

beSocratic - Activity 9 (Class 11) Model organisms

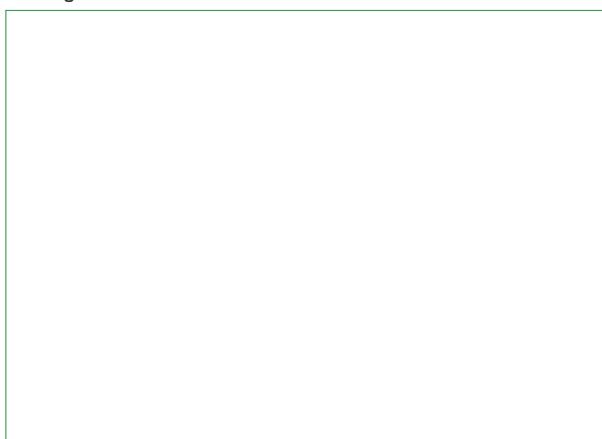
Thinking about model organisms: After reading "Mouse models of human disease", what factors impact comparisons between heart and skeletal muscle function when comparing mice and humans (→)

Based on data in that paper, makes graphs (↓) that compare the heart rate of a mouse (left) and a human (right). Contractions along Y-axis, time along the X-axis

Start with the most obvious ...



How does the inbreeding of animals and the conditions of their housing influence the outcomes of experiments using lab mice?



Organismic size (size associated stresses), contraction rates

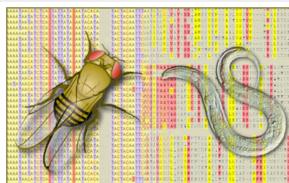
Graphs indicate difference between a heart that contracts ~600 times a minute and one that contracts at ~60 times a minute.

Consider the molecular events (rates at which enzymes, channels are activated, inactivated and move).

A fruit fly has 4 chromosome pairs and about ~15,500 known genes, a *C. elegans* nematode has six chromosome pairs and ~20,500 genes, while *Homo sapiens* and sea urchins have ~24000 genes.

Is there a causal link between chromosome and gene number and the complexity of the adult animal? Explain your reasoning . (↓)

- yes
- no
- maybe
- no idea
- how to answer



We would not expect any simple correlation - the systems are already complex enough to produce an array of cellular behaviors - gene number and such presumably reflect evolution history and various selective pressures

Consider a stretch of genomic DNA, what changes would you (or mutations) need to introduce to generate a gene (draw out ↓ and describe your thinking ↓).



You find yourself thinking back on myosin 1D. If you find a clear homolog of myosin 1D in all metazoans you examine.

You generate animals homozygous for null (amorphic) mutation in myosin 1D, what would you be justified in predicting (→) and explain why (↓)

- early embryonic lethality
- muscle defects
- left-right defects
- organism specific defects
- no idea

Changes needed (by mutation or human engineering):

1. Generation of transcription factor binding sites
2. Generation of translation start site (adequate 5' UTR)
3. Generation of start codon (usually AUG) as well as open reading frame, perhaps as short as 10 to 15 amino acids (30 to 45 nucleotide run).

Plausible response:

Assuming that there are other, similar type I myosins around (like myo1C) that will take over other cellular functions (moving things in cells and such), we might well expect to see only left-right defects.

Consider this explanation for how the elephant got its trunk, would you expect this to be a genetic or an epigenetic effect?

explain your response and indicate what the elephant child's children will look like (↓)



- genetic
- epigenetic
- a combination
- no idea

Plausible response:

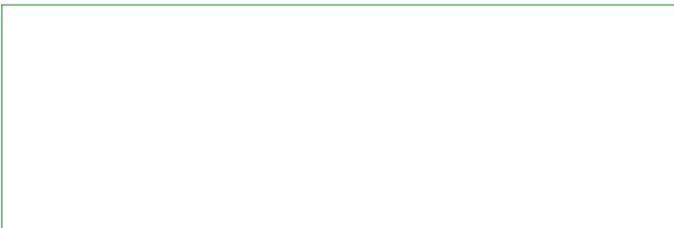
The stretching of the elephant child's nose would not be expected to have genetic effects, so we might expect the elephant child's children would have noses like other elephants – however it is possible that they might have a greater extend of curiosity.

