

<p>MCDB 4650/5651 Developmental Biology Fall 2021 Exam #1</p> <p>are you currently physically in Boulder or surrounding parts of Colorado (→)</p> <p>You have 120 minutes (2 hours) to complete the exam once you begin. Type in the time you started the exam (↓).</p> <p>time exam started</p> <p>An piece of advice: be sure to answer what the question is asking - this often than leads to a shorter answer.</p> <p>if you have no idea how to answer a question - you can write "no idea" in the text box and proceed to the next question. That will get you 1 point.</p>	<p>just info.</p>
<p>Question 1A (4 pts): You are studying a population of social unicellular microbes. How would you characterize, in terms of their behaviors, the difference between an organism that is "un-cooperative" from one that behaves as a "cheater"?</p> <p>Q1B (6 pts): While calling them "cheaters" might be too anthropomorphic, social organisms, both unicellular and multicellular, have to deal with such behaviors. How might the presence of cheaters influence a population (or multicellular organism) and how might a population (or multicellular organism) "defend" itself against cheaters.</p> <p>Q1C (4 pts): A true cheater phenotype always involves (↓ and justify your choice?)</p> <ul style="list-style-type: none"> <input type="radio"/> an adaptive response <input type="radio"/> a noise driven behavior <input type="radio"/> a mutation <input type="radio"/> no idea 	<p>1A: un-cooperative : ignores the presence of others. Cheater: benefit from behavior of other, failure to do their part - make signal or other products</p> <p>1B: Unable to produce a valuable or necessary behavior. Find some way to recognize a cheater, and suppress cooperation (avoid, ignore, attack)</p> <p>1C: A mutation, since we are assuming that it is a permanent, inheritable behavior.</p>
<p>Question 2A (4 pts): We have talked a lot about noise in gene expression, most specifically about the role of noise in the expression of the lac operon of <i>E. coli</i> and variations in gene expression among cells of the "same type" in metazoans, as revealed by single cell RNA sequencing.</p> <p>A feature of the lac operon is that it rarely and briefly "turns on" in the absence of extracellular lactose. Is this behavior advantageous and if so, why? (→)</p> <p>Q2B (4 pts): In the context of the social slime mold <i>Dicytostelium</i>, the choice between differentiating into a spore or into a stalk cell (non-spore) is stochastic. Explain the logic behind this mechanism and why would it be different for a multicellular organism. (↓)?</p> <p>answer for 2B</p>	<p>2A: It can expend energy (turn on the lac operon fully) only when it is beneficial (lactose is present), and can determine, with minimal expense when such behavior is appropriate.</p> <p>2B. In the slime mold, it insures that all cells (with distinct genomes) have a reason to cooperate. In the metazoan, cells are clonally related, maximizing "inclusive fitness"</p> <p>In a metazoan certain structures need to made in particular places at particular times for organismic survival (reproduction) - cellular decision making will be determined by signaling (based on asymmetries). Also, metazoans are clonal.</p>

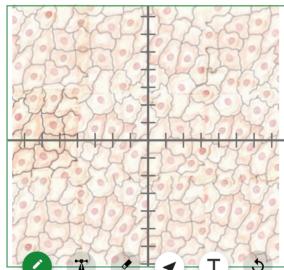
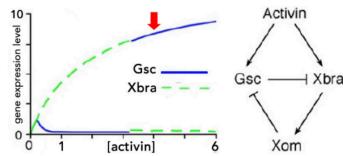
<p>Q2C (4 pts): Briggs et al (2018) analyzed tens of thousands of cells from a stage 22 <i>Xenopus</i> embryo and indicated the different "cell types" present (→), where does the variation come from (↓).</p> <p>Q: What does it mean, in molecular terms, that the cells of a certain type do not map to a single XY coordinate? where does the variation come from (↓)</p> <div style="border: 1px solid green; padding: 5px;">answer 2C</div>	<p>2C: There is noisy (stochastic) variation between cells, this variation is not enough to inhibit differentiation into a particular types cells, but leads to variations in behavior (gene expression)</p>
<p>Question 3A (2 pts): Draw examples of a linear and a sigmoidal dose response curve (→).</p> <p>Q3B (4 pts): Propose a molecular mechanism that would produce a sigmoidal response curve: what are its key features? (↓).</p> <div style="border: 1px solid green; padding: 5px;">answer for 3B</div> <div style="border: 1px solid green; padding: 5px;">3C (4 pts). Suggest how a mutation in one of the components of your model would transform a sigmoidal response into a linear response (↓).</div> <div style="border: 1px solid green; padding: 5px;">answer for 3C</div>	<p>3A: straight vs S curve</p> <p>3B: the initial response to the signal most over-come backreaction</p> <p>3C: A mutation abolishes back reaction</p>
<p>Question 3D (5 pts): Provide a plausible explanation (→) for why decisions controlled by quorum sensing generally rely on threshold response behavior.</p> <div style="border: 1px solid green; padding: 5px;">answer for 3D</div> <div style="border: 1px solid green; padding: 5px;">answer for 3D</div>	<p>3D: because the behavior controlled is counter-productive unless the conditions are right (sufficient number of organisms).</p> <p>No need to make a stalk if there is lots of food around, not possible to make a stalk unless there are enough cells.</p>
<p>Question 4A (5 pts): In the Saka and Smith model, the response to a signal (the relative levels of target gene expression, varies as a function of signal (activin) concentration. Consider a sheet of cells that respond to activin in similar ways (similar activin receptor levels, etc). There is a "special" group of cells, located at the origin (0,0), that secretes activin - the other cells in the sheet do not.</p> <p>Assume that the concentration of activin [activin] in the region of the activin-secreting cells is ~4 unit/ml (red arrow). On the graph (↓), indicate your estimate of activin concentration as a function of distance from the origin - label the axes and describe your assumptions (↓)</p> <div style="border: 1px solid green; padding: 5px;">answer for 4A</div>	<p>4A Bell curve around origin - max level at source, and signal diffuses away (into larger volume) so conc. decreases.</p>

