MidTerm 2 Exam 2020 final

MCDB 4650 Developmental Biology Spring 2020 Midterm exam #2

Question 1A (5 points) Provide a plausible explanation for why a Xenopus egg (~1.2 mm diameter) is so much larger than a human egg (~0.07mm diameter).

answer Q1A here

Q1B (5 points): You are asked to provide a plausible explanation for why gene number is not a good predictor of organismic complexity. First define what you mean by organismic complexity and then explain the relationship between gene number, genome size, and complexity, if any.

answer Q1B here

1A Xenopus develops as a self-contained rapidly developing system. The egg contains all require materials needed up through when the tadpole begins to feed. The mouse egg supports slower development until the time that the embryo implants into uterine wall, after which it receives nutrients for further growth from the mother.

1B We could define organismic complexity many ways, but one could be the number of cell types, tissues or body parts. Genes are defined as DNA regions that encode transcripts (RNAs). Genome size is the total length of the DNA molecules in the cells of an organism. It is an empirical observation that a fruit fly has fewer genes that a nematode, even though it appears more morphologically complex. But with over 15000 genes, many of which can produce multiple gene products (through alternative promoters and gene splicing, and complex combinatorial regulatory networks, there is no (apparently) serious limit to the number of cell types and structures that can be generated. Genome size may reflect aspects of evolutionary history rather than developmental or adult complexity.

Q1C (5 points): In studying embryonic development, it is helpful to have "developmental landmarks", processes that normally occur in a distinctive way so that deviations in specific processes can be identified. Choose **ANY TWO** of the following organisms and propose a developmental landmark & how it might be altered.

Xenopus (↓)	Drosophila (↓)	C.elegans (↓)	Mouse (↓)
answer here	answer here	answer here	answer here

Question 1D (5 points) Consider a late stage developmental process, such as the formation of the eye. Explain why it is unlikely (and in fact probably impossible) that a simple genetic screen could identify all of the genes involved in that process.

answer here

1C Xenopus: asymmetries associated with cortical rotation, onset of gastrulation, formation of neural tube.
Drosophila: in the larva, formation of segments with distinctive patterns of bristles. Anterior-posterior polarity
C. elegans: Distinct pattern of cell division, & cell lineages (specific cells divide / die).
Mouse: The formation of the blastocyst (trophectoderm and inner cell mass); formation of placenta (later could be patterns of HOX gene expression, but these are not (generally) directly visible.

1D Genes are used repeatedly, an in various combinations, as part of general and specific developmental processes. While some of the genes involved in eye formation (and other later stage developmental processes) may be specific for those processes, many will be involved in earlier events. If these genes are mutated (e.g. to null/amorphic) development will likely be derailed before later events can happen.

Question 2A (5 points) In the worm *Ascaris*, the process of genome diminution occurs in somatic cells, but not in the cells that give rise to the germ line. Describe what is going on during genome diminution and identify the molecular processes (including those active during mitosis) that must be involved?

what exactly is your molecular model for the process

Question 2B (5 points) Explain the effects, if, by some mutation, genome diminution occurred within the germ line of an Ascaris embryo. How would that influence embryonic and evolutionary processes

what exactly is your molecular model for the process

2A In genome diminution, parts of the genome in somatic cells are thrown away (degraded - discarded). This requires that there are DNA sequences (and proteins that bind them) that mark genomic regions that are lost and those that need to be maintained in somatic cells.

2B If important genes are lost from germ line cells, then either 1) those cells might be unable to form functional gametes (sterility) or 2) genes needed for embryonic and somatic development (i.e. to form the adult) will be lost, leading to embryonic lethality or sterility. The organism would not be viable (unless gene loss was restricted to non-coding regions of the genome).

