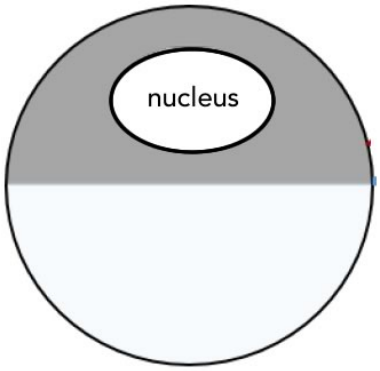
MCDB 4650 MidTerm #2 exam **name:**_____

Read through the entire test first, then start working to answer – it (helps calm one down).
Use the other side of the page if you need more room. If you need to supply details or specific assumptions that make your answer correct, please do. Each question is worth 10 points (and will be normalized to a total of 100 points).



Question 1: Consider the animal-vegetal axis of a *Xenopus* oocyte.
A: In the diagram label the animal (An) and vegetal (Vg) poles of the oocyte and explain (briefly) how the two regions of the oocyte are likely to differ from one another and how such differences could influence the behaviors and fates of embryonic blastomeres.

Top pole An bottom Vg

As reflected by the differentiated pigment in the animal hemisphere, and the asymmetric position of the germinal vesicle (the oocyte nucleus) the oocyte as a whole is asymmetric (animal-vegetal). That means that it is likely that there are different proteins and mRNAs (and other cellular components) localized to different regions of the oocyte.

Because of this original asymmetry, the blastomeres formed after fertilization and cell division (cleavage) will inherit different molecular components, they will be different from one another.

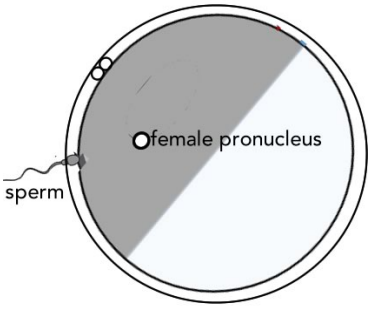
B: In their screen, Wieschaus & Nusslein-Volhard looked to identify genes that influenced the patterning of the early *Drosophila* embryo. Given the details of their screen, would they have identified maternal effect or dominant mutations (YES or NO)? Explain why.

NO, basically because of the way they set up their screen. Their screen identified recessive, zygotically acting mutations. For them to identify maternal effects, they would have had to generate homozygous females - which they did not do (and could not arise in their experiments), since they would not have had an early zygotic phenotype.

[detail: They mutated male flies and crossed them with wild type females to obtain individuals heterozygous for mutations. They then mated heterozygous males and females. By using balancer chromosome, only two F2 populations were possible, heterozygotes (carrying the mutation) and homozygotes (displaying the lethal zygotic phenotype).

C: Explain whether they would or would not expect to find homologs of the *Drosophila* genes they identified expressed in the *Xenopus* oocyte?

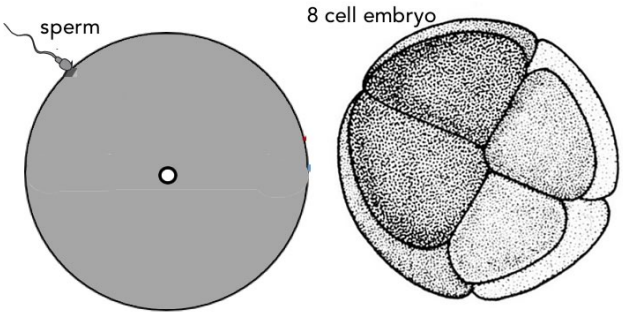
While early development is quite different in Xenopus and Drosophila, these organisms shared a common metazoan (multicellular) ancestor, so we might expect that of some of the genes identified as important in the patterning of the Drosophila embryo will also be used in Xenopus, albeit in different ways. [one such example would be armadillo / beta-catenin]



Question 2: In *Xenopus*, fertilization leads to a number of changes in the structure of the egg.
A: Explain how all fertilized eggs come to have their animal hemispheres pointing upward, no matter what their orientation was before fertilization. Given that eggs are laid and fertilized in water, name a benefit of this post-fertilization reorientation.

