Early events of neural development

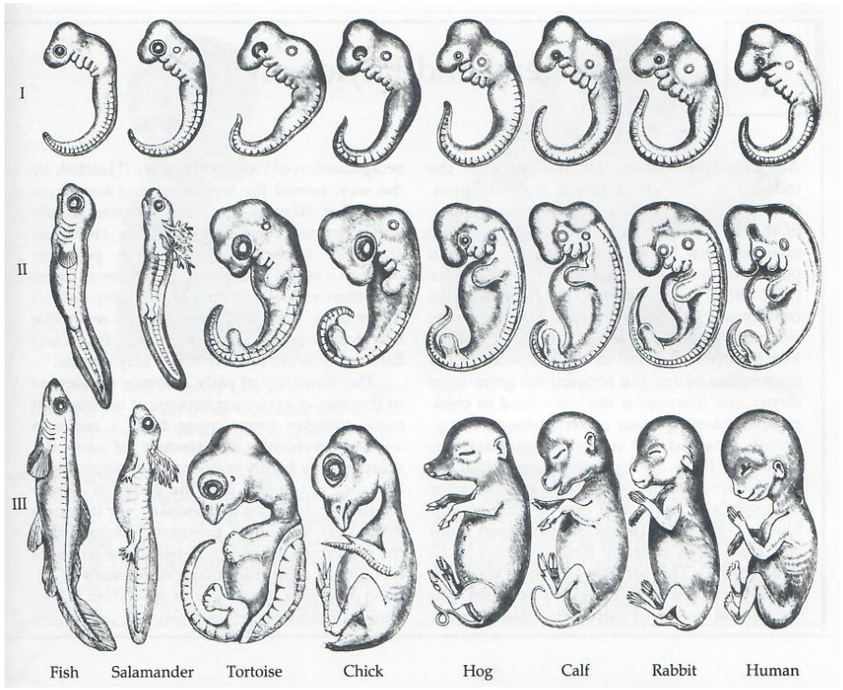
Goals:
1) to discuss the origins of cells in the nervous system
2) to discuss how neural stem cells generate diverse cell types in the nervous system

The next four lectures will cover:
Induction (Jan 22)...emergence of the nervous system
Regionalization (Jan 24)...acquisition of positional information of neural cells
Discussion of a journal article (Jan 26)

Neuronal fate specification (Jan 29)
Cell division and cell lineage (Jan 31)
Discussion of a journal article (Feb 2)

We will deal with glia later in the course!

Some basic concepts



Romanes, 1901

Fundamental mechanisms of neural development are conserved among species.

-Important concepts were first discovered in diverse model systems including invertebrates.

-What makes human brains different from others?

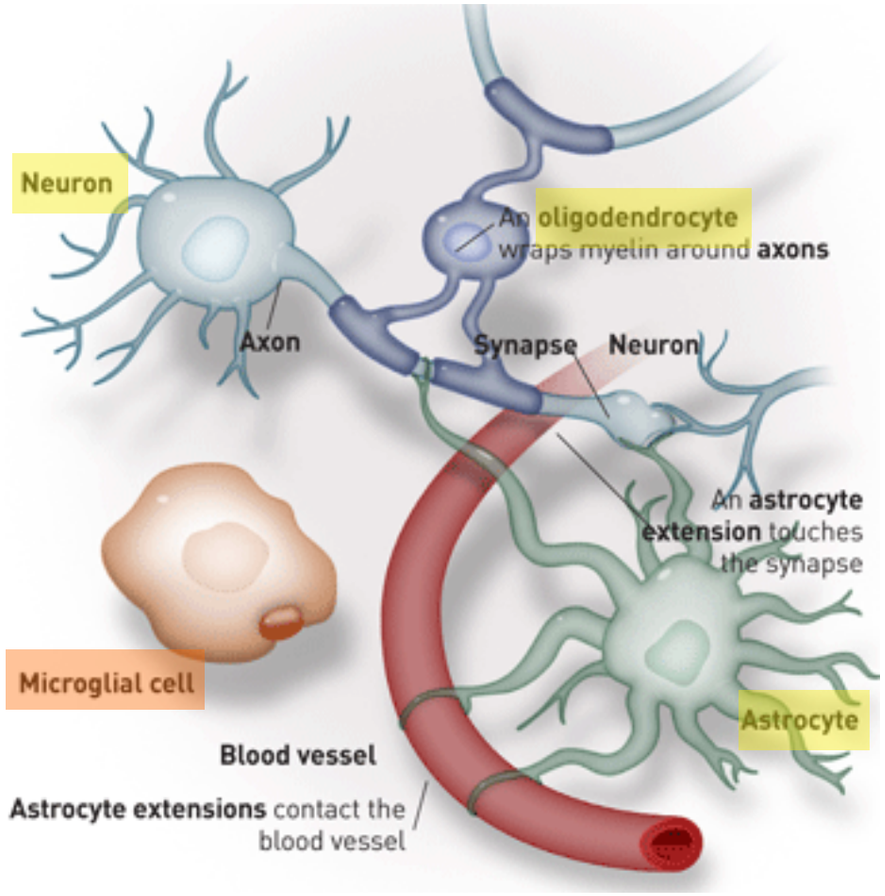
Neural development does not occur in isolation; it occurs in concert with development of other organ systems.

-Molecular mechanisms are also conserved across systems.

Intrinsic vs extrinsic mechanisms

A relatively small number of molecular pathways are used over and over again in different events across life span.

Origins of cells in the nervous system



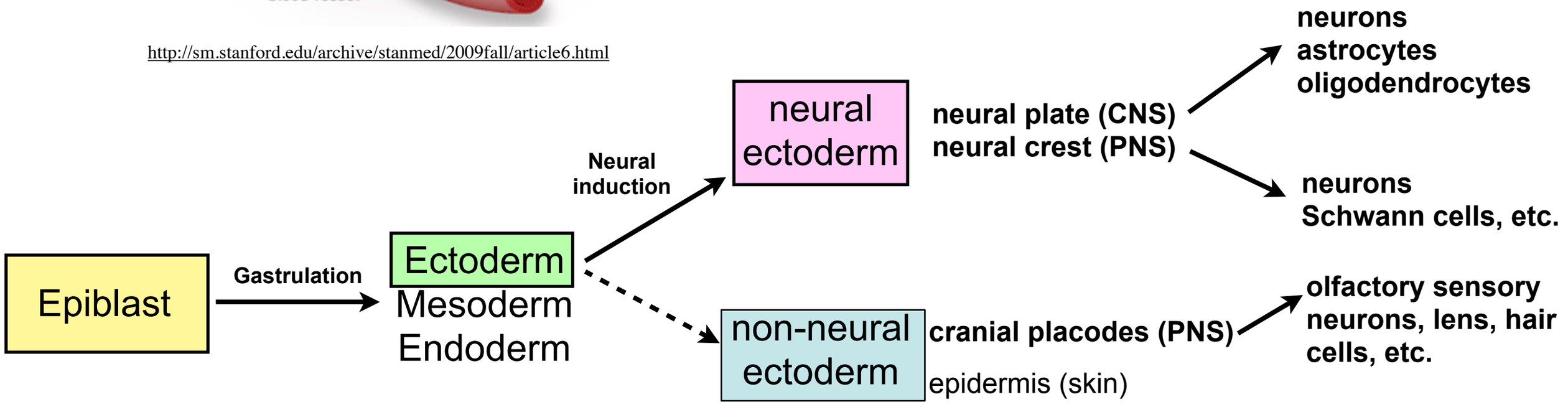
http://sm.stanford.edu/archive/stanmed/2009fall/article6.html

-Neurons and “macroglia” (astrocytes and oligodendrocytes) in the central nervous system (CNS) are derived from neural stem cells, which originate from the neural plate (neural ectoderm).

