Development, Stem Cells, Signaling, and Morphogenesis

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Today's menu:

1 - Developmental Biology matters to Cancer Biology.

2 - Cell lineage/mosaic development vs environment/regulative development and *why signaling pathways*
provide a means to pattern/coordinate cell diversity

3 - Signaling and the Range of a signal.

4 - Cell "competence" to receive a particular signal

5 -Morphogen concept

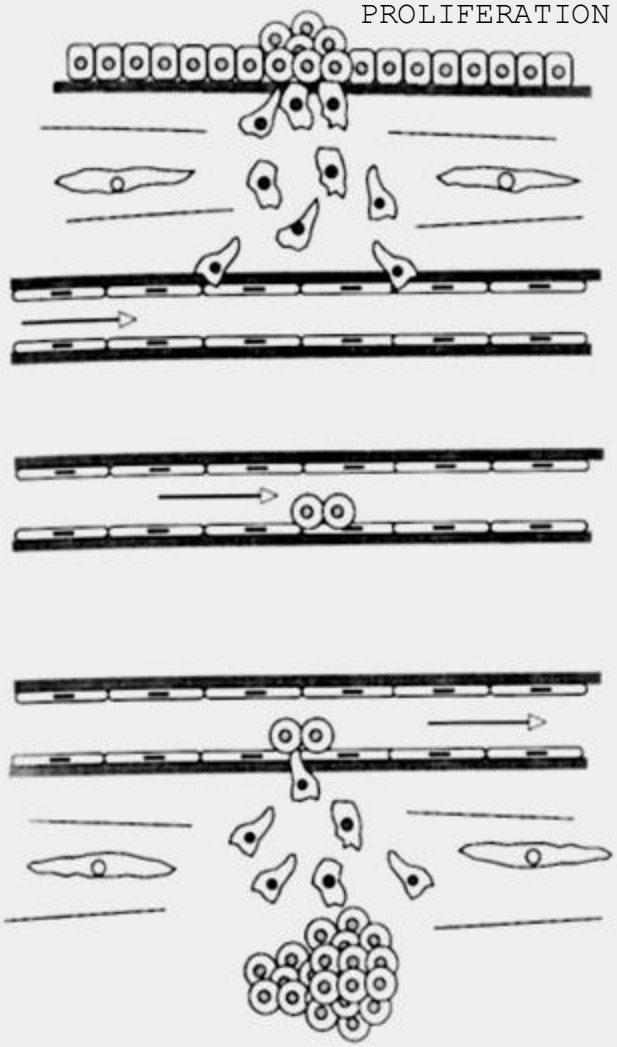
6 - Discuss how cells read and interpret graded information (the discussion paper!)

1. Developmental Biology <-> Cancer Biology

Virchow/Boveri et al. > Cellular Pathology:
drew on embryology to explain pathology-> Oncology

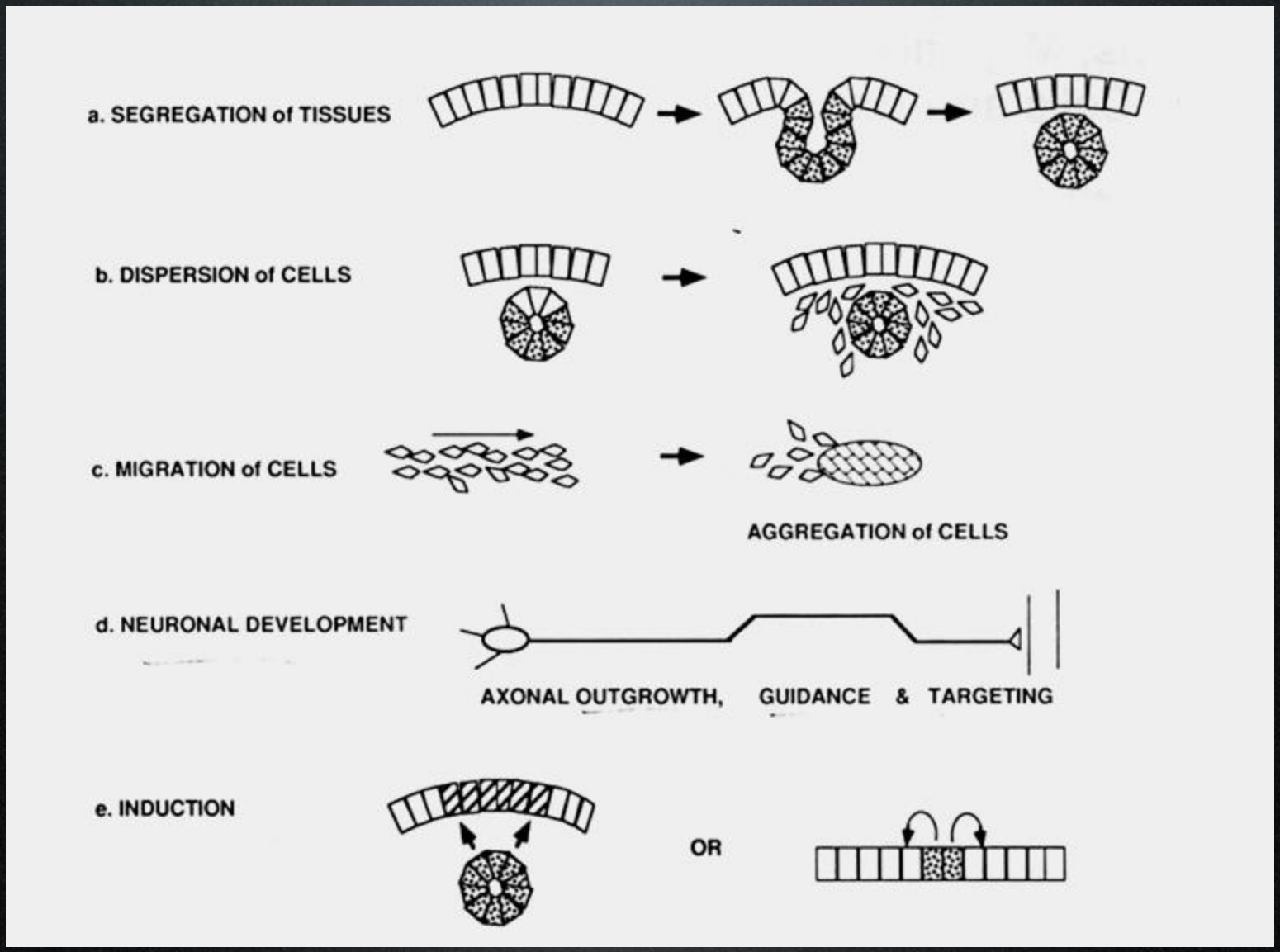
A. What are "normal"cell behaviors? What are "abnormal" ones?
What cell behaviors are found in cancer cells?

Examples of Cancer cell behaviors



1. INITIAL RELEASE
2. INITIAL INVASION
3. INVASION OF BLOOD VESSEL
4. INITIAL ARREST - ADHESION
5. EXTRAVASATION - EXIT FROM VESSEL
6. GROWTH OF METASTATIC NODULE

Developmental Cell Behaviors

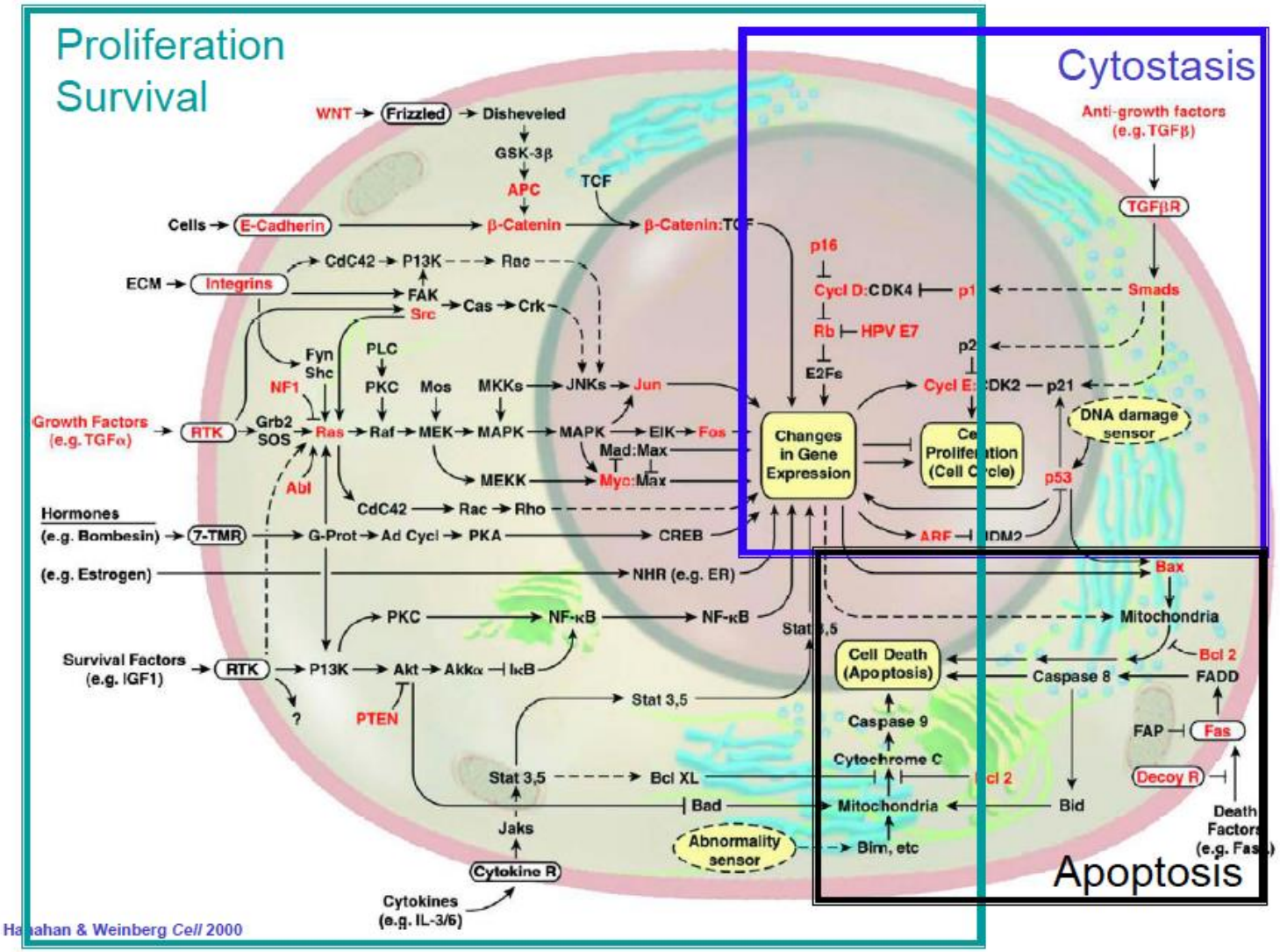


2. Developmental Biology <-> Cancer Biology

A. What are "normal"cell behaviors? What are "abnormal" ones?

B. Context matters.

Context matters.



2. Developmental Biology <-> Cancer Biology

A. What are "normal" cell behaviors? What are "abnormal" ones?

B. Context matters.

C. Logic behind the construction of signal transduction pathways to explain cells, tissues, and organs

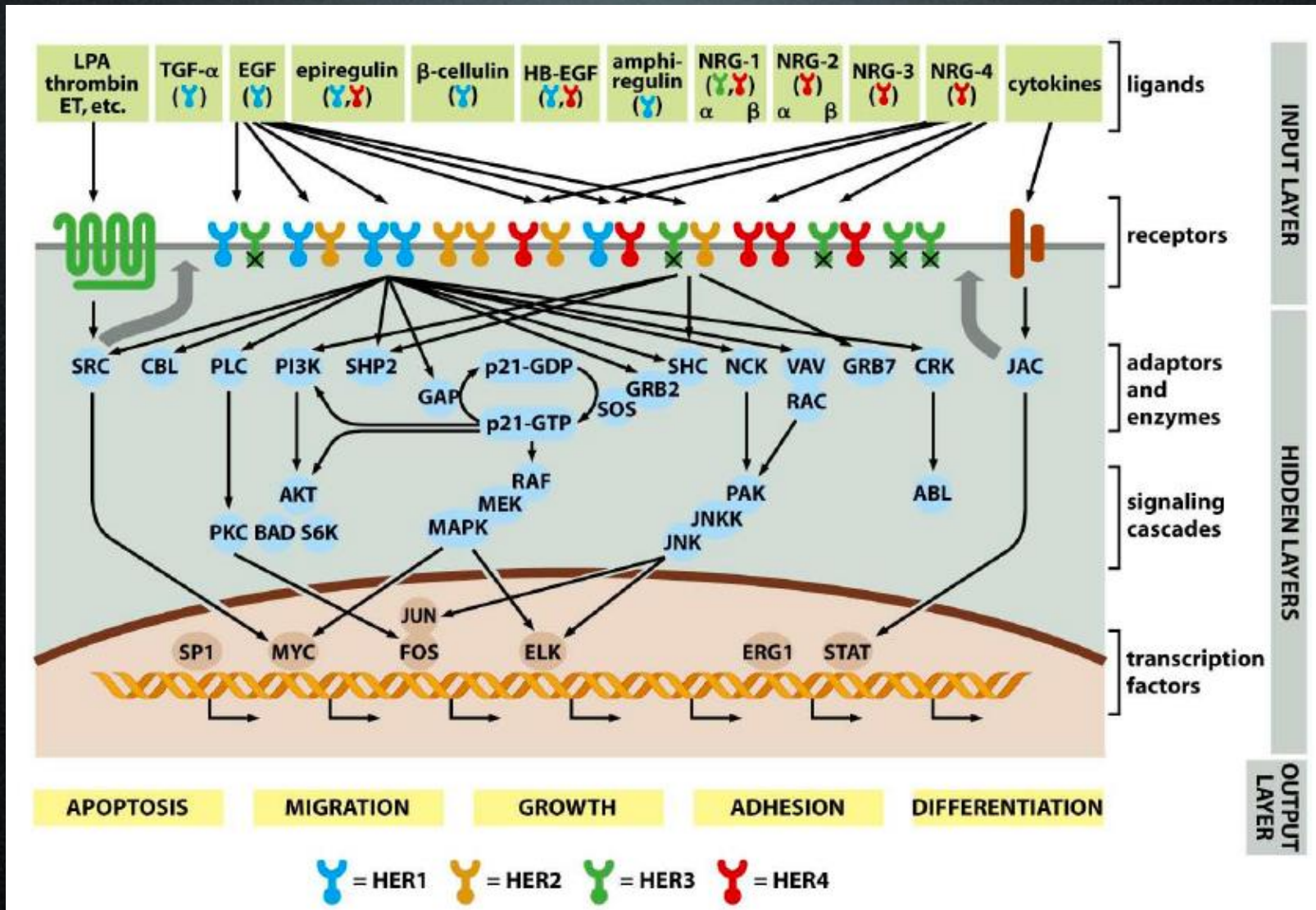


Figure 5.1 *The Biology of Cancer* (© Garland Science 2007)

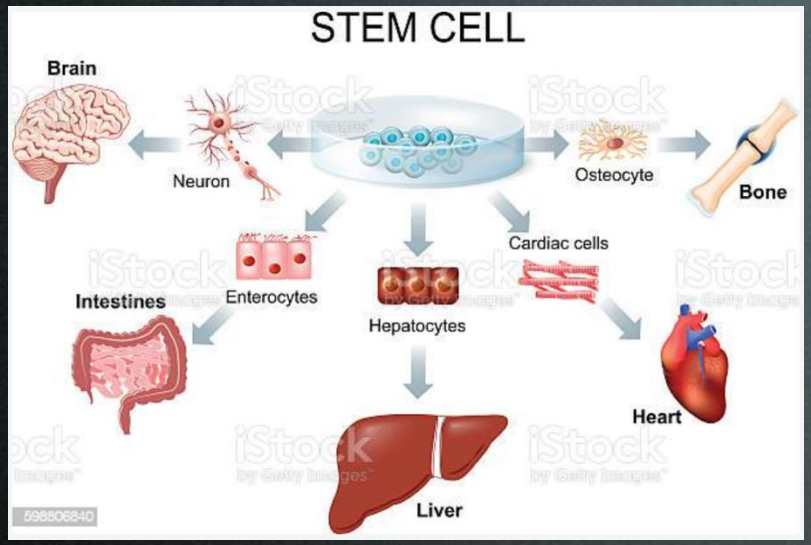
2. Developmental Biology <-> Cancer Biology

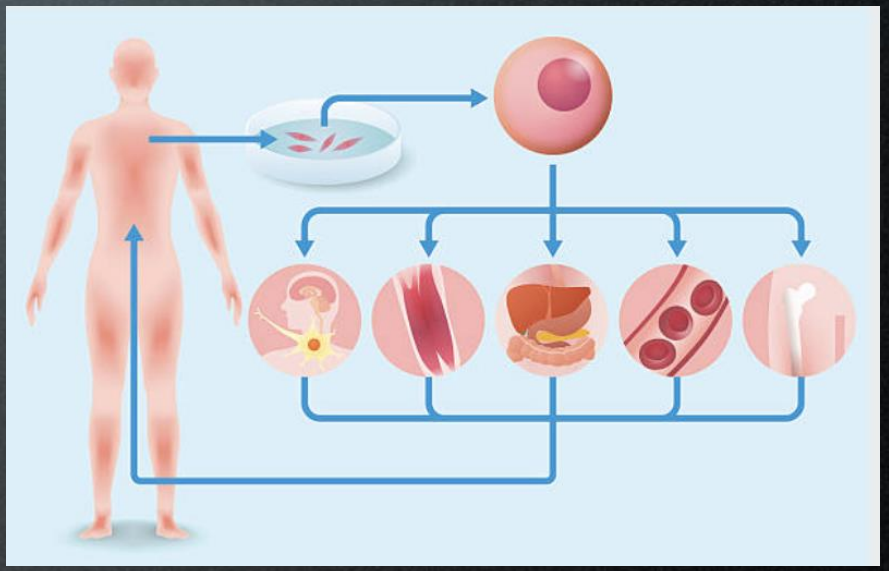
A. What are "normal" cell behaviors? What are "abnormal" ones?

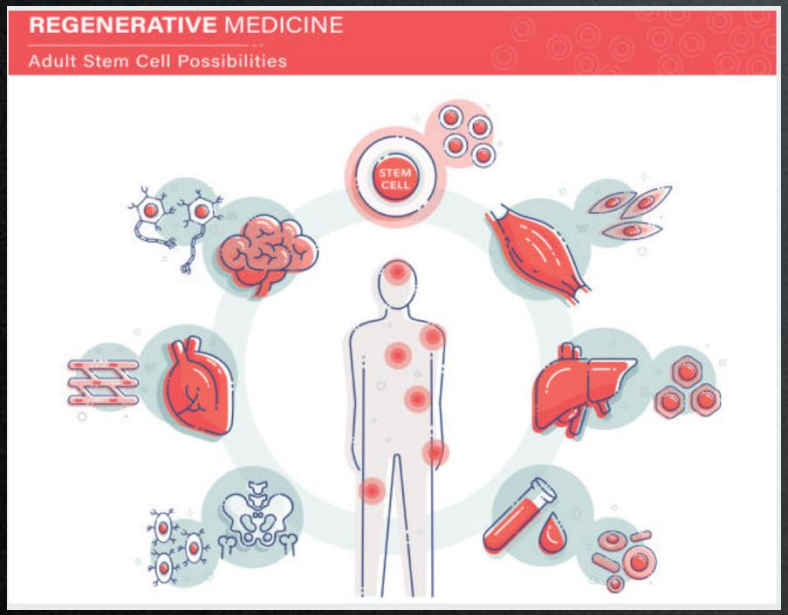
B. Context matters.

C. Logic behind the construction of signal transduction pathways to explain cells, tissues, and organs

D. Nature's Blueprint, Medicine's Promise: Regenerative Medicine


STEM CELL
Brain
Neuron
Osteocyte
Bone
Cardiac cells
Intestines
Enterocytes
Hepatocytes
Heart
Liver




REGENERATIVE MEDICINE
Adult Stem Cell Possibilities
STEM CELL

2. Developmental Biology <-> Cancer Biology

A. What are "normal" cell behaviors? What are "abnormal" ones?

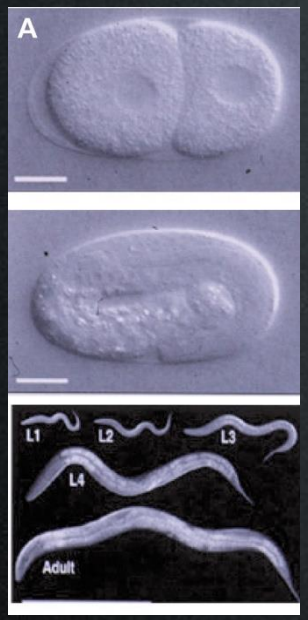
B. Context matters.

C. What is the logic behind the construction of signal transduction pathways ?

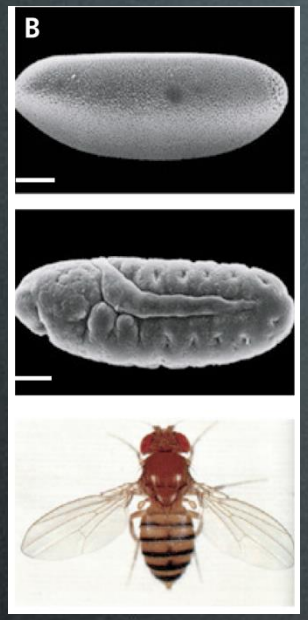
D. Nature's Blueprint, Medicine's Promise: Regenerative Medicine

E. Know your system. Options/the best system to answer your question

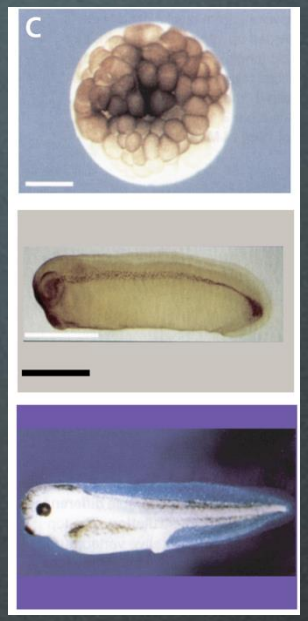
worm



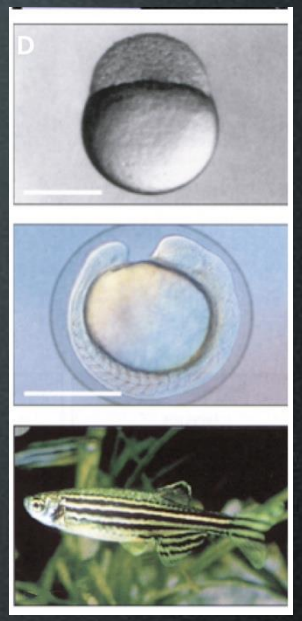
fly



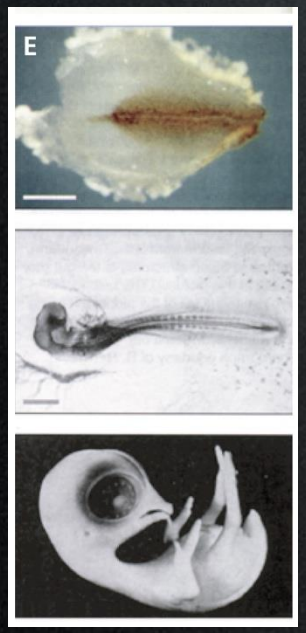
frog



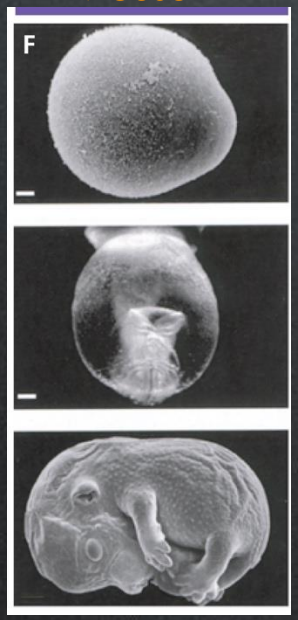
zebrafish



Chick

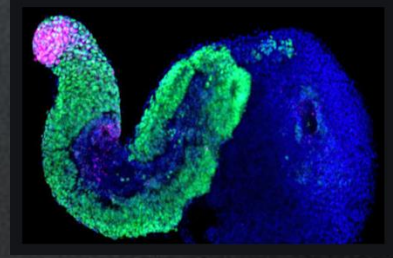


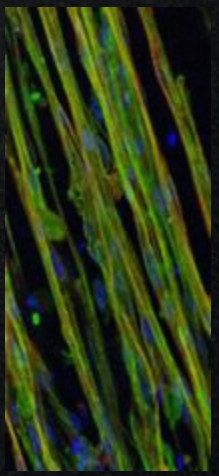
Mouse

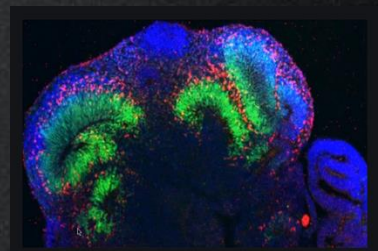


Gastruloids-Synthetic Embryos

Organoids and Stem cells







2. Developmental Biology

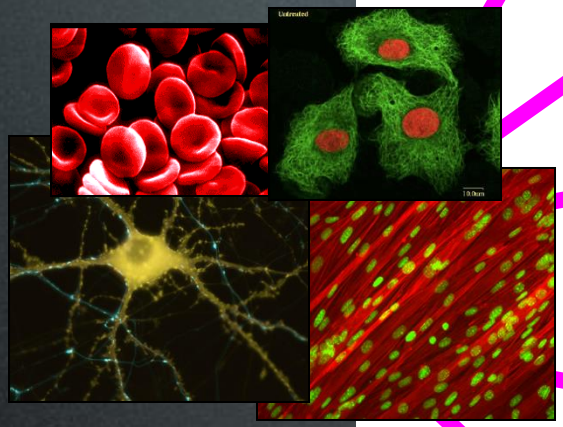
Not confined to a specific discipline

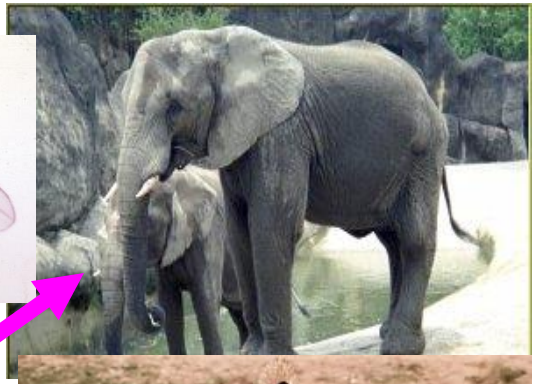
Not confined to any level of organization
(genes, cells, tissues, organs, organisms, ecosystems)

Remains pluripotent– not limited

Has the best questions in science

Fundamental Questions in DB





DB Focuses on relations, process, and context rather than entities

2. Developmental Biology Questions

How do tissues form at the proper place and time? What is the connection between the cell cycle and differentiation?

