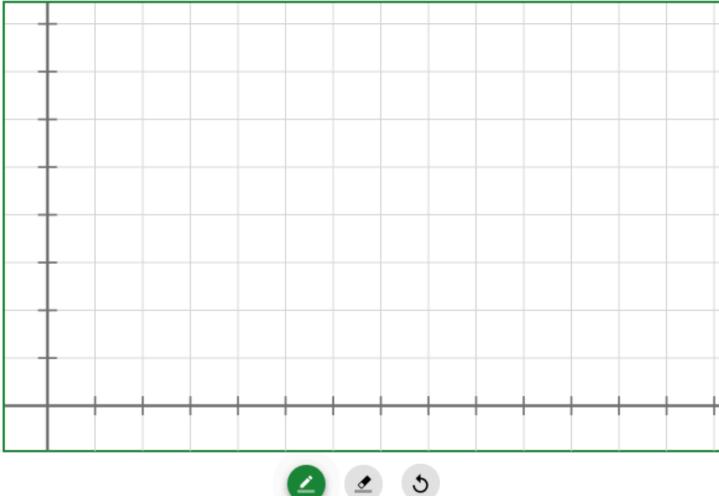


Act 16 Review of thresholds / gastrulation

In terms of increasing concentration of transcription factor [TF] (x-axis), draw your prediction of the expression level (RNA) of a particular target gene.

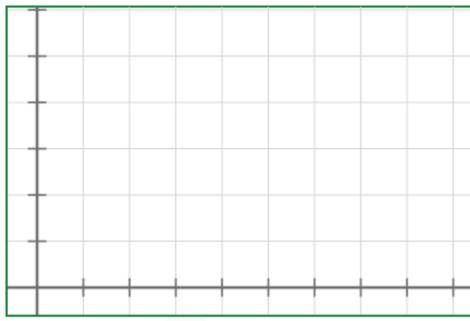


Generally there curve is "S" shaped, with the response (RNA synthesis in this case) starting above a certain value of [TF] and saturating (leaving off) above a certain value. This reflects concentrations of TF needed to occupy regulatory sites, as well as recognizing that there is a maximum number of RNA synthesized per unit time.

Of course RNA levels could increase further (or decrease) if there were changes in the rate RNA degradation.

Often the level of expression "saturates", that is fails to increase further even as the concentration of the transcription factor increases. What would be a plausible mechanisms for that behavior?

how does that work?



how does that work?

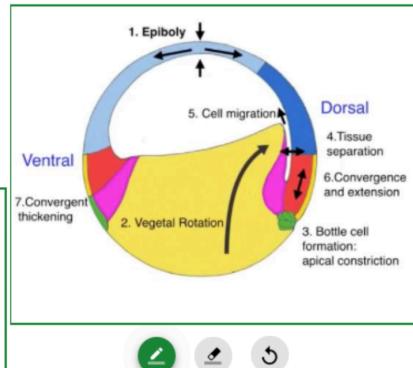
In the graph ↑, how would your system behave if it displayed a "threshold effect"? Can you provide (↔) a plausible molecular mechanism to produce that behavior?

There are only two copies (generally) of each gene, and so only so many polymerase can load on a gene per unit time - once that limit is reached, no more polymerases can load - the synthesis rate is maximum (no matter what the concentration of the regulating transcription factors).

You would get a threshold effect if the concentration for expression onset and saturation are close to one another.

During Xenopus gastrulation, the first visible process to occur is the formation of bottle cells, which allows for the coordination of changes associated with epiboly and convergent extension. Describe what happens (and why) if bottle cell formation did not occur?

how does that work?



Draw (↑) what might happen if epiboly did not occur.

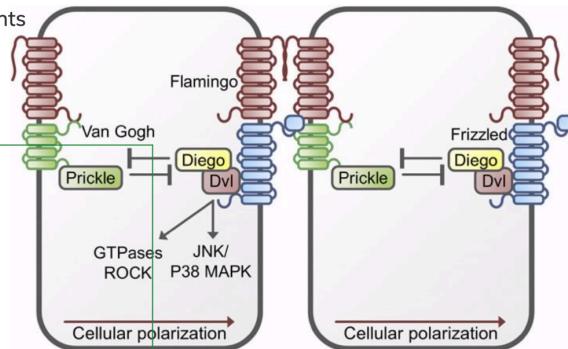
Bottle cell involve changing the shape of cells in the region of the future dorsal lip, so that they are thin on the outer surface and fatter internally. This allows the various movements associated with epiboly and convergent extension to move external cellular regions, internally.

If bottle cell formation did not occur, regions of the embryo would fail to internalize - what is called exogastrulation would occur (but epiboly and convergent extension would)..

Convergent extension movements depend on cellular asymmetry.

predict the outcome if Prickle did not inactivate Diego/Dvl?

what would be the result?