Question 2C (5 points) You are asked to predict whether nuclear replacement experiments, such as those carried out by John Gurdon in Xenopus (in which the nuclei of fertilized eggs were replaced by nuclei from various differentiated cells, producing normal adults) would work in Ascaris. Explain your thinking.

what exactly is your molecular model for the process

Question 2D (5 points) Speculate on whether nuclei of embryonic inner cell mass cells would be more or less effective in supporting normal development of a host embryo compared to nuclei from adult brain cells? Justify your response.

- ○ more
- ○ less
- ○ no idea

what exactly is your molecular model for the process

2C Yes if germline nuclei were used, No if somatic nuclei were used (because of gene loss).

2D (more) because we might expect that more cells are in an open regulatory configuration, and so easier to reprogram. In highly differentiated cells, more genes are permanently "off" (e.g. genes associated with cell division and other developmental fates, and so genes needed for early development may be less readily reactivated (in terms of chromatin organization and DNA modification).

Question 3A (5 points) In Xenopus, embryonic axes are based on egg structure and sperm entry/cortical rotation. Make a diagram of the processes involved (→).

The future dorso-anterior axis is based on the stabilization of cytoplasmic β-catenin involving the Dsh ⊣ Gsk3 ⊣ β-catenin → gene expression pathway. Gsk3 activity depends on the Axin protein. Predict the effect on the embryo if you experimentally inhibited Axin activity. (↓)

explain here

Question 3B (5 points) In zebrafish, asymmetric activation of the maternal protein Hwa leads to the degradation of Axin; You isolate a recessive mutant in zebrafish that encodes an inactive Hwa protein. Predict the phenotypes of offspring from male and female animal homozygous for this mutation (→).

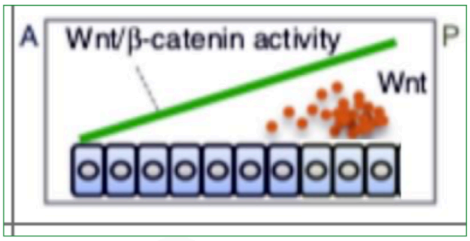
explain here

3A Diagram shows An/Vg asymmetry (pigment), sperm entry, cortical rotation - future dorsal side.

Active GSK3 leads to destabilization of b-CAT; if GSK3 activity is dependent on Axin, in the absence of Axin, GSK3 will be inactive and cytoplasmic (nuclear) b-Cat is uniformly stable, so the embryo is dorsalized (radial head) - ventral behavior is inhibited.

3B If Hwa is not present, then in the absence of other data, we might predict that Axin would be globally stable, and that GSK3 would be globally active, cytoplasmic bCAT would degraded, and the embryo would be ventralized.

Question 3C (5 points) In zebrafish, a Wnt signaling gradient leads to a threshold effect in the accumulation of nuclear beta-catenin. **A.** With a vertical line, Indicate (→) where the threshold effect is and **B** circle the cells that you expect will be eliminated via social interactions.
Explain (↓) how the elimination of "eccentric" cells in a gradient could impact subsequent development...



explain here

3C (line: near second/third cell from the right, cells most left would be eliminated)

Eccentric cells are expressing (or not expressing, and expressing other) genes associated with a specific response, appropriate to their position in the gradient. These cells could go on to produce the wrong structures, or interfere with normal developmental processes.

Their elimination means that the gradient produces a uniform response, at the right place and the right time.

Question 3D (5 points) Consider the process by which eccentrically responding cells are eliminated. How (generally) are such eccentric cells recognized by the experimenter and by the surrounding "normal" cells (↓).

How would you recognized eccentric cells in a Wnt gradient? (↓)

- ○ nuclear β-catenin levels
- ○ β-catenin RNA levels
- ○ no idea

explain here

Question 3E (5 points) Assume that eccentric cells occur within the nascent trophectoderm of the early mouse embryo, how might blocking the elimination process influence mouse development.

explain here

3D There must be surface features of these cells associated with their eccentric behavior (surface proteins expressed or not expressed) that lead to different cell-cell interactions compared to the cell-cell interactions that occur between cell that are responding in the appropriate manor.