Activity 10 ↓ Xenopus

Take a look at: [The Egg and the Nucleus by John Gurdon](#)

In his experiments, Gurdon and colleagues asked whether a nucleus from a differentiated cell (tadpole intestine) could, when introduced into an enucleated egg, support the development of fertile adult.

How did they enucleate the egg (→) and how did that work (↓)

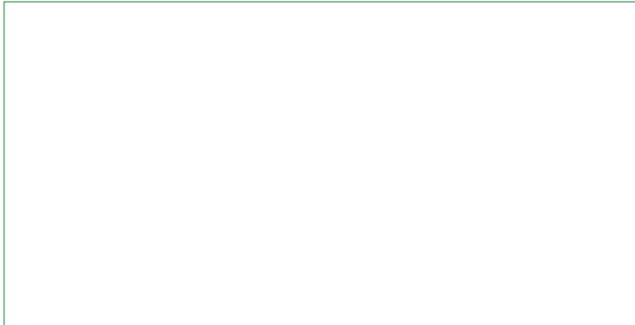


- LiCl
- UV light
- manual removal
- no idea

In his studies, he used UV irradiation of the animal hemisphere, where the maternal pronucleus was located. This caused massive damage to the genomic DNA, essentially removing (functionally) from the cell.

They found that cells from later stages and the adult failed to generate sexually mature adult.

What factors might explain (↓) the difference in the results obtained with tadpole epithelia nuclei and adult nuclei (→).

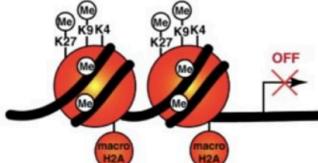
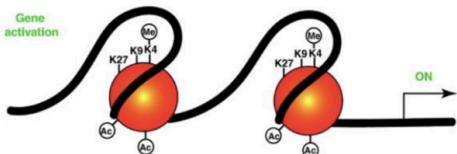


- mutations
- less easily reversible epigenetic changes
- loss of genes during development
- no idea

Plausible response:

We can assume that the nuclei of those cells were more modified by epigenetic factors (chromatin folding, DNA modifications, the presence of various transcription and associated factors so that reversing their effects, back to the totipotent state was less likely to occur.

Through your experiments you discover that transcriptionally repressed chromatin looks like this (→), while transcriptionally active chromatin looks like this (↓)



What processes are involved in the transition between active and inactive chromatin states?
↑→↓

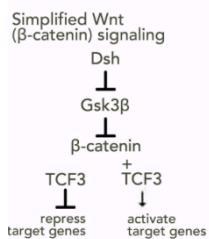


Plausible response:

We can presume that modifications of DNA and histone proteins, together with the presence of various DNA binding proteins, including transcription factors and other structural proteins could act to favor the compact (transcriptionally silent) form of chromatin.

In the same light, other modification and the activation/inactivation of other transcription factors and structural proteins will favor regional unfolding, accessibility, and gene expression.

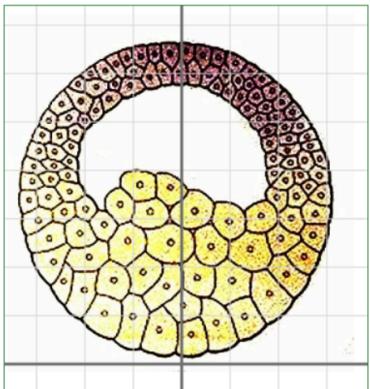
How would inhibiting Dsh activity influence TCF3-targeted gene expression activation



Inhibiting Dsh would (presumably, assuming that Dsh is the only factor involved) lead to the activation of Gsk3 β and the phosphorylation and proteolytic degradation of β -catenin.