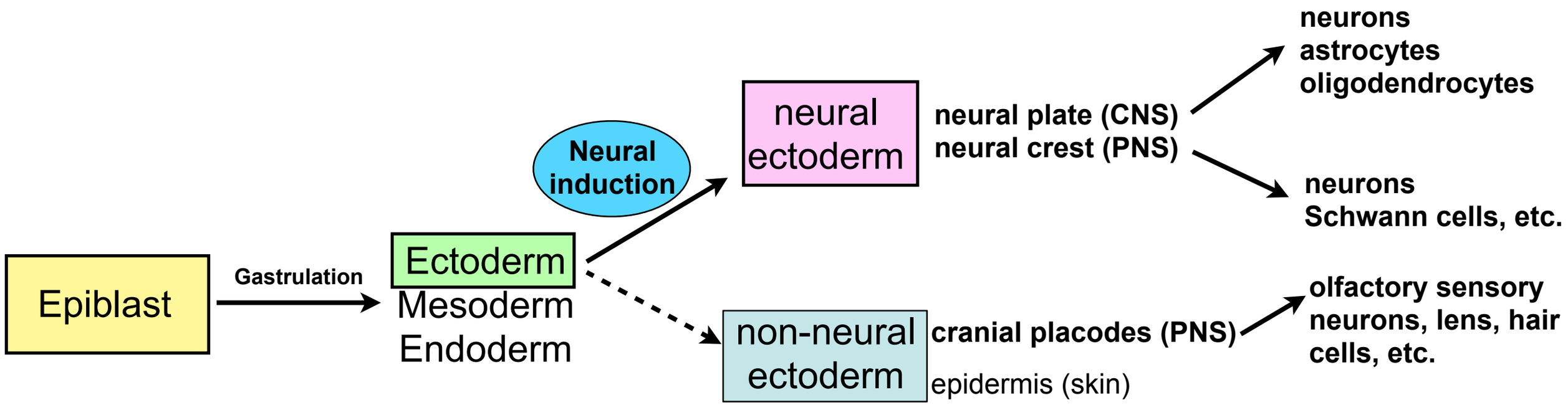
-Microglia are resident immune cells and are derived from the yolk sac (mesodermal origin).

-Cells forming blood vessels are also from the mesoderm.

-Neurons and glia in the peripheral nervous system (PNS) are derived from the neural crest and cranial placodes.



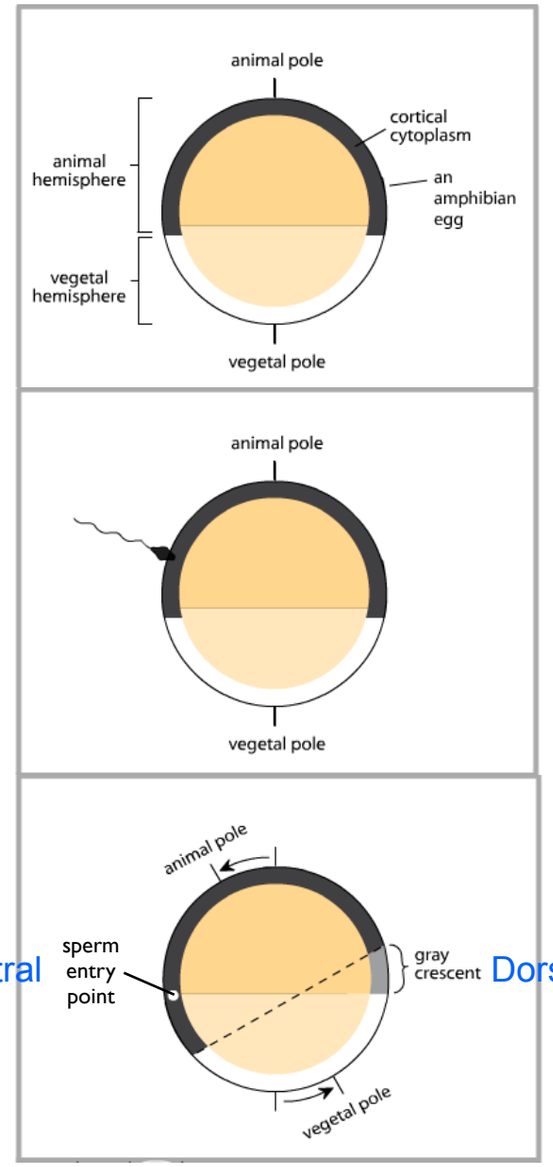
Neural induction causes the ectoderm to form the neural ectoderm



Embryological studies in 1920s found that a specialized, mesoderm-derived tissue (organizer) in amphibian embryos acts on ectodermal cells and make them form the neural ectoderm.

Responsible molecules derived from the organizer tissue were identified in 1990s.

First steps of animal development



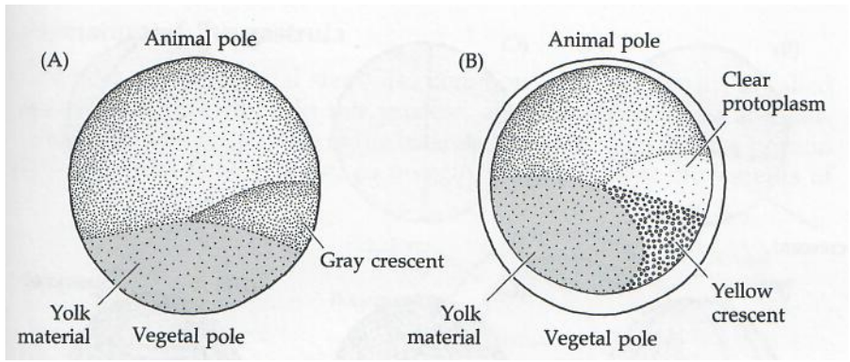
Ventral Dorsal

Egg cytoplasm is asymmetric before fertilization (sperm entry)...animal vs vegetal poles

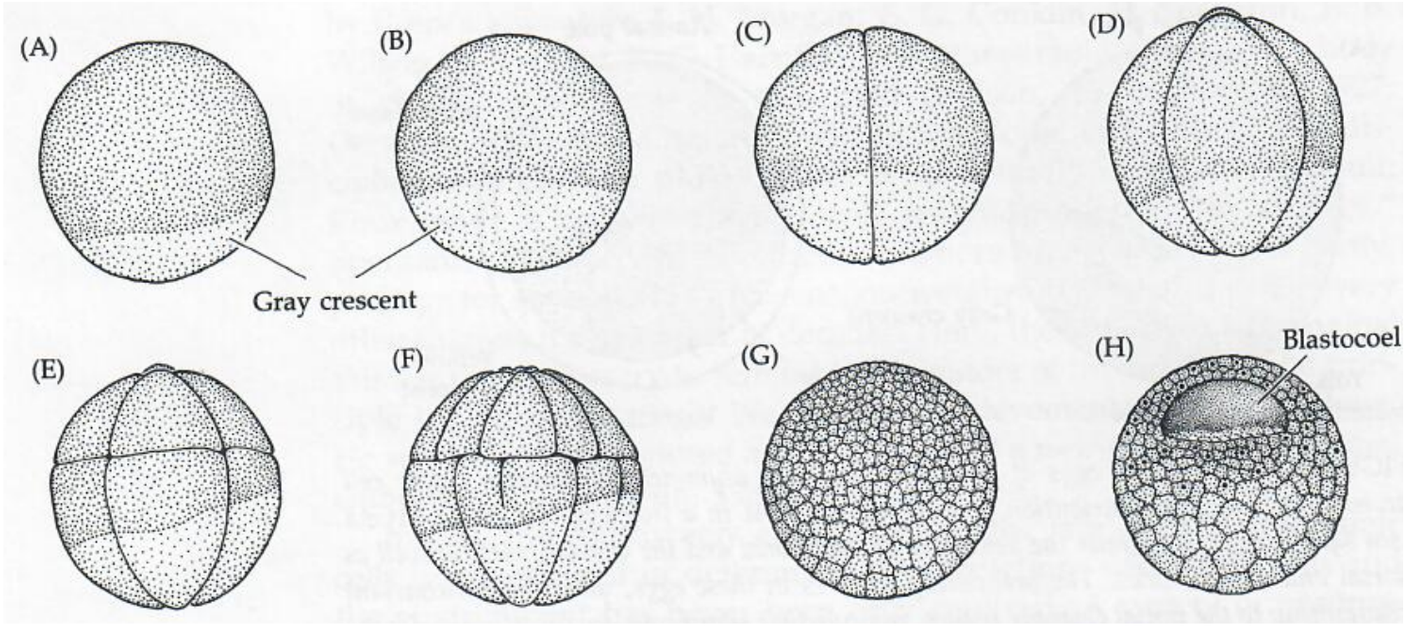
Sperm enters on the animal hemisphere.