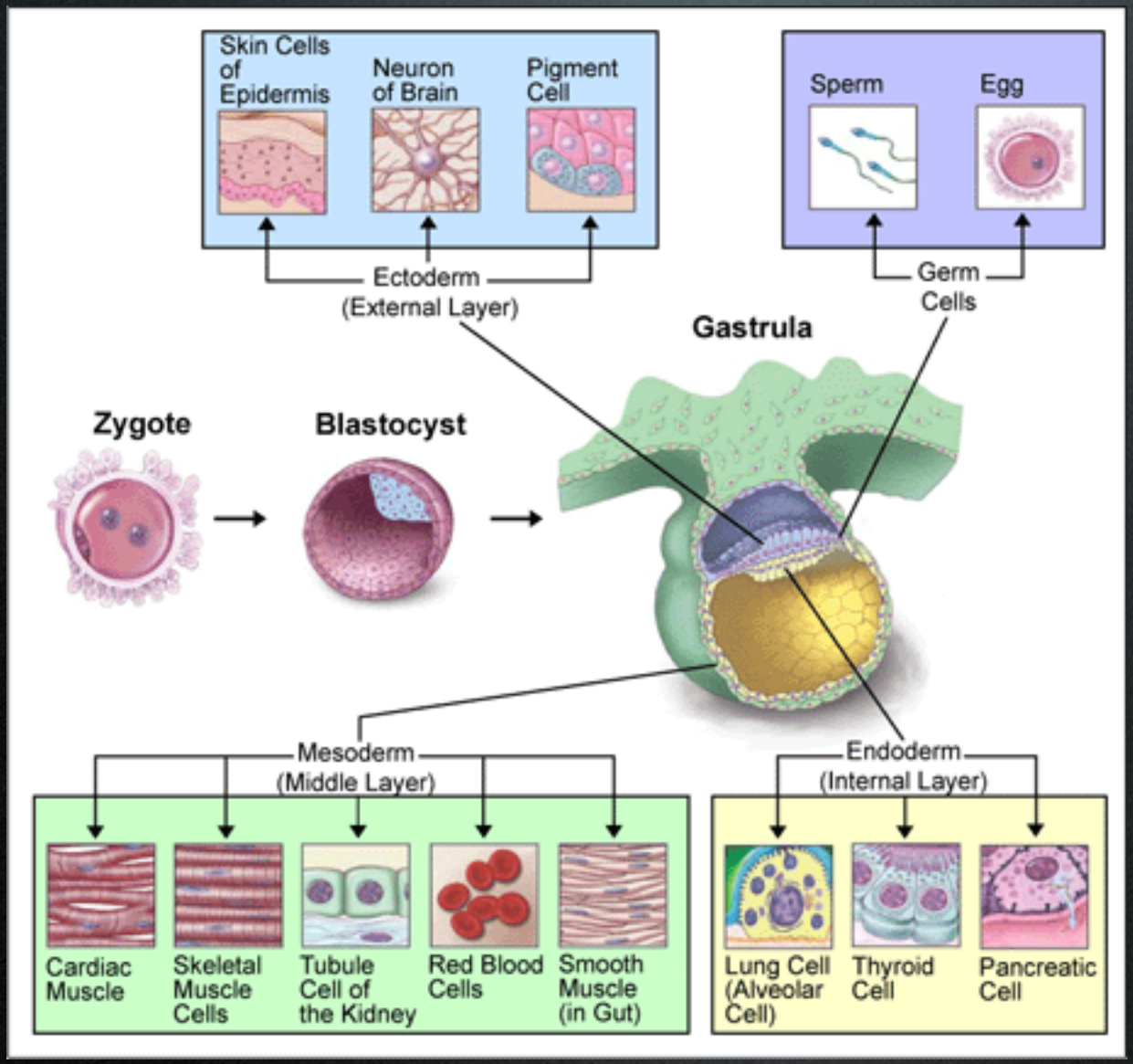
How are tissues/organs made reproducibly, with a specific size, shape, and function?

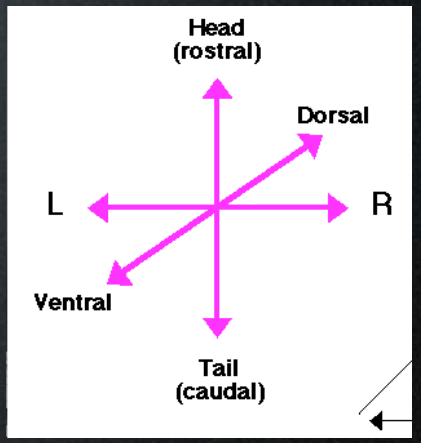
Within an organ, there are many different cell types, including adult stem cells – how do all these types form in the correct temporal and spatial pattern?

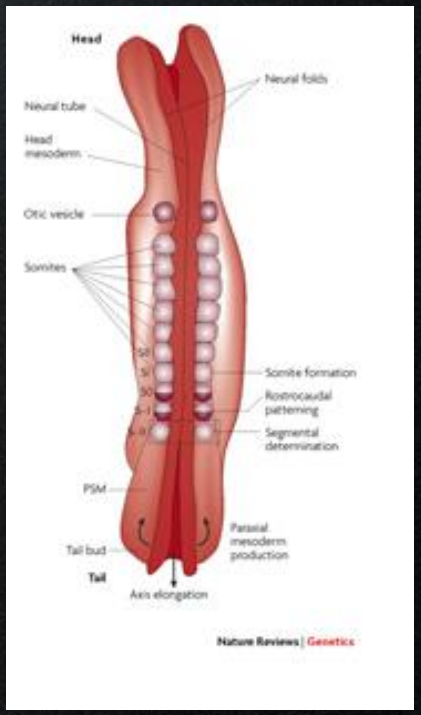
When do the different tissues form? How do different tissues coordinat and communicate their differentiation?

When do organs start functioning? Why can some organs regenerate and others cannot (and BTW…how can salamanders and planaria regenerate everything and we can't?)

Development = cell proliferation + creation of cell diversity organized in space and time







Drosophila Embryogenesis

Narrated by Philipp Keller, PhD
Group Leader, Janelia Research Campus

https://www.youtube.com/watch?v=FChS4KU5jDM


dorsal view
03:00:00
ventral view
50 µm

Two mechanisms to explain cell diversity and organization:

Mosaic development

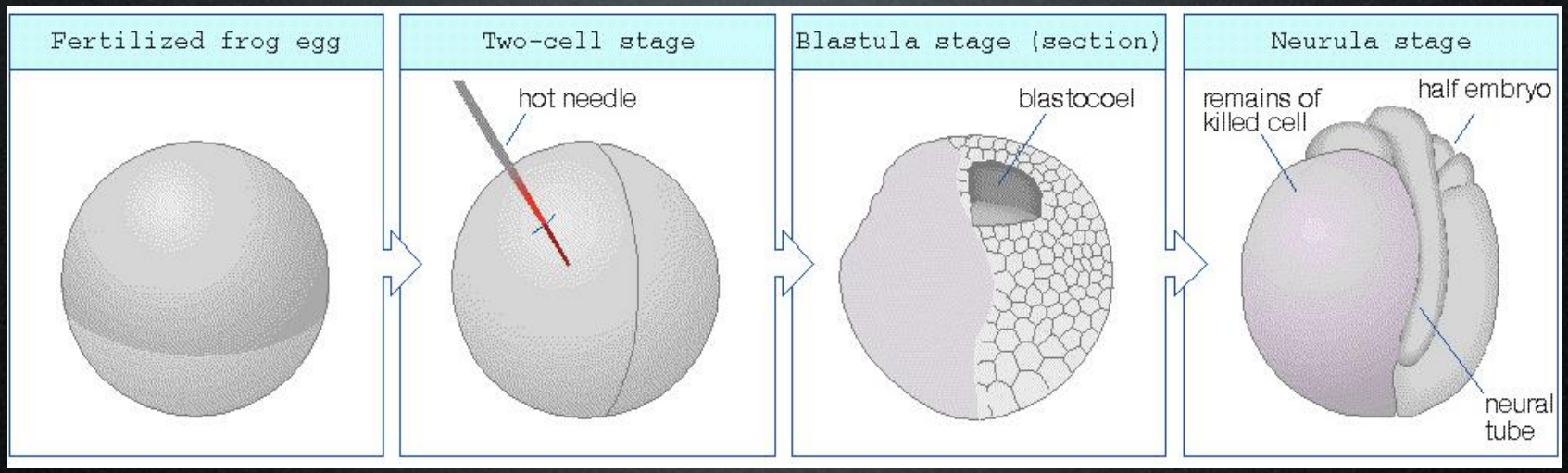
(aka inherited determinants and lineage)

Regulative development

(aka signaling and environment)

Mosaic development

(aka inherited determinants and lineage: Concept)

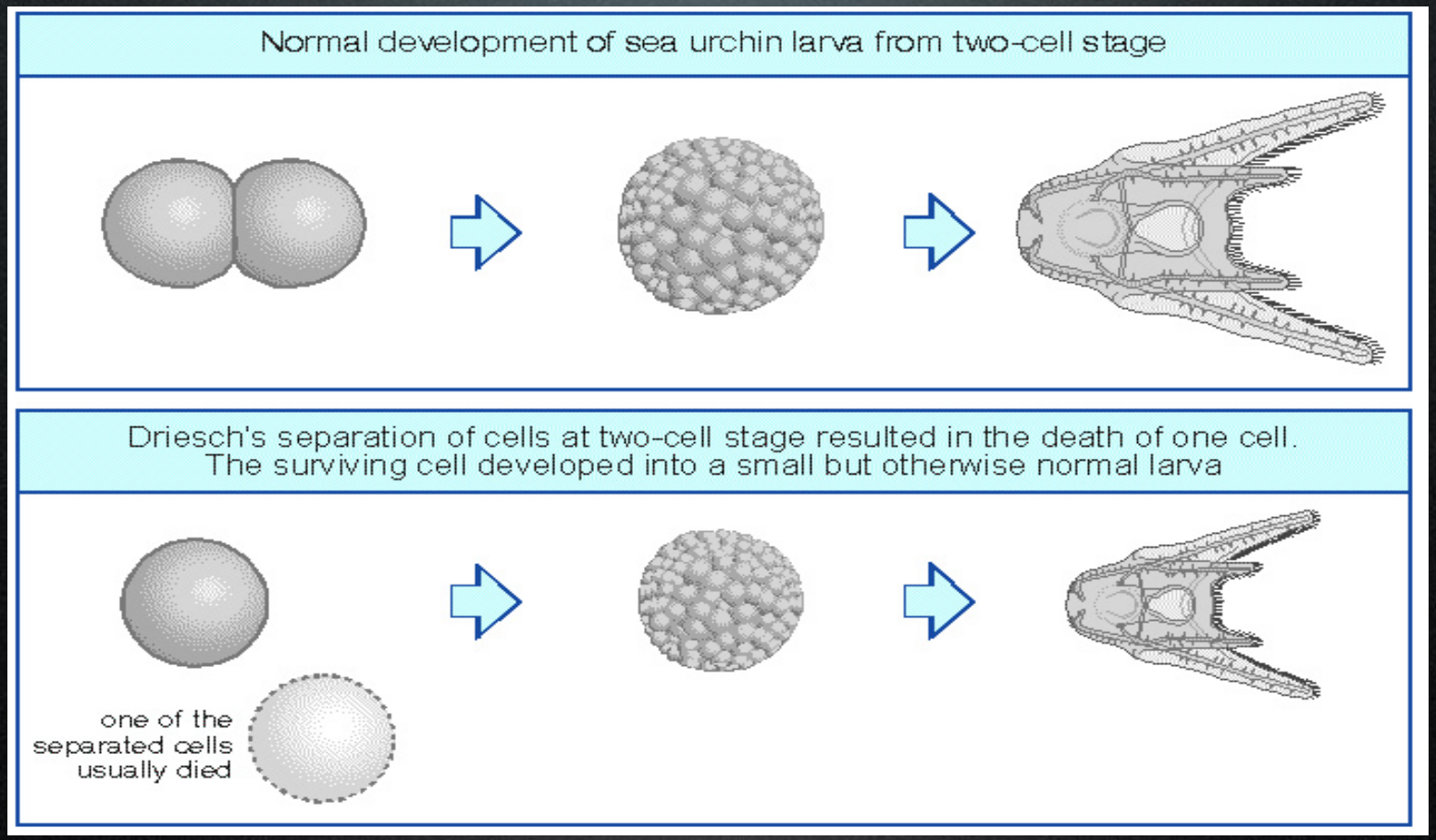


Roux, 1888

http://www.mun.ca/biology/desmid/brian/BIOL3530/DB_Ch01/DBNHist.html

Regulative development

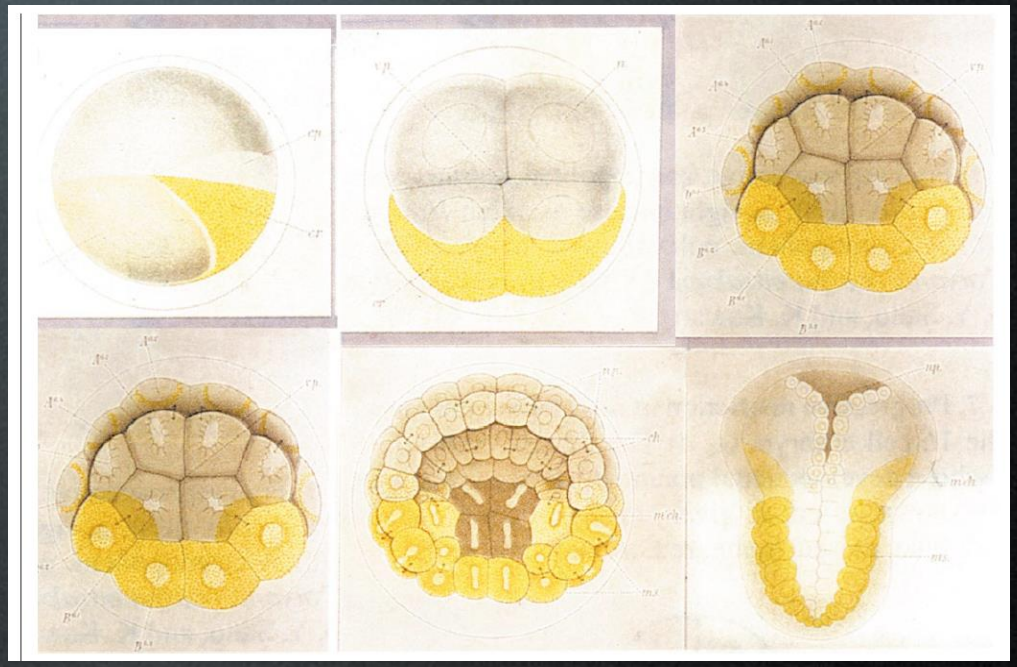
(aka signaling and environment: Concept)



Driesch, 1895

Mosaic development:

Yellow cytoplasm of Styela determines muscle development



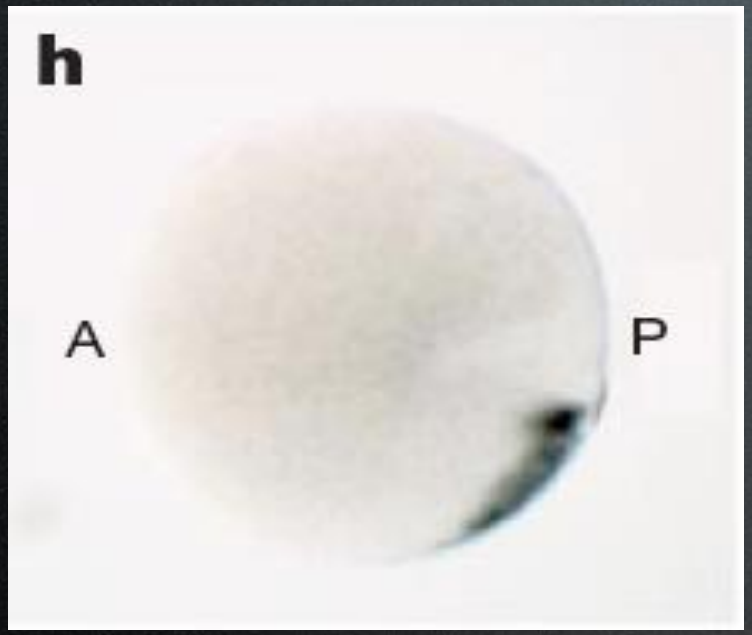
Conklin, 1905

What is the determinant in the yellow crescent?

How do you find this? Circa 1990s? Now?

Mosaic development/Lineage:

Macho 1 RNA as determinant in the yellow crescent?



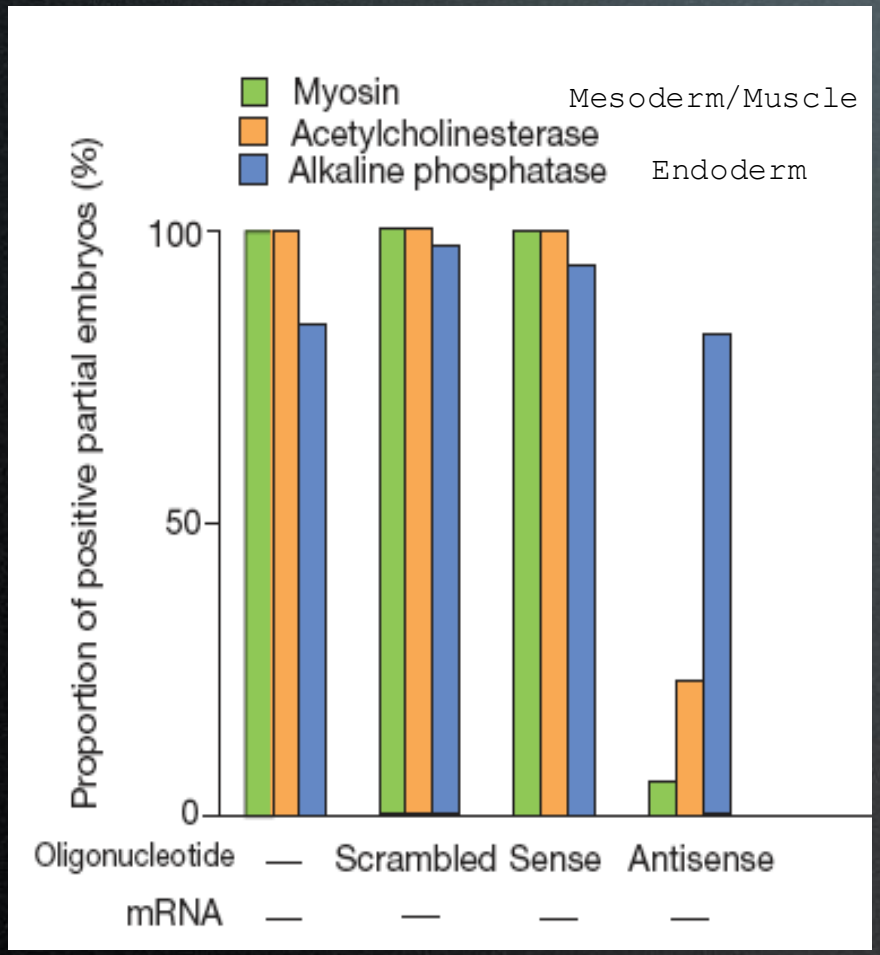
Nishida and Sawada, 2001

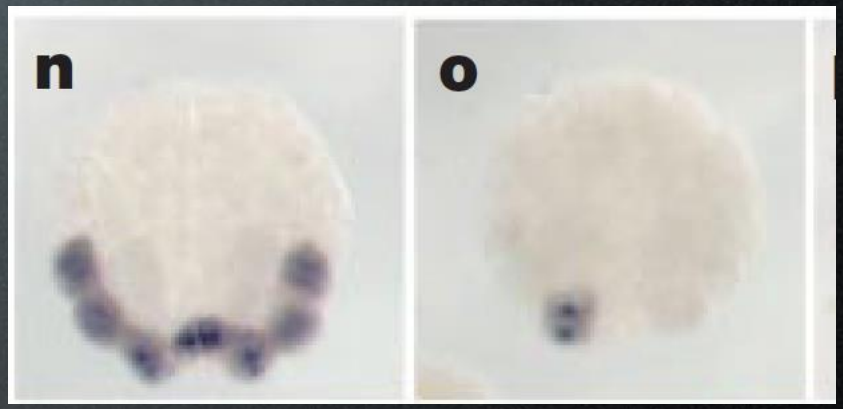
RNA localized!

Subtractive hybridization, insitu

Mosaic development:

Macho 1 antisense





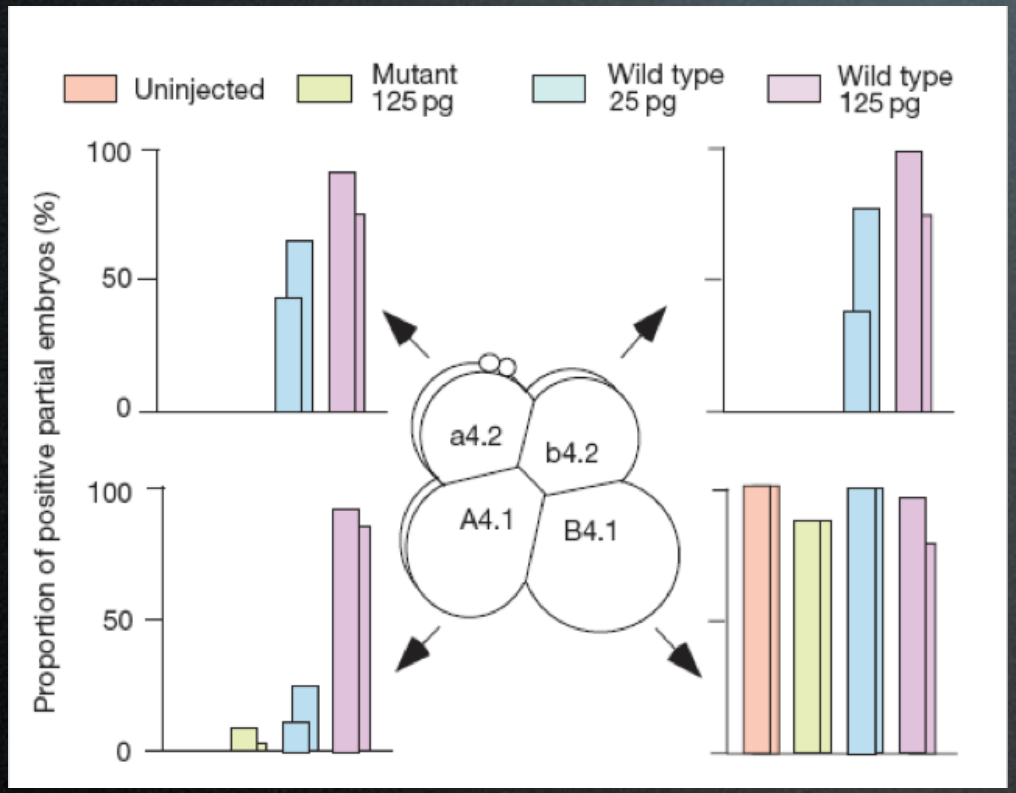
Control
Macho RNA insitu

Antisense injected
Macho RNA insitu

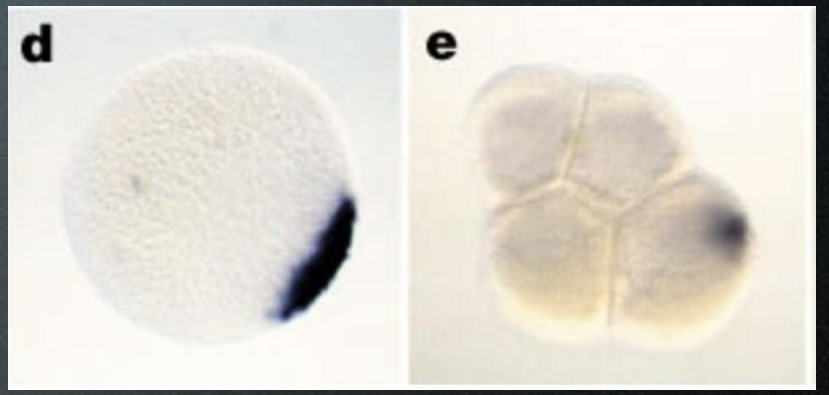
Concept : necessity and sufficiency

Mosaic development:

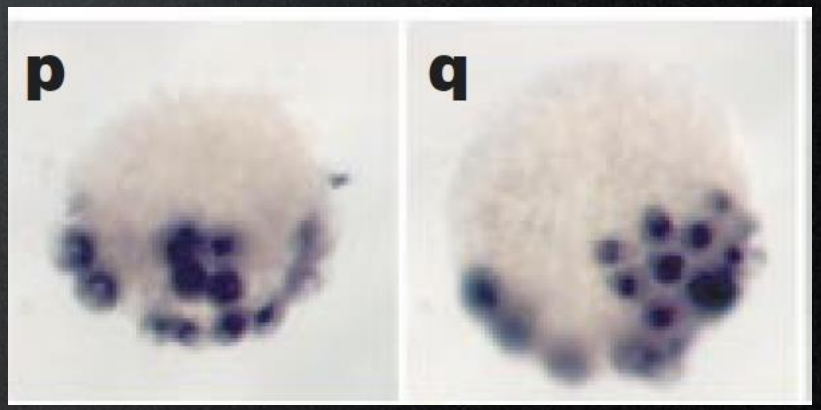
Macho 1 overexpression



Macho 1 expression



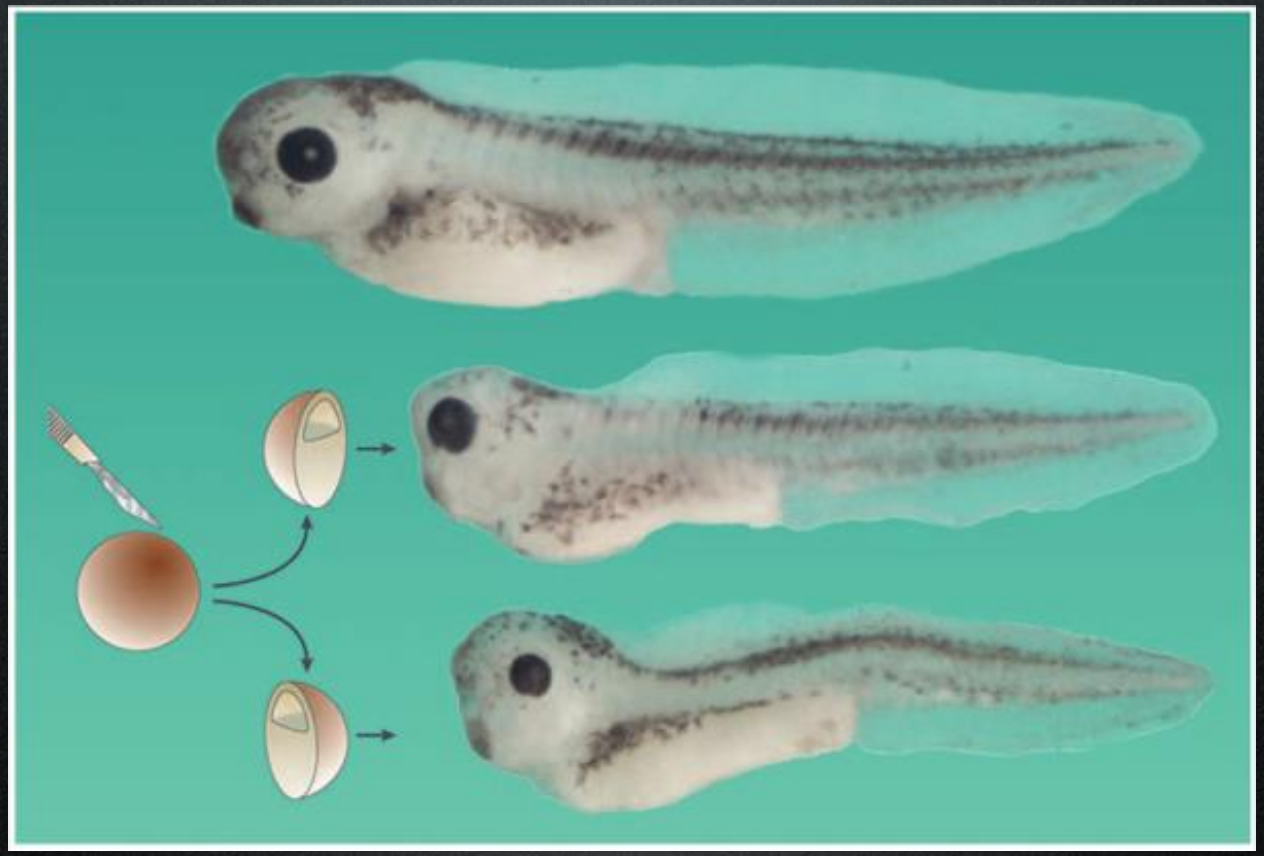
Macho 1 overexpression



Front= myosin; Back AchE

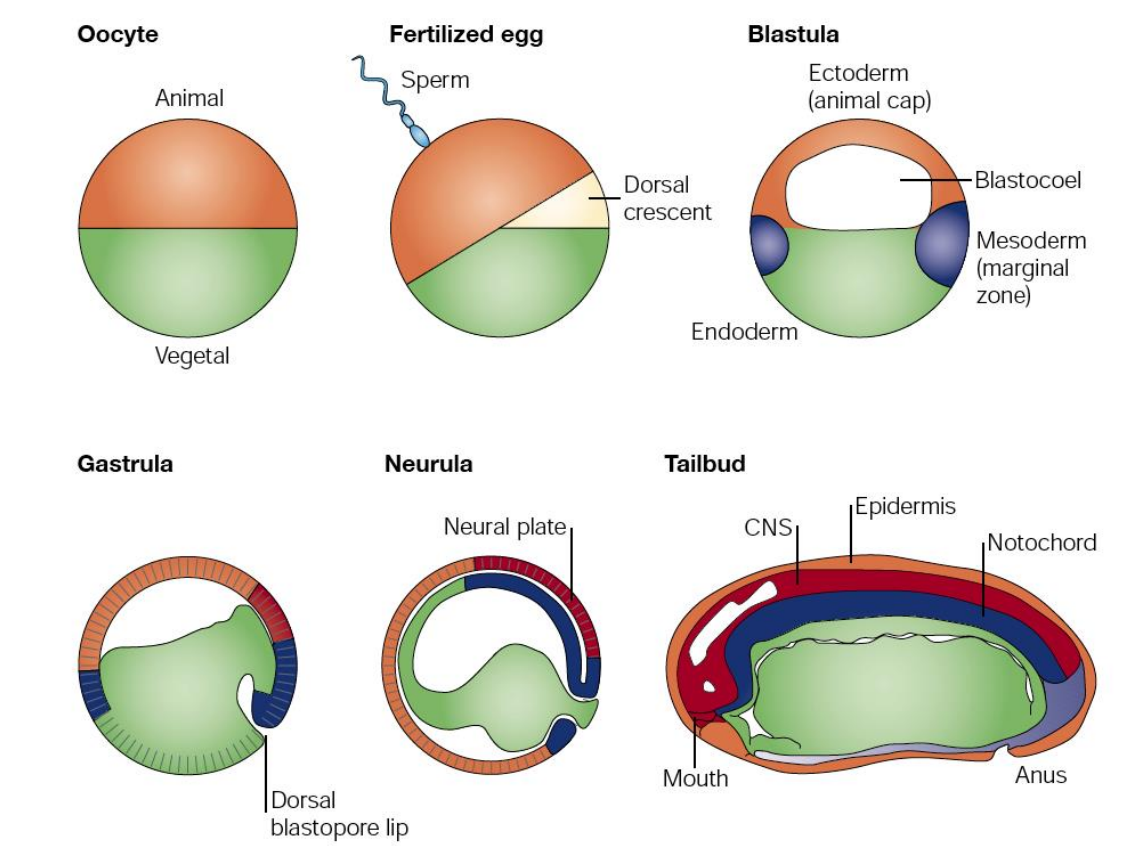
B4.1 usually gives rise to primary muscle
A4.1 aand b4.2 some secondary muscle
A4.2 do not make muscle.

Classical Example of Regulative development: Xenopus

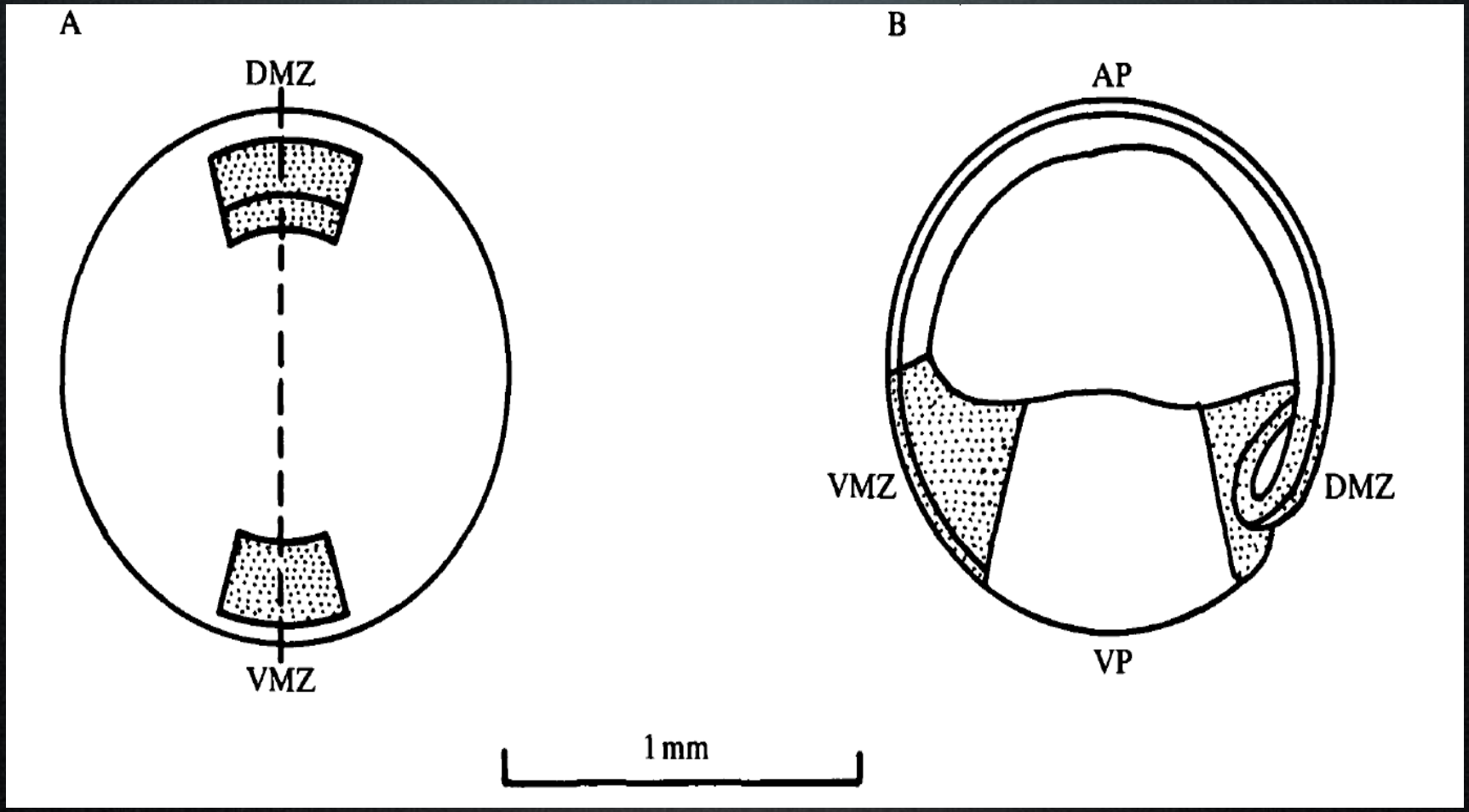


First shown by Hans Spemann 1903

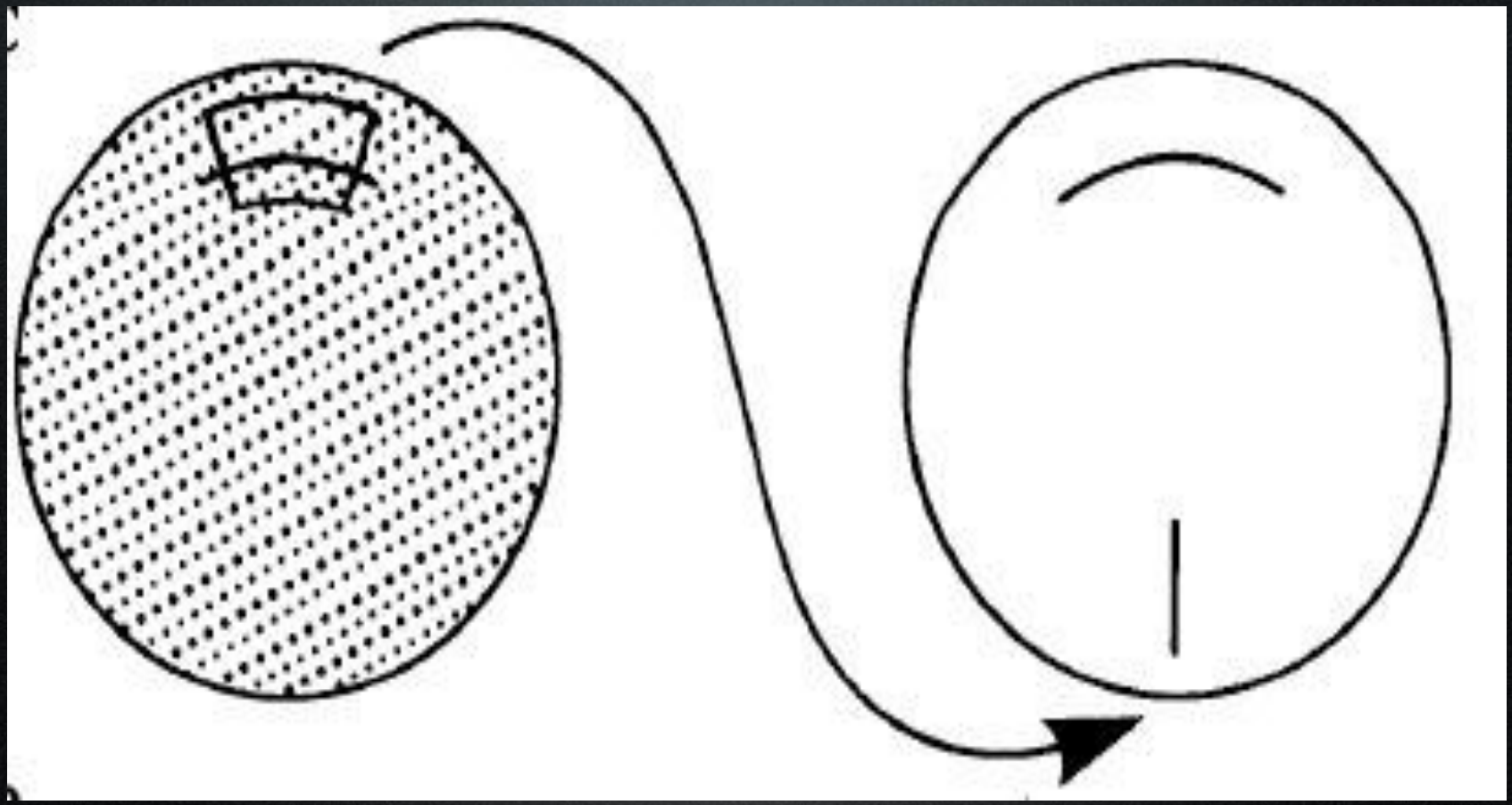
Xenopus embryogenesis



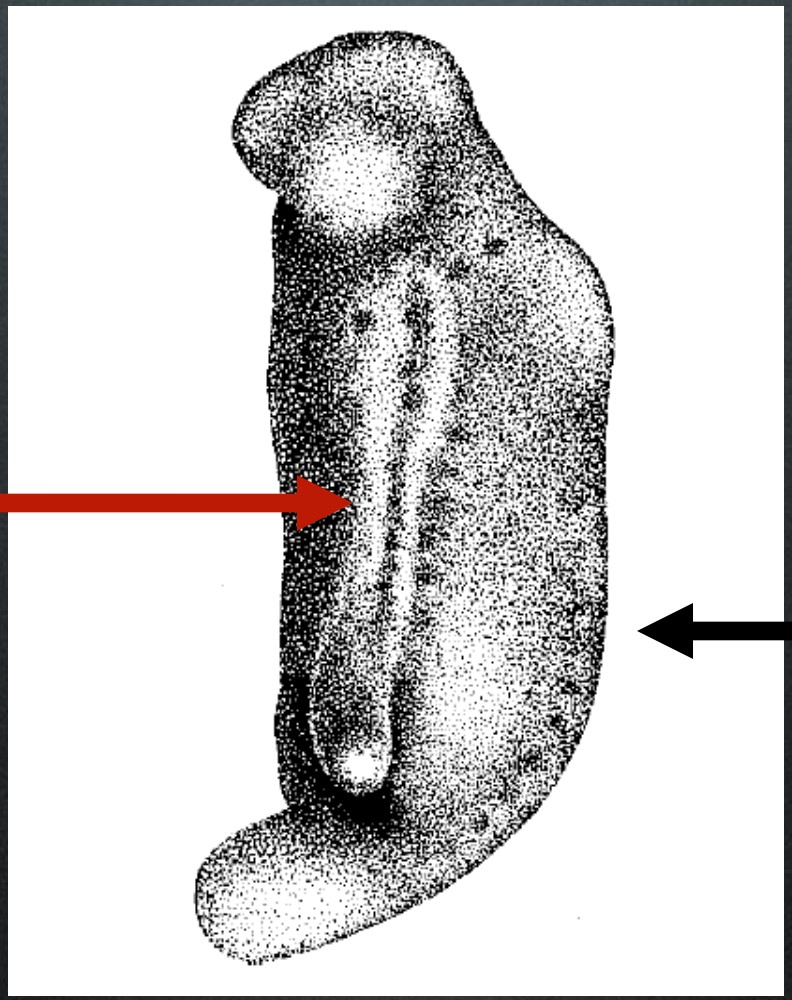
The organizer: the dorsal marginal zone



The organizer: transplantation



Spemann and Mangold dorsal lip transplantation (1923)

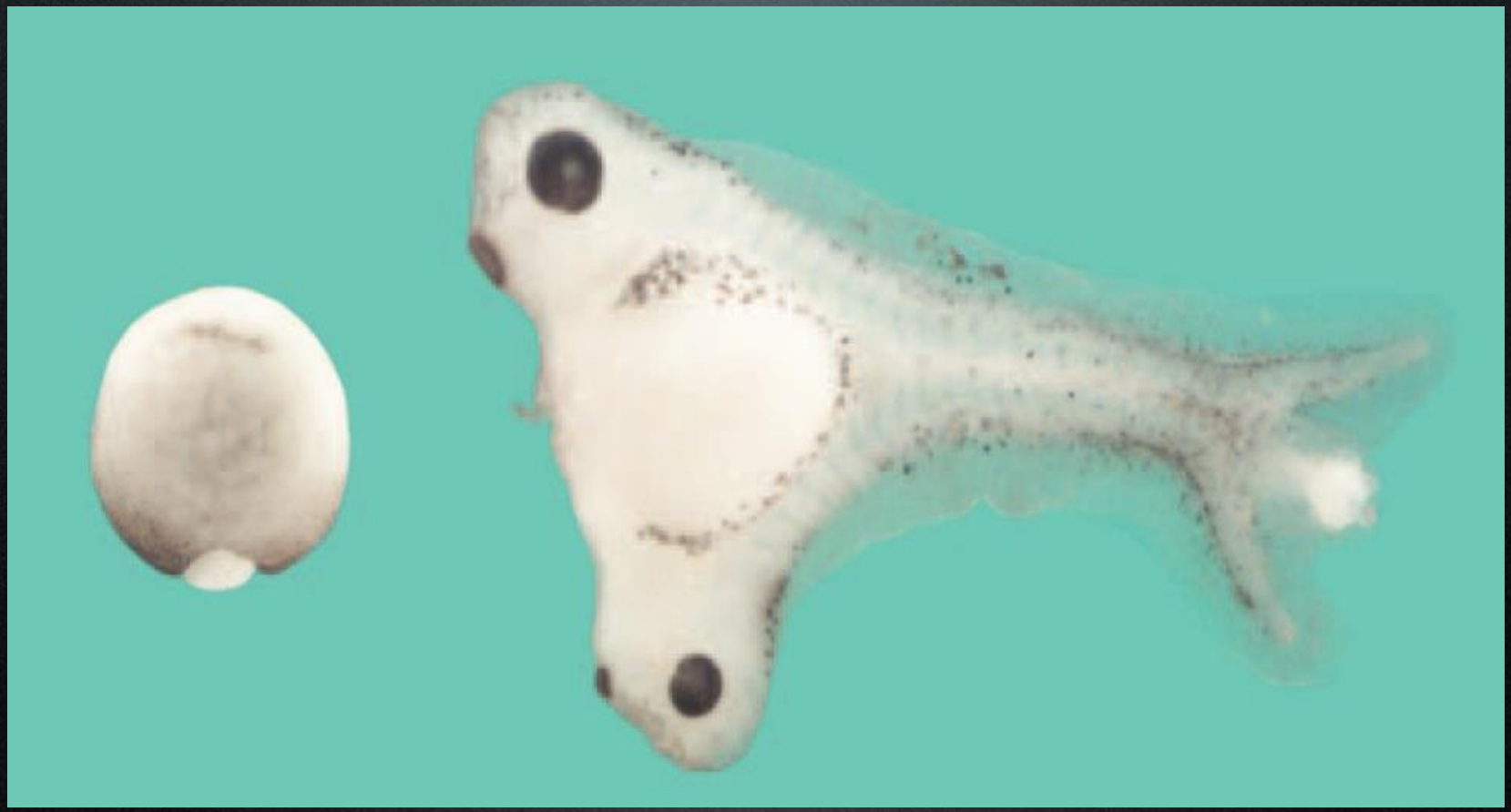


Secondary axis

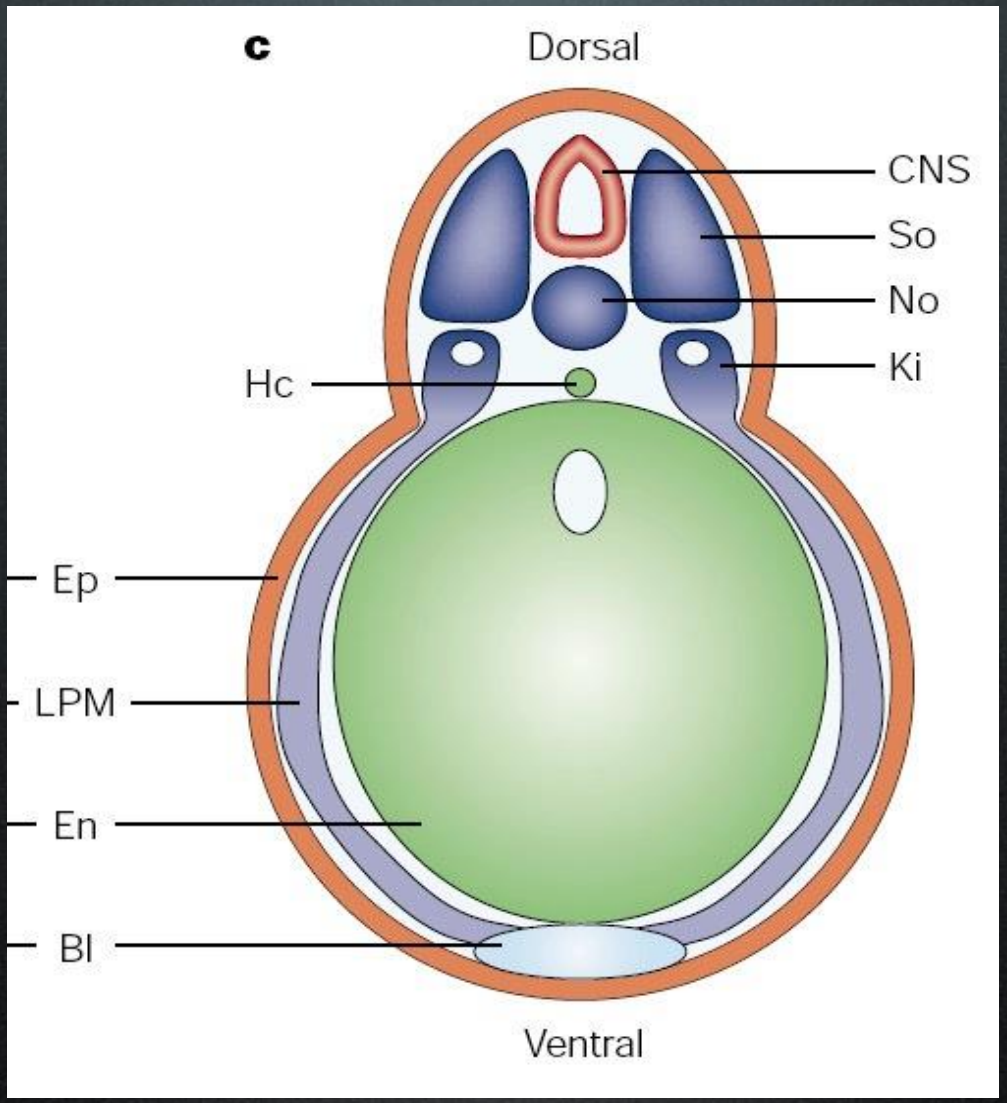
Primary axis

Triton (newt) embryos

Dorsal Lip transplant (Xenopus)



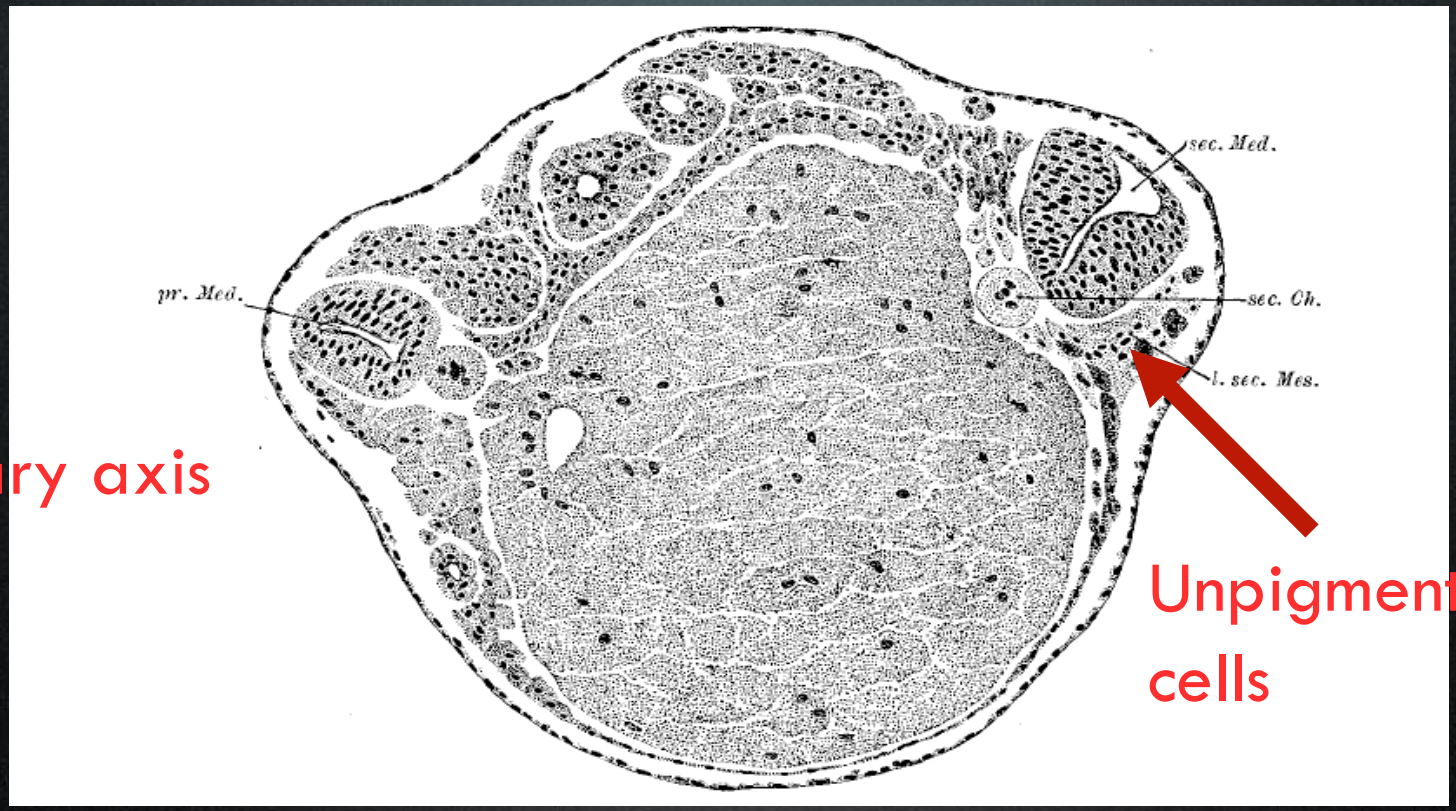
Cross section of amphibian embryo



Dorsal

Ventral

Spemann and Mangold dorsal lip transplantation: The idea of an "inducer"



Primary axis

Unpigmented cells

Pigmented host, unpigmented organizer graft

How could you do this experiment today?