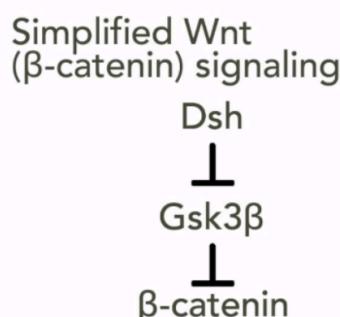
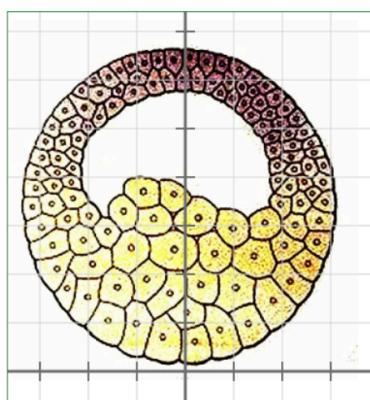
In the absence of cytoplasmic (nucleus) β -catenin, negatively acting transcription factors (like TCF3) will inhibit their target genes.

We introduced (in class) 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 inhibit GSK3 β which inhibits cytoplasmic β -catenin accumulation. How does this influence nuclear β -catenin levels (Y-axis) as a function of position in the embryo (↓) indicate dorsal and explain your reasoning (↓)



We would see a peak of nuclear β -catenin toward the right hand side of the embryo.

Now Indicate the level of transcription of the β -catenin gene as a function of embryo position (graph ↓), and explain how that relates to the nuclear β -catenin and dorsal gene expression. (↓)

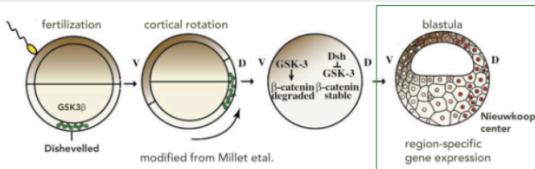


In the simplest case, we assume that expression of the gene encoding β -catenin is uniform in the embryo. Cytoplasmic/nuclear β -catenin only accumulates in regions of the embryo in which GSK3 β activity is inhibited.

Activity 11 - Axis-Zebrafish-Gradient

In the fertilized Xenopus egg, the future dorsal side of the embryo is specified by a sperm initiated/microtubule-dependent cortical rotation.

Normally, the cellular involution associated with gastrulation begins on the future dorsal side of the embryo and finishes later on the ventral side. Predict and explain how the timing of involution changes in embryos treated with Li⁺ versus those in which cortical rotation has been blocked (↓). You may want to watch the Harland video (again).



You block rotation by vegetal UV treatment; predict (↑) the distribution of nuclear β-catenin in the late blastula stage embryo and explain the logic behind your (↓) reasoning.



If we inhibit GSK3 with Li⁺, we would expect the embryo to be dorsalized - so that gastrulation would begin early all the way around the embryo (and not just on the dorsal side, that is the side opposite from the sperm entry induce cortical rotation).

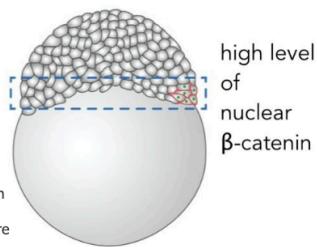
Vegetal UV treatment would block rotation, leading to the ventralization of the embryo - that is, late gastrulation all the way around the embryo. You would likely not see nuclear b-catenin.

Thinking about dorsal specification in the zebrafish, the dorsal stabilization of β-catenin is based on the GSK3β-Axin-APC-CK1 complex. Inhibition of this complex leads to increase in nuclear β-catenin.

Predict what will happen if there is a dominant negative acting mutation of Axin and explain your thinking

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

Predict (and explain) (→) what you might expect to happen if the site on β-catenin that is phosphorylated by GSK3β were mutated to a non-phosphorylateable residue?

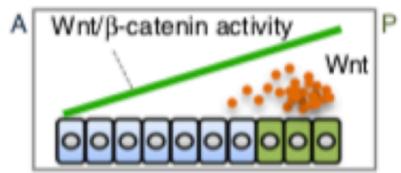


We could make two distinct predictions depending on what we assume the mutation does. The negative Axin mutation might lead to the inability of the GSK3/Axin complex to be inhibited, so GSK3 activity would lead to b-catenin phosphorylation (and degradation), so the embryo would be ventralized. Alternatively, the mutation could act to inactivate the GSK3 complex, leading to b-catenin stabilization and the dorsalization of the embryo.

