

Exam 2

MCDB 4650 Developmental Biology – Exam 2 Fall 2022

proposed answers

→ The exam opens at Noon on Saturday, 10 December and closes at midnight on Tuesday, 13 December.

The exam should not take more than 90 minutes to complete (hopefully less).

You have 300 minutes max to complete the exam once you start.

**DO NOT CLOSE THE EXAM UNTIL YOU HAVE COMPLETED IT,
you will *not* be able to reopen it.**

Q1 (5 pts) In a classical (forward) genetic screen, mutations are generated at random; those mutations that influence the trait of interest are selected for further study.

Such a screen can identify (→)

Assuming that the formation of the trait (e.g. such as the thumb) begins at a certain time of development, describe a strategy (based on modern molecular tools) that could identify all of the genes involved in forming that trait. Assume that you had previously identified all genes in the organism (↓).

answer here please

- all of the genes involved in producing the trait
- some of the genes involved in producing the trait
- the most important genes involved in producing the trait

✓ some of the genes

One approach would be to use RNA sequencing methods to identify all of the genes expressed in the tissues/structures during the period in which the trait forms.

One could then use a Cre-Lox or (easier) an inducible CRISPR-CAS9 approach to generate knock out mutations of each gene (one at a time) to determine their effects on the trait.

(optional The basic problem remains, however, that the formation of a trait is always dependent on pre-existing structures and processes, which complicates any analysis).

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Q2 (5 pts) A valuable aspect of *C. elegans* as a model system is that, in wild type embryos, its development is "invariant". This facilitates the identification of mutations that alter specific traits, cells, structures, and behaviors.

In *C. elegans*, egg laying is controlled by the hermaphrodite-specific neurons (HSNs). A dominant gain of function mutation in the Egl1 gene leads to the death of HSN neurons and an egg laying defect. In animals homozygous for a recessive loss of function (null) mutation in Egl1, the cell death that occurs in a wild type embryo does not occur.

The tra-1 gene is expressed in HSNs (\rightarrow). You have identified a mutant allele, tra1hns, in which tra-1 is not expressed in HSNs.

Predict and explain the phenotypes of a homozygous tra1hns animal that

- 1) carries the dominant egl-1 gain of function allele &
- 2) that is homozygous for the egl-1 loss of function allele (\downarrow).

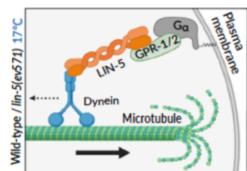


answer here please

Part 1: no effect, HSN neurons die (due to gain of function Egl1 allele), producing an egg-laying defect

Part 2: cell death is blocked in HSN neurons, egg laying normal (wild type).

Q3 (5 pts): In *C. elegans* embryonic asymmetry and cell lineages are determined by the site of sperm entry, which influences the size of the two cells produced at the first embryonic cell division, i.e. the larger AB (anterior) and the smaller P1 (posterior) cell (→).

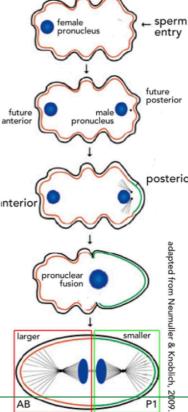


It is possible to alter this asymmetry experimentally.

Consider the case of the protein encoded by the Lin-5 gene. (←) Lin-5 links spindle microtubules to the posterior side of the fertilized egg and recruits dynein. The lin-5(ev571) allele produces a protein that behaves normally at the permissive temperature (17°C), but denatures at the restrictive temperature (27°C).

Predict (and explain) the effect on embryonic asymmetry if, soon after sperm entry, the fertilized egg is transferred from the permissive to the restrictive temperature. ↓

answer here



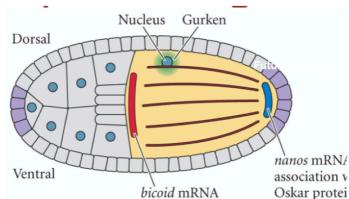
The Lin-5 complex interacts with microtubules and the posterior membrane complex, essentially pulling the mitotic spindle (and chromosomes) toward the posterior end of the fertilized egg. As the position of the spindle influences the site of the contractile ring, the result is an asymmetric cell division (AB larger than P).