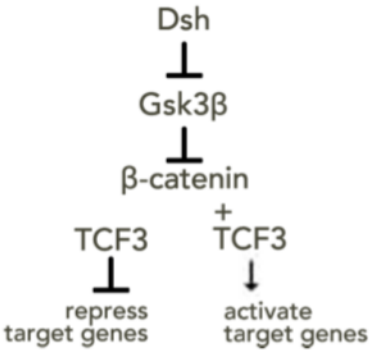
Upon fertilization the vitelline envelop lifts off the egg surface, the egg can then rotate (since the vegetal pole is heavier).

One benefit is that the pigmented animal hemisphere points toward the light (of the sun) minimizing the damaging effects of light (the embryo does not get a sunburn).



B: Sperm entry initiates a second process by which the radial symmetry of the egg is broken. We can see the effect if we look down on an 8-cell embryo. At the molecular level, the process initiated by sperm entry leads to asymmetric protein activity and gene expression.
←**1)**. Label the blastomeres in which Dsh activity is high and low. High on the lightly pigmented side.

There is a maternal mutation in the gene encoding GSK3β, such that GSK3β can no longer be inhibited by Dsh.



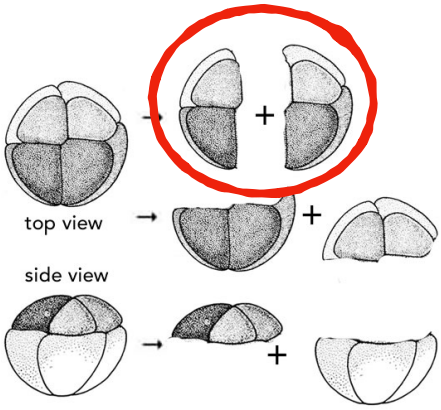
2) Does this influence the asymmetry of Dsh? why or why not.

It doe not, since 1) Dsh is up-stream of GSK3 and 2) the asymmetry of Dsh activity is due to cortical rotation (arising from sperm entry and microtubule driven movement), a process that is not dependent upon Gsk3 activity (or its inhibition).

3) How does this GSK3β mutation influence zygotic gene expression and the formation of the dorsal ventral axis?

GSK3 will be active everywhere, so beta-catenin will be unstable, TCF3 will act as a repressor, so dorsal-ventral asymmetry will not be established. Genes that should be turned on on the dorsal side (by the beta-catenin-TCF3 complex) will not be turned on. The embryo will be ventralized.

Question 3: Eight cell *Xenopus* embryos can be mechanically divided into half embryos. There are three different ways to do this (→).
1) Circle the half embryos that you would expect to give rise to normal looking (twin) embryos and explain your reasoning.

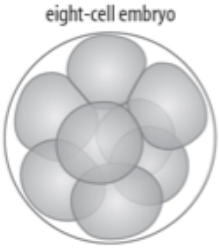


Basically because each half embryo has an intact dorsal-ventral axis (as well as the asymmetries of the oocyte), and could be expected to produce a normally (but smaller) embryo.

2) For the other types of split embryos, predict their phenotypes and explain your logic.

The middle set would be either dorsal or ventral, and would likely to produce head only and belly/tail only "half-embryo".

The bottom would be missing the animal-vegetal asymmetries (proteins / RNA) of the oocyte, and probably would not have full dorsal-vegetal asymmetries.



Question 4: Now consider a (pre-compaction) 8-cell mouse embryo. Assume that you can divide it into two four cell embryos that had the ability to complete normal development.

1) Would it matter (YES or NO) (circle) how you divided the embryo and 2) explain why your predicted result for the mouse is the same or different from the situation in *Xenopus*.