Q4B (5 pts): Consider a sheet of cells that respond to activin in similar ways but do not secrete activin, together with a distinct group of activin-secreting cells located at the origin (0,0).

Assume that, at steady state, the concentration of activin [activin] in the region of the activin-secreting cells is ~4 unit/ml (red arrow).

On the graph indicate using the drawing and text tools (\rightarrow) which cells will express Gsc and/or Xbra as a function of distance from the origin and explain your assumptions (\downarrow). Note that the X and Y axes indicate distance from the origin.

answer for 4B



4B: Region fo Gsc expression surrounded by region of Xbra expression - directly derived from graph behavior, behavior changes as activin conc. decreases (move to the left).

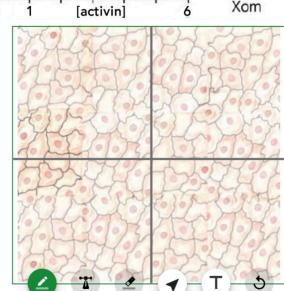
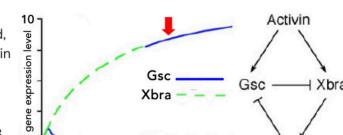
There could be region where both are expressed (at low levels), far from the source.

Q4C (5 pts): Now assume that these cells are ciliated, and that their ciliary beating is coordinated resulting in a flow of fluid across the sheet of cells from left to right.

Using the drawing module (\rightarrow), indicate whether the pattern of Gsc and Xbra expression in the cells will change, and if so, how? You can assume that the system has come to steady state.

Explain the assumptions you relied on below (\downarrow).

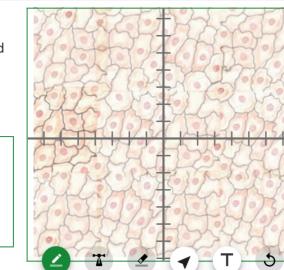
answer for 4C



Q4C: Shifted to the right (more oval) - due to flow (asymmetry in activin concentration)

Q4D (5 pts): Now assume that these cells have a mutation such that the coordinated movement of cilia is disrupted and there is no longer any net flow of fluid across the cell sheet. How does this influence the distribution of Gsc and Xbra expressing cells? (\downarrow) (no need to indicate the effect on the graph)

answer for 4D



Q4D - System reverts back to the original activin distribution and Gsc/Xba expression patterns.

Uncoordinated ciliary motion might (or might not) increase diffusion rate - diffusion will still occur.

Q5E (5 pts): If the handedness of an organism depends upon a cilia-driven signaling asymmetry, a mutation that abolished ciliary movement would (\rightarrow): explain your logic (\downarrow)

answer for 4E

- produce a symmetrical adult
- adults with reversed L-R asymmetry
- no effect, there are other factors involved
- both normal, reversed and mixed individuals

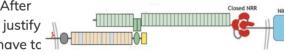
Q4E Most likely D (A also acceptable) - all types of organization possible (depends on argument proposed)

There remain asymmetry producing processes (remember Myo1D)

Question 5 (6 pts) Signaling by Notch involves the Delta-binding induced proteolytic cleavage of Notch, generating the notch intracellular domain (NICD) that regulates gene expression. After the initial signaling event, which is most likely to be true (\rightarrow): justify your answer (more than one could be justified, but you only have to do one) (\downarrow).

answer 5

- the NICD is reattached and the receptor is active
- new receptors have to synthesize
- the receptor could act as
- an inhibitor of Delta signaling
- no idea



Q5: B or C could be justified. Signal leads to inactivation of receptor - inactive receptor could act as inhibitor.