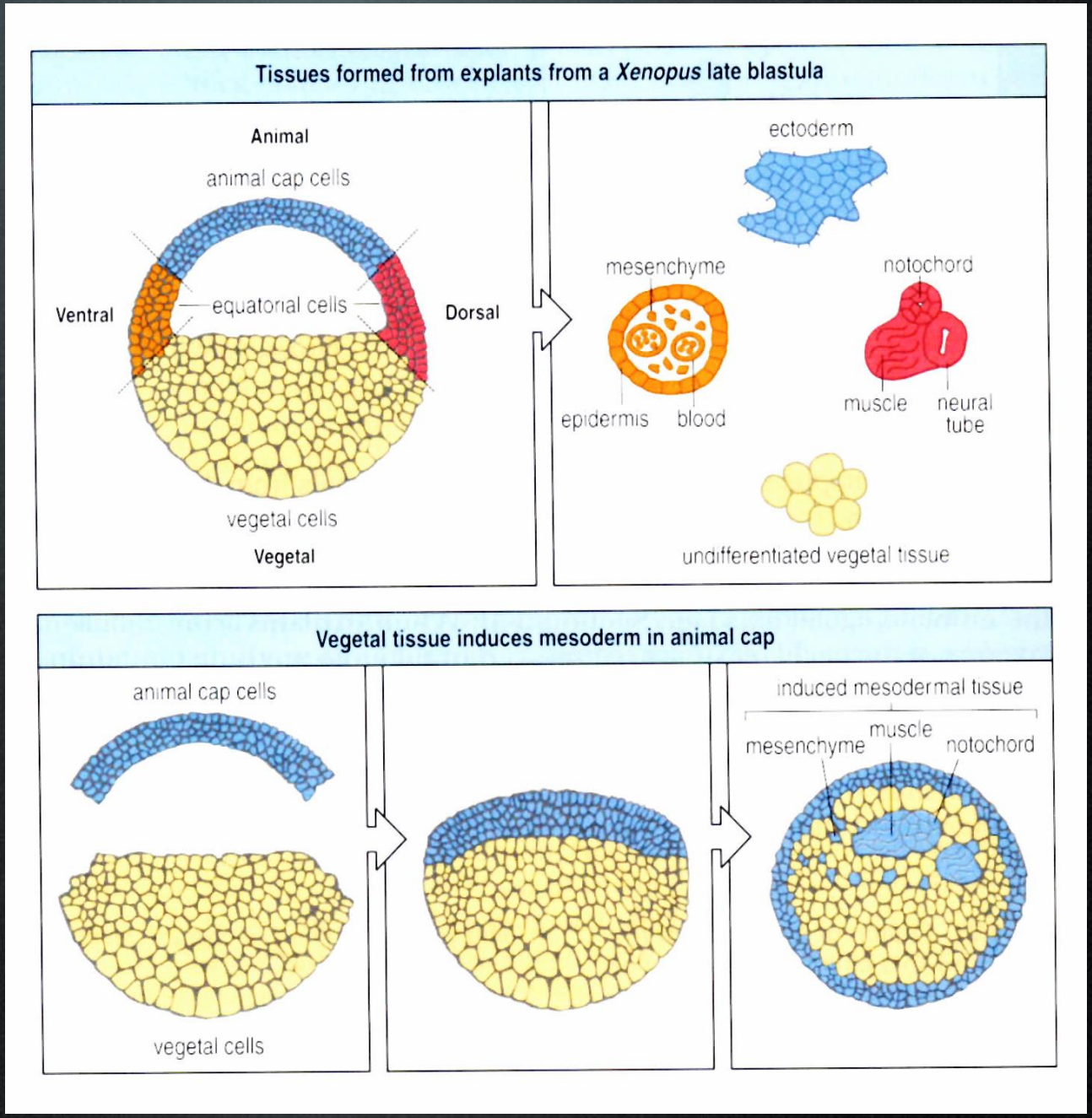
Environment/ Regulative development: Concepts

Induction: Process whereby a cell(s) instructs a program of gene expression or activity on other cells.

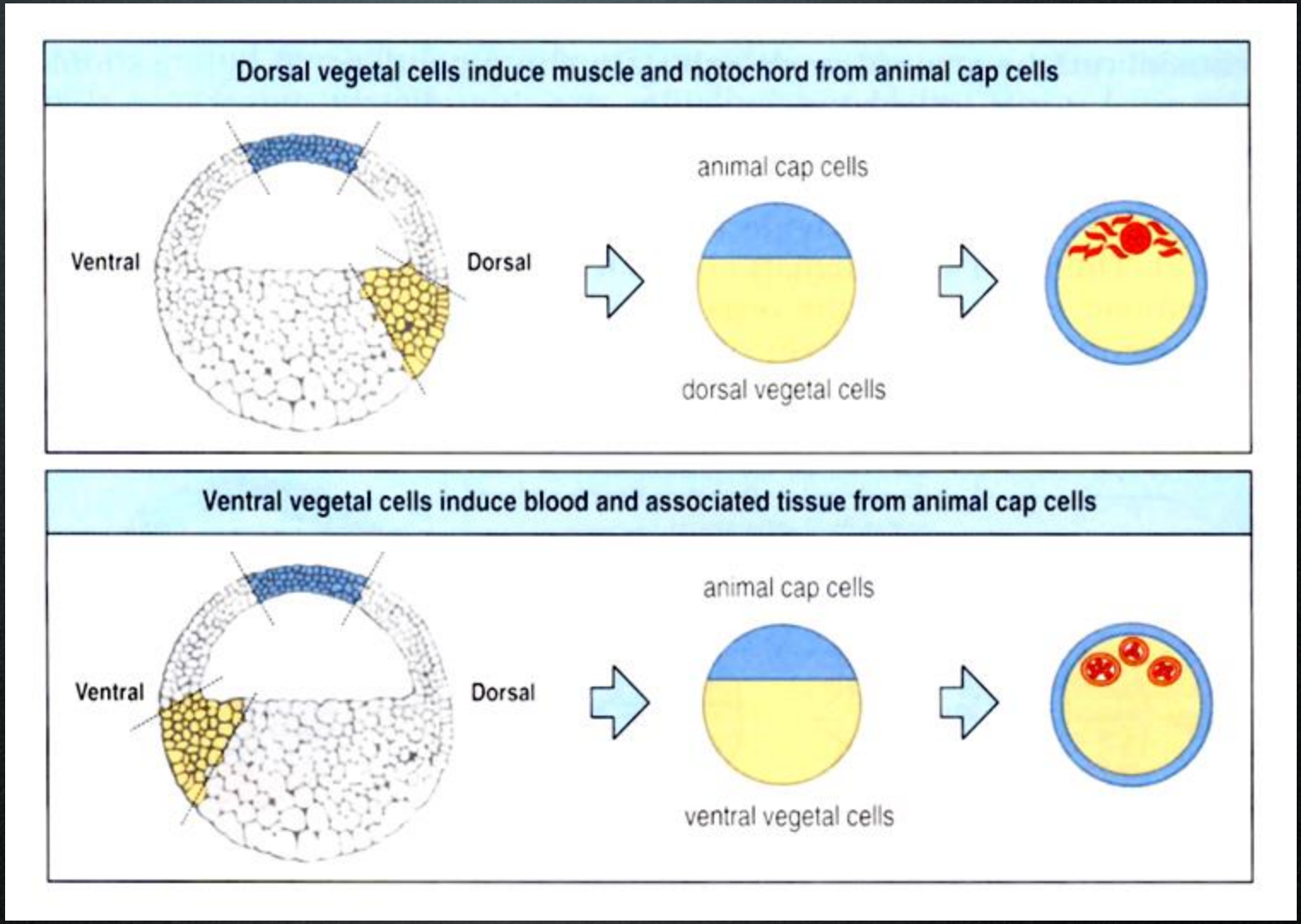
Competence: the ability of cell(s) to respond to an inducer

Classical Example of **Induction**:

Xenopus Mesoderm

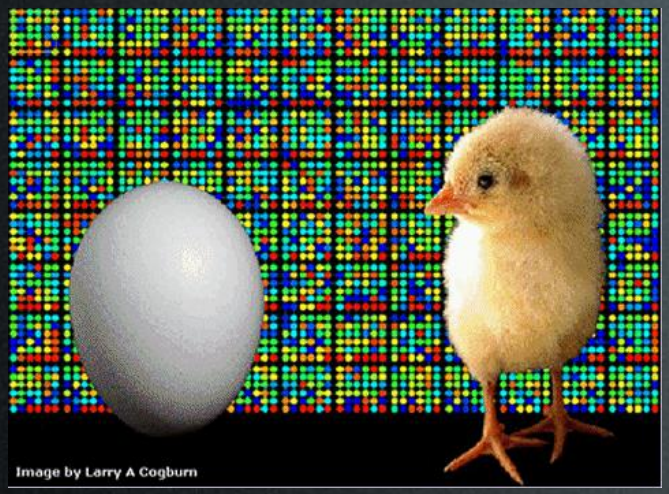


How do you interpret this experiment?

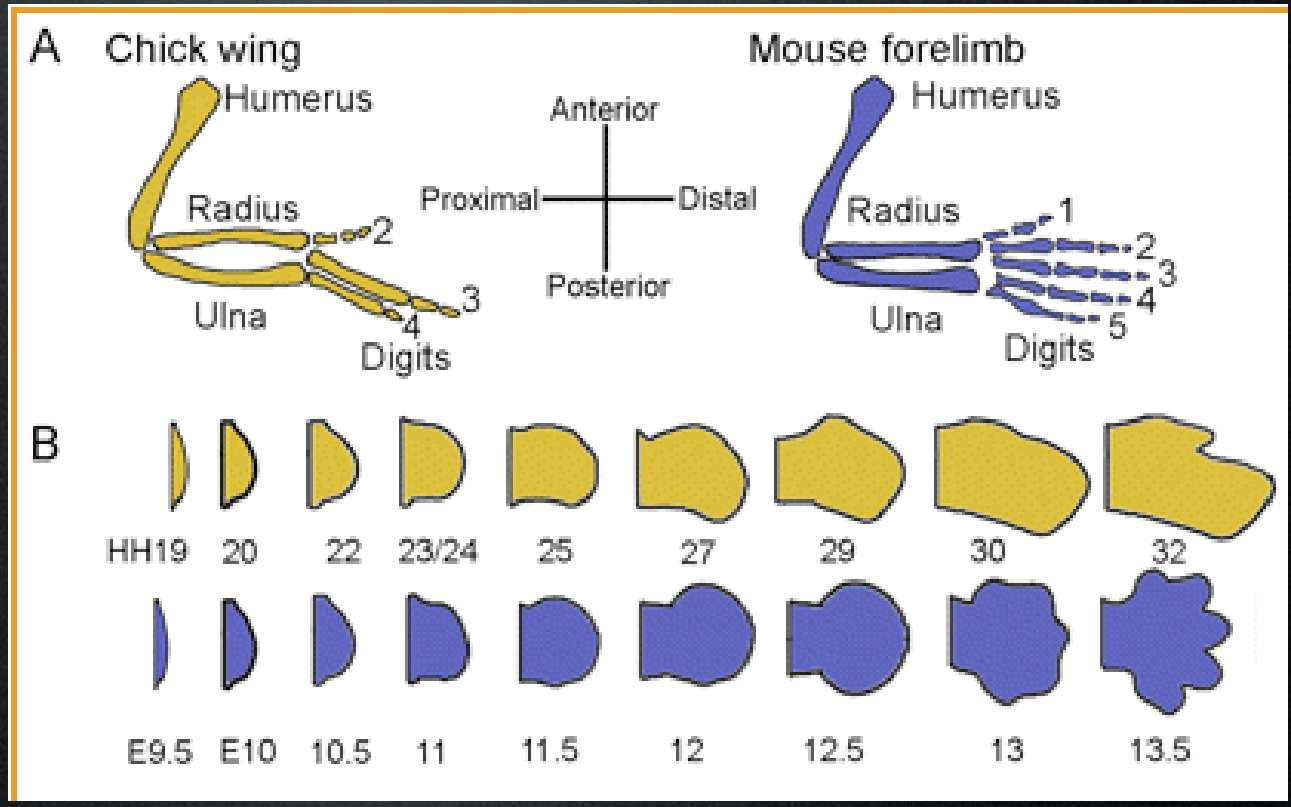


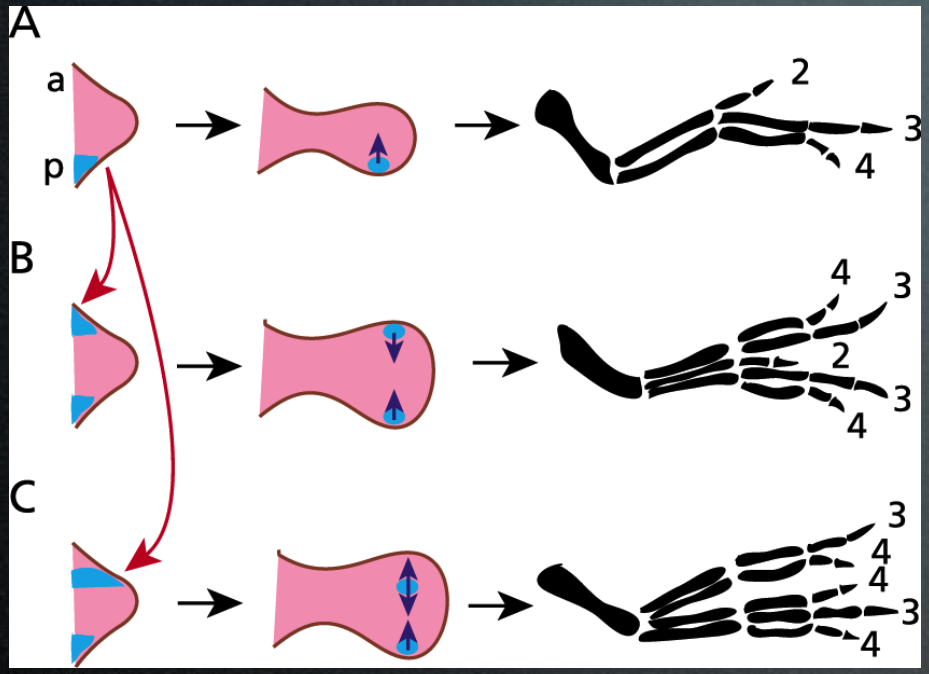
1. Different inducers come from the dorsal and ventral vegetal regions.

Or 2. Same inducer different amounts

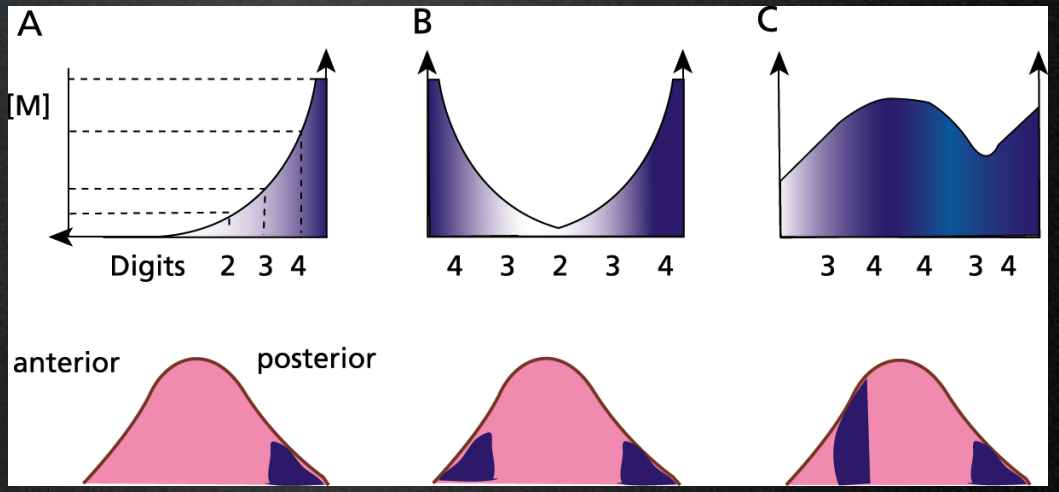


Example of a Long range Instructive process: Limb Development

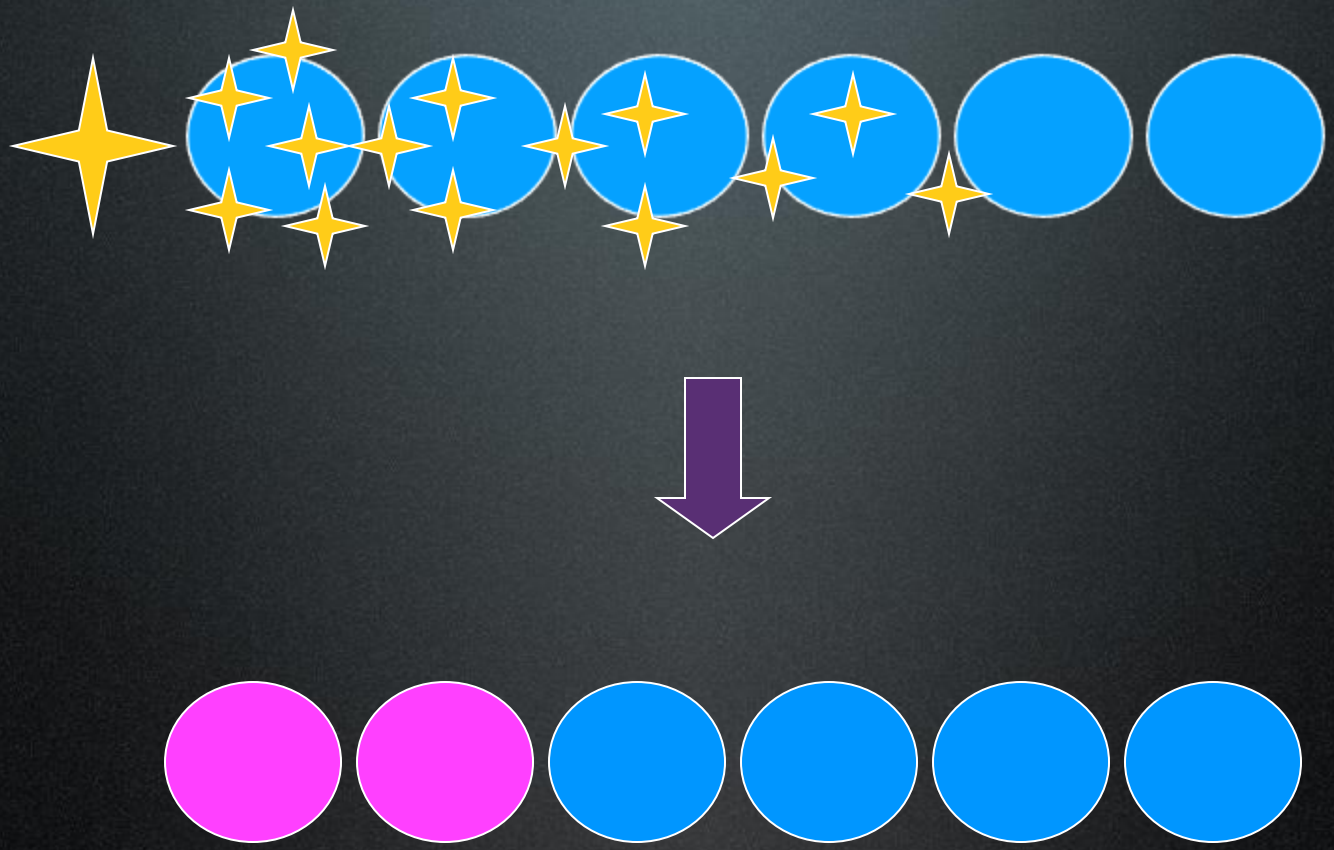




Example of a Long range Instructive process



Concept: Positional Information: cell fate determined by position in a field relative to an instructive substance.



Cells read a long range instruction and respond OR cells read a series of short range signals.

Concept: Signaling shapes development

Why signal transduction pathways have the "perfect" qualities to be inducers*

1. Directional : source ---> target

2. Amplification: transfer of info in several steps allows for potential to amplify "signal", especially if the steps operate with different efficiencies.

3. Diversification: steps serve to connect different pathways
   e.g. Dsh/Dvl – Notch/Wnt signaling

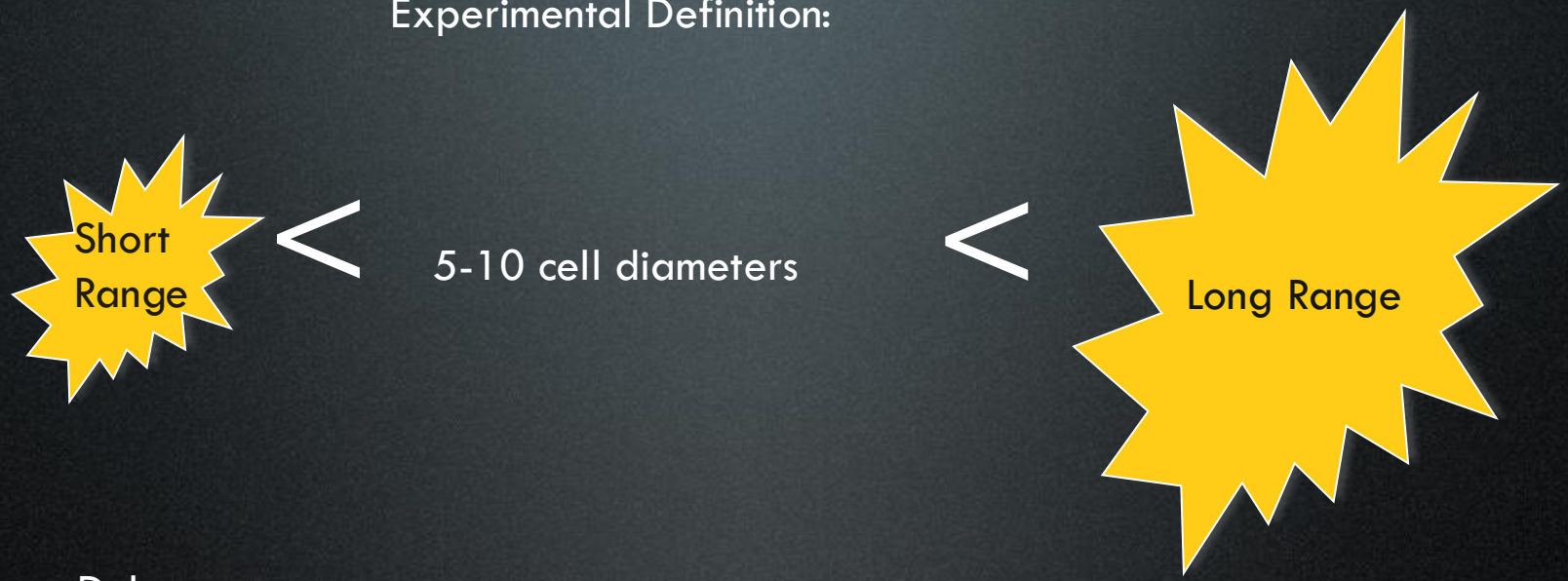
4. Integration: info from more than one signal produces a single response

5. "Controls" : mechanisms to turn signal flow "on" or "off"
   e.g. feedback loops

*but don't rule out transcription factors!!

Signals can be SHORT or LONG Range

Experimental Definition:

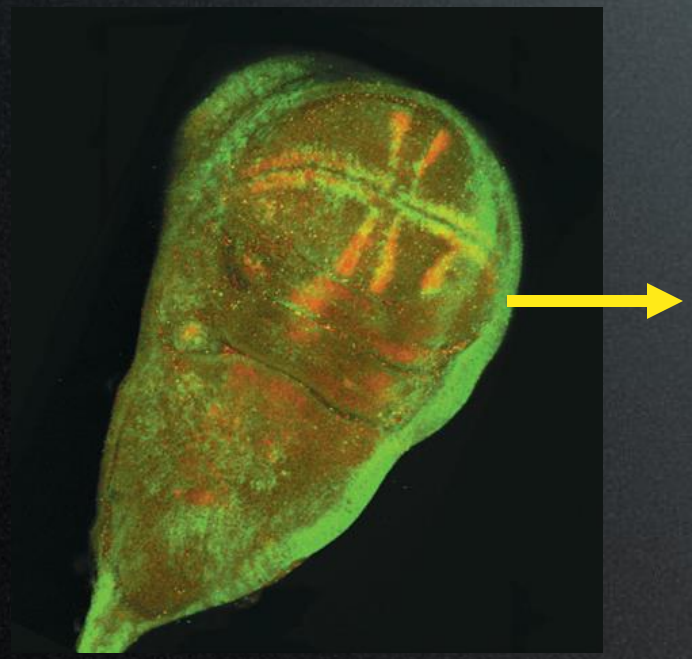


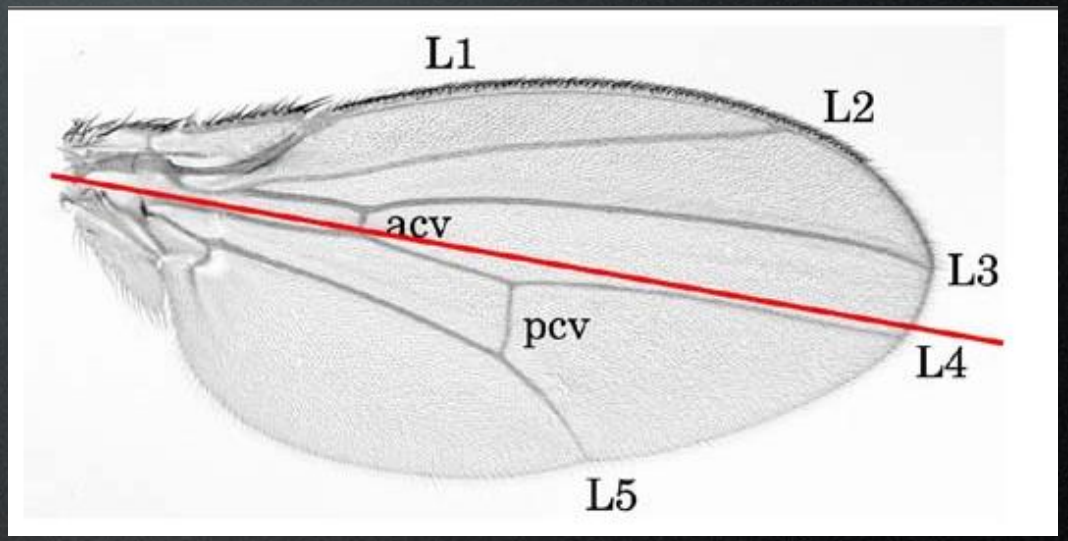
e.g. Delta
Serrate
Ephrins
EGF
FGF
Hedgehog

e.g. Retinoic Acid
BMPs
Wnts
Hedgehog
Hormones

Signals can be LONG or SHORT Range

1. Experiments to determine Range: example wing of the fly

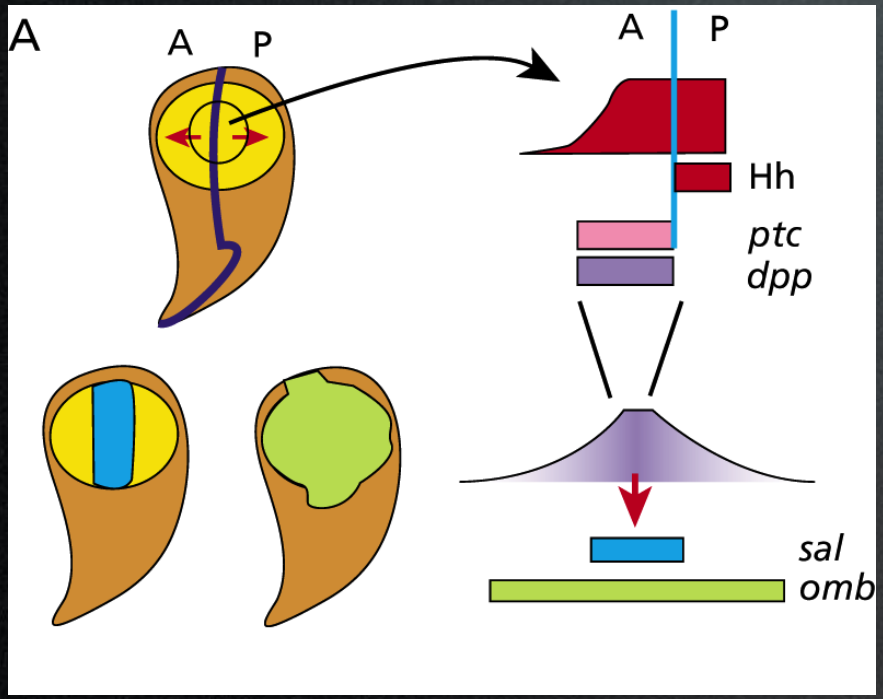


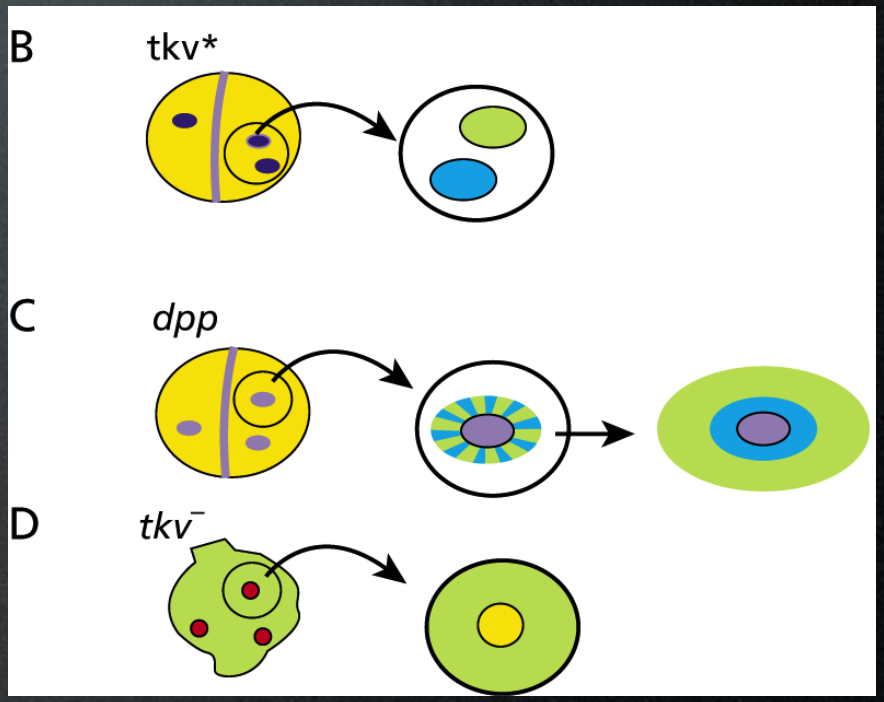


Signals can be LONG or SHORT Range

1. To determine Range, one needs:

   *good markers of function (e.g. gene expression, changes in adhesion)
   *localized signal (e.g.signal pattern itself, beads….)
   *field of cells that doesn't change significantly in dimensions during signaling





Concept: mosaics/clones to understand signaling

2. Visualization of Range:

*Label signal with radioactivity or gfp,
*Provide from a localized source and analyze distribution: Wnt, Activins.