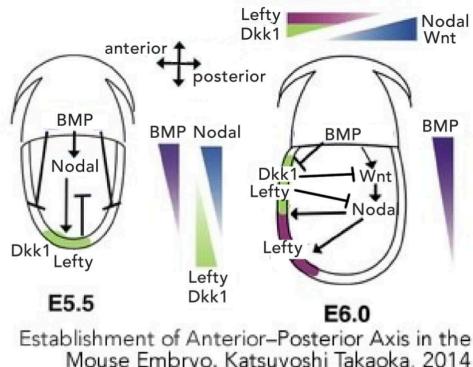


Most likely cell would not be polarized, but display uniform migration movements.

The sheet of cells might well not elongate (fail to undergo convergent extensions)

In mouse, the embryonic axis form in relation to the site of implantation. At embryonic day 5.5, There is a BMP signaling gradient arising from the extraembryonic region. BMP signaling activates expression of a second signaling molecule, Nodal. Nodal in turn activates expression of the Nodal signaling inhibitor Lefty and the Wnt inhibitor Dkk1. Half a day later, the system has gotten more complex with asymmetric expression of Wnt and the Wnt antagonist Dkk1.

Make a plausible prediction of the outcome if, for some reason, Dkk1 expression was not inhibited by BMP signaling.



We might expect to see the embryo develop with radial symmetry, since the Wnt signaling on the future anterior side of the embryo would not be inhibited by Dkk1.

Enter one question or call for clarification for the reading paper
"The mechanism of somite formation in mice" by Yumiko Saga.

Activity 17

Activity 17: Somites

How are somites (and vertebrate) similar to and dissimilar to segments in a Drosophila embryo.

answer here

What is a plausible (and general) model for how HOX gene expression is controlled?

answer here

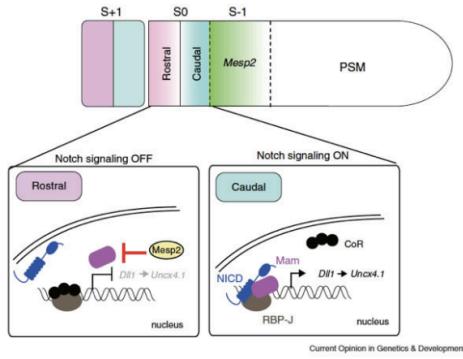
Similarities: repeated structure, differentiated cell fates influenced by HOX gene expression

Dissimilarity: somites form sequentially through a progressive gene expression network (clock and wavefront model). Drosophila segments form more or less simultaneously based on maternal asymmetries.

Hox gene regulation is controlled by various underlying asymmetries in signaling and transcription regulatory networks active in the tissue. Interactions within the HOX cluster influence the order of gene expression (anterior to posterior)

What would happen if Notch signaling were inhibited

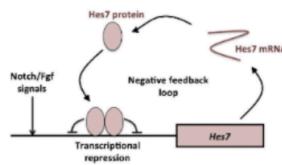
answer here



Since caudal development of the segment is dependent on Notch signaling (formation of NICD), the segments would be uniform - no proximal-caudal distinction.

Consider the Hes7 regulatory network

Draw (↓) the level of Hes7 protein as a function of time, assuming that Notch/FGF signaling is turned on at t=0.

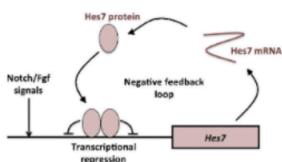
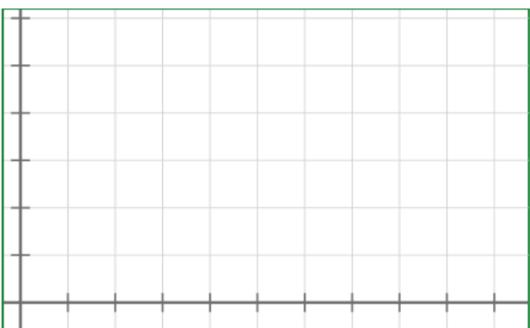


Explain what ideas you considered when making your graph.

answer here

Hes7 protein would, after a delay for transcription and translation, increase - as it increased, it would pass through a threshold concentration at which it would form dimers and bind to the Hes7 gene regulatory region and block Hes7 gene expression. Assuming a constant rate of Hes7 RNA/protein degradation, Hes7 protein levels would then drop. When Hes7 levels dropped below the inhibitor threshold, Hes7 inhibition of Hes7 gene expression would be removed, and Hes7 protein would increase - the system will cycle.

Indicate how your graph (↓) would change if the Hes7 protein had a much longer half-life?

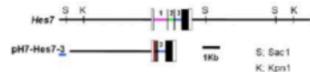


Explain in words why (and whether) yours graph changed?

answer here

The first part of the system will be as before, but once the level of Hes7 protein rises to the level needed to inhibit Hes7 gene expression, the repression of Hes7 expression will last for a longer time, so that the period of oscillation in Hes7 protein will be longer.

