Exam 1 Answer Key

Thursday, October 12, 2023 9:53 PM

Q1A (5 points): Molecules and macromolecules, like DNA, RNAs and proteins, interact through surface-surface interactions. The strength of these interactions is a function of the complementarity of molecular surfaces, electrical charges (often partial), hydrophobic and H-bonding interactions. Once formed, why do these interaction "come apart" (→)
Brownian motion -- molecules are in constant motion; inevitable that there will be some stochastic association/dissociation with receptors.
Stochastic changes in external environment -- changing temperature, pH, etc. causes protein folding to change.
Enzymatic activity -- enzymes directly target complexes and dissociate them

Q1B (5 points): Post-translational modifications and allosteric effectors can alter the binding affinities between a molecule and its "binding partners". In simple general terms, how does such a change in binding affinity come about (→)
Binding affinity: the strength of the interaction between receptor and ligand.
Modifying polypeptides changes their chemical structures -- this changes electrostatic interactions, steric hindrance, etc. precluding

Q2A (4 points): Various forms of cooperation between unicellular organisms are observed. For example, behaviors such as programmed cell death or digestive enzyme secretion, occur only when cells are present in high concentrations. Explain why such community-regulated behaviors are less effective when the organism is "alone." (→).
Less effective when the organism is alone because of quorum sensing. Quorum sensing relies on bacteria producing signaling molecules that are secreted into the environment. These molecules help bacteria assess population density in their general vicinity. A sufficient concentration of these signaling molecules in the environment induces specific sets of responses in the bacterial population (an example of the threshold effect, whereby an exponential response is induced once the concentration of a particular signaling molecules reaches a particular level.) In a solitary individual, there is a significant dearth of these molecules produced, making it much harder for the molecule to reach the concentration necessary to induce a response.

Q2B (6 points): Under certain conditions, individual cells in the slime mold *Dictyostelium* will aggregate. After migration towards the soil surface, some cells in the aggregate will differentiate to form non-reproductive stalks, while others will form reproductively capable spores.
Explain why both quorum and nutrient sensing are used to coordinate and control this behavior.?
Quorum sensing: involves the production and detection of signaling molecules based on population density that enable cells to differentially regulate gene expression and to communicate with each other, contributing to the success of the population. Using quorum sensing, bacteria can detect, when a secreted signaling molecule reaches a certain threshold, how "many" individuals there are in a population, and what the "state of the union" is. Once a critical mass is reached, some bacteria will differentiate to form non-reproductive stalks so as not to overcrowd the area and completely deplete it of resources.

Nutrient sensing: allows cells to interrogate and respond to the availability of resources in their environment. Cells can use this information to time reproductive events: if nutrients are scarce, a cell might differentiate to form a stalk; if nutrients are readily available, a cell might form a spore.

Q2C (5 points): In evolutionary terms, how might a Dictyostelium "cheat" and explain why such a strategy is like to be unproductive over the long term (→).
"Cheating" refers to a strategy where individual cells exploit the cooperative behavior of the population for their own benefit. In the short-term and under stable conditions, cheating might not have a significant effect on a bacterial population, but will benefit the individual. Ways in which the individual benefits include biofilm exploitation (provides protection and adherent surface), energy saving (cheating cells siphon energy that could have gone to the collective to their own reproduction and growth), survival during stress, and saving metabolic energy. However, in the long term cheating is detrimental to both the individual and the population. Cheating decreases the overall group fitness by depriving populations of productive/cooperative members, make the population less adaptable, and increases its vulnerability to environmental changes.