Outer layer of the cytoplasm rotates toward the side of sperm entry.

This rotation creates a visible band called the grey crescent, resulting in the formation of the dorsal-ventral axis.



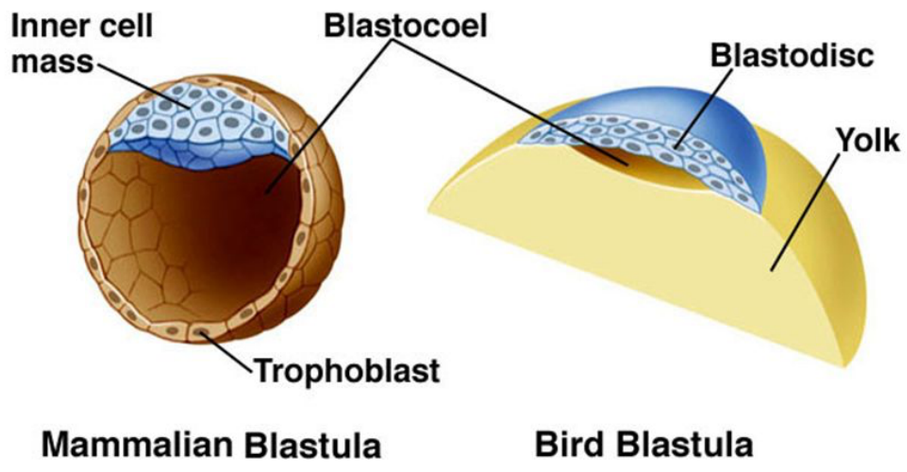
Formation of blastula



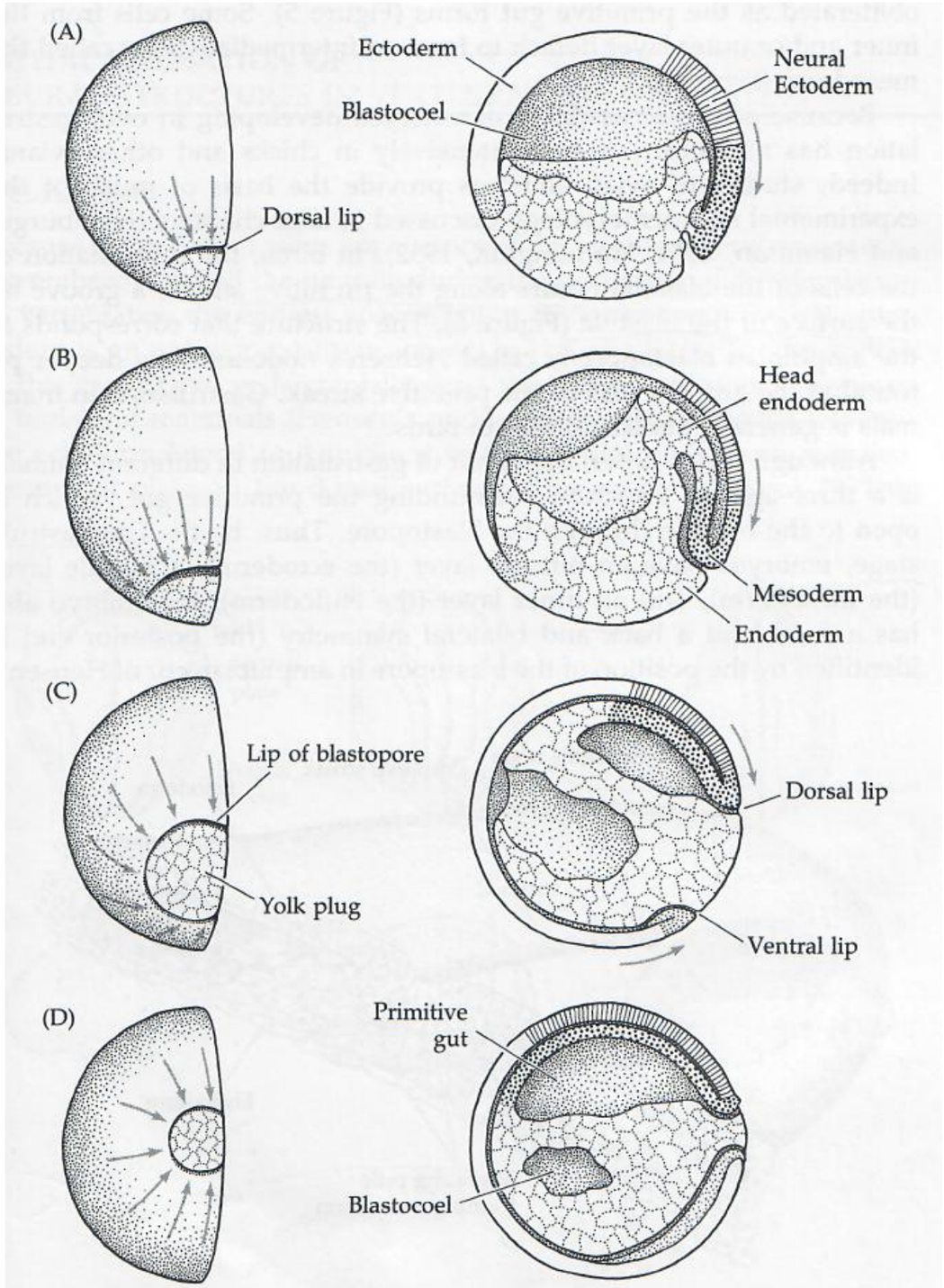
http://www.youtube.com/watch?v=IjyemX7C_8U

A fertilized egg quickly undergoes divisions (cleavage) and forms a blastula in ~6 hours. An inner cavity (blastocoel) is formed.

This process is shared among reptiles, amphibians, fish, birds and mammals, although the shape of blastula differs between species.