Assuming that the **only** effect of the mutation is render b-catenin unphosphorylatable, b-catenin would be stabilized, leading to dorsalization (activation of target transcription factors)

In Zebrafish, there is a wnt gradient associated with the dorsal axis. As we discussed in class, 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, which leads to the development of a second anterior-axis (a second head).

Provide a model (\rightarrow) that explains why these cells do not die.



A simple model would be that the cell death effect depends upon differences between β -catenin levels in a cell compared to surrounding cells.

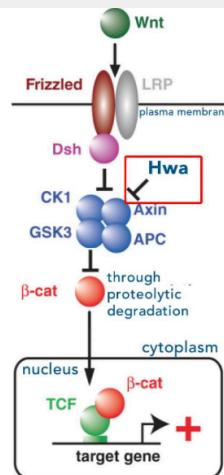
When we inject β -catenin RNA, all of the cells in the neighborhood have (similar) high levels of β -catenin - so there is no death signal generated.

In the zebrafish, when active, the product of the Hwa gene lead to the inhibition of Axin, which is part of the **conserved** macromolecular complex involved in regulating the degradation of cytoplasmic β -catenin.

Predict (\downarrow) and explain) the effects of these mutations on the level of β -catenin regulated gene expression...

1. a mutation in GSK3 β that renders it constitutively active.
2. a mutation in APC that leads to its failure to associate with GSK3, Axin, and CK1
3. a mutation in TCF that weakens its interaction with β -catenin.
4. a mutation in β -catenin that inhibits its ability to enter the nucleus.

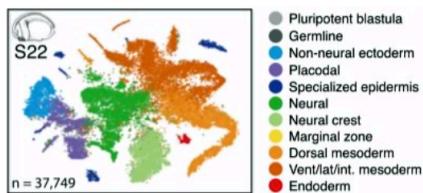
four explanations



1. Ventralization (β -catenin is destabilized globally)
2. It is possible that GSK3 is inactive in the absence of APC, so that β -catenin is stabilized) - dorsalinization
3. Genes that should be activated by high levels of β -catenin remain inactive, since β -catenin does not interact with TCF effectively (a more complex, less predictable phenotype)
4. Genes that should be activated by nuclear β -catenin remain inactive, leading to ventralization

Activity 12

When we consider complex data sets, such as those associated with single cell RNA SEQ analysis of developing embryos, the results are often analyzed by using principle component analysis so that the observations can be displayed in meaningful ways. In this case from Briggs et al (2018), the analysis of ~38000 cells from a stage 22 *Xenopus* embryo is displayed in terms of cell types (→).

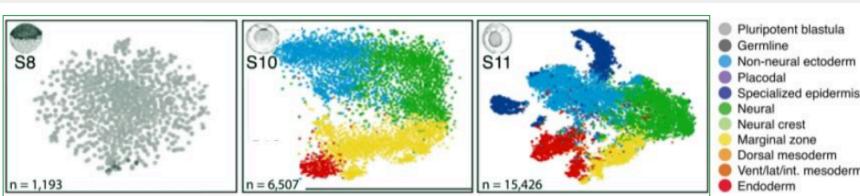


There is clearly differences in the expression of various genes that either
1) does not effect the terminal phenotype significantly (or is compatible with it),
2) is modified later in development,
or 3) perhaps the cells are removed (die) or
adopt a different fate.

Q: What does it mean, in molecular terms, that all of the cells of a certain type do not map to a single X,Y coordinate? (↓)

explain

Provide a model that explains the variability between cells of a certain type (↓).



Consider the changes in gene expression during the early stages of development. Circle (for stage 8 ↑) the first specific cell type to appear during development. What cell type is it, and why might it appear early? (→)

By stages 10 and 11 there are a growing number of cell types, including some that appear to be in the "wrong" places (circle some in stage 10/11 ↑).

