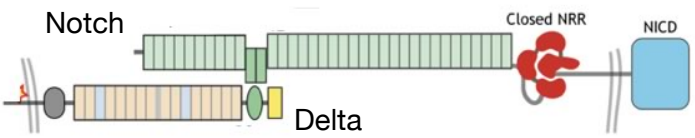
C. How is a threshold response in a signaling system like quorum sensing, how is it different?

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?



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.

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 cel