Gastrulation generates three germ layers

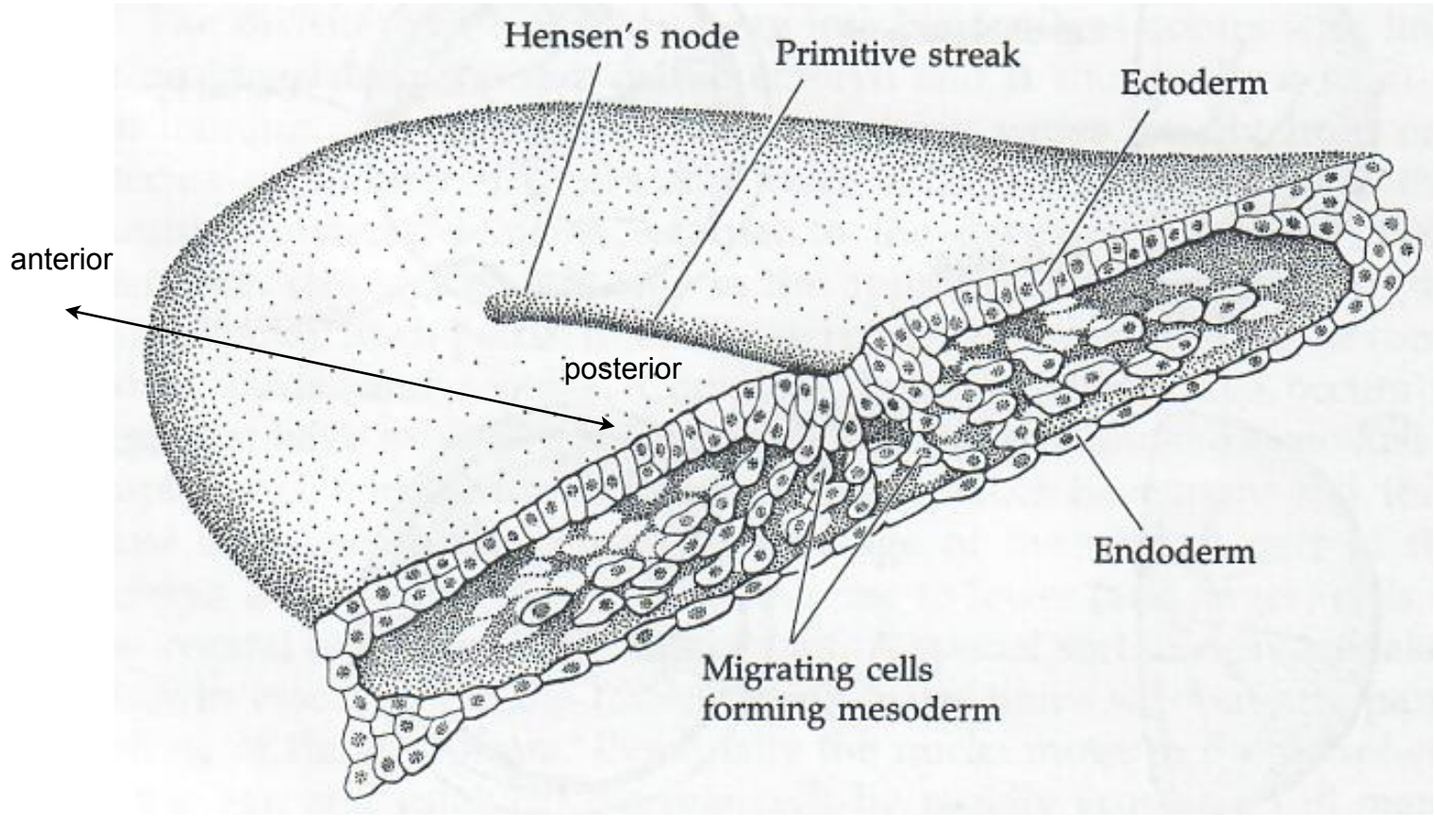


-An indentation (blastopore) forms on the surface of the blastula (where grey crescent was) (A).

-A portion of the embryo begins to invaginate (B).
Cells that go inside form the endoderm.
The outer layer becomes the ectoderm.
The intermediate layer called the mesoderm is also formed.

The blastocoel is obliterated as the primitive gut (also callee archenteron) forms (C,D).

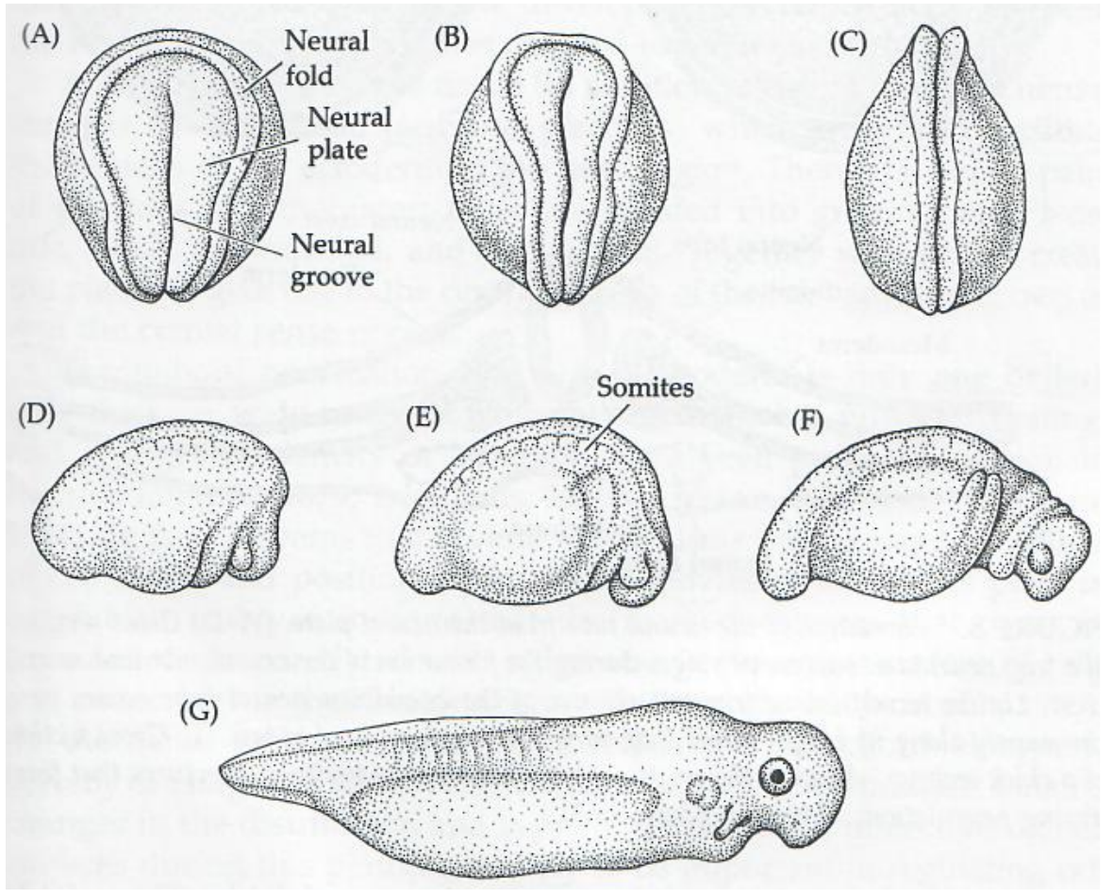
Gastrulation in birds

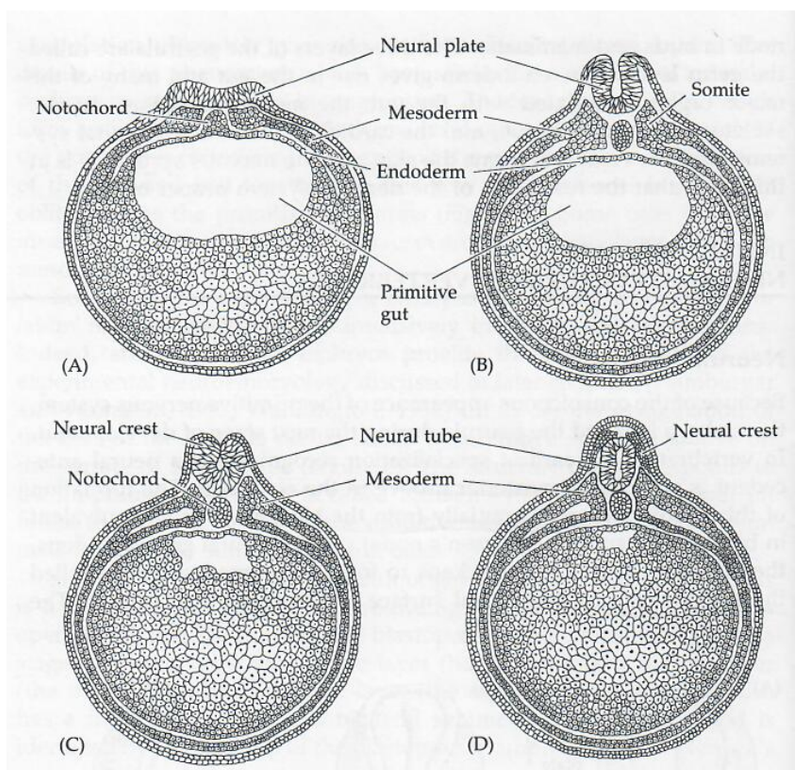


-Accessibility of embryos in ovo has made avians a good model of early development.
-In birds, invagination of cells of blastula occurs along the primitive streak.
-Hansen's node is a deeper pit at the anterior end of the primitive streak, and corresponds to the amphibian blastopore.
-Gastrulation of mammalian embryos is generally similar to that in birds.

-In all animal species, gastrulation results in:
1) formation of three germ layers; ectoderm, mesoderm (muscle, bone, blood, etc.) and endoderm (digestive organs, lung, etc.)
2) formation of front and back (posterior end: blastopore or Hansen's node) and bilateral symmetry

Neurulation





-During the next stage, the embryo is called the neurula due to the conspicuous appearance of the nervous system.

