

Tuesday, 5 Oct '21

13. REVIEW



- On the web site - notes from Zach's review session + Zoom recording (on the zoom page)
- Exam 1 next Thursday (on beSocratic)
 - **Opens at 10AM on Thursday** and remains open until Noon on Friday
 - You have **120 minutes, once you start**, to take the exam
 - If you have an accommodation - send me email and I will set up your exam

DEVO@CU MCDB 4650

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Tuesday, 28 September 2021

Topics requested from students to discuss on Tuesday:

- Points of the dose-response curve: Pre-threshold, threshold, saturation
- Lac operon – molecular level noise
- Establishment of the A-P axis during early embryonic development
 - Wnt/BMP antagonistic signaling and effects on axis development
 - Quorum sensing
 - 3D graph interpretation
 - Two headed embryo experiment – axis regulation/determination

- **5.1 What factors may have driven the evolution of multicellular organisms (animals)?** I'm not sure if I understand the predator/prey example in class. Is this because cells were in the presence of predators, or is it more having to do with mutations that arose?

- The one we talked about (experimentally) is escaping predators, basically by becoming larger through simple colonial multicellularity. Once there is simple multicellularity, other factors can lead to the ability to better integrate cooperation between cells (such as the differentiation of cells in different ways to carry out different function). That can lead to the germ line-soma split, and can enable the organism to exploit new ecological niches.

- **6.2 How might left-right asymmetries arise at the cellular level ?** I understand general cellular level asymmetries but got lost at the L-R axis. Is this an induced symmetry?

- This arises from the handedness of various cellular structures. Think about the organization of microtubules in many cells, radiating out from the centrosome (MT-organizing center). If they have a bias to bend to the left (or the right), the organization or behavior of the cell can have a handedness.

- **8.3 How do evolutionary adaptations influence development processes, such as the speed and mechanisms behind early embryonic development?** I'm just unsure about this one.

- Consider the adaptation to make lots of offspring externally. That leads to a vulnerable stage, that can be minimized by rapid development - and such rapid development can be facilitated by building what is needed into the oocyte/egg.

- **9.1 How is a homeobox-containing gene different from a Hox gene?** Is a homeobox-containing gene a transcription factor for a Hox gene?
 - Close - the homeobox is a DNA binding motif of a protein; there are a number of homeobox-containing transcription factors. HOX genes are homeobox-containing genes organized into a cluster, a cluster in which the order of the genes corresponds to the position of their expression along the anterior posterior axis of the embryo / developing organism.
 - **10.2 How does sperm entry interact with egg asymmetries to establish A-P axis?** Is this having to do with the sperm entry point only being on the animal side of the egg? And does the A-P axis form along the entry point, or is it an indirect effect (like when there is rotation)?
 - To specify the position of any cell in an embryo, we need two axes. The oocyte/egg (in *Xenopus*) the A-V axis is established during oogenesis with the pigmented animal hemisphere and the yolk vegetal hemisphere. The second axis is supplied by the sperm entry point and the "opposite side of the egg" axis. Sperm entry then leads to a series of event that results in cortical rotation (away from the sperm entry point), which establishes an asymmetry leading to the formation of the Nieukoop center and Spemann organizer).
 - **11.1 How did early embryonic asymmetries aid in identifying (natural) inductive molecules in *Xenopus*?** Can you clarify what inductive molecules are?
 - Molecules that can induce the embryonic anterior-posterior / dorsal-ventral axis. Because the embryo can be ventralized, but still response to dorsalizing signals, injecting RNAs encoding those proteins can be use to identify specific gene products that act as induces (are inductive).

What features of *Xenopus* development would lead you to predict that the axes of the embryo are predetermined (at least in part) by the structure of the oocyte?
 (pick all that apply) (+)

Explain your reasoning (1).

Focus is what think is the "best" predictor.



- large egg
- rapid development
- no zygotic transcription early in development
- external development
- asymmetric pigmentation and yolk
- no idea

Why would rapid cell cycles during early development suppress zygotic gene expression (1).