It would likely not (NO) matter how you divided the embryo, since at this stage all of the blastomeres would be equivalent. The asymmetries found in the Xenopus embryo at the 8-cell stage (maternal animal-vegetal and zygotic dorsal-ventral) are not present.

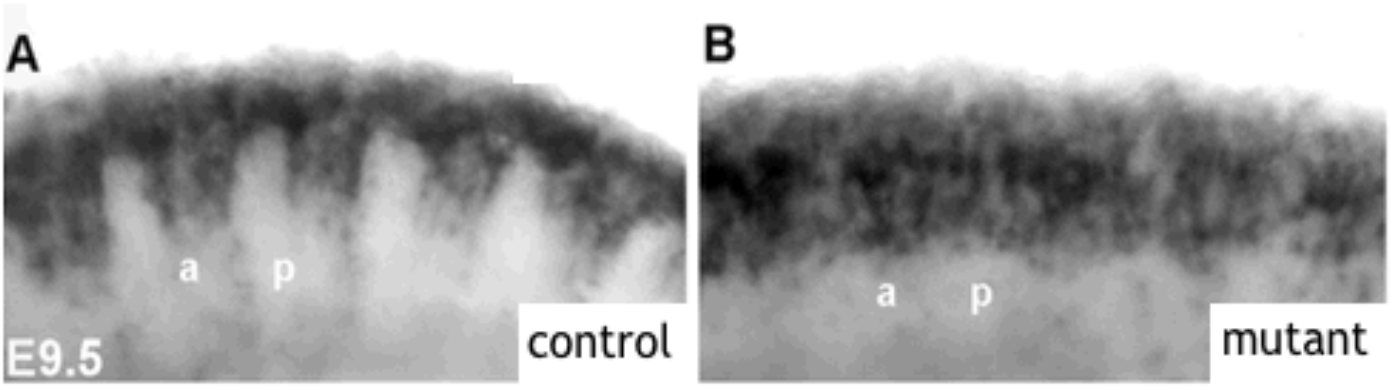
2) Assume for the moment you could do a similar experiment in *C. elegans* or *Drosophila*, would the result be similar to the result in the mouse or *Xenopus*? explain.

More similar to Xenopus, since these organisms rely on axes established maternally and by a controlled sperm entry point.

Question 5: You identify a new gene, describe how you would determine 1) whether it is a member of the homeobox transcription factor family, 2) whether it is likely to be functional (able to bind DNA) and 3) whether it is likely to be a HOX gene.

1) Does the gene have the sequence that encodes a homeobox (DNA binding) domain in the protein?
2) Are the conserved amino acids within the within the homeobox domain present? If so, it is likely to bind to DNA in a sequence specific manner.
3) Is the gene located with a HOX cluster region of a chromosome?

Question 6: In the mouse, the trunk neural crest normally migrates out preferentially over the anterior aspect of the somites (**panel A**). You discover a mouse mutant in which the neural crest cells move uniformly over both anterior and posterior aspects of the somites (**panel B**). ("a"-anterior somite, "p"-posterior somite)



A+B) Develop a hypothesis as to what type of gene product or process might be defective in the mutant and why. Predict where you think the normal gene acts, in the neural crest or somite (or both) and explain your reasoning.

The migratory components are intact, since the crest cells migrate, but how they sense and respond to signal in the environment they are migrating through are defective.

Some possibilities:
Repulsive (to migrating neural crest cells) signals located on the posterior aspect of the somites may be missing,
Attractive (to migrating neural crest cells) signals normally restricted to the anterior aspect of the somite may be misexpressed on the posterior aspect as well.