-The neural groove forms near the blastopore and extends anteriorly in the ectoderm.

-The ectoderm lateral to the neural groove widens and forms a flattened structure called the neural plate on the dorsal surface of the embryo (A).

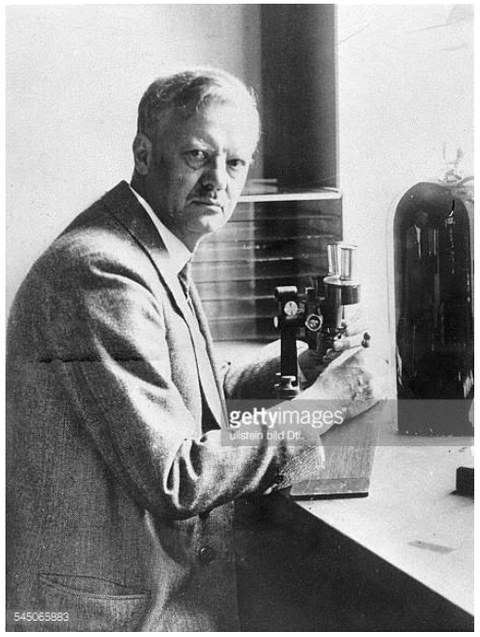
-The ridge of the neural plate (neural folds) thickens and bends towards the midline (B), where they meet and fuse (C). This process is called neurulation and gives rise to the neural tube.

-Some mesodermal structures are also formed during neurulation, including the notochord (midline; axial mesoderm) and somites (lateral; paraxial mesoderm).

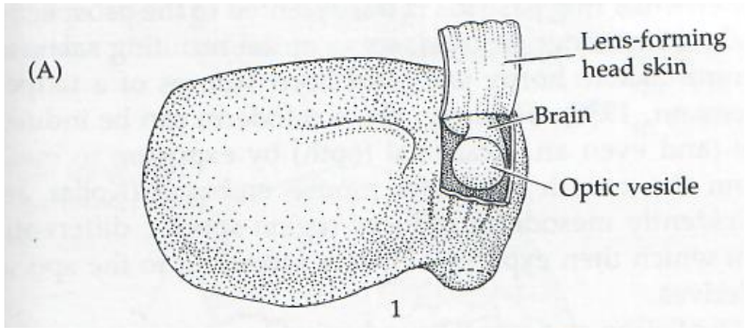
-Neural crest arises from the neural plate but separates from it at the lateral border of the neural folds.

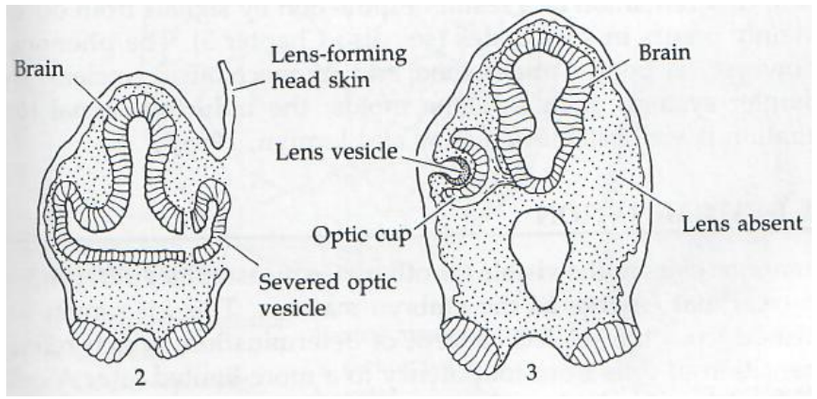
Mechanisms of neural induction

Hans Spemann (1869-1941) studied cell-cell interactions during early embryogenesis.



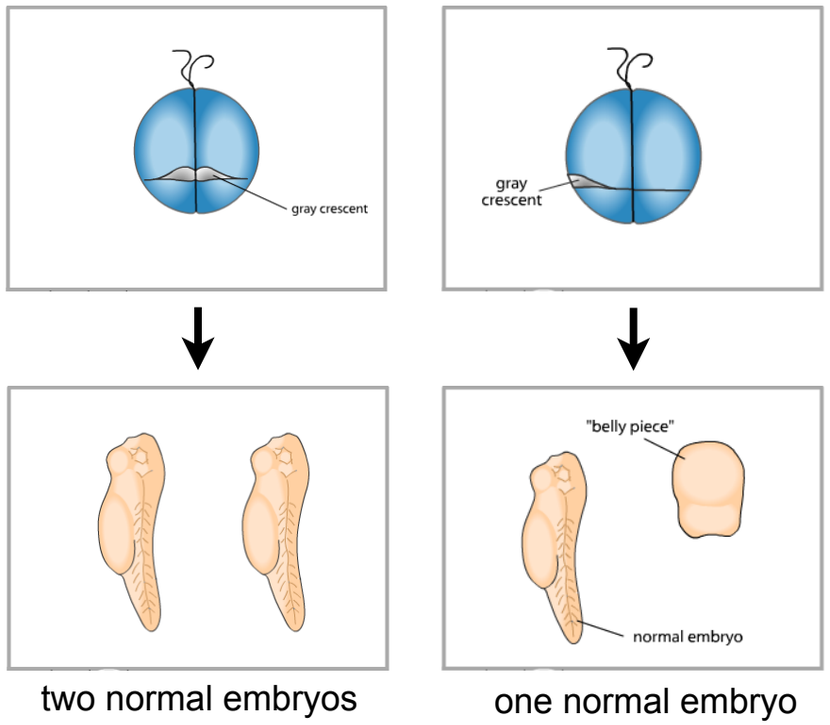
1. lens induction by underlying optic vesicle





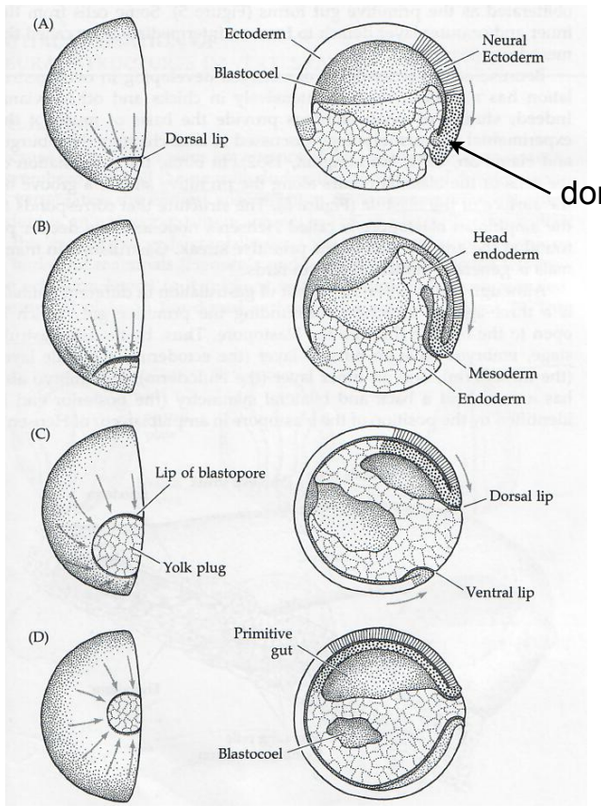
2. separation of blastomeres

It was necessary to include the grey crescent (future position of the dorsal lip of the blastopore) for the entire embryo to form.