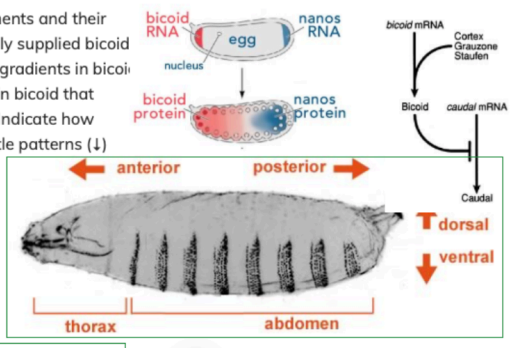
(nuclear b-catenin levels)

3E One plausible scenario would be that these aberrant trophectodermal cells might behave like inner cell mass cells, and begin to differentiate, forming embryonic-like structures, disrupting the normal formation of the placenta leading to aberrant (abortive) development ...

Question 4A (5 points) In *Drosophila*, larval segments and their distinctive bristle patterns, are based on maternally supplied bicoid and nanos RNAs. Regulatory interactions lead to gradients in bicoi and nanos proteins. You are studying a mutation in bicoid that reduces its repressive effect on caudal synthesis. Indicate how such a mutation would influence segemental bristle patterns (↓)
Will the mutation have a maternal or a zygotic phenotype (→)

- ○ maternal
- ○ zygotic
- ○ no idea

Explain your thinking and be clear on your assumptions (how are maternal effects different from zygotic effects (↓).



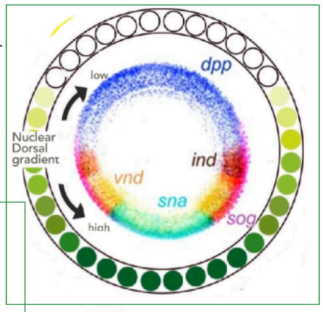
explain here

4A We would expect anterior segments to assume a more posterior phenotype.

We would expect it to have a maternal effect

Because bicoid is supplied to the eggs by the mother (and is active before zygotic transcription begins)

Question 4B (5 points) In *Drosophila*, there is a gradient of the transcription factor dorsal, with high concentration on the ventral side of the early embryo. The embryo displays multiple gene expression response thresholds to this gradient. Predict the relative affinities of dorsal protein binding sites in the genes dpp, sog, sna and vnd,
Which genes have high affinity and which have low affinity binding sites? and explain why a simple response to dorsal protein concentration would fail to explain the observed behavior,e.g. pattern of gene expression (↓).



explain here

Question 4C (5 points) Predict (←) and explain (→) how zygotic gene expression would be expected to change if dorsal was not expressed at all? Specifically, how would expression of dpp and sna change?

- ○ no change
- ○ no sna expression
- ○ no dpp expression
- ○ no idea

explain here

4B We would expect that genes with the lowest affinity dorsal binding sites would be the most ventral, so in terms of affinity (low to high) it would be sna, sog vnd, and dpp.

If the system was based only on dorsal-DNA site affinities, we would expect all genes to be expressed in the region of high dorsal concentration, while only dpp (for example) would be expressed in the region of low dorsal concentration.

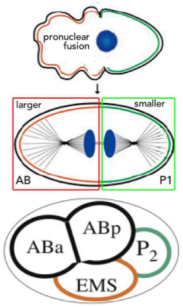
4C Assuming that their expression were totally dependent on dorsal, neither Sna or Dpp would be expressed, so the embryo would fail to form a dorsal-ventral axis.

(I think this is a confusing question, so any reasonable answer will be accepted).

Question 5A (5 points) You are studying development. Explain how it is possible to use the specific features of *C. elegans* development to identify genes involved in the regulation of programmed cell death.

explain here

Question 5B (5 points) You discover a way to move the position of the first cleavage furrow (cell division plane) of the *C. elegans* embryo. Predict (and explain the basis of your prediction), plausible effects on later development if the AB/P1 cleavage furrow is moved dramatically to the left, so that the AB cell is half the size of the P1 cell (↓).