If you think there is an effect, describe a plausible mechanism

In the course of their nuclear replacement studies Gurdon and colleagues replaced the nucleus of the fertilized egg with a muscle cell nucleus. They found that embryonic cells that normally did not express muscle-specific genes expressed such genes.

The diagram shows a 'Muscle cell' with a nucleus being transferred into an empty egg cell, which is labeled 'Nuclear transfer'. The resulting embryo is a 'Blastula', consisting of a central cavity and a layer of cells. A bracket below the blastula is labeled 'Transcription' in red, with the text 'No transcription' written in red next to it. To the right of the blastula, arrows point to three layers of the embryo: 'Neuroectoderm', 'Mesoderm', and 'Endoderm'. Above these layers, a bracket is labeled 'High expression muscle gene' with two values: '56%' and '52%'. Below the diagram, a box contains the following text:

Provide a plausible mechanism that could lead to that behavior; how might it be relevant to the efficiency of "nuclear reprogramming"? (1)

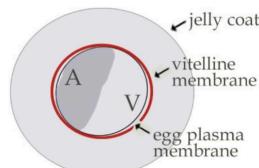
What types of processes might be involved?

described in: [The Egg and the Nucleus by John Gurdon](#)

Inside an adult female *Xenopus*, oocytes are oriented in all types of directions, yet RNAs and proteins are asymmetrically distributed along the animal-vegetal axis.

What does that tell you about the mechanisms involved in establishing the A-V axis?

What types of processes might be involved?



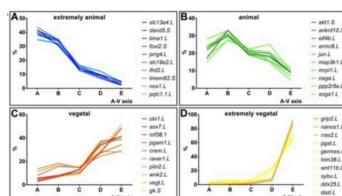
Moving from the animal to the vegetal pole of the mature oocyte, one discovers that different cytoplasmic RNAs are distributed asymmetrically within the oocyte.

Answer below (↓)

A. What type(s) of cellular mechanisms might produce such an outcome, what will be needed?

B. How might this asymmetry influence the distribution of proteins in the pre-embryo?

What types of processes might be involved?

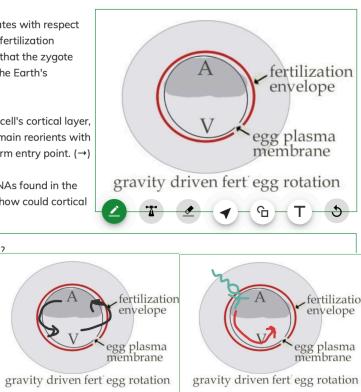


After fertilization, the pigmented cortex rotates with respect to the yolkly inner region of the egg and the fertilization envelope moves away from egg surface so that the zygote reorients so that the A-V axis is parallel to the Earth's gravitational axis.

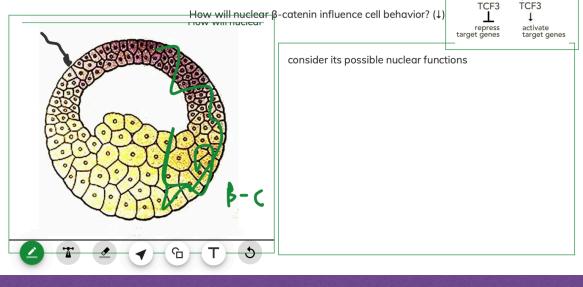
If the egg's pigmentation is located in the cell's cortical layer, indicate how the pigmented cortical domain reorients with respect to the sperm entry point. (\rightarrow)

If some of the asymmetrically distributed RNAs found in the oocyte are also localized to the egg cortex, how could cortical rotation influence embryonic cell fate?

What types of processes might be involved?



We introduced the effects of various manipulations on dorsal-ventral axis formation in Xenopus. The cortical rotation that occurs after fertilization leads to an asymmetry in Dsh activity. Active Dsh inhibits GSK3 β which inhibits the accumulation of cytoplasmic β -catenin. Indicate the position of the original sperm entry point, and the region of the embryo where nuclear β -catenin will be found be found in late blastula stage embryo (1).