The wild type Hes7 gene has multiple introns. You delete all but one, how does this change the level of Hes7 protein as a function of time (compared to your original graph)?



Explain in words why (and whether) yours graph changed?

answer here

Without the introns, the time taking to synthesis and process the RNA (into a mature mRNA) will be shorter, so the appearance of Hes7 protein will be quicker (the response between activating the gene and the appearance of protein will be shorter).

This would like influence the rate of oscillations in Hes7 protein concentration over time.

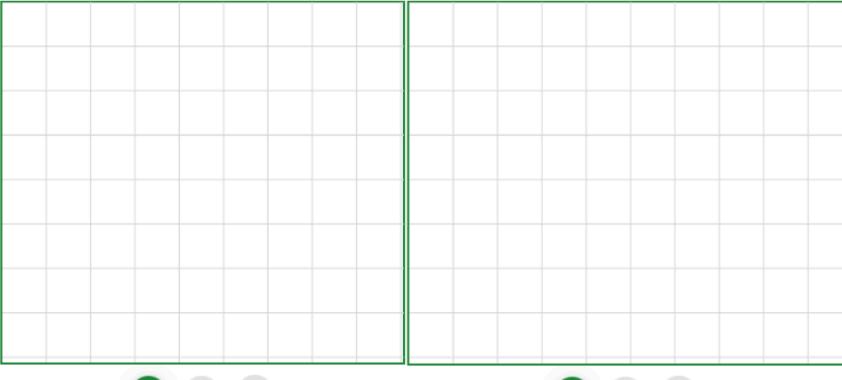
answer here

Do you a specific questions about the Sagner & Briscoe paper

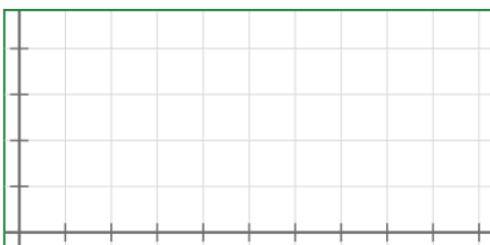
Activity 18 Neural Tube

Neural tube: Consider how the neural tube is formed and comes to have its dorsal-ventral axis. Draw a schematic (based on your notes) on how the ectoderm comes to be divided into ectoderm and neuroectoderm and then develops dorsal-ventral variations in cell type., and indicate primary the signaling systems involved.

Early (before tube forms) (↓) and later (↓) after the tube has closed.

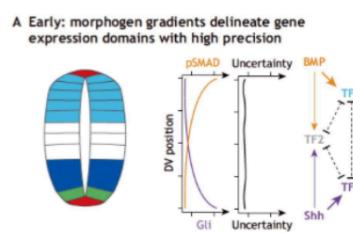


Gradients: In the early stages of the neural tube's dorsal-ventral specification, there are two opposing gradients, generated by BMP and Shh signaling. In the graph, the X axis goes from extreme ventral (at 0) to extreme dorsal extreme (at the right edge). As a function of axis position, blot the ratio of pSMAD to Gli.

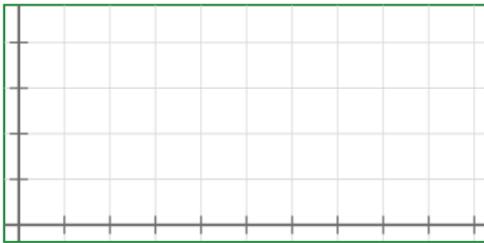


How then can a cell determine where, exactly, it is along the neural tube's dorsal-ventral axis how does its D-V position alter the cell (→)

how does it work?

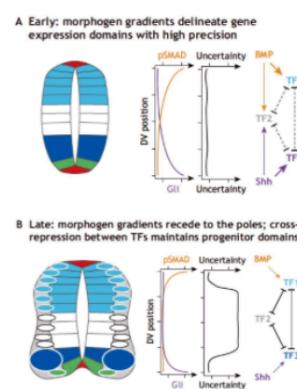


As development proceeds, the gradients recede. In the graph indicate how the ratio of pSMAD to Gli changes.



Explain how is it that cells in the central region of the neural tube can come to adopt specific differentiated states, and form specific types of neural progenitor cells (→)

how does it work?



The neural tube is formed by the folding of the ectoderm, driven by inductive signals from the underlying mesoderm (specifically the notochord).

As the folding continues, the ectoderm/epidermis will fuse and disconnect from the underlying neural tube.

BMP signaling will be inhibited in the neural region, with Shh signaling from the notochord (ventral neural tube floor plate).

The pSMAD (active BMP signaling) and Gli (active Shh signaling) will change from low to high (pSMAD/Gli) as we move from extreme ventral to dorsal.