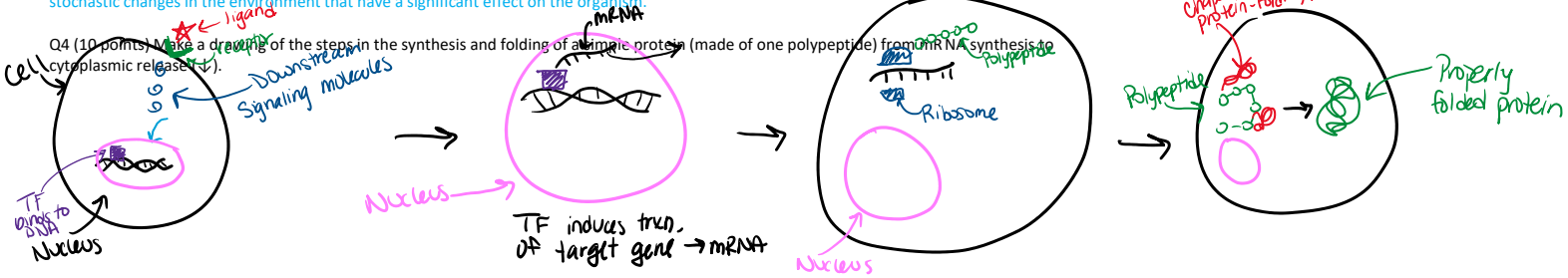
Q3A (4 points) Skeletal muscle nuclei, transplanted into enucleated one cell Xenopus embryos, continue to express muscle specific genes in the gastrula, well before muscle cell differentiation or gene expression normally occurs. What molecular processes are likely to be involved in this behavior? (→).
Epigenetic memory -- DNA in the muscle cell nucleus is already "primed" to support skeletal muscle, not embryos. Therefore, it likely retains many of the epigenetic modifications that make muscle-related genes more accessible to transcription factors.

Transcription factors -- transcription factors for muscle-specific genes could be present in higher amounts in a nucleus derived from this cell type. As such, when transplanted in the embryo, it is already "primed" for muscle cell gene expression.

Timing is everything -- skeletal muscle is much further on in the developmental pathway. Because the embryo is still at such a plastic state, it is easier to get these cells to express muscle-specific genes than it would be to get the muscle cells to express embryonic-specific genes.

Q3B (6 points) Consider the various epigenetic changes involved in cellular differentiation, for example from an early embryonic cell to a muscle cell or a neuron. Define what is meant by an "epigenetic changes" and suggest how might they be reversed. (→)
Epigenetic changes: modifications to DNA that may influence gene expression but which does not include changing its underlying structure. Examples of epigenetic changes include methylation and histone modification. These changes might be reversed using enzymes (demethylases or helicases) or stochastic changes in the environment that have a significant effect on the organism.

Q4 (10 points) Make a drawing of the steps in the synthesis and folding of a simple protein (made of one polypeptide) from mRNA synthesis to cytoplasmic release (↓).



Then i) describe what is it about the way polypeptides typically fold that facilitates the construction of "chimeric" proteins, e.g. those with functional distinct domains, and ii) (finally) how might a chimeric protein form "naturally"? (↓).

Q5A (5 points) In their experiment, Boraas et al cultured the unicelllular algae *Chorella* together with its unicellular predator *Ochromonas vallescia.* Over a period of time (40 days), a multicellulloar form of *Chorella* appeared. How might you determine whether this was an adaptive or a potentially evolutionary change, perhaps leading to the formation of a new species)(↓)
Adaptive: culture *Chorella* together with *Ochromonas* and observe the appearance of a multicellualar *Chorella.* Remove *Ochromonas.* If multicellular *Chorella* vanish, you can identify it as an adaptive response (when the predator is removed, the phenotype reverts back to "normal".)

Evolutionary: culture *Chorella* together with *Ochromonas* and observe the appearance of a multicellualar *Chorella.* Remove *Ochromonas.* If multicellular *Chorella* persist from generation to generation, you've observed a potentially evolutionary change (when the predator is removed, the "new" phenotype persists.)

Q5B (5 points) What types of evidence would you cite as evidence that sponges and other metazoans (like people) shared a common ancestor with the a single celled choanoflagellate? (↓)
Genetic similarities/conserved gene sequences (homeobox genes)
Similar cell morphology, cell types, and cell structures
Similar reproductive trajectory (cell fate determination)
Conserved signaling pathways (Wnt, Notch, etc.)