two normal embryos

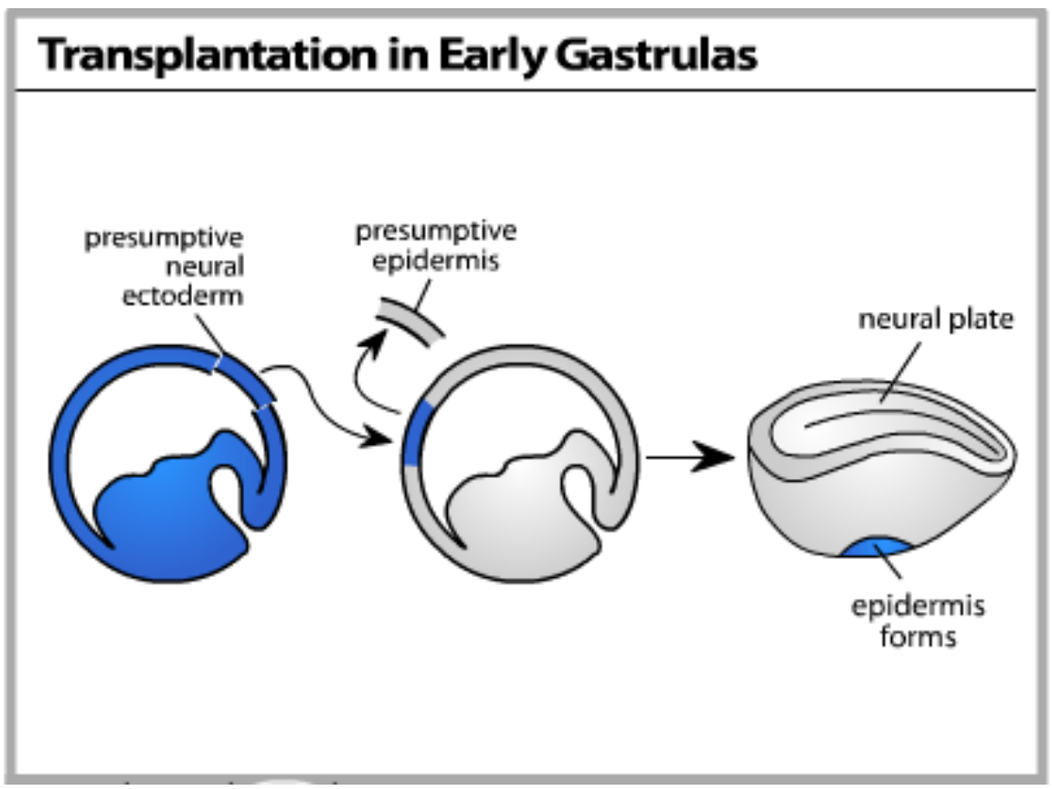
one normal embryo

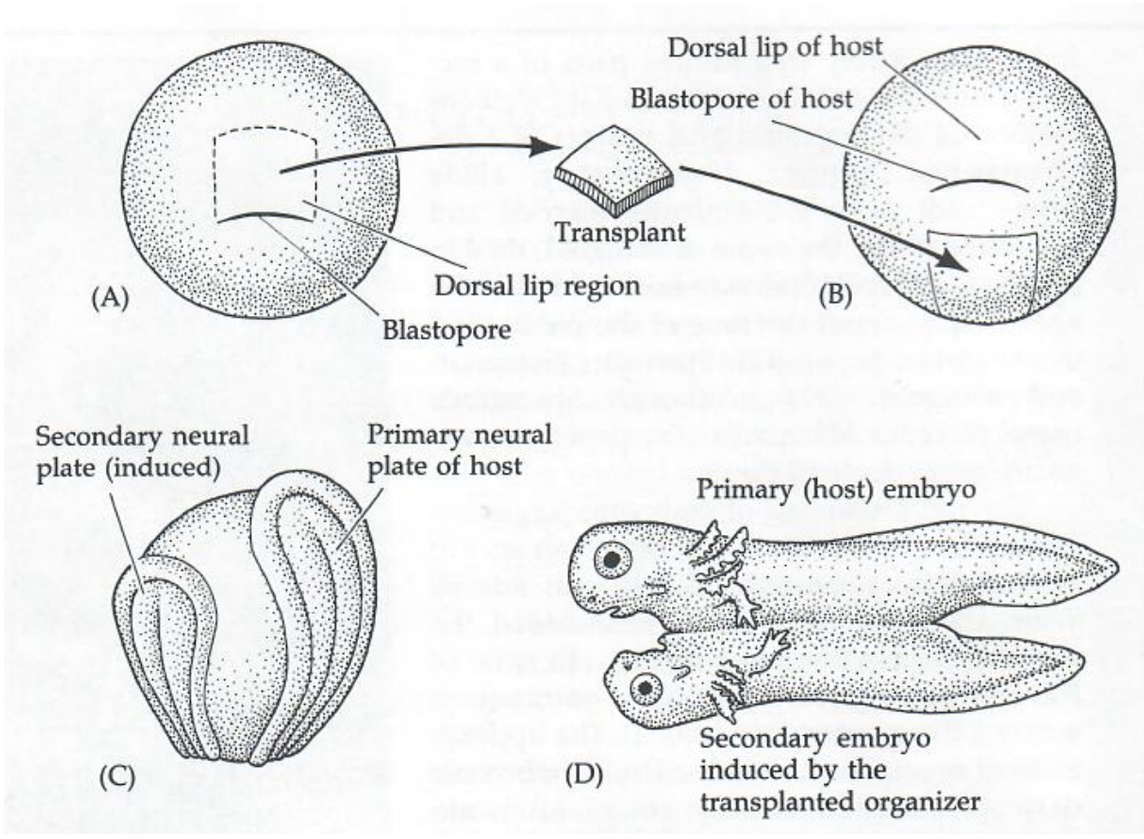


Mangold/Spemann's transplantation experiments

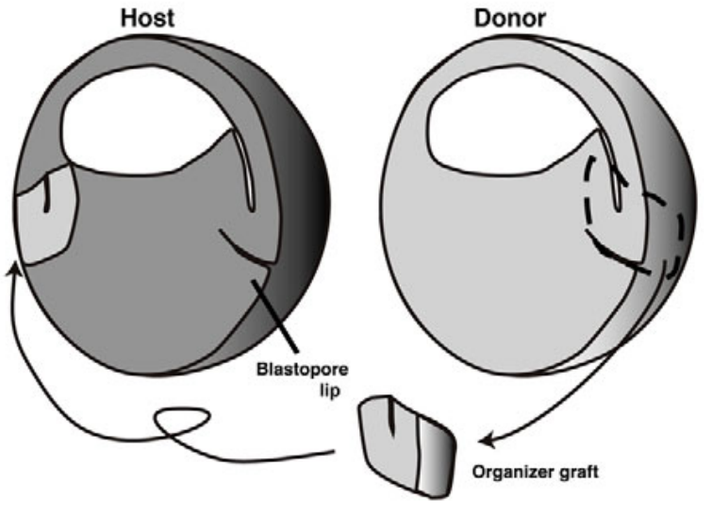
Hilde Proescholdt (Mangold) transplanted various parts of newt embryos into the host at early gastrula stage:

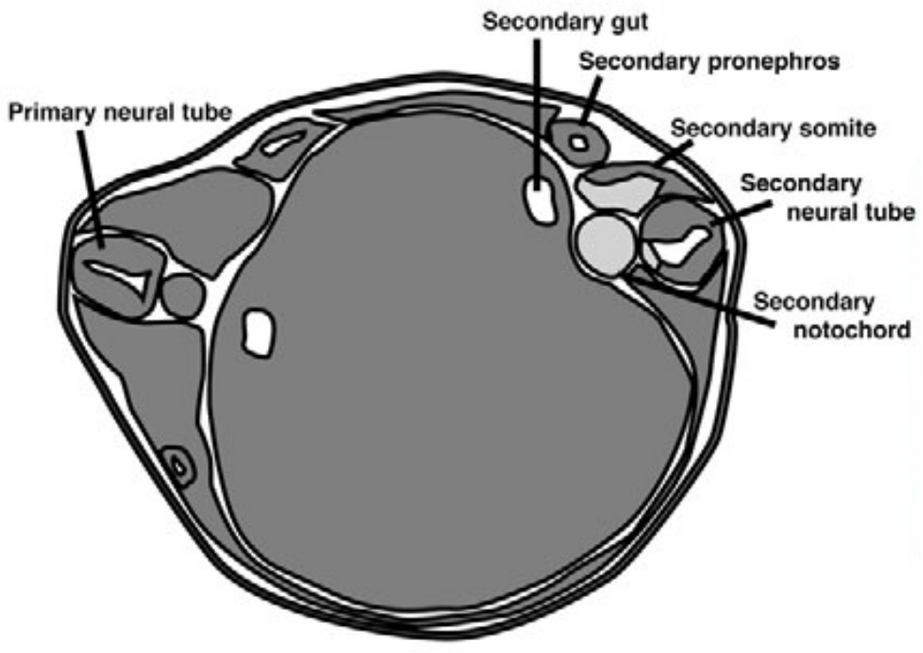
-Most pieces adopted the fate of the host tissue.
-An exception was the dorsal lip transplant. The dorsal lip did not adopt the host fate and induced a variety of unexpected tissues like the neural plate, notochord and somites.