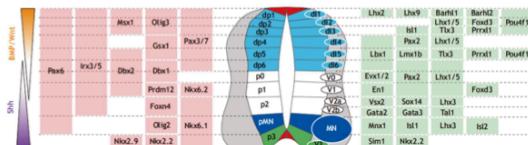
Since pSMAD and Gli activate different sets of genes (at different concentrations), a cell's developmental fate (what it becomes) reflects the pSMAD/Gli ratio.

Note we are talking about activated GLI (GliA) which reflects Shh signaling activity.

There will be a long region in which the pSMAD/GLI ratio remains low and constant - in this region it become more ambiguous where the cell is within the developing neural tube.

However earlier, which position more unambiguously defined, different sets of genes were turned on (and off), so that later, cells can rely on patterns of gene expression (established earlier) to make differentiation "decisions".

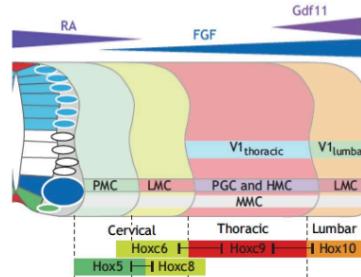
Imagine that this is the pattern of gene expression in the rostral region of the neural tube (→). How would HOX gene expression influence gene expression and cell differentiation in more caudal regions? (↓)



how does it work?

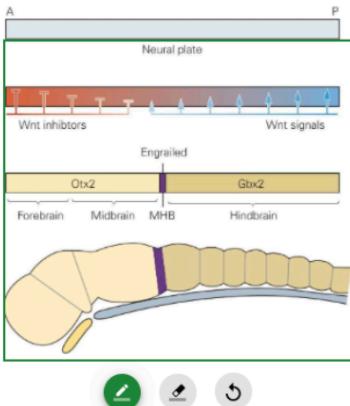
Because HOX proteins are transcription factors, and the pattern of their expression influences anterior-posterior specification, if there are anterior-posterior differences within the neuronal cell types of the neural tube, we would expect changes based on which Hox genes are expressed.

Predict how and explain why (↓), the system would change if the HOX genes did not act to inhibit the expression of neighboring HOX genes (→).



how does it work?

We might expect ambiguity in the factors determining cell fate associated with specific anterior-posterior positions within the neural tube. Cells might be getting "mixed" signals.



The rostral-caudal patterning of the neural plate is based on Wnt and anti-Wnt gradients. The position of the midbrain-hindbrain boundary (MHB) is marked by expression of the transcription factor engrailed.

Indicate in the picture (←) 1) how the Wnt signaling would be expected to change if expression of the Wnt inhibitor was inhibited and 2) indicate how this would be expected to influence the expression of engrailed. Explain your reasoning (↓).

how does it work?

Suppressing expression of a wnt inhibitor would be expected to lead to the expansion of the Wnt gradient (higher levels of Wnt signaling in more anterior positions).

This would lead to the movement of the midbrain-hindbrain boundary toward the anterior end of the neural axis.

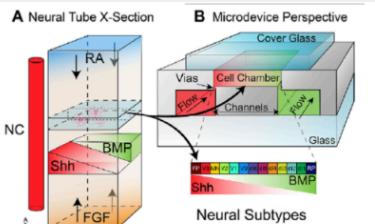
Activity 19 Neural Tube and Crest

19. Neural tube / neural crest

Consider the in vitro system designed to mimic the signaling gradients involved in the patterning of the neural tube (→).

(↓) Explain the difference between in vivo versus in vitro.

and then make a prediction, what while the pattern of HOX gene expression look like in this system, what factors might be expected to influence it, and explain why (→).



in vivo versus in vitro

Hox gene expression

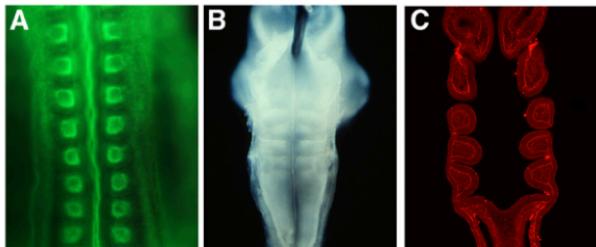
In vitro means out of the body, typically in glass (or in the modern world, in plastic). An artificial system of varying levels of complexity. In vivo (or in situ) refers to within a living (intact) organism

We might expect that this system would mimic one anterior-posterior position in the developing embryo (but we cannot be sure which one).

There are three segmentation processes occurring within the vertebrates involved in the formation of mesodermal somites (A), neural rhombomeres (B), and the pharyngeal arches (C).