Mechanism for re-attachment is particularly unlikely, since NICD is in cell while Notch extracellular domain is outside.

Question 6 (5 pts) Using *Xenopus*, John Gurdon found that nuclei from early larval gut cells efficiently replaced the nucleus of the fertilized egg and supported normal development. In contrast, nuclei from later muscle cells did not. Embryos made using muscle cell nuclei were expressed muscle specific genes NOT normally expressed at the embryonic stage examined.

How would you explain this observation? (→)
Which is true (↓)?

- Muscle cells have different genes than early embryonic cells
- Various epigenetic factors have led to difficult to reverse expression of muscle-specific genes
- DNA encoding inhibitor enhancer sequences have been deleted during development
- no idea

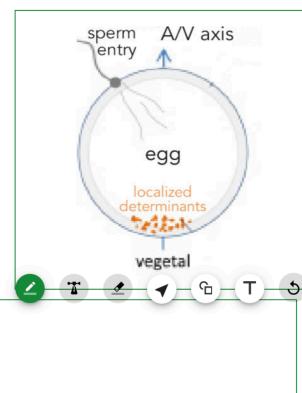
explain 6

Q6 B Indicates that it is difficult to reverse all gene expression (epigenetic) decisions.

No genome diminution in *Xenopus* (somatic nuclei can induce viable clones, albeit not as efficiently).

Q7 (6 points) In *Xenopus* the oocyte (and egg) are asymmetric along the animal vegetal axis. One example of this asymmetry is the distribution of cytoplasmic RNAs, some RNAs are concentrated in the animal hemisphere, different RNAs in the vegetal.

After fertilization the cortex, with extreme vegetal RNA attached, moves with respect to the egg's inner region. Indicate the distribution of extreme vegetal RNAs in the early embryo (following sperm induced rotation) (→) and explain how their movement could lead to asymmetrical behaviors later in development. (↓)



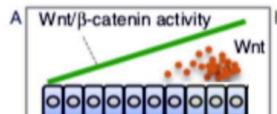
answer 7

Q7 The RNAs will move to the right (with the cortex) leading to asymmetry in signaling and responses to signaling.

Question Q8A (6 pts) Zebrafish has two experimental advantages over *Xenopus*, it is transparent and more readily amenable to genetic modification. To explore the behavior of the "eccentric cells" associated with dorsal axis formation (cells that display more or less nuclear β-catenin than their neighbors), you have developed a zebrafish line in which the sequence encoding β-catenin has been replaced by a sequence encoding a GFP-tagged version of β-catenin.

For this line to be useful, it should be the case that (→). Describe a simple (genetic) experiment you could determine whether the GFP-β-catenin transgene acts in a wild type manner (↓).

answer 8A



- the expression of the β-catenin gene is unaltered
- the stability of the GFP-tagged β-catenin is not regulated by Wnt signaling
- the GFP-tagged β-catenin no longer interacts with transcription factors
- the GFP-tagged β-catenin behaves like the untagged polypeptide
- no idea

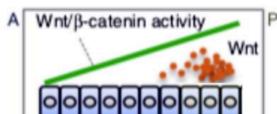
Q8A A and D

Question asks for genetic experiment - Take transgenic zebrafish, mate male and female, determine if homozygous b-cat-gfp embryos develop normally, and are viable and fertile.

Question 8B (4 pts) Let us exploit the advantages of zebrafish to study the behavior of "eccentric cells" associated with dorsal axis formation.

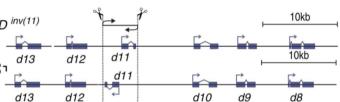
Using the "well-behaved" GFP-β-catenin zebrafish line, describe how you might determine whether eccentric cells become "less eccentric" over time (more like their neighbors), migrate to another region of the embryo, or undergo apoptosis and disappear (↓)

answer 8B



Q8B: Because embryo is transparent, and the b-catenin-GFP behaves as does beta-catenin, it is possible to watch the behavior of the eccentric cells within the embryo to determine whether they disappear (die) or their expression levels changes (they become normal), or even if they migrate out of the region.

Q9 (6 points) You flip the orientation of one of the genes in a HOX cluster and observe that neither developmental timing nor the expression domain of the gene are altered - what could you conclude about the regulatory elements that control the expression of the flipped gene?



Answer 9

q9 All regulatory elements are within the reversed region, so the switch has no effect.