Neural induction by the dorsal lip





Harland (2008)

The use of pigmented donor and unpigmented host allowed the identification of the origin of the new tissues.

Notochord: donor-derived

Neural tissue: host-derived

Conclusion:

The dorsal lip of the blastopore (they called it the organizer) induced the formation of neural tissues from the ectoderm that would have otherwise become epidermis.

This experiment demonstrated that cell and tissue fate can be determined by signals received from other cells (embryonic induction).

Hans Spemann was awarded with the Nobel Prize in 1935 on his work on embryonic induction.

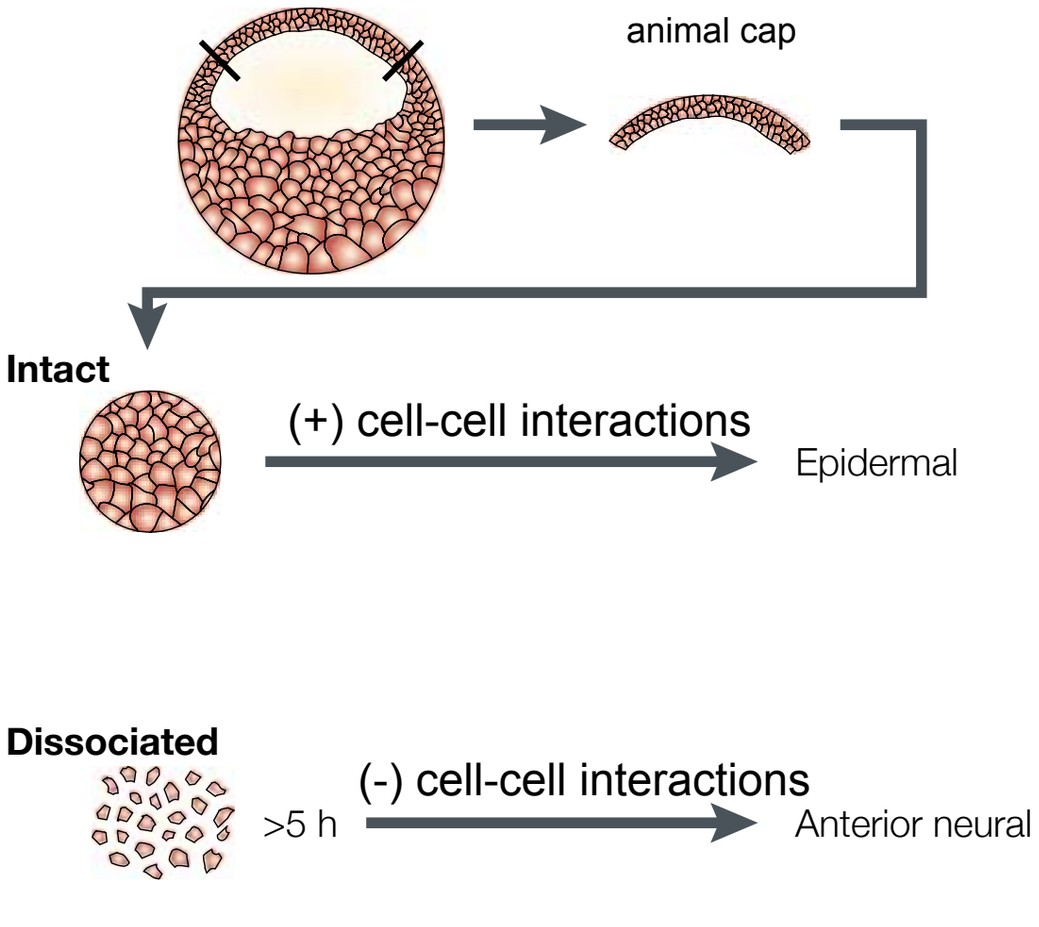
Soon after Mangold/Spemann's findings, Waddington showed that the rostral tip of the primitive streak (=Hansen's node) has similar properties to Spemann's organizer (1930, 1934).

What are the molecular substances of the Spemann organizer?

Does the organizer secrete soluble molecules that instruct the ectodermal cells to take on the neural fate instead of epidermal fate?

No one could identify such molecules until the 1990s.

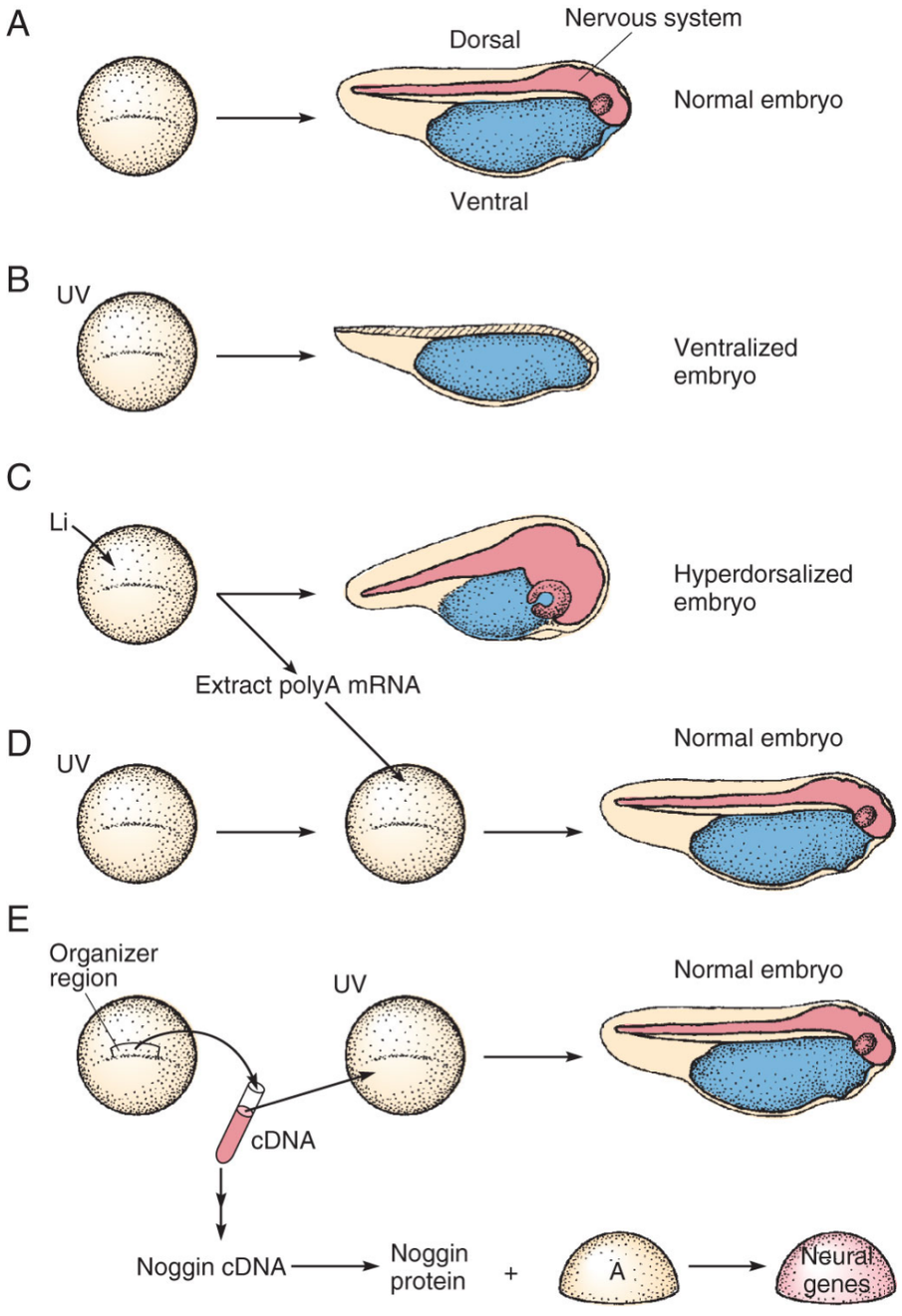
In vitro experiments suggested a “default model” of neural induction.