3. Specificity of a long range signal,
i.e. Is signal truly long range?

As shown in previous slide: *Removal of receptor in cells at a distance from the source.

*Inhibition of protein synthesis and measurement of target gene transcription to exclude intermediates.

Range is influenced by:

* Intrinsic biochemical properties of signal

* Interactions with other molecules

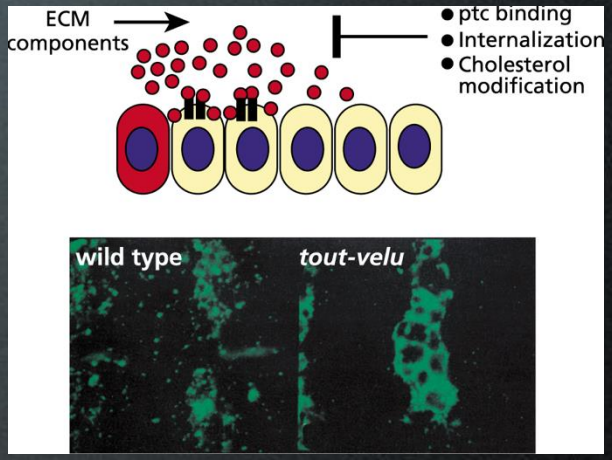
Concept: diversity of responses from one signal

Specifically

(7 different possibilities as of today):

1. Ligand modification

   e.g. Hedgehog -- proteolysis and cholesterol addition



2. Interactions with Extracellular Matrix/Proteoglycans

e.g. Wingless (Wnt)-- heparin sulfate interactions/Dally

Hedgehog -- "tout velou" regulates synthesis of proteoglycans

affects RANGE over which Hh signals

3. Secretion

e.g. Porcupine [encodes an ER protein] regulates Wg secretion

4. Receptor-signal interactions are complex

Sequestering -- Hh and Patched

Removal of Ptc leads to greater range of Hh protein and hence signaling

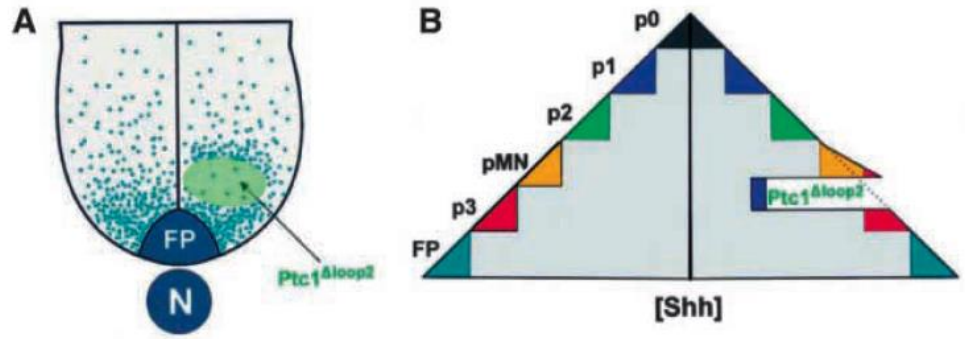


Fig. 6. A model for effects of Patched (Ptc) on neural patterning. (A; left half) Shh emanating from the notochord (N) induces formation of the floor plate (FP), and subsequent *shh* expression in the FP generates a ventral-dorsal activity gradient of Shh (as indicated by the density of the blue dots). (B; left half) The activity gradient of Shh promotes the specification of a series of ventral cell types: p0, p1, p2, pMN and p3, which are progenitor domains from which distinct V0 neurons, V1 neurons, V2 neurons, motoneurons and V3 neurons are generated respectively. Production of a mutated form of the Shh receptor Ptc ($Ptc1^{\Delta loop2}$; A; right half; light green), which does not bind Shh but antagonizes its signaling, causes cell-autonomous abnormal dorsal spread of Shh and (B; right half) ventral-to-dorsal switches in neural progenitor identity. Modified with permission from Briscoe et al. (Briscoe et al., 2001).

5. Ligand turnover -- endocytosis

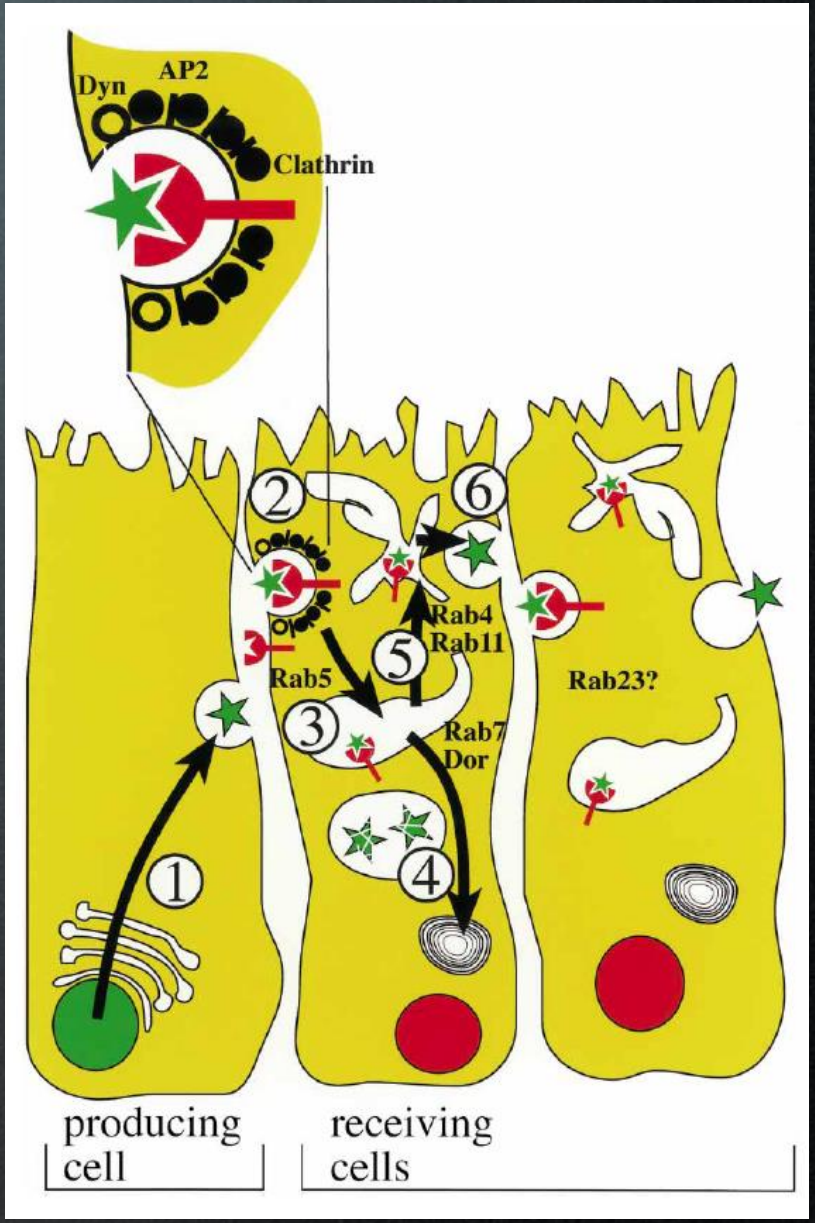
Distribution of signal is modulated by rate of lysosomal degradation.

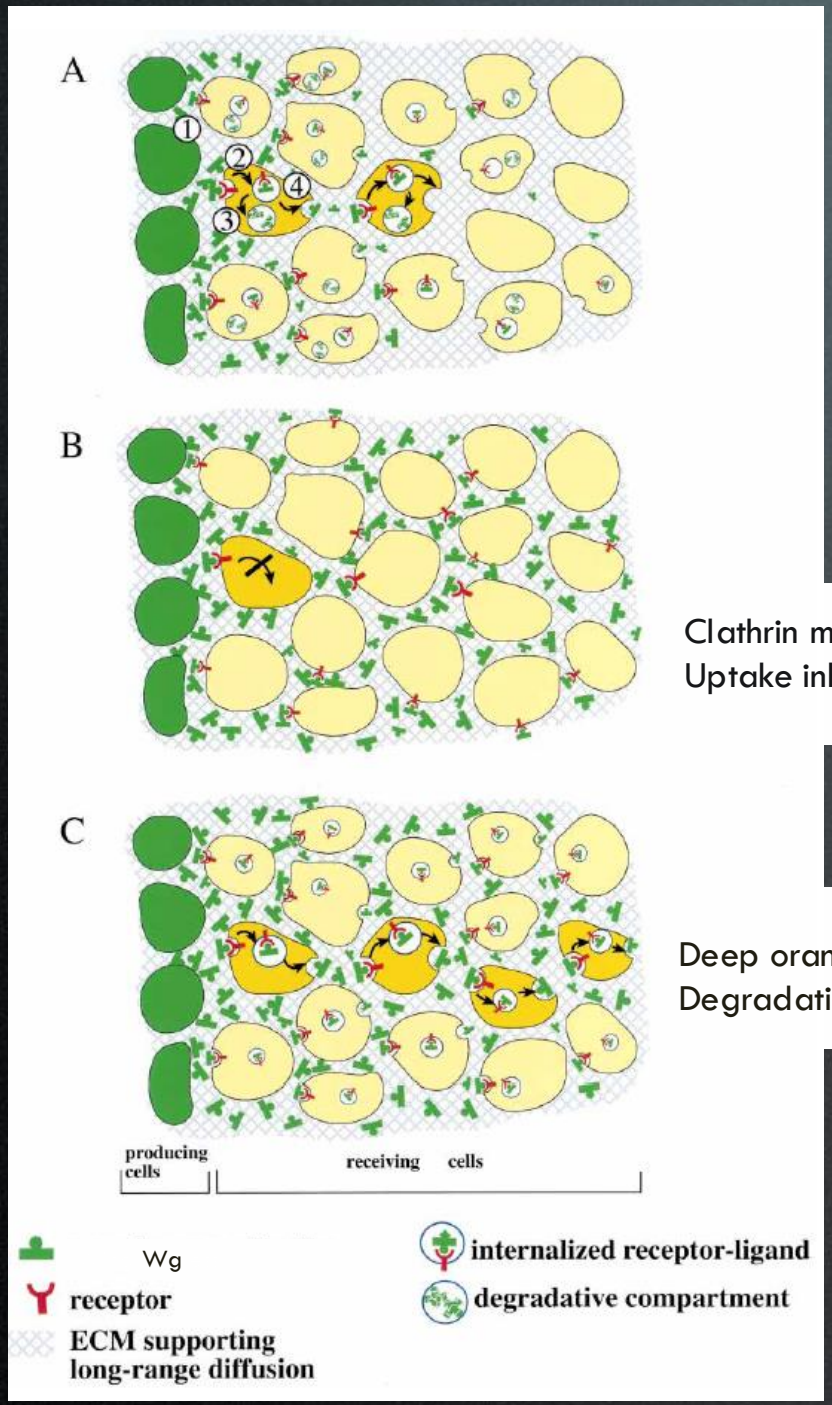
Signals like Wg (WNT)/Dpp (TGFB) are found in endocytic vesicles

Some evidence:

a. Block lysosomal degradation with
   mutants: e.g deep orange/ dominant active RAB7 [small GTPase required for endosomal sorting]
   or
   drugs e.g. Chloroquinine

-> Changes range of signaling





Example: Wingless (aka Wnt)

Lysosomal degradation restricts Wingless signaling

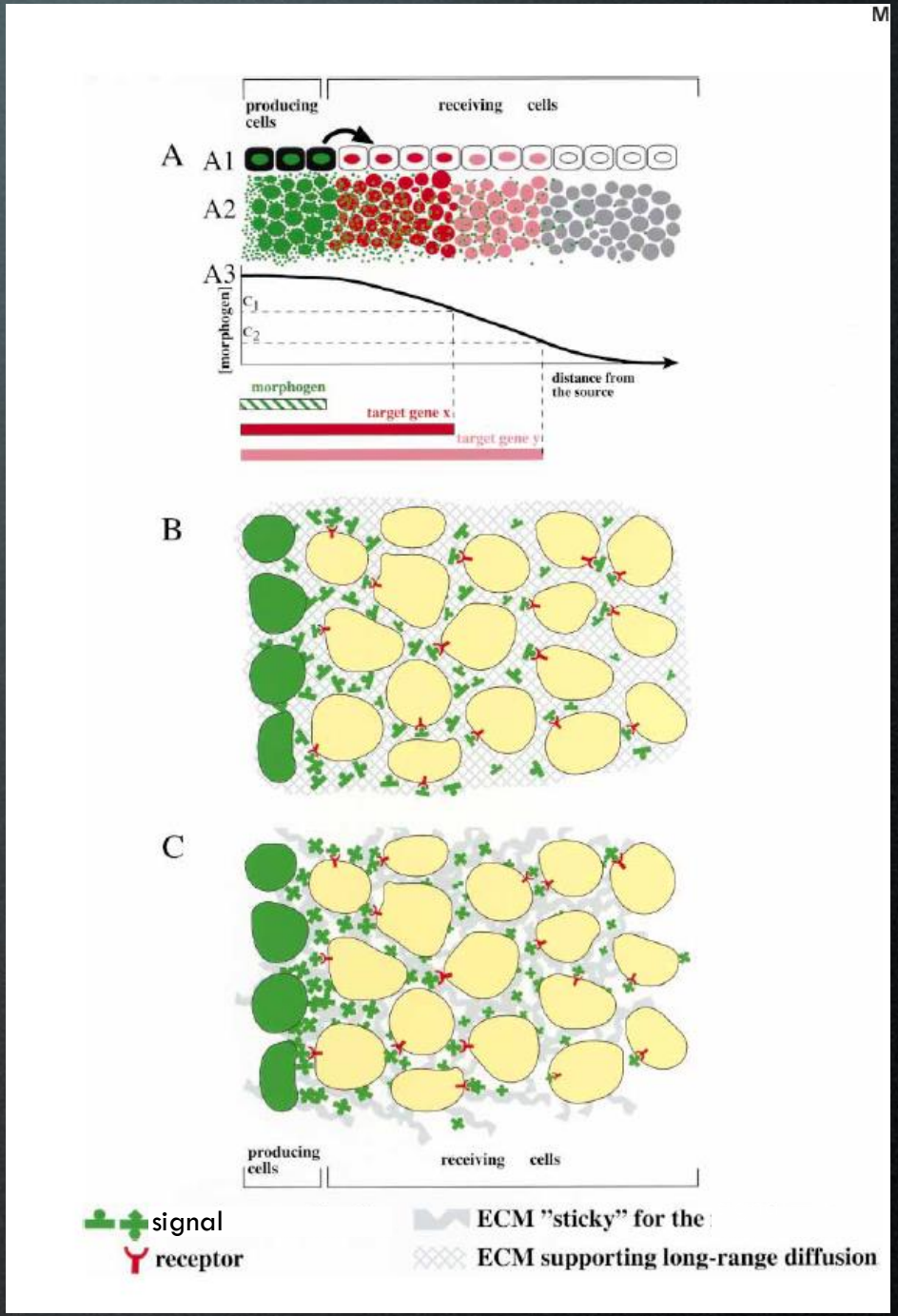
Clathrin mutants
Uptake inhibited

Deep orange mutants
Degradation inhibited

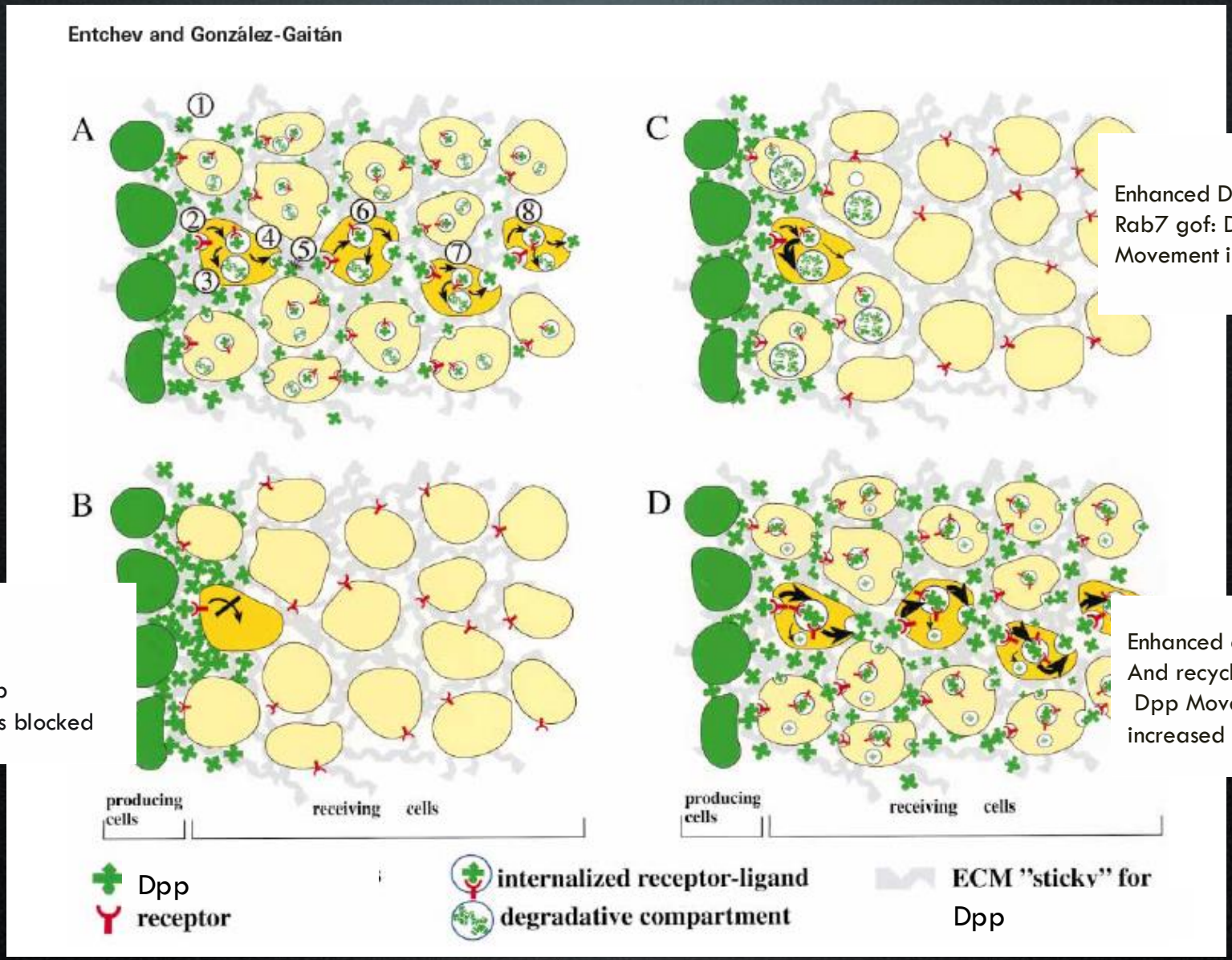
6. Mechanism of Transport

"Passive" Diffusion versus
"Active" Transport

a. Consider Diffusion as passive



b. Consider mechanisms of "Active" Transport: Planar Transcytosis of Dpp (aka TGF B superfamily member)



Enhanced Degradation:
Rab7 gof: Dpp
Movement is blocked

Endocytosis
Defective
Mutant: Dpp
Movement is blocked

Enhanced endocytosis
And recycling (Rab5):
Dpp Movement is
increased

Other modes of transport:

a) Cytonemes --cytoplasmic "fingers" that deliver signal at a distance (Dpp signaling).

b) Argosomes -- exovesicle packets delivered to cells at a distance (Wingless signaling)

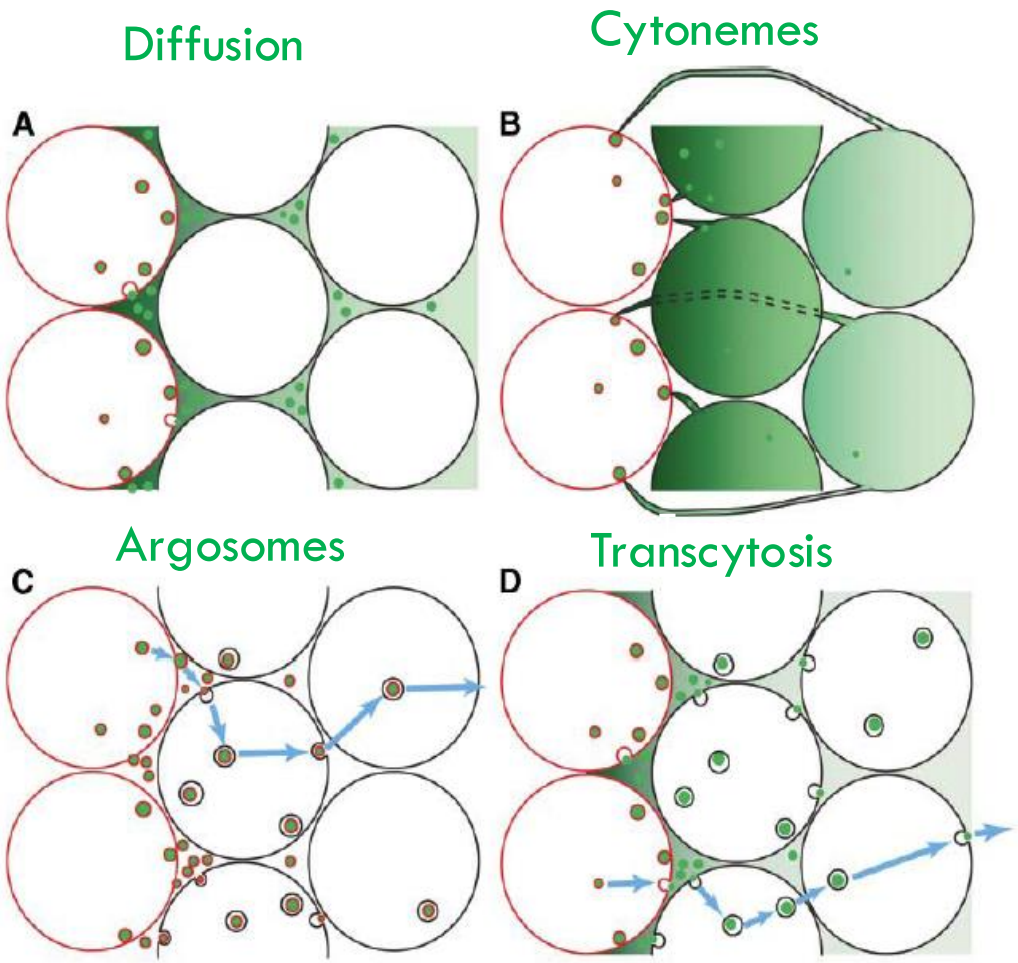


Figure 1. Mechanisms for Morphogen Gradient Formation

Red membranes represent expressing cells, black membranes represent receiving cells, and green shading represents ligand.

(A) Diffusion through extracellular space.

(B) Cytonemes project from distant to expressing cells and thereby produce an intracellular gradient of ligand [3]. An alternative mechanism involves the extension of projections from expressing to nonexpressing cells [4].

(C) Argosomal transport [5]. Membrane from expressing cells "chaperones" ligand through receiving tissue in "exovesicles."