When the Lin-5 protein is inactivated (in response to increased temperature), the lin-5 dependent "pull" will disappear, so the mitotic spindle and contractile ring will remain at the center of the fertilized egg, leading to AB and P blastomeres that are more equal in size, disrupting the lineage specification pathway.

Q4 (5pts) In *Drosophila*, gurken plays multiple roles. During oogenesis, bicoid RNA is localized to the future anterior pole of the oocyte in wild type females. In gurken^{-/-} females bicoid is found localized to both the anterior and posterior ends of oocytes.

Propose a plausible model by which the absence of gurken could produce such an effect, what cellular systems might be involved in bicoid RNA localization (↓)

Answer 4

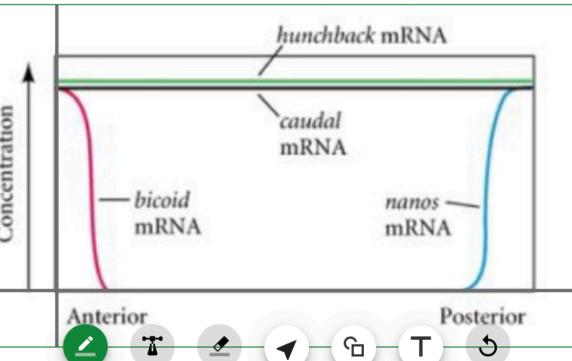


One model could be that the anteriorly localized gurken gene product (or even the gurken mRNA), directly or indirectly, acts to stabilize bicoid RNA.

Alternatively, it could influence the organization of the microtubule system (e.g. so that MTs are oriented in both directions), so that bicoid RNA is transported to the posterior pole.

Q5 (10 pts) In *Drosophila*, bicoid and nanos mRNAs are localized to the anterior and posterior poles, respectively, of the egg. On fertilization, translation begins. The bicoid protein inhibits the translation of the caudal RNA, while the nanos protein inhibits the translation of hunchback RNA.

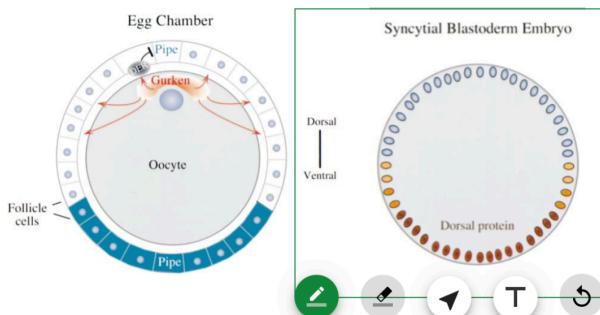
In the graph → indicate how the distribution of bicoid, nanos, caudal and hunchback proteins changes over time after fertilization (and the onset of translation). Explain your reasoning ↓



you answer here

We should see (on the graph) appearance and movement of bicoid and nanos proteins from the anterior and posterior positions, respectively.

And then the appearance of caudal and hunchback protein gradients, reflecting the negative effects of bicoid and nanos on the translation of caudal and hunchback RNAs.



answer here ; explain your reasoning

Q6 (5 pts) Secreted gurken protein binds to EGF receptors expressed by the surrounding somatic follicle cells; this represses expression of the pipe gene.

Later in development, by which point embryonic nuclei have moved to the embryo surface, secreted pipe protein activates follicle cell Toll receptors, activating expression of the dorsal gene, which encodes a transcription factor.

(→) How (and why) would the expression of genes regulated by dorsal be altered in a *gurken*^{-/-} embryo and how would it differ from a *gurken*^{-/+} embryo?

You can include a drawing if you want (optional).

Pipe would be expressed in all follicle cells (instead of the ventral cells), leading to activation of the Toll receptors and the nuclear localization of the Dorsal protein.

In a heterozygote, assuming that the gurken mutation is recessive, we would expect that one copy of the wild type gurken gene would be sufficient to produce enough gurken gene product to inhibit the local (dorsal) expression of the pipe gene – producing a wild type embryo.