Q: What does it mean, "to be in the wrong place" in terms of patterns of gene expression, and what might be the fate of such cells? (→)

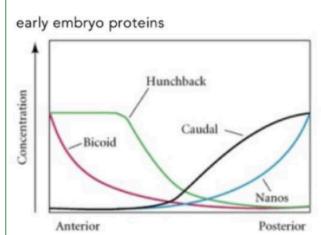
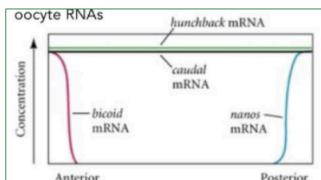
The earliest cells to appear appear to be germline cells (bottom dark grey cells at stage 8).

These cells are set aside early to form the ovary/testes. Reduce the number of cell division, and so possibility of mutation and such).

These are dots of one color in regions primarily of another. They may change their patterns of gene expression or perhaps undergo apoptosis.

In Drosophila, the late stage oocyte already has some asymmetrically distributed RNAs, while other developmentally important RNAs are distributed uniformly (\rightarrow). After fertilization these RNAs are translated and their gene products interact.

Consider the network by which bicoid regulates the embryonic concentration of the caudal protein; predict (graph \rightarrow) what would happen to [caudal] in an embryo derived from a mother that failed to synthesize bicoid RNA in its germ line and explain your reasoning (↑)

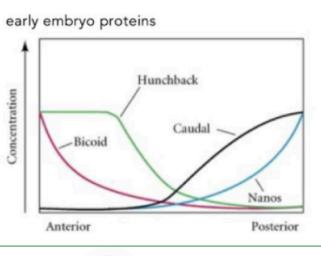
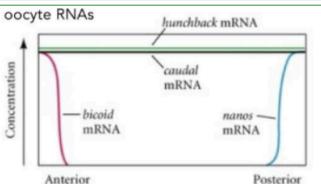
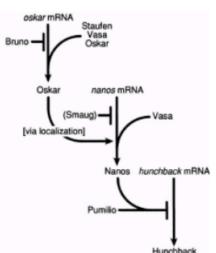


The level of caudal protein would increase in the anterior region of the embryo

The nanos RNA is localized to the posterior end of the oocyte, and nanos protein appears in the early embryo (\rightarrow).

Consider the network by which nanos cooperates with other proteins to regulate the concentration of hunchback protein.

Predict (graph \rightarrow) what would happen to [hunchback] in an embryo derived from a mother that failed to synthesize the pumilio protein and explain your reasoning (↑)

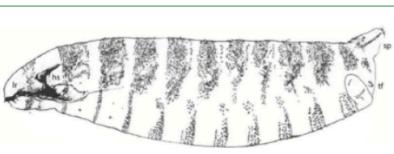


Since pumilio works with nanos to inhibit hunchback RNA translation / protein levels, we would expect the level of hunchback protein to increase in the posterior region of the embryo.

Activity 13

The patterning of the larval cuticle in *Drosophila* is highly stereotyped. In this drawing indicate the anterior-posterior and dorsal-ventral axes (→).

Given what you know, predict the phenotype of an embryo from a mother that is homozygous mutant for both bicaudal and dorsal. Make a sketch below (↓) and explain what the embryo would look like and why (↓).



explain here (please!)



- previously isolated a "fascinating maternal mutation, bicaudal, causes the formation of larvae with two rear ends in mirror-image symmetry"
- "a new maternal mutant, dorsal, was discovered with very specific loss of ventral pattern elements such that the entire cuticle appears dorsalized"



Consider the expression of HOX genes in a wild type embryo. Predict the pattern of HOX gene expression in an embryo from a homozygous bicaudal mother (→)

Explain your prediction (↓)

explain your prediction



- the same as in bicaudal
- no effect on HOX expression
- impossible to say
- no idea

← Now predict the expression of HOX genes in an embryo from a homozygous dorsal mother; explain the logic behind your choice (↓).

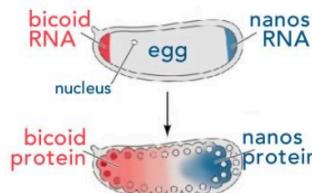
why?

We would expect early embryos with duplicated posterior regions (leaving unclear how the sperm enters, since it normally enters through an anterior structure).