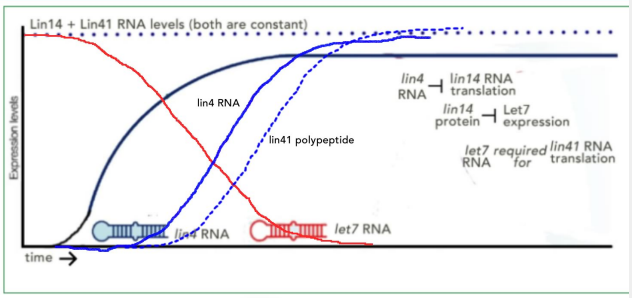


explain here

5A Because cell lineages are invariant in C. elegans, and because are a small number of cells, it is possible to identify genes that change lineage behaviors. Perhaps the easiest changes to recognize would be the survival of cells that would normally die (by apoptosis) or that should not die, but do.

5B Assuming that cell division and differentiation decisions are made on the basis of inherited cytoplasmic components, and cell-cell interactions, we might expect to see changes arising from how cells interact with one another and the cytoplasm that they inherit, in the patterns of cell division (cleavage sites and timing) and different developmental differentiation outcomes. For example, perhaps the P2 cell would be larger and the EMS, ABa and ABp cells smaller.

Question 6A (5 points) Studies have revealed that small RNAs can regulate the stability of target mRNAs. Consider the case in which *lin4* RNA inhibits the translation of *lin14* RNA, while the *lin14* protein inhibits *let7* expression. *Let7* RNA is required for the translation of *lin41* RNA. Indicate the level of *lin14* (solid line)(→) and *lin41* (dashed line) proteins as a function of time.



6B (5 points) By what mechanisms could *lin4* RNA influence the intracellular concentration of *lin14* protein (→)?

explain here

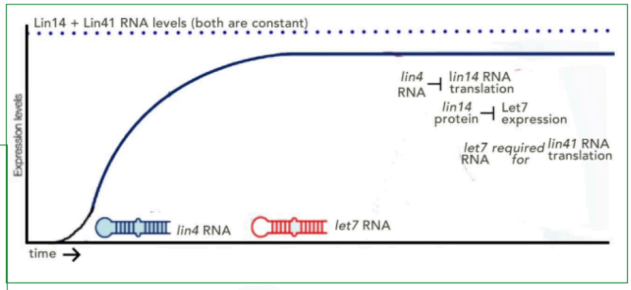
6A As lin4 RNA increases, lin14 protein decreases - As lin14 protein decreases, let7 expression increases, as let7 RNA increases, lin41 translation (and so polypeptide level) increases

6B Since lin14 RNA level appears constant (not influenced by lin4 RNA level), the most likely mechanism would be that lin4 RNA binds to lin14 RNA and blocks its translation, either by blocking initiation or elongation (but not effecting its stability or synthesis)

Question 6C/D (5 points)
Consider a mutation in the lin4 gene that abolishes the negative effect of lin4 on lin14. First, (→) explain your proposed mechanisms (what does the mutation do exactly) and then,
Q6D (5 point): in the graph (→), predict the behaviors of lin14 and lin41 proteins.
extra credit: Can you propose an evolutionary logic for why small RNAs would be used to regulate polypeptide synthesis?

explain here

explain here



6C A mutation in the Lin4 RNA (changing its nucleotide sequence) could abolish binding to a complementary sequence in the lin14 RNA, and so abolish lin4's effect on lin14, which requires a physical (RNA-RNA interaction). Alternately, the mutation could disrupt the folding of the lin4 RNA, influencing the binding of molecules normally associated with it, and required for its function).

6D lin14 polypeptide would stay high, leading to inhibition of let7 expression and so no translation of lin41 RNA, no lin41 polypeptide.

Plausible explanations ...