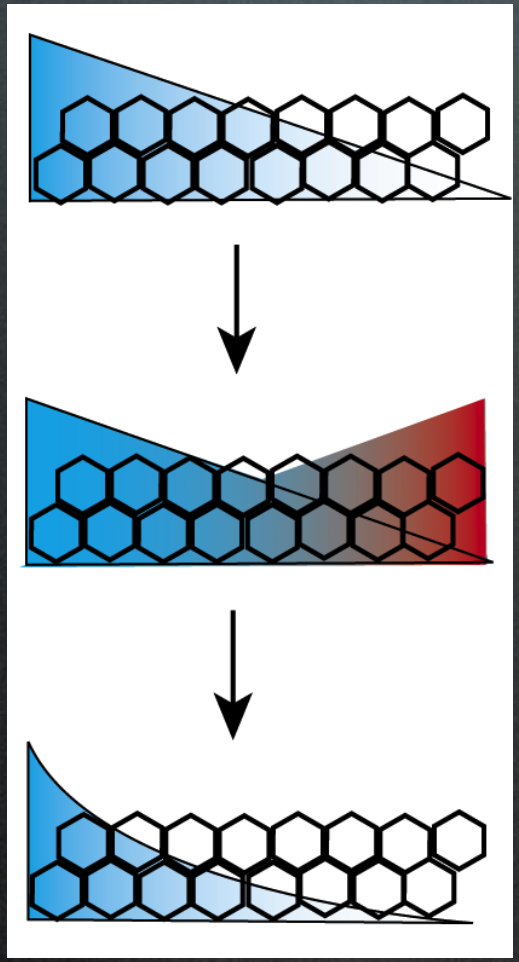
(D) Transcytosis [6]. Ligand moves across receiving tissue by serial recycling through endocytosis and exocytosis.

7. Secreted antagonists

e.g. Dpp -- short gastrulation (sog) = BMPs -- chordin

e.g. Wnt -- Frbs, Cerberus,Dkk

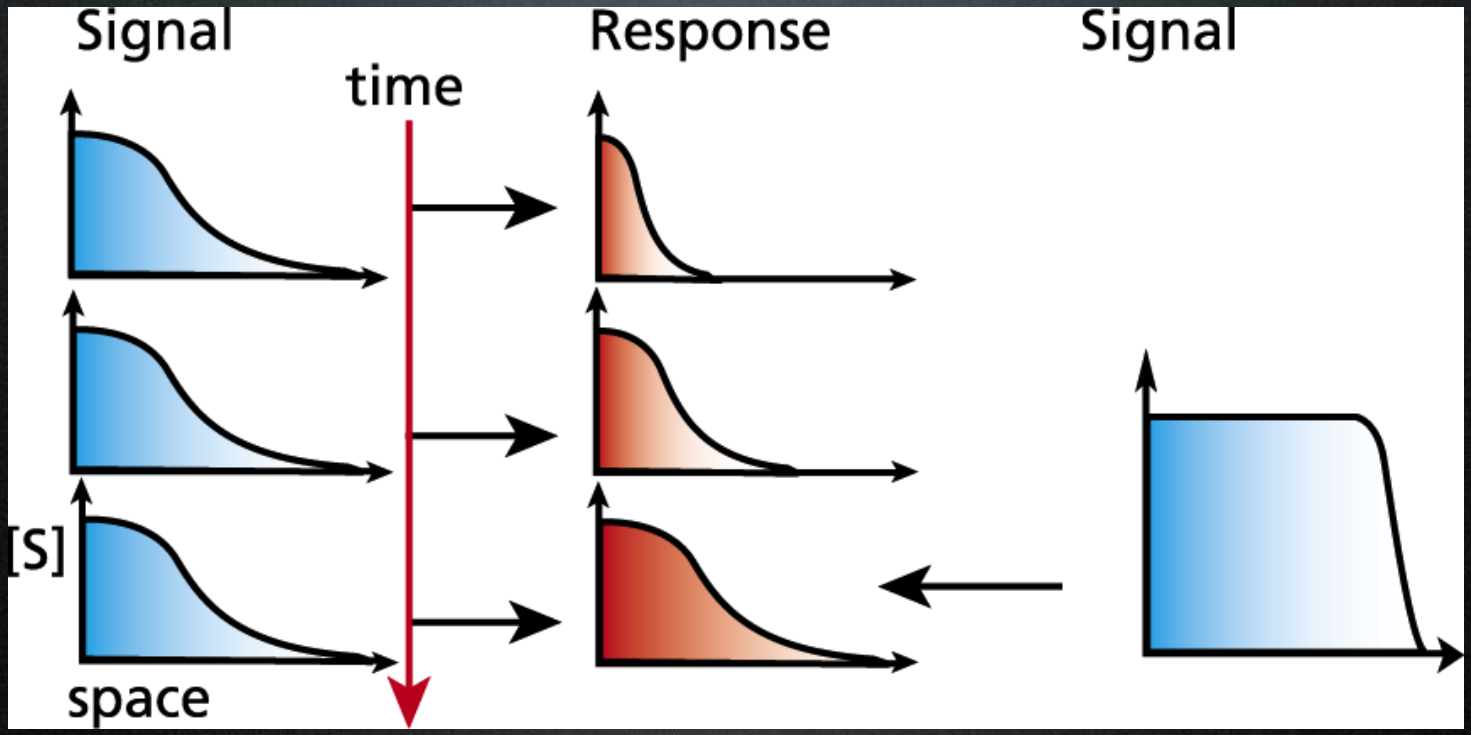
Notum--represses Wg signaling in Drosophila



RESPONSE =

Strength of Source +

"Diffusion" parameters + Time that cells can respond.

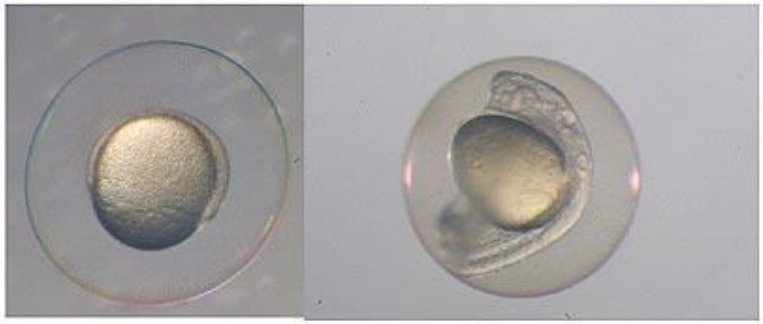


7. **THE THREE FUNDAMENTAL LAWS OF DEVELOPMENT**

1. TIMING IS EVERYTHING.

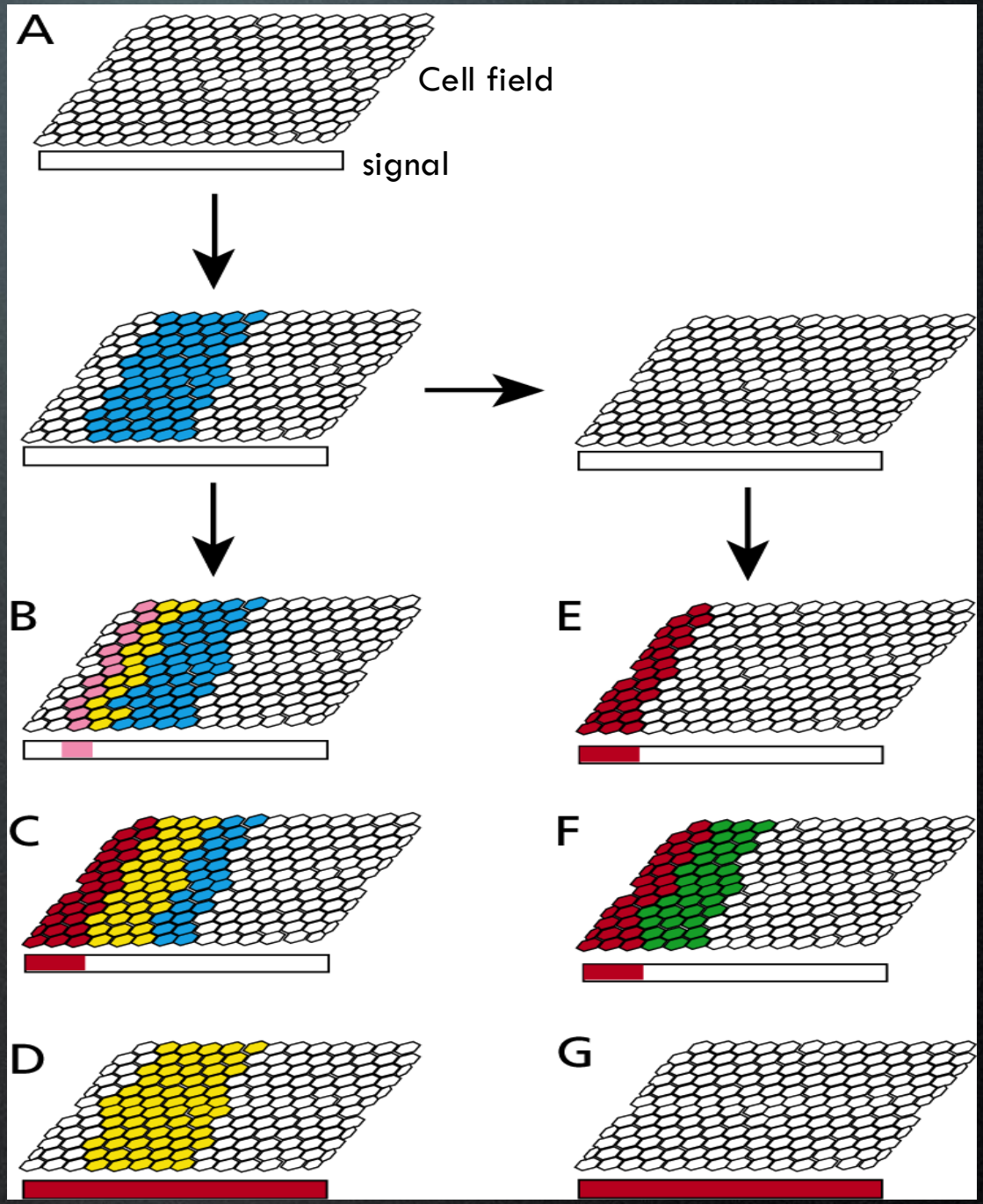
2. VALUE IS CONTROLED BY THREE THINGS: LOCATION, LOCATION, and LOCATION.

3. BOTH THE ABOVE ARE TRUE.



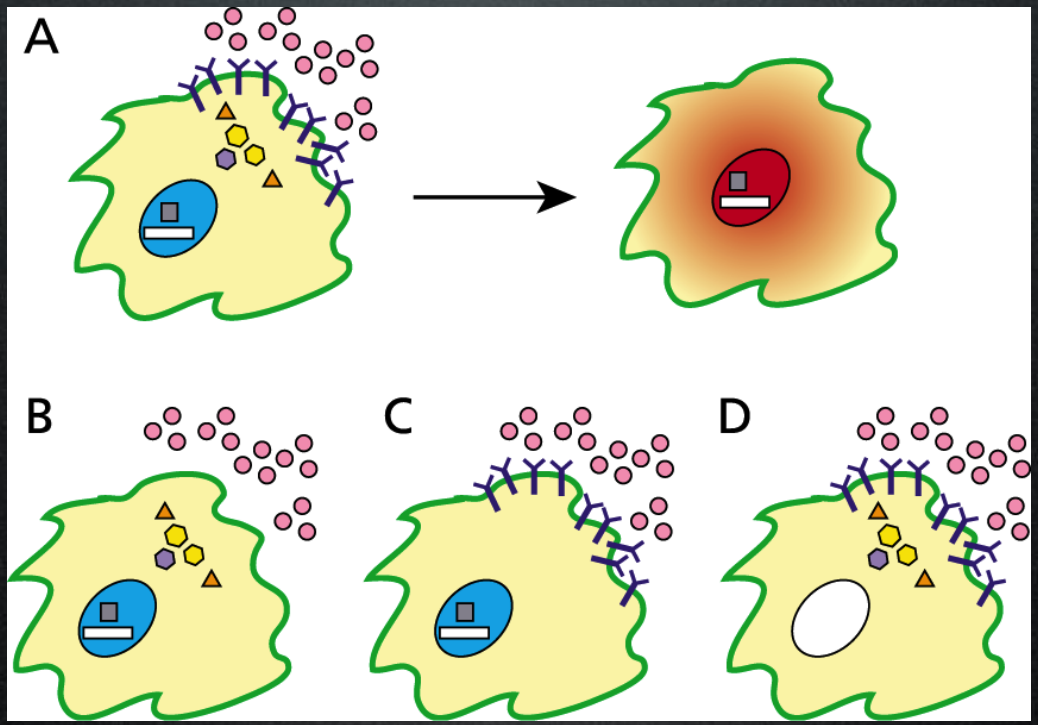
In a systems approach, development is 100% controlled by spatial parameters and 100% controlled by temporal parameters.

COMPETENCE:
the ability of a cell to RECEIVE and INTEPRET a signal

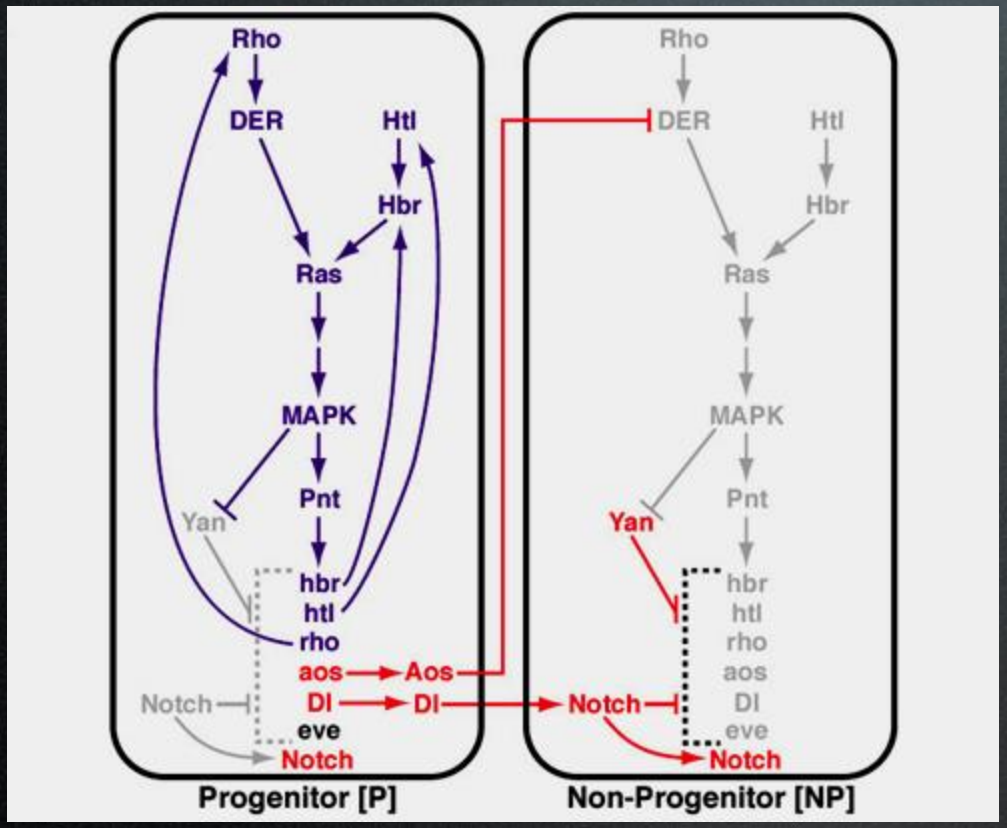


Competence in Molecular Terms:

* depends on developmental history
* in molecular terms:
  - correct receptor?
  - the pathway components?
  - can the cells execute the response?



Once a cell receives a signal, it may then itself become an active player in determining the "outcome" of the inductive process.

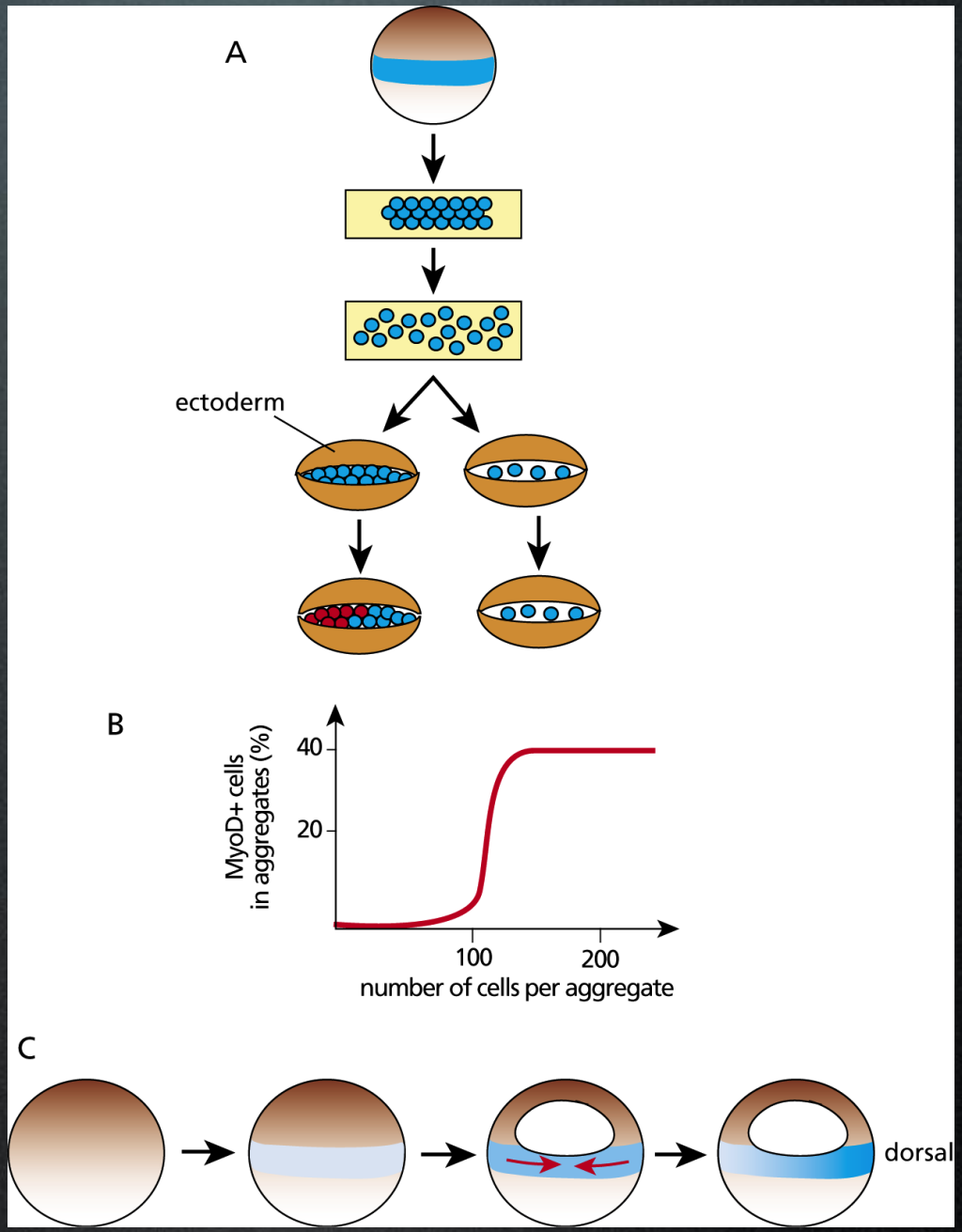


Cross Talk and Feedback Mechanisms assure the Specification of Founder Cells.

* changes in stability or concentration of pathway components e.g. + or - feedback loops that increase or decrease receptor levels

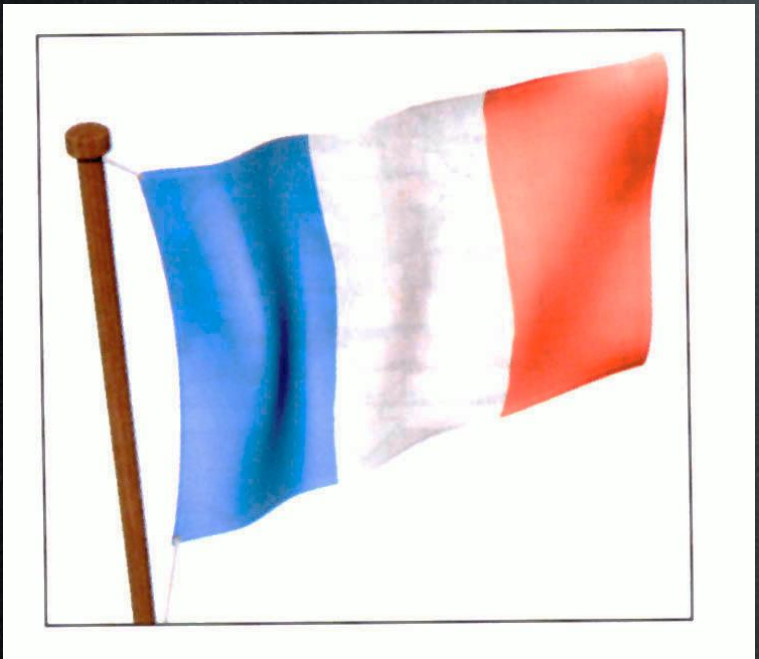
Community effect

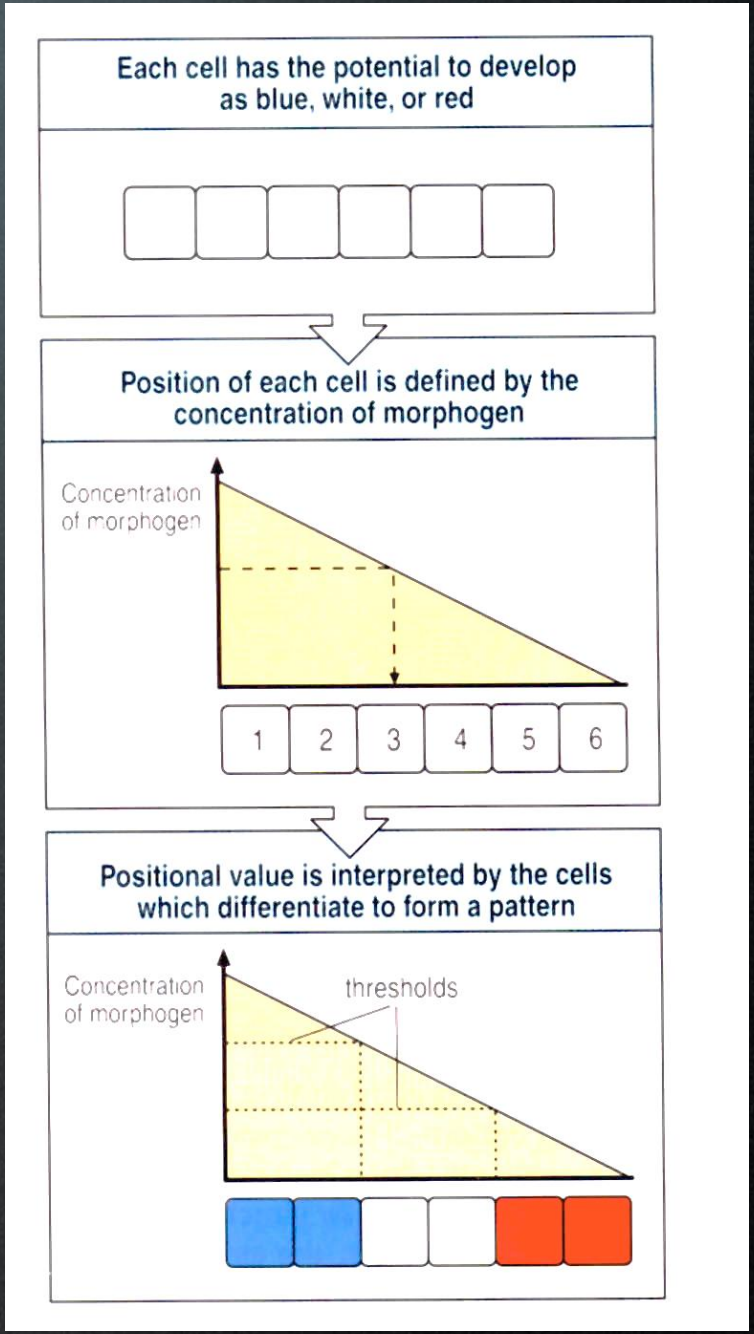
#s of receiving cells matter



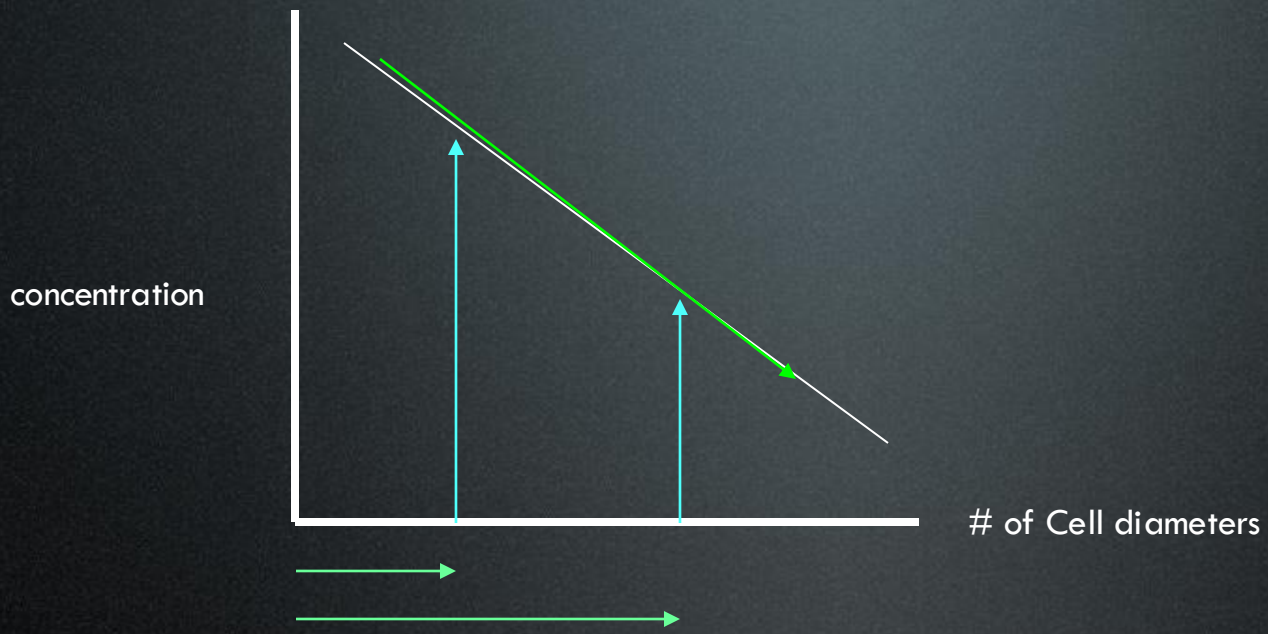
Morphogen concept (Turing 1952 + Wolpert 1969 + Crick 1970)

Morphogen = Diffusible inducer that can elicit different responses and so instruct developmental fates in a concentration dependent manner.