Somehow mutations in bicaudal are acting to replace anterior determinants (e.g. like bicoid) with posterior determinants (e.g. like nanos).

Consider the following situation. Instead of a cell-less syncytium, assume that a complete cell division occurs followed each nuclear division in the early *Drosophila* embryo. What aspects of the normal system involved in establishing embryonic axis and segment identity would no longer work.

Propose a model by which gradients of bicoid and nanos proteins could be established (↓)



Since it is based on diffusion gradients of RNAs and proteins, we would expect cell membranes to block such movements and so larval patterning would not occur.

An alternative model would involve signaling systems across the membranes, which would (of course) be slower (and probably incompatible with the *Drosophila* life style).

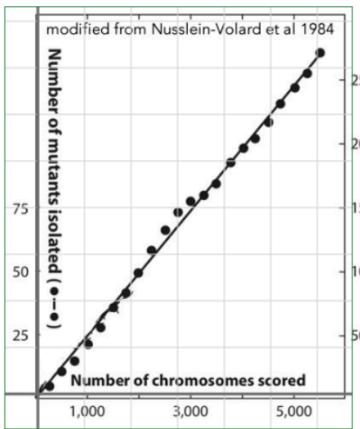
You are carrying out a screen, such as that performed by Christiane Nusslein-Volhard and Eric Weischaus. Draw the curve (\rightarrow) that describes the relationship between the number of unique loci (genes) identified and the number of chromosomes scored.

Explain the shape of your curve; how does this relationship differ from the one that describes the number of mutants as a function of chromosomes scored (\downarrow) **AND explain** why the relationship the relationship between the number of mutants as a function of chromosomes scored is a straight line.

Are all of the mutations identified unique?

- yes
- no
- impossible to tell
- no idea

explain



One way to map mutant alleles with respect to one another on a chromosome is to look for recombination events, events in which two mutant alleles, originally on different (maternal and paternal) chromosomes come to reside on the same chromosome - a lack of such recombination events suggests that the two mutant alleles are in the same gene. Why is that (\rightarrow)

We find two mutant alleles, m1 and m2, that produce (as homozygotes) the same phenotype. Complementation analysis is based on the assumptions that when two mutant alleles are in the same gene, they will fail to complement each other, that is, the phenotype m1/m2 will be the same as m1/m1 or m2/m2. If they are in different genes, then m1/+ m2/+ animals will have a wild type phenotype.

YET, it is possible to find examples in which mutant alleles in the same gene complement each other, so that m1/m2 has a wild type phenotype. Describe ($\downarrow\rightarrow$) a model that could explain how such an outcome is possible.

how might it work?

- recombination occurs when alleles are near one another
- recombination occurs with a gene
- the frequency of recombination increases with separation of the alleles along a DNA molecule
- no idea



The more chromosomes screened the more mutations identified, but as the number of chromosomes screened increases, more and more of these mutations will be in the same genes (and may even be the same mutation).

In your curve, the number of unique loci genes will saturate, that is, no longer increase in number (plateau) once all (most) genes that can be mutated to produce a recognizable phenotype have been identified.

Recombination frequency is a function of distance between genes / sites of sequence variation.

No (very low) recombination suggests that alleles are located near one another - e.g. in the same gene.

We can assume that the gene encodes a polypeptide with multiple functional domains, so that a functional variant in one allele can complement a dysfunctional variant in the other (and visa versa).

Activity 14

Which (pick one →) do you think is the most important aspect of *C. elegans* that makes it a powerful (and unique) model system for the study of embryonic development?
justify your answer in terms of uses and uniqueness (↓)

what so special about the worm

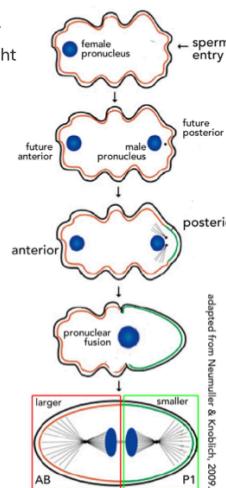
- genome size
- some scientists picked it, and people began working on it
- just another metazoan (evolutionary relationships)
- invariant cell lineage
- that it is a hermaphrodite
- its transparency
- number of cells in the adult
- speed of development
- no idea

I would suggest that invariant lineage is the most important, in that it leads to the ability recognize changes in specific cell fates

That said the other traits are also useful.