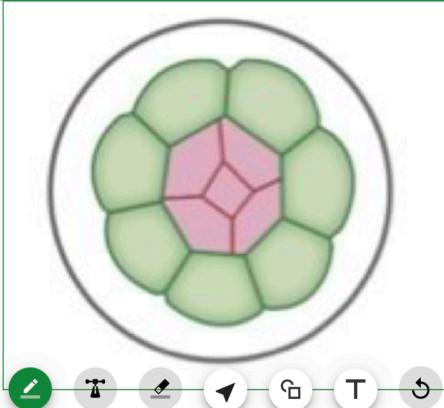
If it were not, the gurken mutation would be haploinsufficient (i.e. dominant).

Q7A (5 pts) In general terms, the patterns of early development reflects adaptations to the environment of the early embryo. Summarize briefly which factors favor having embryonic asymmetries "built into" the egg versus those that favor the emergence of asymmetries during embryonic development (↓)?

7a

Q7B (5 pts). During mammalian development, the process of "compaction" is critical to the "decision" of cells to differentiate into either the inner cell mass or the trophectoderm. What general cellular processes and structures (as opposed to specific molecules) are involved and how do they work (↓)? You can used a drawing (→) if you want (not required).

7b



A: When development needs to be rapid, as when eggs are fertilized and/or develop externally without parental protection (i.e. vulnerable), building oocytes/eggs with pre-existing asymmetries leads to more rapid development. Emergent asymmetries (changes in gene expression, cell movements, etc), take time, time available when embryo is protected (internal).

B: Basic processes, associated with compaction (formation of adhesive junctions/permeability barriers between cells), lead to cells and cell surfaces that experience different environments. These environmental differences (and associated signals from neighbors) leads to changes in protein activity and gene expression - differentiation.

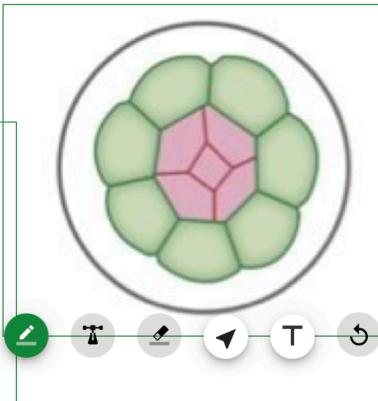
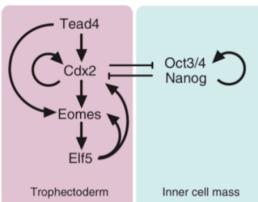
As an example, polarization of cell layers can lead to the directed transport of nutrients and signaling molecules (apical to basal, etc), followed by osmotic movement of water.

Q8 (5 pts) There is a gene network involved in the trophectoderm-inner cell mass distinction. Cdx2 drives trophectoderm development, while Oct3/4 expression, in particular, is required for cells to be totipotent or pluripotent.

Originally all of the cells in the early (pre-compaction) mouse embryo express both Tead4 and Oct3/4. Tead4 acts as an transcription activator in the absence of Hippo signaling, and a repressor when Hippo signaling is active.

If Hippo signaling is regulated in part by intracellular O₂ levels, how might that explain the pattern of Hippo signaling in the post-compaction

answer here



Q9 (5 pts) In the early mouse embryo, inner cell mass cells with mitochondrial defects disappear.

What type of experiment would reveal whether such cells died because of the direct effects of defective mitochondria (a form of necrosis) or because they were induced to die (undergo apoptosis) by their "more normal" neighbors? (→)

answer here

If we assume that Hippo signaling is off in the surface layer of the embryo, and on in the internal cells, then Tead4 would be off in these internal cells, leading to the absence of Cdx2 expression and the loss of Cdx2 inhibition of Oct3/4 and Nanog.

Oct3/4 and Nanog expression would inhibit Cdx2 in inner cells.

One approach could be to compare the behavior of a fertilized eggs with wild type versus mutant mitochondria, so that all cells in the early embryo / inner cell mass are the same.

If cell death is the direct result of the mitochondrial defect, all inner cell mass (mutant) cells should die.

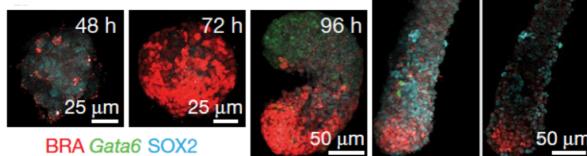
If it involves interactions between cells, the mutant cells should survive, since all neighbors are the same (all mutant).