(↓) Explain the difference between analogous and homologous structures and then describe how you would might decide whether these processes have a common evolutionary origin (→).

homologous versus analogous

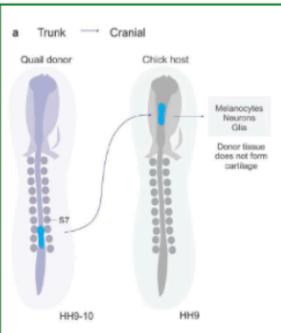


what evidence / predictions

When we talk about homologous processes, we assume that the process was present in the common ancestor.

Analogous processes are similar, but arrived at independently (but because there are a limited number of components from which build processes, there may be components in common).

In the vertebrate case, there are significant differences in these three processes, so we might assume that they are analogous.



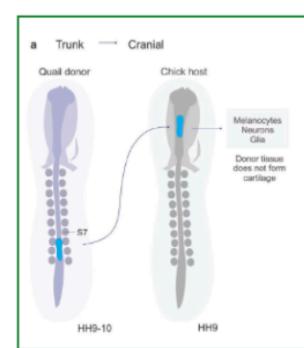
Normally cranial neural crest cells can differentiate into cartilage as well as melanocytes, neurons, and glia (Schwann cells). In transplant experiments, LeDouarin and others found that trunk neural crest cells, transplanted into the cranial region failed to make cartilage ... but formed the other cell types, whereas transplantation of cranial neural crest cells into the trunk produced cartilage

← Draw what is happening in the trunk to cranial transplant experiment and explain then provide a plausible model for why the two types of neural crest cells differ, indicate what your model predicts, how you might test it (↓)

model / predictions

It is possible that trunk crest cells have lost the ability to respond to the cranial environment, lost the ability to form cartilage.

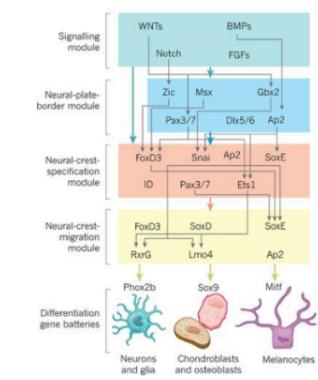
They could well differ in terms of the Hox genes they express, alternatively, since trunk crest forms later, different signaling-gene network events that occurred earlier could lead to different "molecularly accessible" behaviors. The cranial crest cells may respond differently to the signals found in the trunk (in part because they express different Hox genes (would be interesting to see if there are changes in Hox gene expression)).



How might changes in HOX gene expression figure into the differential behavior of different (pre-migratory) populations of neural crest cells.

← In your drawing compared to the wild type, indicate how the cranial and trunk neural crest might differ in terms of HOX gene expression and explain how this might influence their behavior (↓)

Hox effects

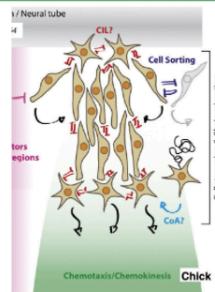


In this gene expression network, there is something conspicuously missing (or at least I think so). Can you explain what it might be (one thing)? (↓)

what is missing (and is present in every other gene regulatory network we have considered)?

In this diagram of a migrating stream of neural crest cells, provide a model for how the "leading cells" might differ from the "followers" (↓)

what is changing?



At different levels of the A-P axis, we would expect that different Hox genes would be expressed, leading to different "downstream" genes. Their expression would influence cellular behavior.

Part I: There are few negative or positive feedback interactions shown. Such feedback interactions are key to determining the behavior of most networks, insuring that "choices" are made between different cell types.

Part 2: The leading cells are interacting asymmetrically with various matrix/cellular elements that they are moving through. This will lead them to express different genes and different behaviors.

Following cells will have a different set of asymmetric interactions, leading to different behaviors.

Activity 20 Limb

Activity 20: Reviewing limb formation (part 1)

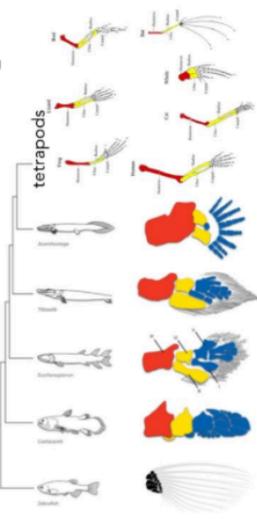
Based on anatomical comparisons between tetrapod vertebrates & various existing (coelacanths) and fossil (e.g. Acanthostega, Tiktaalik, Eusthenopteron) "fish", explain why developmental (and evolutionary) biologists are not surprised to discover similar molecular mechanisms are involved in fin and limb formation (↓)

explain

You identify an similar DNA sequence region in coelacanth and mouse. The coelacanth sequence drives reporter gene expression in the mouse limb bud, that would prove that it is (choose →) for normal limb formation? Explain your reasoning and does it matter whether the sequence is located near related genes in the chromosome? (↓)