Simplified Wnt (β -catenin) signaling

Dsh

Gsk3 β

β -catenin

TCF3

TCF3

TCF3

TCF3

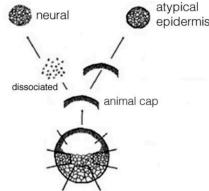
How will nuclear β -catenin influence cell behavior? (4)

consider its possible nuclear functions

Drawing conclusions. Here is an experimental observation. It is possible to dissect out the cells that form the roof of the blastocoel (the fluid cavity of the blastula state embryo) to produce what is known as an "animal cap".

On its own, without other inductive signals, the animal cap will form a ball of atypical epidermis. BUT if the cells of the animal cap are dissociated to form isolated cells, which are then cultured (again without external signals), those cells will form neural tissue. In the embryo, the neural tube is derived from the ectoderm (which also forms the epidermis / skin).

Suggest a plausible mechanism for how neural development might be regulated during early development (4).



What types of processes might be involved?



In zebrafish, the product of the maternally expressed Hwa gene is necessary for the inhibition of Axin in the developing embryo (\rightarrow). Predict (1) (and provide your logic) the effects of these mutations on the level of β -catenin regulated gene expression...

1. a maternal null mutation in Hwa.
2. a mutation in TCF that weakens its interaction with β -catenin.
3. a mutation in β -catenin that inhibits its ability to enter the nucleus.

three explanations

Dorsal specification in the zebrafish involves the stabilization of β -catenin due to the inhibition of the GSK3 β -Axin-APC-CK1 complex.

Predict (\rightarrow) and explain (1) what you might expect to happen if the site on β -catenin that is phosphorylated by GSK3 β were mutated to a non-phosphorylatable residue?

universal increase in nuclear β -catenin
 no nuclear β -catenin anywhere
 impossible to predict
 no idea

high level of nuclear β -catenin

universal increase in nucle...
no nuclear β -catenin anywhere
impossible to predict

In zebrafish, when explanted 256 cell stage enveloping layer (EVL) cells have been in contact with the embryo's yolk region, the explant develops with an asymmetry, even if the explant is dissociated and then the cells are reassociated.

What does that imply about the processes that establish the embryo's dorsal-

A 256 Cell Stage EVL Media 1hpc 2hpc 3hpc 7hpc

LTS + 3% FBS + PenStrep
B 7hpc C 2hpc D 3hpc E 3hpc F 7hpc

TonicGra

Predict what might happen if the explant were treated with Li+ (1)

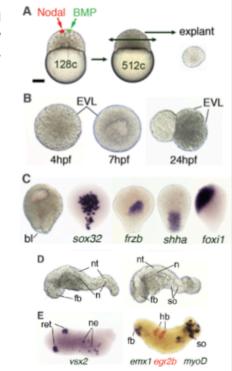
What types of processes might be involved?
What might happen and why?

When regions of the enveloping layer (EVL) are explanted earlier (so that they are NOT in contact with the (vegetal) yolk region) they fail to develop an axis.

However, if such EVL explants are derived from embryos in which one cell was injected with RNA encoding Nodal and another cell with BMP RNA are cultured, complex "picoids" developed with a clear anterior-posterior axes. What does that imply about the role of localization of beta-catenin in axis

How might you test your model?

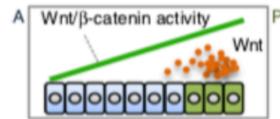
Construction of a Zebrafish Embryo from Two Opposing Morphogen Gradients



In zebrafish, there is a Wnt gradient associated with the dorsal axis. As we discussed in class, "eccentric" cells in this region of the embryo that display either too little or too much nuclear β -catenin, compared to their neighbors, are induced to undergo apoptosis.

Yet, it is possible to inject RNA encoding a stable form of β -catenin into the opposite side of the embryo; this leads to the development of a second anterior-axis (a second head).

Provide a model (\rightarrow) that explains why the cells receiving the β -catenin RNA do not die (or do they?).



what do you think happens to β -catenin and why