Q10A (5 pts) It is possible to culture mouse embryonic stem cells such that they aggregate and begin to "self-organize".

Early on (48h) a sub-set of cells within such an aggregate begin to express the "posterior" marker gene Bra. Propose a model by which these Bra+ cells appear and then aggregate at one end of the gastruloid (by 120h)(↓).

answer here

- 3D-dimensional renderings and confocal sections of gastruloids at different times showing the elongation and expression of BRA, SOX2 and Gata6H2B-Venus (green).



Q10B (5 pts) How might you decide, experimentally, whether Bra+ cells migrated to the posterior pole of the embryo or whether only posteriorly located Bra+ cells survived (avoided apoptosis)? (↓)

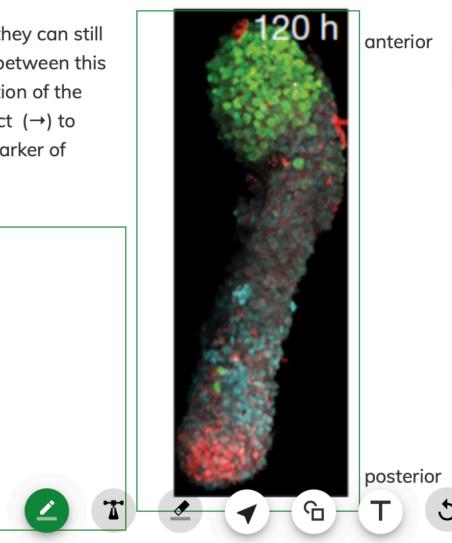
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A: We may assume either that Bra+ cells appear in particular regions of the inner cell mass (ICM), for example at the periphery, or that they appear stochastically - that once Bra+ expression reaches a threshold level in a cell, the cell is "committed", and acts through various processes to migrate (aggregate) toward each other, establishing the posterior pole of the gastruloid.

B: You might suppress apoptosis and directly observe whether all Bra+ cells aggregate. Alternatively, you could construct a reporter like (e.g. Bra-GFP or some other gene co-expressed with Bra) and, if the behavior of the transgene is normal, directly watch the movement of Bra+ cells within the gastruloid.

Q10C (5 pts) Although gastruloids develop differently from embryos, they can still form an axially polarized structure. Assuming a simple relationship between this axial organization and the antero-posterior/rostral-caudal organization of the embryo, predict the pattern of HOX gene expression you might expect (→) to observe. Explain your assumptions (↓). Remember Bra (red) is a marker of posterior and GATA (green) marks anterior in this system.

answer here).



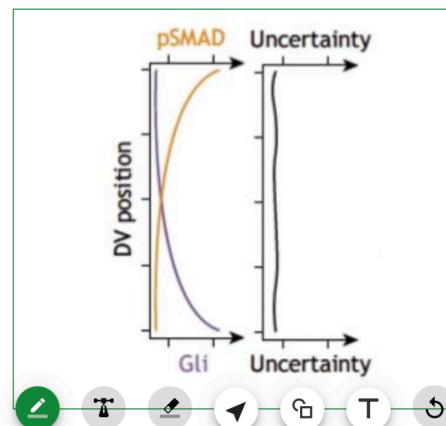
We would expect to see expression of anterior Hox genes first, at the anterior end of the gastruloid. Over time we might expect to see more posterior Hox genes expressed toward the posterior (Bra+) end of the gastruloid.

Q11 (5 pts) Within the vertebrate neural tube, different cell types occur at different dorsal-ventral positions.

This patterning is based on a ventral Shh signaling gradient (associated with active Gli) and a dorsal BMP signaling gradient (associated with pSMAD). Based on these two gradients cells can estimate their position along the neural tube's D/V axis.

How would uncertainty in D/V localization change if the BMP gradient were reduced or absent (indicate on the graph →) and explain, mechanistically, why changes in the BMP gradient will influence cell fate determination. Explain your reasoning (↓)

answer here



We would expect to see an increase in uncertainty toward the Dorsal region of the neural tube, because of the relatively flat level of Shh signaling /Gli activation in that region.