explain

- necessary
- sufficient
- both
- more experiments needed
- no idea



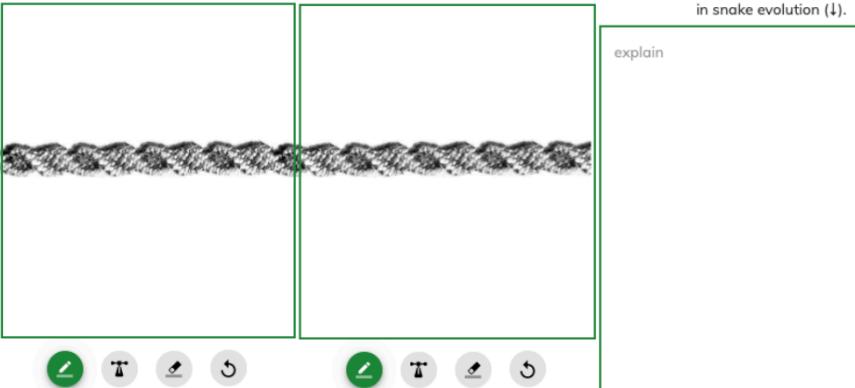
These observations suggest that limbs and fins are homologous structures, that they major change involved repurposing bones present in the "wrist" structure present in lobe fin fishes.

Such an observation would only indicate that the enhancer element responds to signals /TFs present in the mouse limb bud. We would expect the sequence to located near homologous genes in both organisms (otherwise it could just be a fortuitously similarity)

To prove necessity or sufficiency, one would have to show that removing the mouse sequence blocked limb development, and that its ectopic expression produced a limb

As we discussed in class, there is a similar limb enhancer region in coelocanth, python, and mouse. Generating a 17 bp deletion in the mouse enhancer, similar to the sequence found in the python, leads to the loss of limbs in the mouse. In the diagrams below, make a cartoon-ish model (mouse on the left, python on the right) for how the two enhancer element differ.

Explain the logic of your drawing and then predict (and justify) whether such a mutation would be an early or late event in snake evolution (↓).



explain

The major difference between the two would be expected would be the absence of a DNA binding protein that recognizes the sequence in the mouse but does not bind to the python DNA (since that sequence is not there).

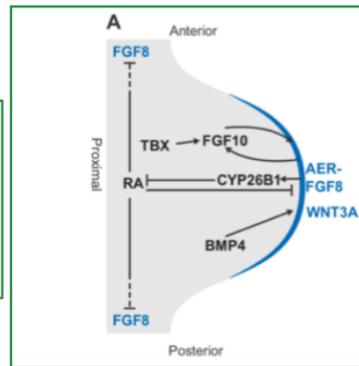
We might argue that the loss of limbs BEFORE the snake ancestor had adopted a particular lifestyle would be deleterious - we would predict that as snakes became less dependent upon their legs, their loss (and so the mutation) would have less and less of an effect on their reproductive efficiency (evolution).

In the lateral regions of the embryo that will form a limb, there is the regional induction of retinoic acid (RA) signaling. Indicate a plausible model by which regional activation of limb development occurs ($\downarrow \rightarrow$).

explain

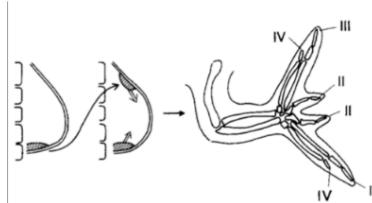
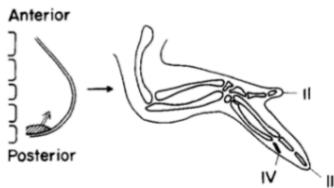
For the system to work, there needs to be a time delay in the expression of FGF in the AER. Explain why and what would happen if there was no such delay (\downarrow).

explain



The simplest model would be a signaling system, based on HOX gene expression would lead to turning on RA signaling system at specific anterior-posterior positions.

The major reason for the delay involves (at least as shown) the fact that FGF turns on CYP26, which negatively regulates RA signaling. As the limb grows out, the effect of FGF becomes more distant from the RA signaling region, there are two gradients of RA and FGF signaling involved in limb growth and patterning.



How is this classic experiment in the chick limb (\uparrow) similar to or different from the Xenopus organizer transplant experiments we talked about early in the course (\downarrow).

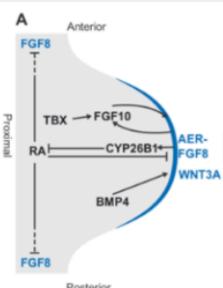
Based on the experimental outcome, which parts of the limb are affected? (\downarrow)

explain

explain

They are similar because the transplanted region acts as a source of signals that influence the behavior of the surrounding cells (tissue).

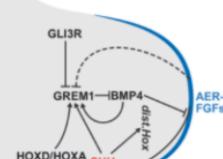
Looking at the diagram, we expect to see the effect primarily on the autopod (wrist, hand and fingers)



Something that is missing from many (all) diagrams of the limb development is the regulatory network that determines the asymmetric and regionally restricted Shh expression.