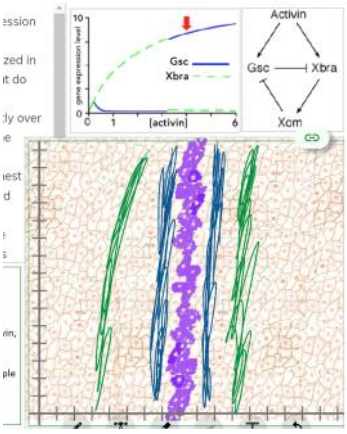
Q6A (4 points) The late stage *Xenopus* oocyte (and later the egg) are asymmetric, with a visible animal-vegetal axis. At the molecular level, specific RNAs and proteins are asymmetrically distributed along this A-V axis. Indicate the effects of sperm entry on cortical vegetal RNA and protein distribution (→)
Cortical rotation -- Fertilized egg rotates and repositions the cortical cytoplasm relative to the internal cytoplasm. This helps establish a new AV axis.

Asymmetric localization of signaling molecules -- Rotating the cytoplasm results in differential distributions of molecules that provide the cell with positional information (morphogens or RNA molecules, for instance.) Eventually, this induces differential gene expression/translation to help different regions of the embryo specialize in function. This also helps pattern the DV axis.

Q6B (6 points): Describe the major developmental role(s) of sperm entry and how the disassembly of cortical microtubules in the fertilized egg will impact subsequent development (↓).
Roles of sperm entry
1. Activates the egg, inducing changes in membrane potential, metabolic activity, and cell cycling
2. Cortical rotation
    a. Helps establish the AV axis and redistribute signaling molecules (proteins and RNA)
3. Combines genetic information, precipitating a genetically similar, yet distinct, organism
4. MT reorganization
5. Aligns axes (DV)

Disassembly of cortical microtubules
1. Disrupts axis formation because MT's are critical for maintaining proper cellular organization
2. Loss of cellular organization
3. Prevention of cortical rotation/distribution of cytoplasmic determinants
4. Abnormal cell division

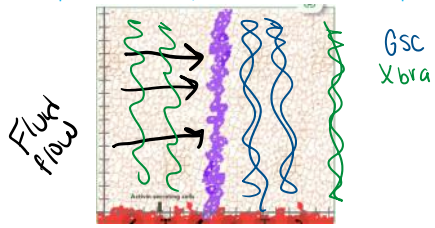
In the Saka & Smith model, the response to a signal (the expression of target gene) varies as a function of signal concentration. **Consider a sheet of cells**. Cells that secrete activin are organized in a vertical row (purple / dark) - neighboring cells respond to, but do not synthesize or secrete activin.
Q7A (3 points) **Assume** that the concentration of activin directly over the activin-secreting cells is ~4 unit/ml (red arrow →). Using the drawing and text tools indicate the cells that you predict will express Gsc and which will express Xbra. Indicate where highest level of stochastic variation in gene expression will be expected to occur.



Blue = Gsc
Green = Xbra

Q7B (5 points): Explain the logic behind your predictions (state your assumptions! Don't assume that we know exactly what's going on in your brain!)
From the graph, we can see that 4 units/mL is the threshold concentration required for Gsc expression. Therefore, in cells close to those secreting activin, we can expect to observe Gsc expression (in blue). However, as you move further away from the activin-secreting cells in the sheet, the activin signal becomes weaker. Therefore, we can expect cells further away from the purple ones to revert to Xbra expression (in green) because the activin signal is insufficiently strong to induce Gsc expression.
Highest level of stochastic variation will be right where Gsc expression "ends" and Xbra expression "begins" -- there may or may not be a sufficient concentration of activin to produce more Gsc to inhibit Xbra.

Q7C (2 pts): Indicate how your predictions would change if the activin-secreting cells had cilia that drove extracellular fluid flow from left to right. Describe your assumptions.
If there was fluid flow from left to right, you would expect to see more Gsc expression in cells to the right, and it would extend further out. There would be minimal Gsc expression to the left; most of those cells would express Xbra.

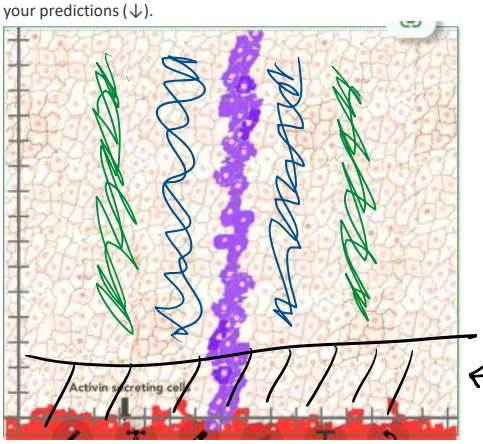


Assumption: fluid flow is not strong enough on the left to completely eliminate some small level of activin.