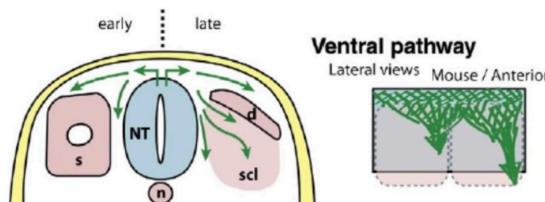
The combination of BMP and Shh signals will turn on various (not overlapping) sets of genes, and the interactions between the gene products of these expressed or inhibited genes can be expected to result in different "types" of neural cells.

Q12 (5 pts) Migrating neural crest cells can follow a number of different routes (green arrows) through the embryo. In the ventral view, a pair of somites are indicated,

In general terms, what factors influence the routes that the neural crest cells take and the types of cells that they differentiate into (↓).

How might Hox genes influence neural crest migration and their differentiation ?

answer here



Migration would be influenced by both the size of cell-free pathways, through which the cell can move, and the presence of various "chemotactic" signals that encourage or discourage cell movement, through these pathways.

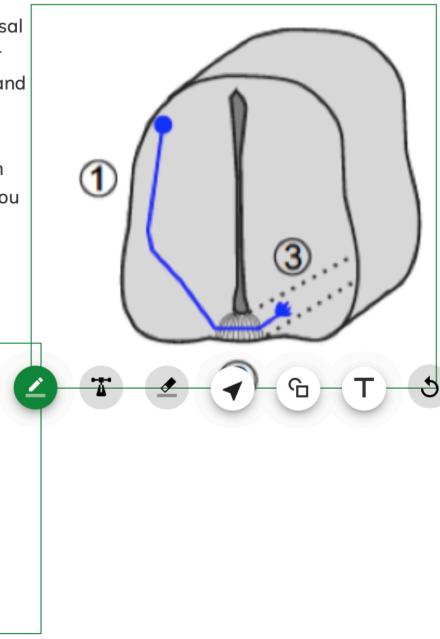
Both would be influenced by the presence of spaces between somites, and signals, associated with anterior-posterior HOX-dependent / influenced signals. Neural crest cell (survival) differentiation will be influenced by their original A-P position (HOX gene expression), their migratory pathway, interactions between migrating and surrounding cells, and interactions with cells and signals at their final destination.

Q13 (5 pts) In the neural tube, axons from neurons located in the dorsal region grow toward the ventral floor plate. Once they reach the floor plate, they cross over (from one side of the neural tube to the other) and then migrate rostrally to connect into the brain.

Indicate (and explain) based on processes of attraction and repulsion how the behavior of the growth cone changes during this process. You can use a drawing (\rightarrow) if you want (not required).

Finally, since the neural tube is an elongated structure, propose a mechanism by which neurons in a specific region of the spinal cord "know" which regions of the brain they should connect to (\downarrow)

answer here



Neuronal growth cones (growing tips) will interact with various signaling and adhesive molecules expressed on or made by the cells that they encounter. These signals can either encourage continued growth (moving "forward" or discourage growth (retreat and redirection).

As the growth cone moves, its response to signals can change - an attractive signal can become repulsive. These changes are influenced by other signals impinging on the growth cone.

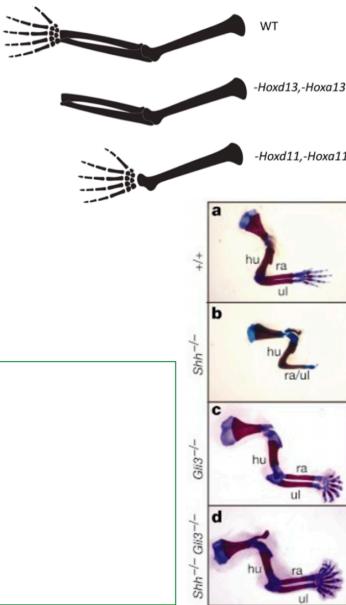
The behavior of neurons within the spinal cord will be influenced by their position along the A-P axis, which is influenced by differences in HOX gene expression.