Morphogen gradients: three variables



Scalars: define threshold of response

Vector: defines direction for response (orients cells in a field)

Slope: defines the size of the field

Qualities of a Morphogen:

1. Diffuse over a distance

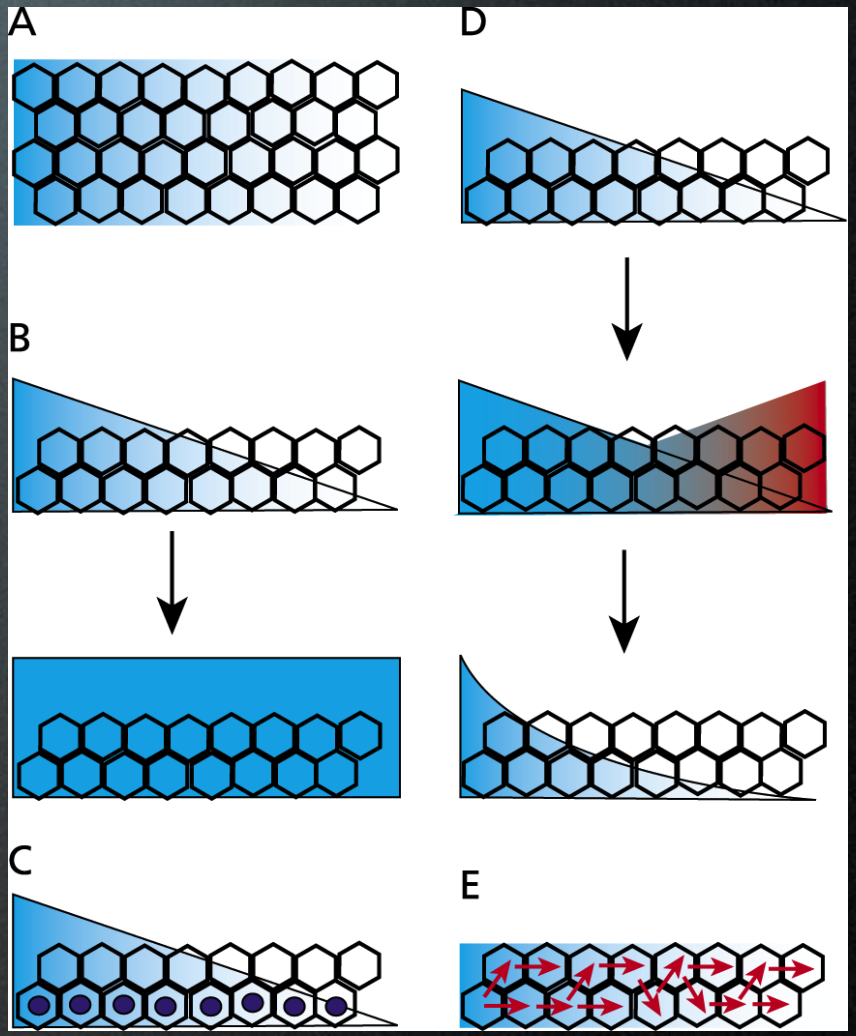
2. Elicit different responses directly in a concentration dependent manner

3. 1&2 have to shown to be true *in vivo*

4. Cells upon which the morphogen acts have to be functionally equivalent so that the different responses can be related solely to the differences in morphogen concentrations

How does graded information get established?

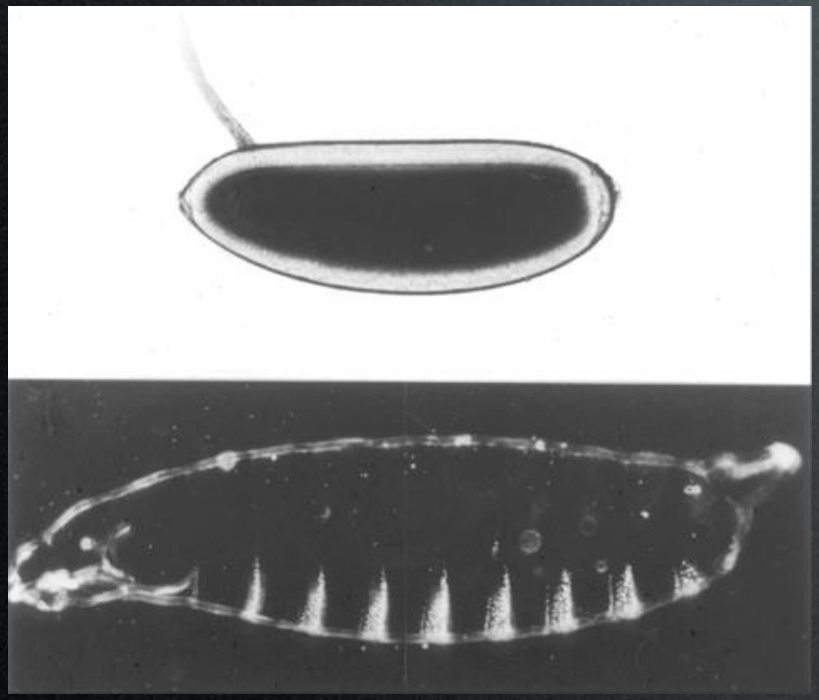
* Transport with Active sinks (B/C)
* Interactions with ECM, other receptors.. (Hh) (E)
* Antagonistic gradients (Dpp in the embryo) (D)
* Keep time/cell proliferation in mind!

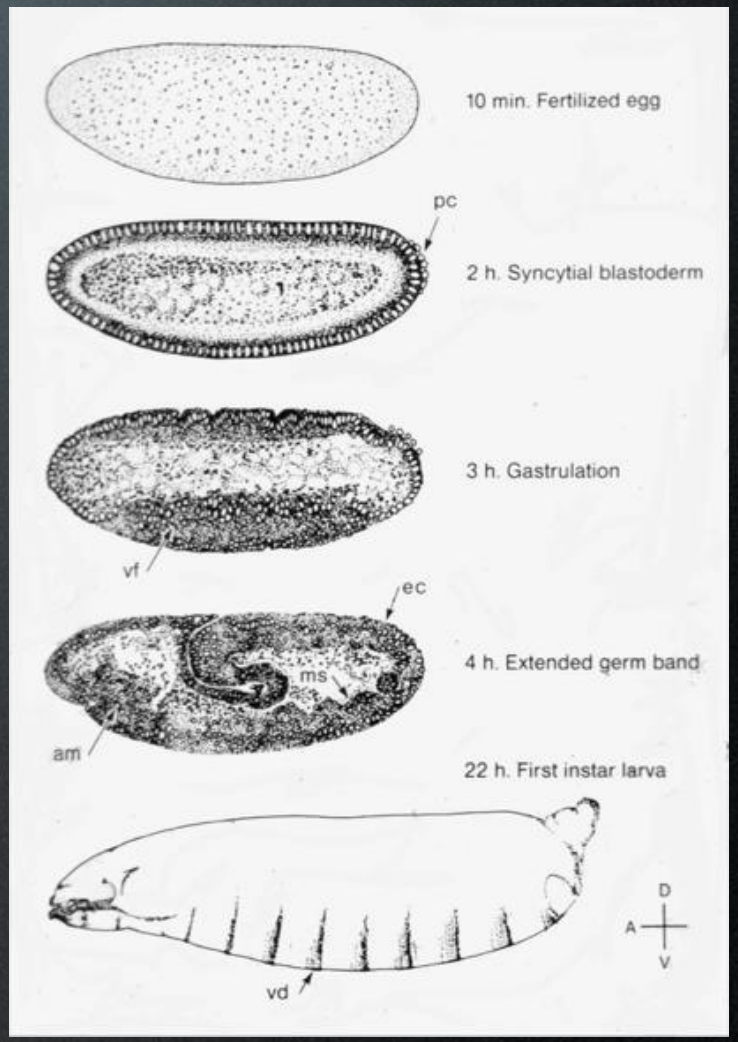


Examples of Morphogens:

Best Defined:

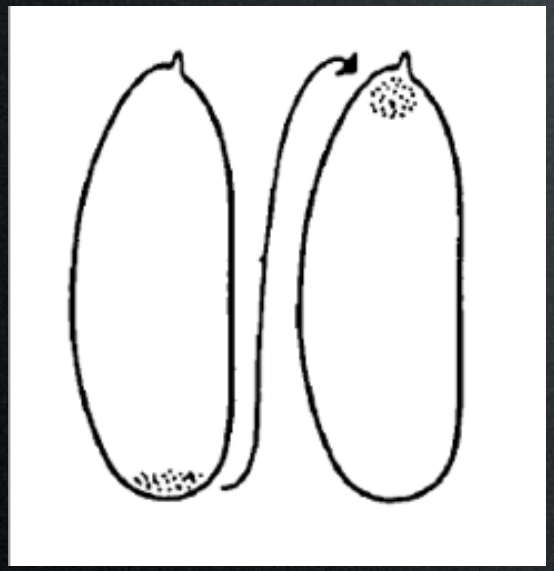
Bicoid : Transcriptional Regulator in the fly syncyticial blastoderm (bicoid)

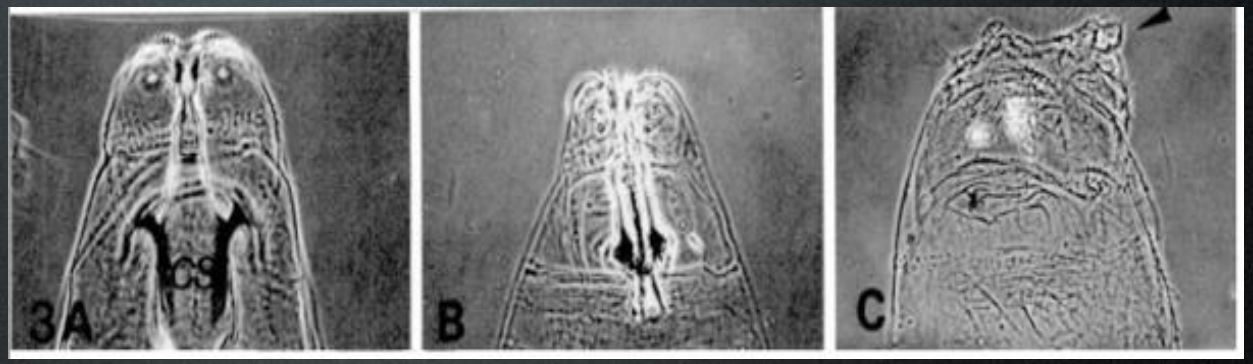




Cytoplasmic transplantation experiment demonstrate

Anterior and posterior determinants in the *Drosophila* embryo



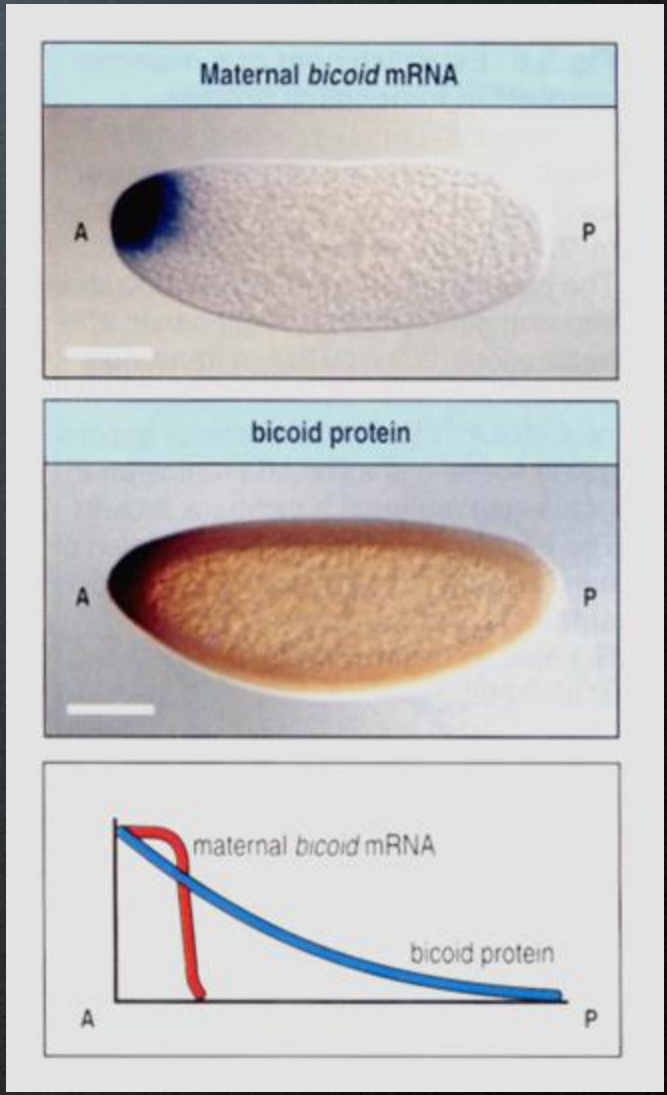


Posterior cytoplasm promotes tail development

Similar experiments indicated that anterior cytoplasm promoted Head and anterior development.

Bicoid -- homeodomain protein; RNA deposited at anterior pole

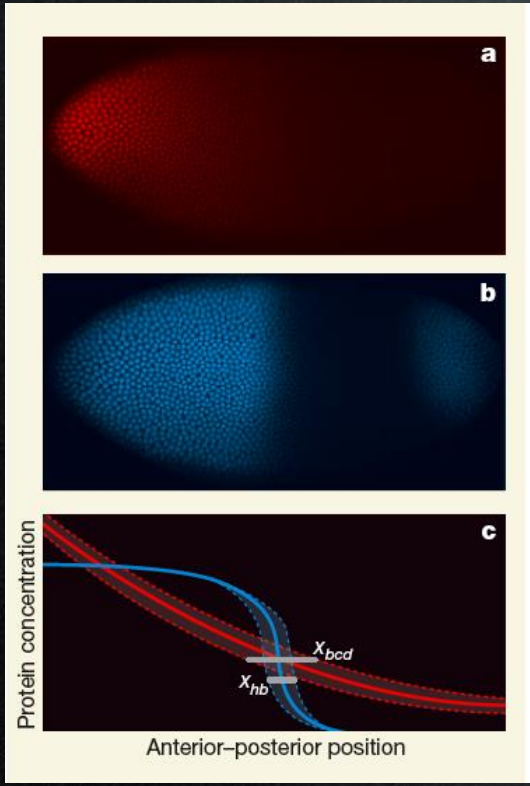
-- protein diffuses through early embryo, creates a concentration gradient across a field of nuclei that instructs cells to adopt different developmental fates along the A--> P axis.

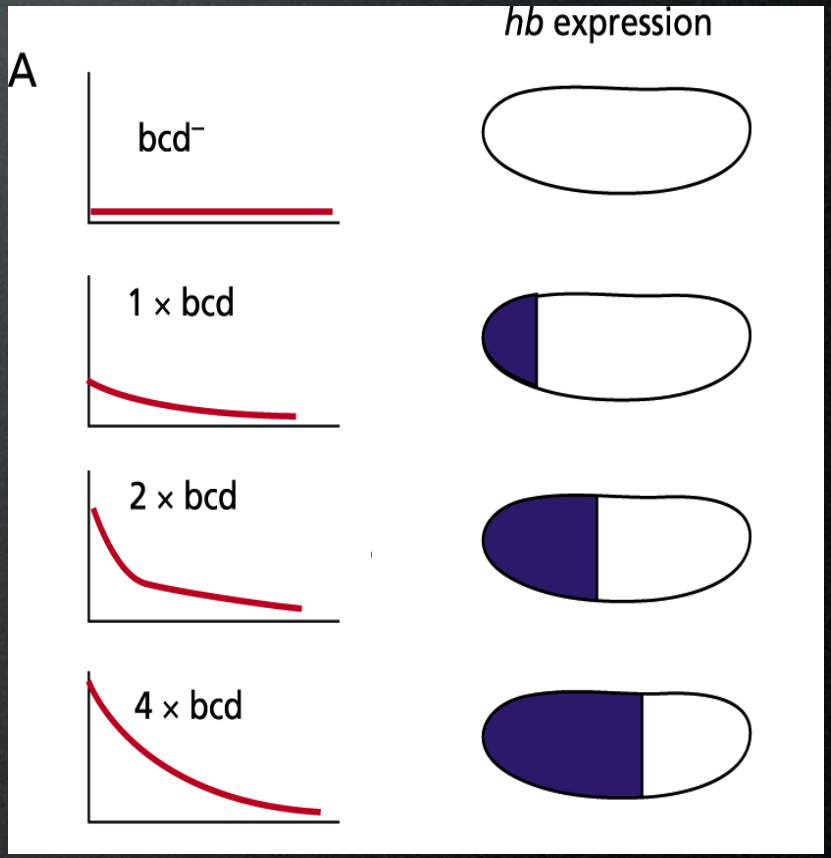


Bicoid facts continued:

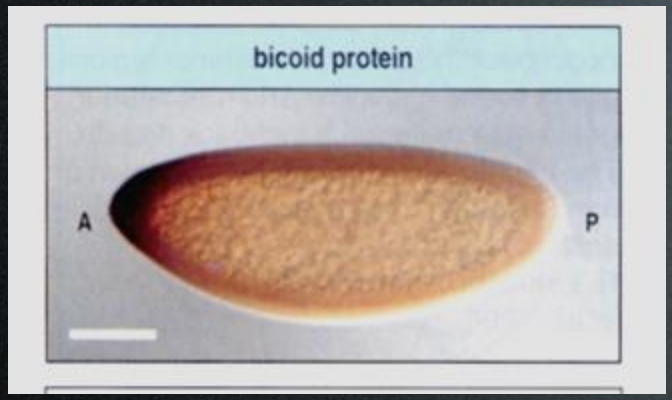
*lowering the dose of bicoid shifts pattern anteriorly whereas increasing the dose shifts the pattern posteriorly (morphological assay)

*hunchback expression is a response to bicoid (molecular assay)

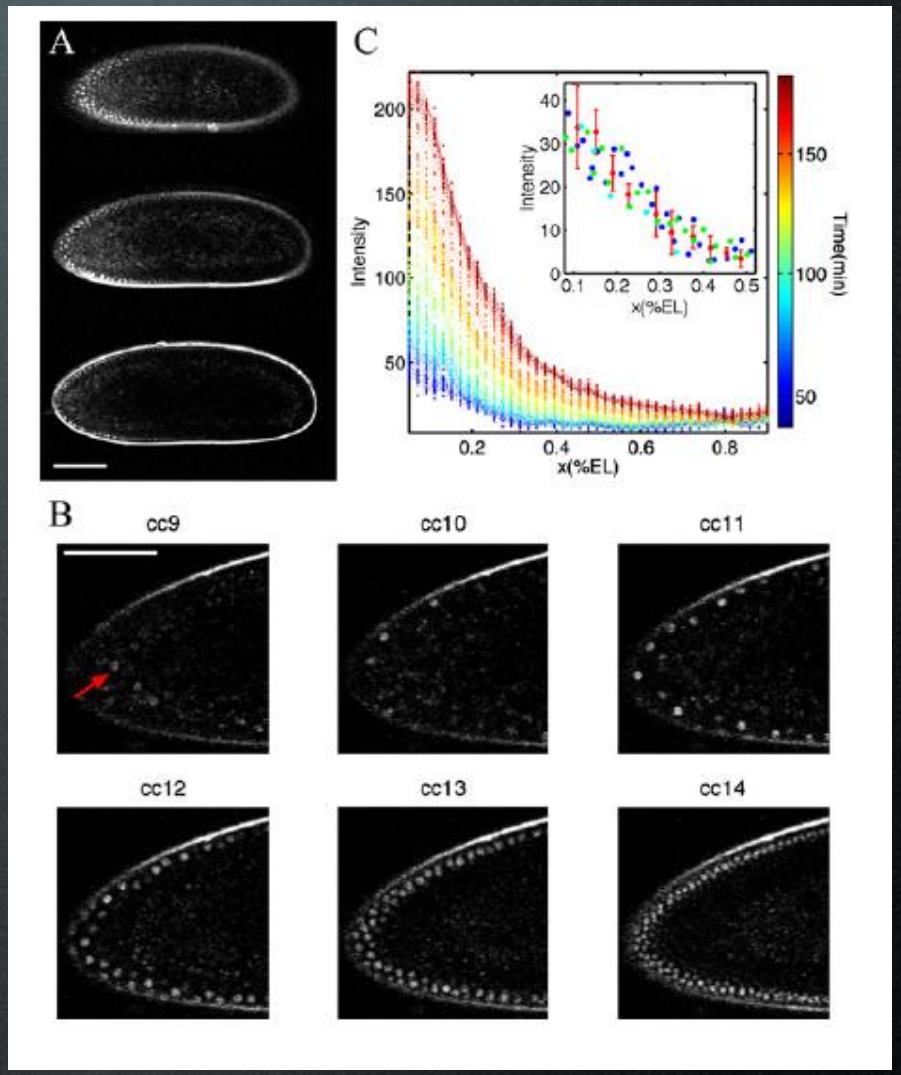




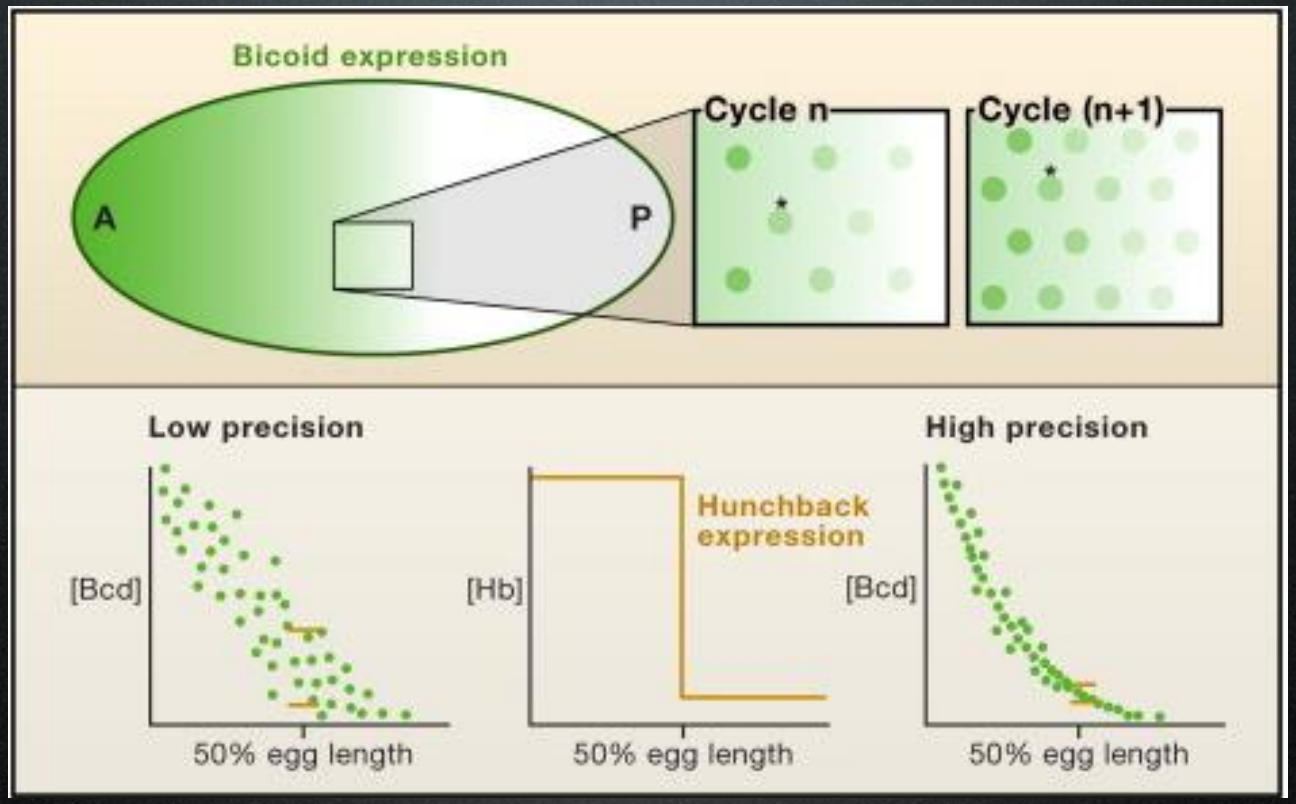
Visualizing the Bicoid Gradient with actively dividing nuclei



Fixed Preparations
Reinitz, J. 2007

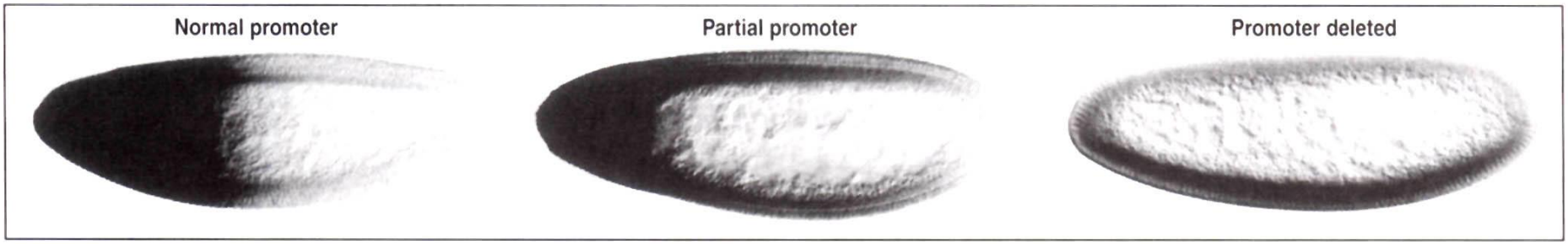


Live Preparations-- bicoid egfp
Gregor et al., 2007


Bicoid expression
A
P
Cycle n
Cycle (n+1)
Low precision
[Bcd]
50% egg length
[Hb]
Hunchback expression
50% egg length
High precision
[Bcd]
50% egg length

Gibson, 2007

How is the bicoid gradient translate into different amounts of hunchback RNA?