Q7D (5 pts): Now assume that the row of cells at the bottom of the sheet secretes a protein that binds to and inhibits secreted activin. Indicate how the presence of these of "anti-activin" secreting cells influences you previous predication of Gsc and XBra expression and d**escribe** the assumptions behind your predictions (↓).

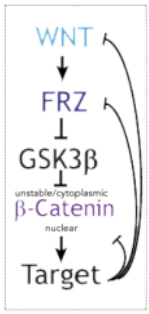
No activin at bottom = no Gsc or Xbra produced near the bottom. However, as you

Q7D (5 pts): Now assume that the row of cells at the bottom of the sheet secretes a protein that binds to and inhibits secreted activin. Indicate how the presence of these of "anti-activin" secreting cells influences you previous predication of Gsc and XBra expression and d**escribe** the assumptions behind your predictions (↓).



No activin at bottom = no Gsc or Xbra produced near the bottom. However, as you moved further up, you would see Gsc and Xbra expression as in question 7A. Assumptions: activin-inhibiting protein cannot pass the black line, so is ineffective above it. Below the black line, activin is **completely** inhibited.

Q8A (5 pts) What type of mutation, in the gene encoding β-catenin, would lead to the nuclear localization of β-catenin in all cells in which β-catenin is normally expressed. Explain the logic behind your suggestion? (↓).
Hint: Gsk3β is a protein kinase, which means it can phosphorylate other proteins.



Mutate the gene so that the β-catenin protein is no longer phosphorylated by GSK3B; this should render it stable in the cytoplasm (and able to enter the nucleus). Since β-catenin is widely expressed, we will see nuclear β-catenin in all cells in which the gene is expressed..

Q8B (5 pts) Akieda et al (2019) studied the fate of cells that expressed "inappropriate" levels of nuclear β-catenin. In their studies, they used a transgenic zebrafish line in which the sequence encoding β-catenin-GFP replaced the wild type β-catenin protein. These transgenic fish were heterozygous with a genotype of β-catenin wt/ β-catenin-GFP. What types of studies should they do to demonstrate that β-catenin-GFP did not disrupt normal developmental processes?

The simplest approach is to generate embryos homozygous for the GFP-tagged β-catenin allele; does this embryo show any abnormal phenotypic effects, if not, we can assume that GFP-tagged β-catenin behaves like β-catenin.

Q9 (5 pts) In zebrafish, a dorsal cap explants using cells that have not been in contact with the yolk region of the egg fail to form axial structures in vitro. In contrast, explants from earlier stage embryos using cells that *have* been in contact with the yolk region do form axial structures in vitro, even if the cells of the explants have been separated from one another and then reassociated. What does this observation suggest about the mechanisms involved in Zebrafish axis formation?
The yolk might produce signals critical in axis formation; therefore, cells in contact with the yolk may receive these signals and induce them to form the axis. Additionally, the yolk contains various maternal factors (such as RNAs and proteins,) which influence axial patterning.

Q10 (5 points): In single cell RNA sequencing studies in both zebrafish and Xenopus (shown here), different cells of the same "type" are found to express (transcribe) similar but not identical levels of various genes. What does this variation mean, in terms of cellular behavior, where does it come from, and how and why might it change over time in a particular cell?
Where does it come from: stochastic events during transcription and translation, cells at different stages of the cell cycle may exhibit different expression profiles, and DNA may be differentially epigenetically modified depending on the cell state.

In terms of cellular behavior:

- Functional diversity: cells expressing different genes will exhibit different cellular behaviors
- Adaptability: cells may be differentially able to adapt to their environments when faced with a significant change, leading to evolution or acclimatization
- This could also lead to disease. Mis-regulation of gene expression can result in cancer.

How it might change over time: as cells are faced with different environmental conditions, they may have to turn on different genes to compensate for these changes. Evolution -- results in different genes having preferential expression. Developmental stages might result in different genes being expressed at different times.