Propose (and explain) a plausible signaling system that would lead the observed pattern of Shh expression (\downarrow)

explain

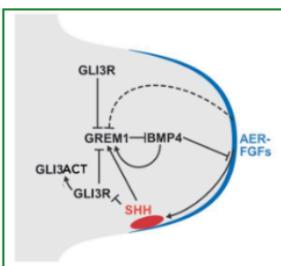


It could involve either HOX gene expression, or an asymmetry in the expression of FGF8 in the flank of the animal.

One question is the timing of Shh expression. In the second diagram, the role of HOX genes is indicated, so there would be a difference between the anterior and posterior sides the early limb bud.

Activity 21 Limb + IPSC

Activity 21: Finish Limb and start ES & IPS cells



Indicate the proximal distal axis (↑)

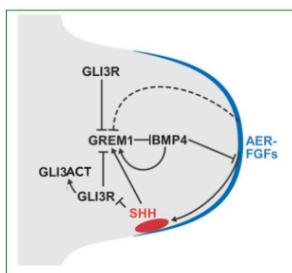
In the mouse limb bud, expression of FGFs in the apical epidermal ridge leads to limb bud growth in the proximal → distal direction. Imagine a mouse in which the Shh gene has been deleted in the cells forming the limb.

- Predict how this will influence limb growth. (↓)
- Explain why a Gli3 null limb grows out normally (what can you conclude about the primary function of Gli3 in the outgrowth of the limb bud).

explain here

In a similar model, we might expect that BMP inhibition of FGF is restricted to the region of the limb bud "below" (more proximal) to the AER (where FGFs are expressed).

In this case, in the absence of Shh, growth would be unaffected, what would be affected would be the regulation of BMP signaling levels, which would influence the differentiation of the mesodermal tissue (bone/muscle) of the limb.



Indicate the proximal distal axis (↑)

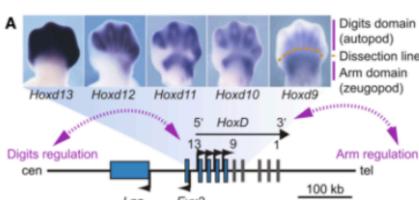
A Gli3 null limb produces a symmetric and polydactyl autopod. How is the function of Gli3 different in the context of autopod formation compared to limb bud outgrowth.

explain here

In the autopod, GLI expression is necessary for Shh to be able to produce apparent asymmetry. Without GLI, the digits would be symmetrical (no thumb-little finger axis), and there might be more depending upon the size of the AER region that the strength of lateral inhibition between digit forming regions.

If the proximal - distal axis of the limb is analogous to the anterior-posterior axis of the body, predict which Hox genes would you expect to be expressed first during limb development?

explain here (please)

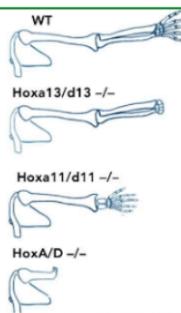


Here are phenotypes associated with null mutations in various Hox genes. Is Hox13 expression independent on Hox11? (→)

- yes
- no
- no idea

Explain your reasoning, what if anything can you conclude as to the expression of Hox13 genes in a Hox11 null limb (↓)?

explain here (please)



The role of Hox genes during vertebrate limb development
José Zañón* and Denis Duboule**

The most proximal (the genes at the 3' end of the Hox cluster, the genes with the lower number).

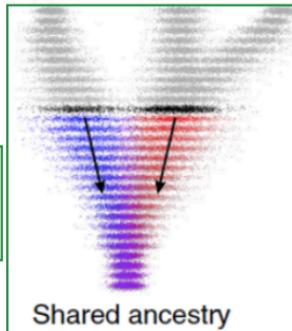
We would expect that Hox13 expression is independent on Hox11 and vice versa.

In this case it looks like the expression of the genes are independent, controlled by regional signals.

Activity 22 Organoids

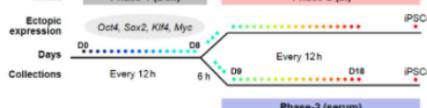
In their analysis Schieberger et al characterized and mapped the changes in gene expression as cell are reprogrammed from somatic fibroblasts to various cell types, including iPS cells. Indicate in the graph the general direction of time (\rightarrow). Explain, in molecular terms, your understanding for why, exactly, all of the original cells do not become iPSCs (\downarrow).

answer here please



In their reprogramming method, these researchers used cells from a transgenic mouse in which the Yamanaka factors are expressed from a DOX-regulated gene cassette. The expression of this gene cassette is turned off after 8 days. Why (\downarrow) don't the cells revert back to embryo fibroblasts?