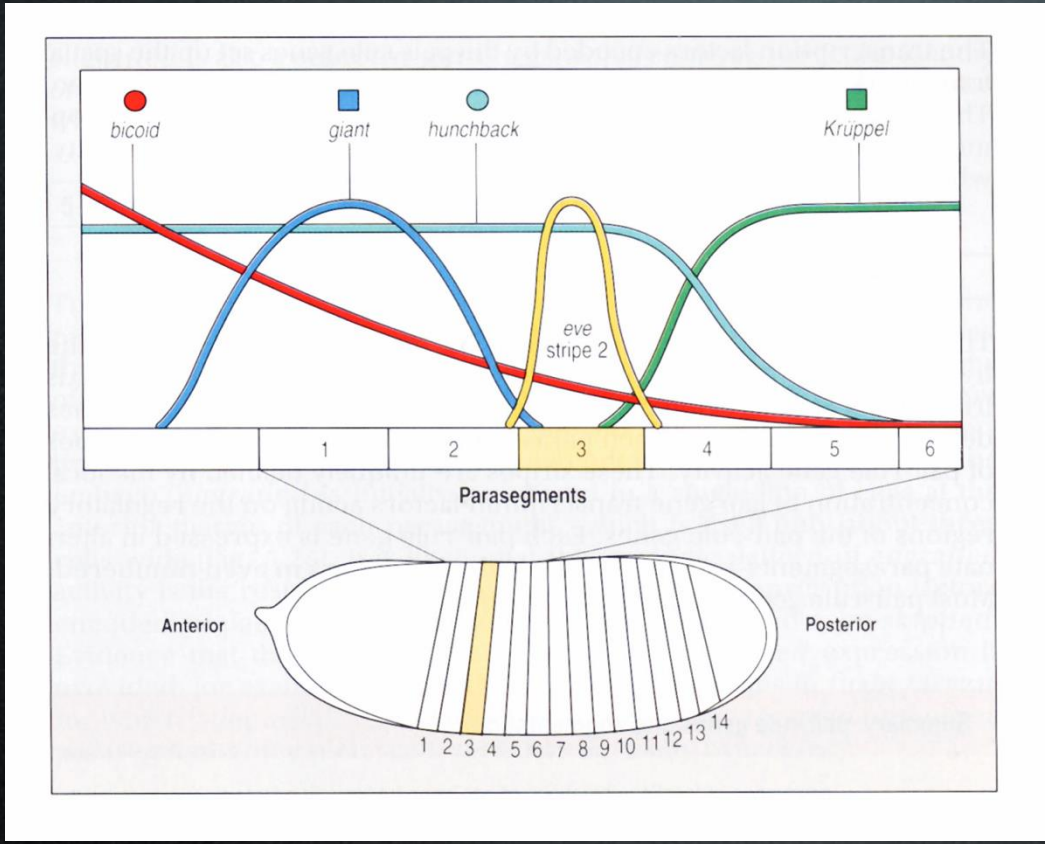


Promoter-LacZ fusions reveal

*number of bicoid binding sites in hunchback promoter determines output

*affinity of binding sites (low affinity sites in high bicoid levels--restricted to anterior end; high affinity sites at low levels --provide broader pattern of expression)

Further elaboration of the Bicoid gradient:

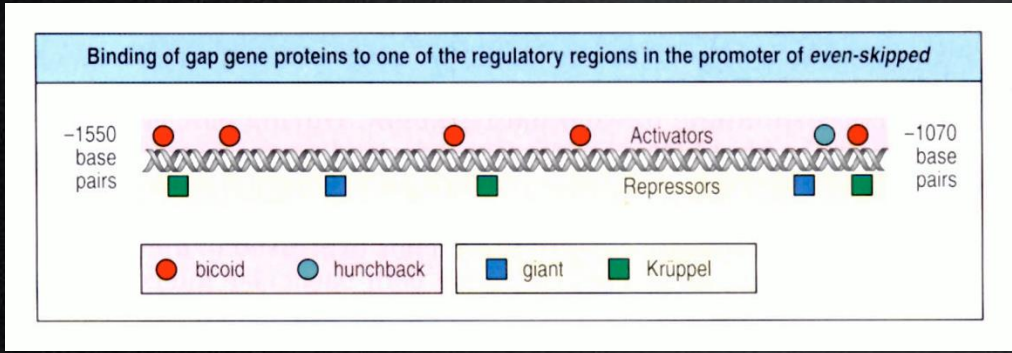


*Number of different sites

*Affinity of sites

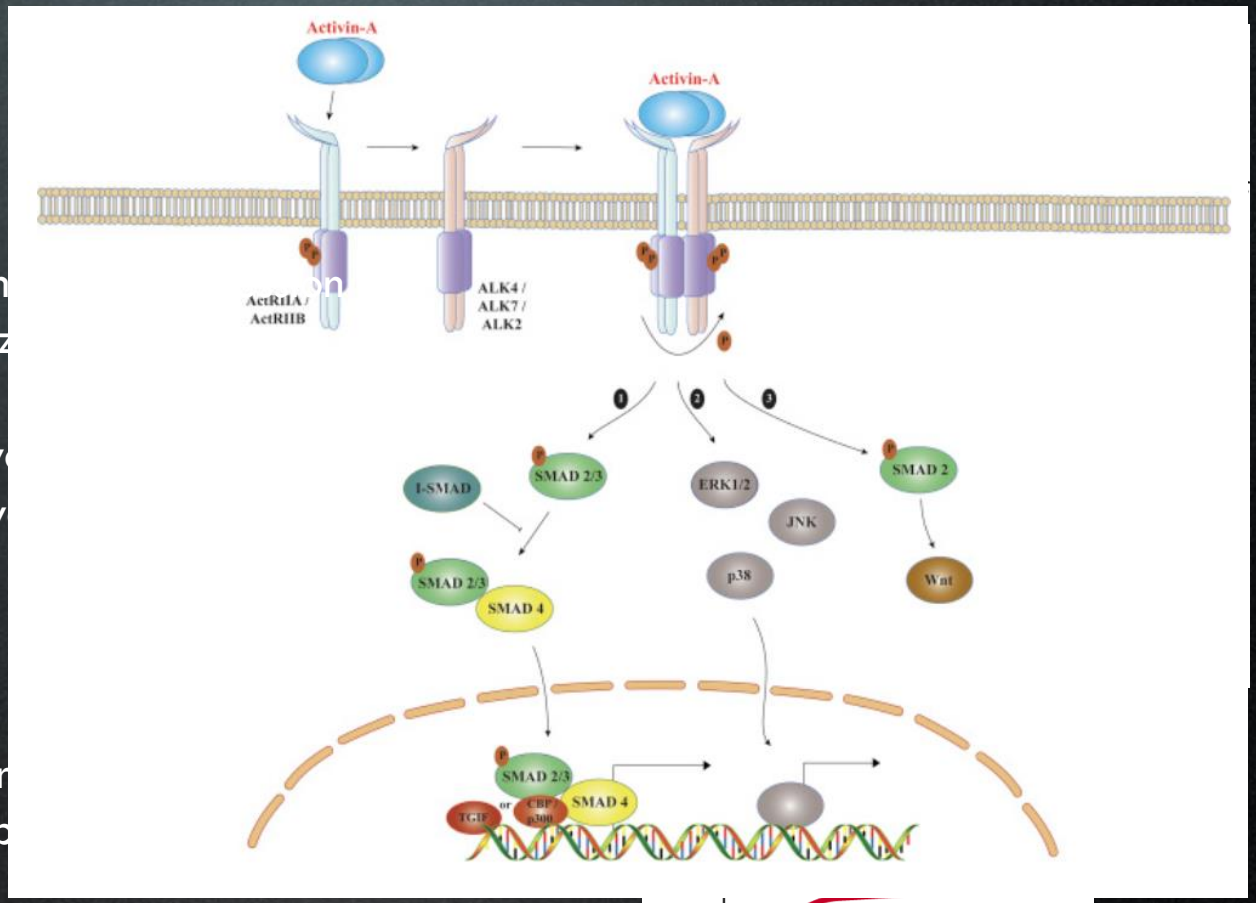
*Combinatorial interactions

*Cooperative interactions between bicoid molecules



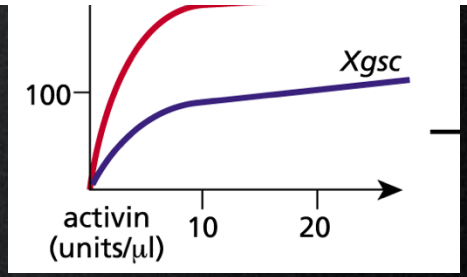
A critical question inherent to the Morphogen concept:
How do cell read and interpret graded information?

Activin as a morphogen:



Different levels--> differen
*gradient visualiz
* two responses
- hi lev
- lo lev

Asked "Does the cell measur
relative # of occupied recep



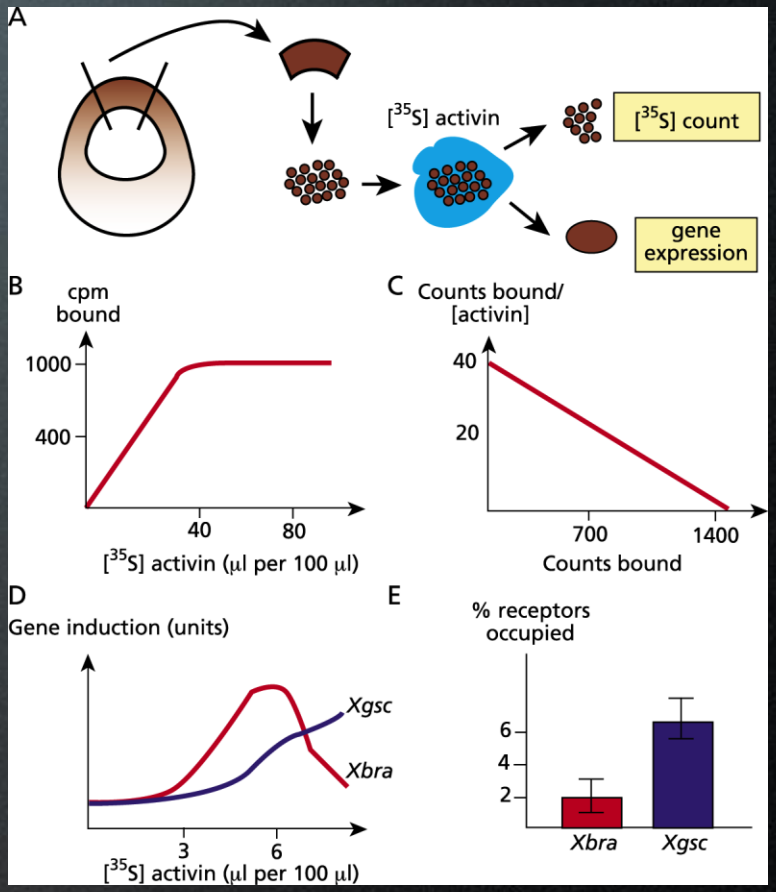
Dyson and Gurdon, 1998

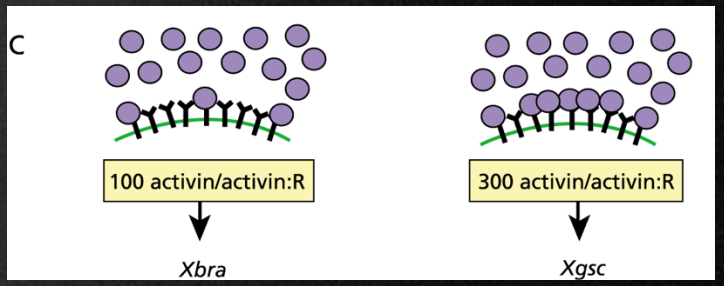
Determined amount of activin required to saturate receptors (B) and amount of
Activin required to induce different targets (D) to find:

*only 2% receptor occupancy --> Xbra
*only 6% receptor occupancy --> Xgsc

In other expts:
Determined that wildtype cells have ~5000 activin binding receptors.

So ~100 filled receptors->Xbra; 300filled -> Xgsc

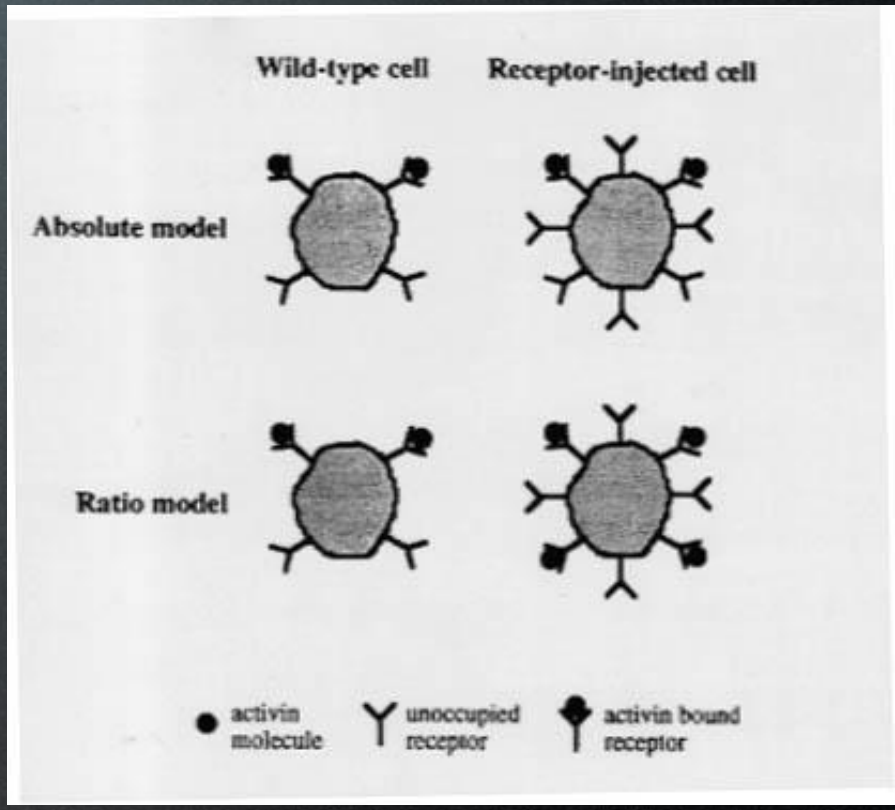




Dyson and Gurdon, 1998

Does the cell measure the relative or absolute numbers?

Test: inject more receptors (increase from 5000 to 35,000)



Find: values correspond to 100 and 300 receptors for Xbra and Xgsc respectively

<u>So cell measures absolute numbers!</u>

Gone on to correlate receptor occupancy with transcriptional alterations
(the # of active SMAD complexes)

Developmental Biology:

A pluripotent science

induction, competence, positional information

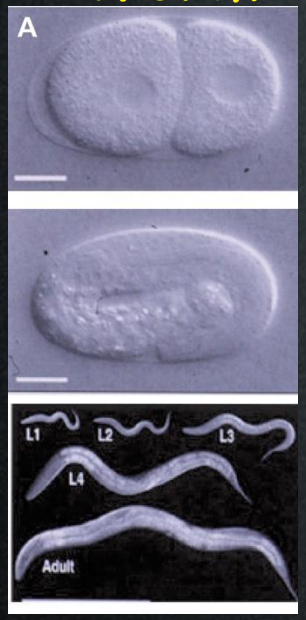
Signals are the perfect tool used to organize
cells in space (proliferation and death)

Ways to regulate signaling in vivo both the range of activity as well as the "type" of activity (collision of cell biology, signaling and development).

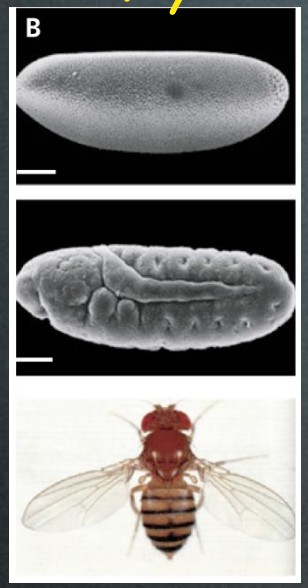
Special inducers = morphogens

Molecular definitions of gradients/signaling activity…
more complexity revealed…

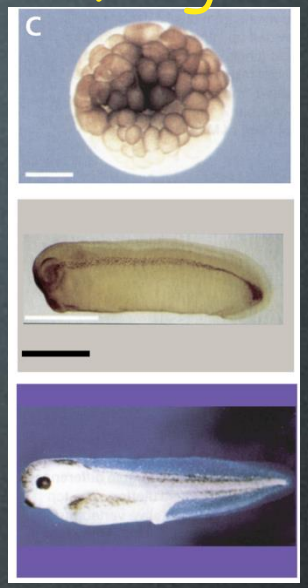
Worm



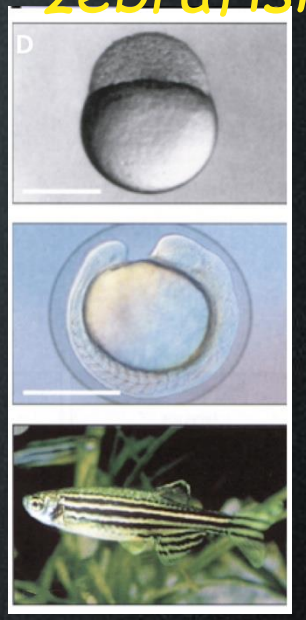
fly



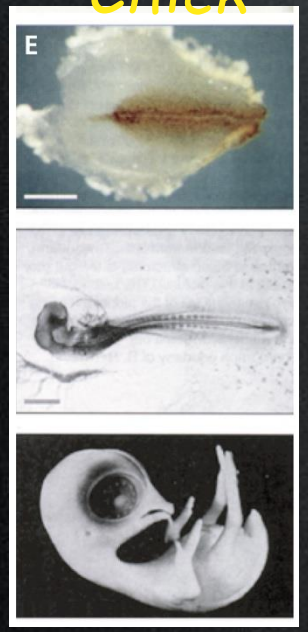
frog



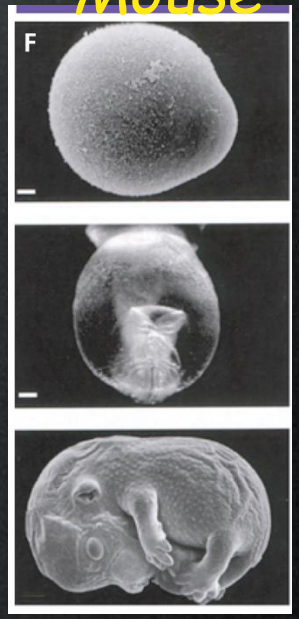
zebrafish



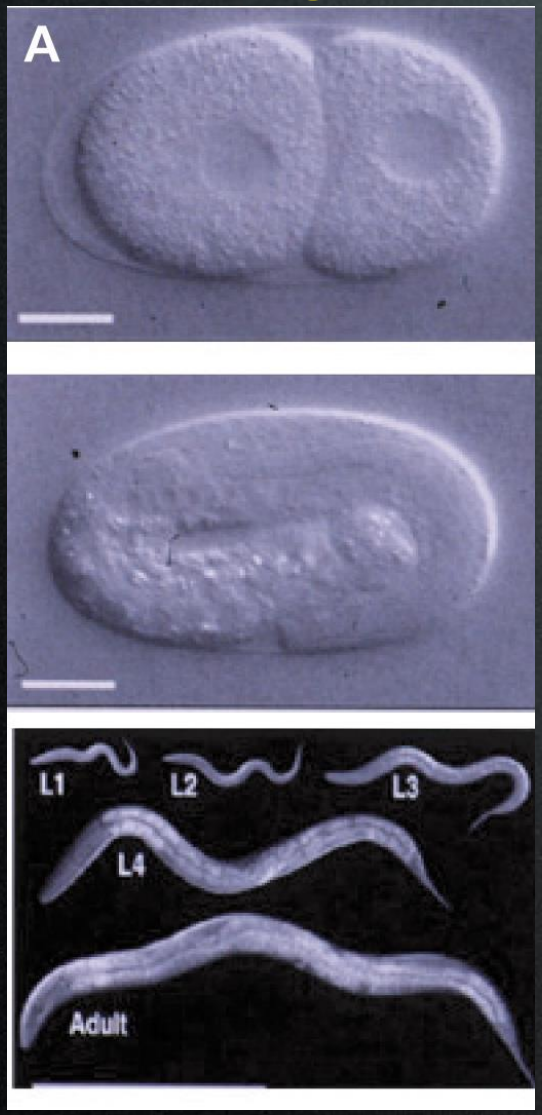
Chick



Mouse



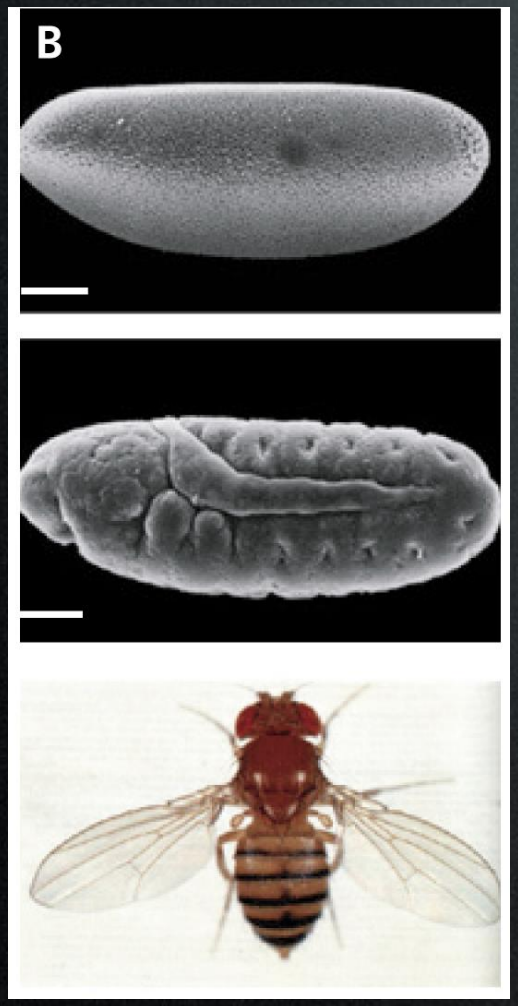
C. elegans



Plus:
+ lineage map of every cell in the body
+ transparent embryo and adult
+ genetic analysis available
+ rapid development : from zygote to adult
+ cheap

Minus:
-small
-Limited insitu/antibody approaches
-Hermaphrodites make genetics tricky
-No trangenesis
-invertebrate

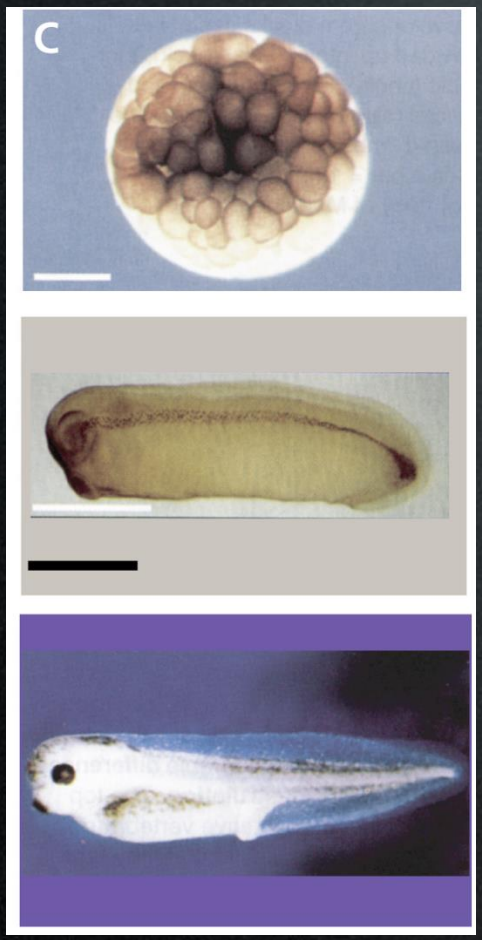
Drosophila



Plus:
+ genetics, genetics, genetics!!
+ easily control spatial and temporal expressio
+ rapid development: from zygote to adult in 1
+ lots of embryos outside the mother
+ cheap
+ awesome (personal bias)

Minus:
-small cells
-invertebrate
-multiple phases of development (embryo to la
pupa to adult)

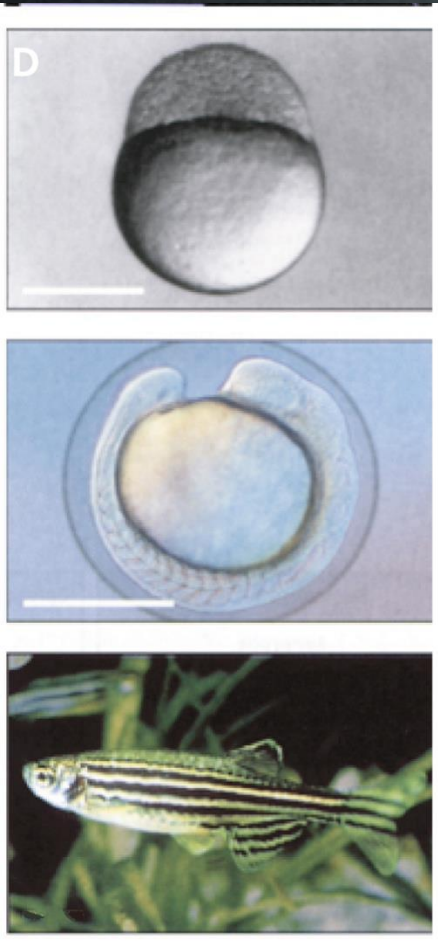
Xenopus



Plus:
+ large eggs and zygotes
+ eggs laid outside the mother
+ great for experimental manipulation (cut and
+ extensive embryology
+ egg will translate RNAs for expression studi
+ vertebrate

Minus:
- no genetics in a classical sense, although antisense, RNAi and morpholino technol available
-Some times of year produce better oocytes t
-Year to get fertile adult from zygote

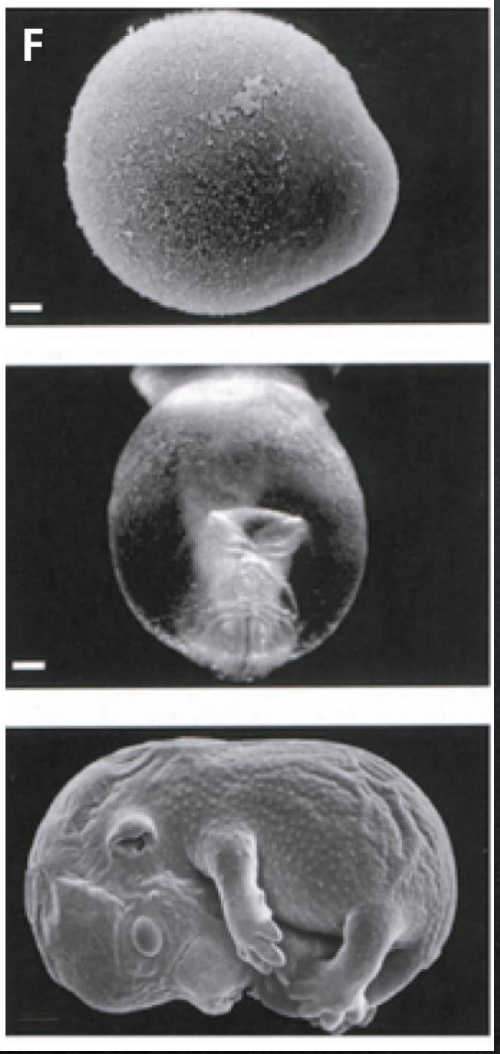
Zebrafish



Plus:
+ large optically clear eggs and zygotes
+ large numbers of eggs laid outside the moth
+ vertebrate
+ genetics available

Minus:
- 3 months to get from zygote to adult
-Pseudotetraploidy makes genetics….interesti
-No targeted knockouts ….but now crispr!
-Lots of fish tanks needed: more expensive th

Mouse



Plus:
+ the model for mammalian development
+ genetics with knock-in/transgenic technolog
+ extensive embryology

Minus:
- 3 months sexual maturity cycle
- low litter sized makes genetics more of a ch
- in utero embryonic development
- expensive

1 - Developmental Biology matters to ALL Biology.*

(DB is the pluripotent stem cell of scientific disciplines).

Embryology/Developmental Biology (back in the early 1800s)

Cell Theory (1862) embryo constructed of Cells -> Cell Biology

Cell Formation (late 1800s)

Metchnikoff et al. :mesoderm->macrophages-> Immunology

Gene theory (early 1900s)

Morgan and Wilson -> Genetics "transmission of inherited traits"

Evolution (early 1900s)

Darwin/Huxley/Haeckel > differences between modifications of development -> Evo-Devo

*Gilbert , 2017

2- Developmental Biology matters to ALL Biology.*
(DB is the pluripotent stem cell of scientific disciplines).

Nerve cells (early 1900s)

Harrison/Cajal> axonogenesis/synapse/neural connectivity

-> Neurobiology

Tumors (early 1900s)

Virchow/Boveri et al. > Cellular Pathology –

embryology to explain pathology

-> Oncology

Stem Cell Biology (late 1900s)/ Regenerative Medicine

Single Cell Transcriptomics (2000s)

*Gilbert , 2017