In *C. elegans* embryonic polarity is re-established in the egg. If you wanted to try and reverse the polarity of the embryo, or create a two-headed embryo how might you go about it? Think back to experiments from Xenopus and generate and describe (↓) a plausible experiment and explain why it just might (but probably wouldn't) lead to a two-headed worm,

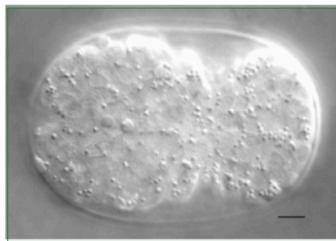
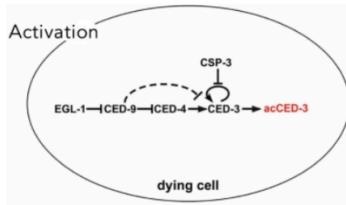
how might you manipulate the early embryo to reverse or alter the adult.
How might it be possible to generate a two headed adult



We might extract cytoplasm from the prospective posterior domain and inject into the prospective anterior region of another embryo (assuming that could get through the egg shell, and that the material was cytoplasmic rather than associated tightly with the embryonic plasma membrane).

Probably could not move membrane associated factors.

In *C. elegans*, cells undergoing programmed cell death change their morphology and refractivity; these changes can be observed in living animals using Differential Interference Contrast (DIC or Nomarski) optics. There are three distinctive apoptotic cells in this image (→); circle them.



answers here please

The apoptotic process in the dying cells is controlled by the regulatory network outlined above (↑).

Q1: based on this network, predict whether Egl1 is expressed universally or only in cells that are fated to die and

Q2: Predict what will happen if there were a mutation leading to the absence of Ced9 activity, (→).

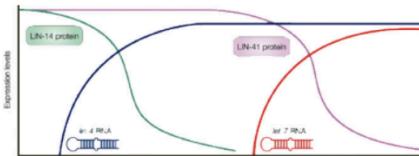
Given what we are shown, and the role of Egl-1 inhibition of CED9, without which Ced3 will be active and inducing cell death, we might predict Egl1 is expressed only in certain cells.

As above, we might expect Ced3 to be active everywhere - leading to global cell death.

Genetic studies in *C. elegans* discovered a role for "microRNAs" in regulating gene expression. This figure (→) shows the effect of the microRNAs produced by the lin-4 and lin-7 genes on the levels of lin-14 and lin-41 proteins over developmental time.

Make and explain a model that leads to this behavior; draw (↓) and explain.

how does your model work?

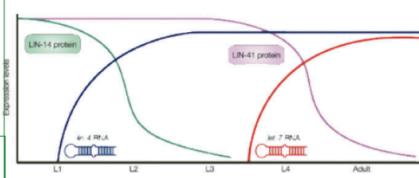


The expression of lin4 and lin7 leads to the degradation of or the inhibition of the translation of lin14/lin41 RNA - so their expression leads to reduction in polypeptide levels.



Assume that a mutation changes the binding affinity between lin-4 and lin-14 RNAs. What type of mutation would produce such an effect and how would it influence the level of lin-14 protein over time (→) and explain your reasoning. (↓)

what happens and why?



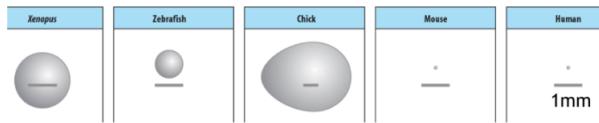
We would expect a less effective inhibition, the levels of lin-14 protein would increase (or decrease less rapidly as a function of lin-4 level).

Since lin4 negatively regulates lin14 which negatively regulates FOXO, we would expect a lin4 mutation would lead to an increase in lin14 and so a decrease in FOXO expression.

how does FOXO expression change?