Q14 (5 points) Hox genes are involved in the proximal-distal patterning of vertebrate limb. A (homozygous) deletion of *Hoxd13* leads to loss of the more distal autopod (hand/foot), while deletion of *Hoxd11* leads to the absence of the zeugopod (radius/ulna) (→).

Shh signaling is also involved in limb development. A (homozygous) deletion of Shh leads to abnormal zeugopod and autopod development, while deletion of Gli3 leads to excess digits (autopod defects) (→).

Based on these observations, what can you conclude about the similarities and differences in the molecular interactions between *Hoxd13* and *Hoxd11* and Shh and Gli3 in the limb? (↓)

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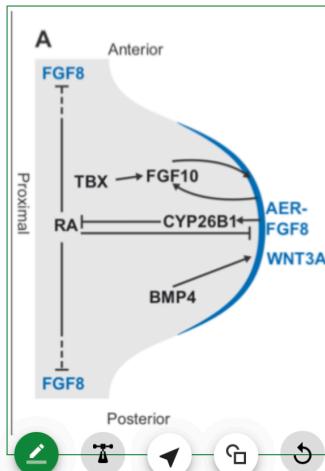
Q15 (5 points) The outgrowth and differentiation of the limb bud is controlled by an interaction network involving the expression of genes involved in RA synthesis and FGF8 and Wnt3A signaling factors expressed in the apical epidermal ridge (AER) (→).

Surgical Removal of the AER inhibits outgrowth of the limb bud.

Predict (↓) what would happen to RA synthesis after removal of the AER.

answer here

Indicate, in the graph, in the where the ZPA (the site of Shh expression) normally occurs and what would happen if the normal anterior-posterior pattern of HOX gene expression (in the trunk) was reversed.



We would expect that expression of the most distally expressed hox gene (*hox11*) is independent of the more proximal hox gene (*hox13*).

In the case of the Shh pathway, the Shh pathway involves the Gli3 gene product. In the absence of Shh, Gli3 acts as a repressor, which must be inactivated (i.e. eliminated or turned into an activator). The fact that eliminating Gli3 produces a relatively normal limb (with extra digits), indicates that Gli3 activation (via Shh signaling) is primarily involved in patterning the hand (autopod).

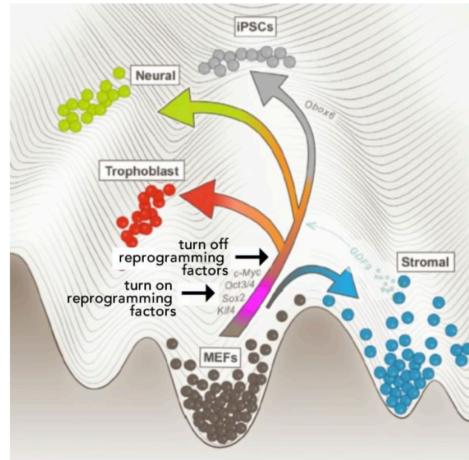
In the simplest model, the repressive action of CYP26B would remain (not inhibited by signals from the AER) so RA signaling would be inhibited.

In the graph, there is a wild type Shh expression domain in the posterior ZPA. We might predict that reversing the normal pattern of HOX expression would (perhaps) lead to a similar region of Shh expression on the previously "anterior" side, and the absence of the "normal" posterior Shh expression domain.

Q16 (5 points) Using a DOX-inducible cassette that expresses the Yamanaka factors cMyc, Oct4, Sox2, and Klf4, mouse embryonic fibroblasts (MEFs) can be reprogrammed to produce iPSCs.

Provide an explanation for why, when the expression of the reprogramming factors are turned off, the cells 1) do not return to behaving like MEFs? and 2) why not all become iPSCs (\downarrow)

answer here



Once reprogramming has started (with the activation of the Yamanaka factors), the cells move into a progression of gene expression/protein activity states. Those states go through their own progressions of regulatory changes, leading to various cell types. The changes associated with these cell types are not readily reversible, since that would require altered signaling and changes in gene expression.

The response of cells to the Yamanaka factors depends on the original state of the cell, and there appears to be variability between cells in those states, often associated with low variable concentrations of specific proteins, and previous DNA, histone, and protein modifications, leading to differential and stochastic choices between responses to the reprogramming factors.