Or the receptors (on neural crest cells) or down stream components necessary to respond to the repressive (somite) signals could be missing (in the neural crest cells)

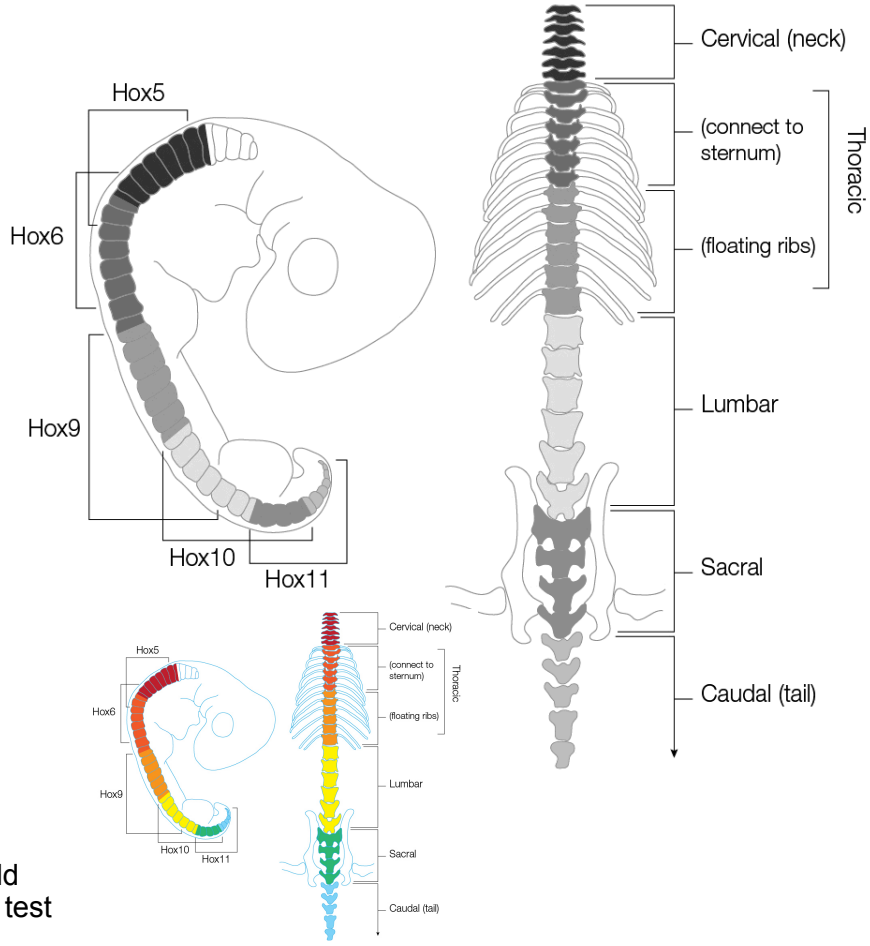
C) How might examining the behavior of the neural crest in other regions of the embryo (e.g. the cranial region) inform (alter) your model?

One could, for example, move truck crest to the cranial region - if it migrated normally, one might conclude (tentatively) that the defect involved genes expressed in the somites.

[optional: one might transplant crest from mutant embryos into wile type embryo or visa versa]

Question 7: You find a mutation that alters the expression pattern of the HOX gene Hox6. Hox6 is normally expressed in the thoracic region of the spinal cord; in the mutant, Hox6 is expressed in both the thoracic region and lumbar regions. **A:** What type of phenotype might you expect to observe and why?

One might expect to see (floating) ribs appearing in the lumbar region, because ectopic Hox6 expression could cause a homeotic transformation of lumbar to thorasic spinal cord



B: You discover a null mutation in the Hox5 gene, which is normally expressed in cranial neural crest and the cervical region of the spinal cord, In the absence of Hox5 expression, Hox6 is expressed throughout the anterior (cervical) region of the embryo - a region in which it (Hox6) is not normally expressed.

Draw up a plausible gene network that would explain this observation and suggest a way to test it.

A simple model would be that Hox5 normally represses expression of Hox6.
But because Hox5 and Hox6 are normally co-expressed in a small over-lapping region, other factors might also be involved, or the effect could be due to a gradient in Hox5 expression or activity.