Activity 15



Provide a plausible explanation for why it is that the eggs of various vertebrate animals vary so greatly in size (↓).

Provide a model that explains why the first cellular differentiation event in the mouse/human involves the formation of the trophectoderm & the inner cell mass (↓).

part 1

part 2

Part 1: Egg size reflects the amount of nutrients needed to support zygotic development until such time that it can feed on its own.

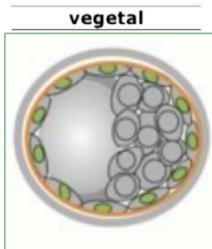
Growth of mammalian zygotes is supported early through interactions with the mother through the placenta. No need for large stores of nutrients in the egg.

Zygotic growth is totally dependent on establishing the interaction between zygote and mother, an interaction based on specialized tissues, specifically the trophoectoderm and functioning placenta. This makes subsequent development dependent on these tissues (so they form first).

animal

D1.1
D2.2

vegetal



You carry out a fate mapping experiment in the D1.1 and D2.2 blastomeres of a Xenopus embryo. Predict the outcome (and explain your reasoning). Will the cell types that arise from these two blastomeres be the same or different (↓)?

consider what is meant by cell types

Now consider a similar experiment in the mouse, in which you carry out a fate mapping study of two cells in the inner cell mass. Start by indicating which cells you are targeting (←) and then explain your predicted result (→)

what is the expected outcome?

Because they come from different parts of the embryo, and these parts are different in terms of maternal / zygotic gene expression events (including the dorsal rotation), the cell types produced by these different blastomeres will be different.

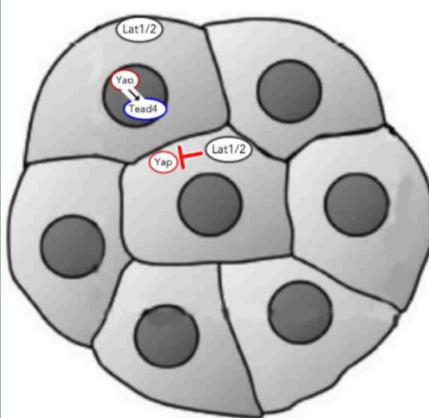
In the mouse, we expect the cells of the (early) inner cell mass to be equivalent, so different cells will produce overlapping (similar) types of cells.

As development proceeds, they will begin to diverge in terms of the cells they will produce, as they themselves differentiated (because they come to be in different environments, receiving different signals).

If we think about the regulatory network involved in trophectoderm/inner cell mass differentiation, there is clearly a cellular decision switch. Looking back over your notes, predict which cells will normally become trophectoderm (→).

Generate a plausible model by which i) differences in cell surface interactions could regulate the activity of Lat1/2 and ii) what do you expect will happen if there were a mutation in Yap that inhibits its inactivation by Lat1/2 (↓).

what is happening?



Surface cells will become trophectoderm.

Inhibition of Lat1/2 through binding to free surface proteins allows Yap to enter nucleus, activate Tead4 and start trophectoderm differentiation.

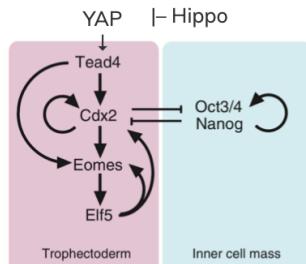
We might expect that all cells of the embryo will become trophectoderm

Normally Yap enters the nucleus and interacts with the transcription factor Tead4 to activate Cdx2.

Activation of Cdx2 leads to the increased Cdx2 expression in trophectodermal cells. Oct3/4 is a marker of totipotent cells.

What would be the effect of Cdx2 expression on totipotent cells? Do you expect the percentage of totipotent cells to increase or decrease during development and why? (↓)

what is going to happen with Oct3/4 expression over developmental time?



We would expect that the expression of Cdx2 would inhibit Oct3 leading to inhibition of the totipotent cell phenotype.

In general, we would expect that the number (percentage of total cells) of totipotent / pluripotent cells would decrease as development proceeds because cells (tissues) are becoming more and more specialized.

Out of all of the concepts and review questions for midterm 2, which are you the most confused by?

let us know....