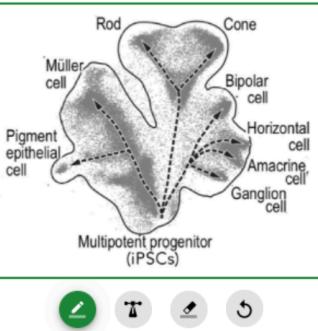
answer here please



Time is running from bottom to top.

We can assume that each of the yamanaka factors (which are transcription factors) are regulating a number of genes, and it is the overlap of these regulated genes that determines cellular behaviors. Because of the noisy nature of gene expression, and the fact that as gene expression patterns emerge they lead to further downstream changes, the process leads to multiple patterns of gene expression.

Once the process starts, simply turning off the the cassette does not return the system to its original state. Different sets of TF and regulated protein activities are present - the system has changed.



In the retinal organoids studies - Cowan et al (2019) tested 21 human iPS cell lines, of which only 6 were found to form retinal organoids with a layered appearance. One of these lines, F49B7, formed organoids with all five layers seen in intact adult retinas. How would you explain the observation that not all iPS cell lines produced "good" retinal organoids (\downarrow).

answer here please

In this diagram, indicate (\uparrow) with a circle where progenitor cells "decide" to become a bipolar, horizontal, amacrine, or ganglion cell. With a square indicate there cells "decide" on becoming neuronal or non-neuronal. Explain what happens when a cell "decides", how does this "decision" influence the eventual fate of the cell?

answer here please

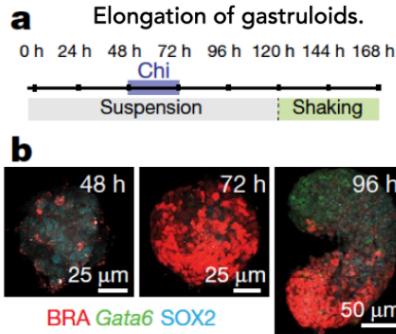
iPSCs are defined by the expression of certain markers, as well as their ability to differentiate into multiple other cell types. But that does not mean that they are identical, it is possible that different sets of genes (or even different alleles - think monoallelic expression) are active at various levels. This will lead to changes in their behavior.

The neural non-neuronal choice appears to occur early (as does the choice to become a rod or cone, as opposed to some other neuronal cell type. The choice between neuronal (non-photoreceptor) cells occurs later.

A cell fate decision involves turning on (and off) specific genes associated with differentiation into specific cell types. This also involves activation and inactivation of proteins and macromolecular systems. Generally, one choice excludes other - it is largely irreversible (unless forced).

In a gastruloid generated from embryonic stem cells, the originally spherical aggregate of 200-300 cells was first treated with a Wnt agonist (Chi) while in suspension culture & then placed in a shaking culture. During this period asymmetric gene expression appears. Propose & explain a model (considering class materials) for how the initial asymmetry of the gastruloid appeared and is maintained? (4)

answer here please



Based on the Beccari et al paper (and your class notes) as in vitro gastruloid development proceeds, describe the expression of the Hoxd genes, and explain why their expression is similar to or different from the expression in the developing embryo. (→)

answer here please

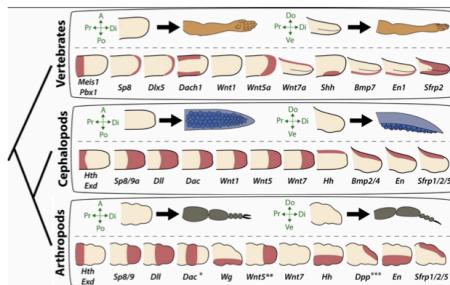
This could be a stochastic event, in which a group of cell cells come (by chance) to express certain genes (e.g. Bra). There can be communications between these cells (similar to quorum sensing) so that if enough cells in an area express the gene, they positively reinforce the expression, induce neighboring cells to express the gene(s), and inhibit expression in cells located further away.

Once the signaling center is established, it patterns the rest of the gastruloid. The expression of the Hox genes then emerges based on that regulatory axis....

Consider the expression of related genes during limb formation in vertebrate tetrapods, cephalopods (squid and their relatives), and arthropods.

Would you argue that this data is evidence that
 1) the common ancestor of all three had limbs or
 2) the common ancestor had a limited set of signaling molecules from which to choose (to build a limb)?
 Explain(↓)

answer here please



This is a tricky one, since that ancestor existed a very long time ago. At the same time, what is reasonably amazing is the relative conservation of signaling system - we can certainly expect that the ancestor had a set of conserved signaling system (Hh, Wnt, BMP, FGF, RA, etc). There are two choices, the limb formation system was present in the ancestor, or that given the signaling systems available, there are only limited number of ways to build a cellular system that leads to the formation of limb, a system that can drive organismic movement. Hard to distinguish, unambiguously between the two choices.