1) isolated ectoderm explants (animal caps) differentiated into epidermal tissue in vitro when the tissue was kept undissociated.

2) when isolated animal cap cells are kept dissociated, they differentiated into neural cells

Identification of endogenous neural inducers



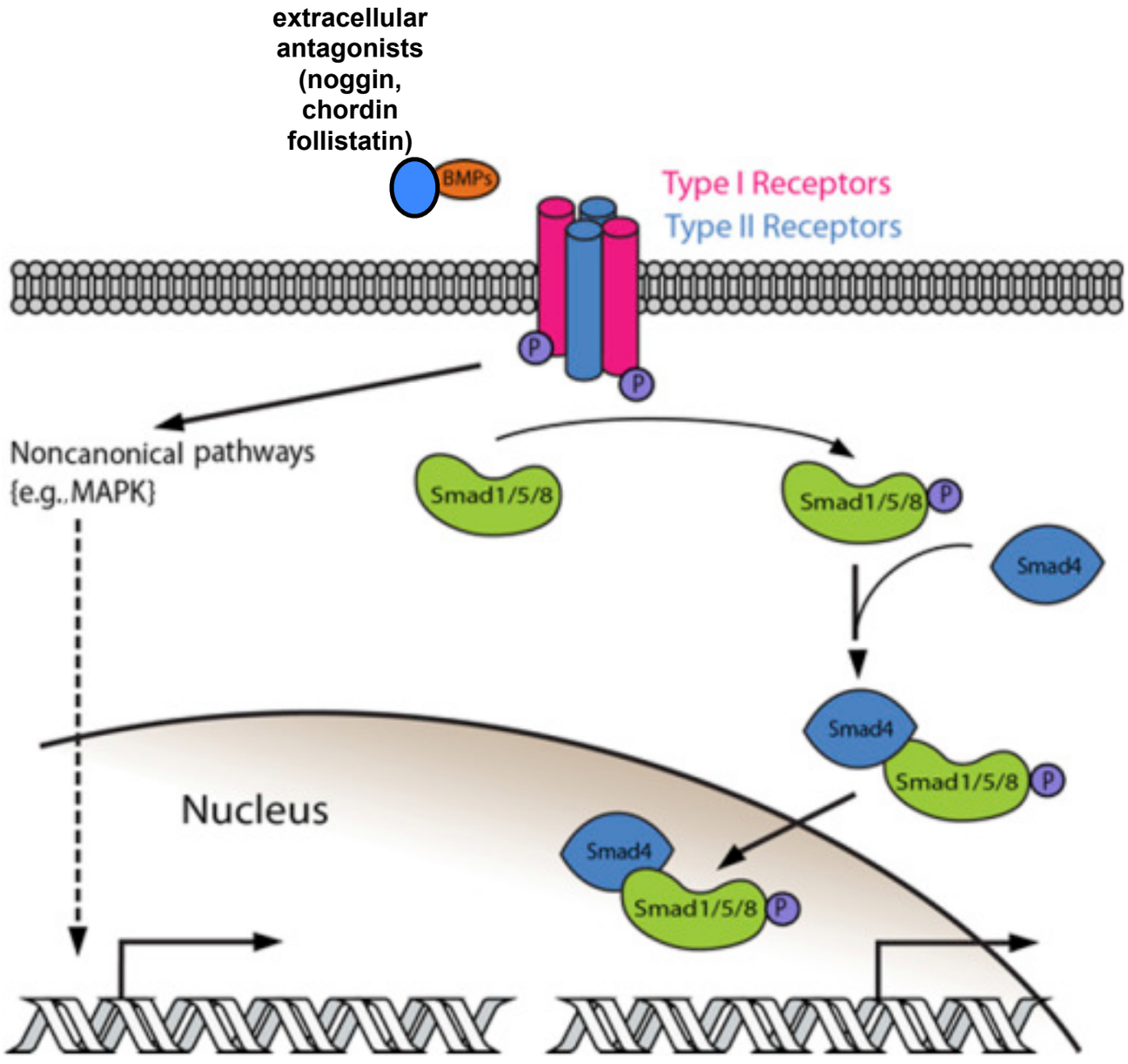
mRNAs that encode neural-inducing molecules should be expressed in the organizer. How can you get a tissue that is enriched in such mRNAs?

Richard Harland's group used "expression cloning" strategy to isolate noggin as a neural inducer expressed in the organizer region (1993). Other groups found follistatin and chordin.

-These molecules induced the neural plate without requiring the presence of mesoderm.
-They were expressed in the dorsal lip and the notochord (=derivative of the dorsal lip).

Later experiments indicated that they do not act in an instructive manner to induce the neural tissue; instead, they act indirectly by inhibiting the bone morphogenic protein (BMP) pathway.

BMP signaling pathway



Type I and type II receptors form a heterotetramer.

Binding of BMP to receptors is inhibited by extracellular antagonists like noggin, chordin, etc.

Binding of BMP to the receptors results in phosphorylation of type I receptors

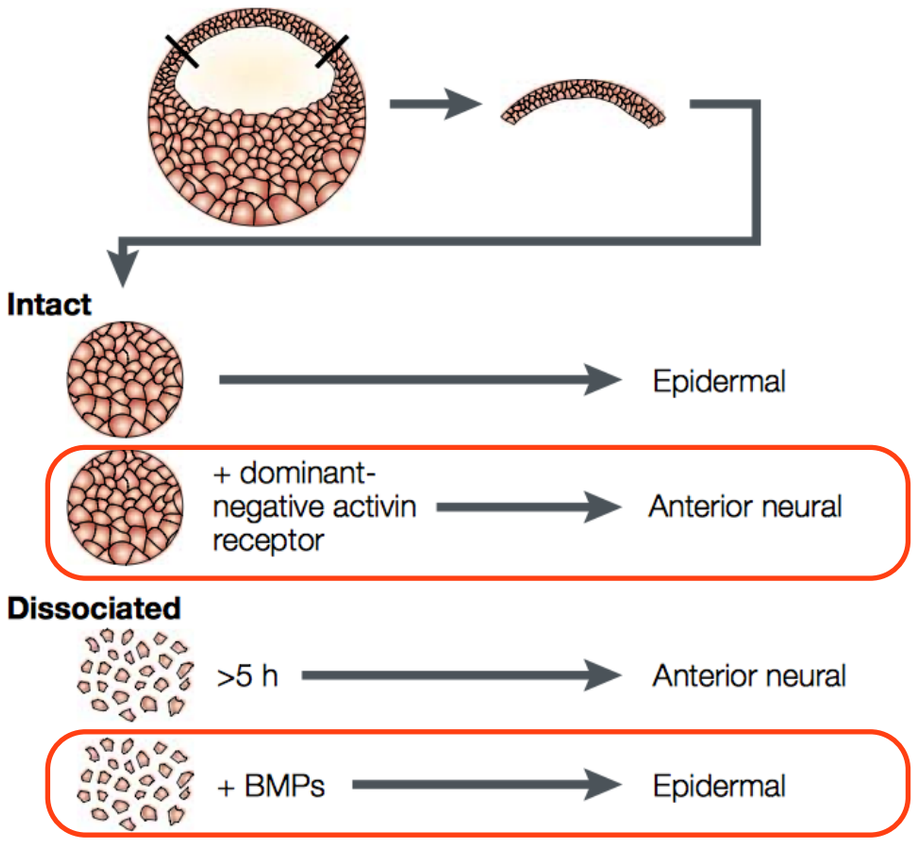
Phosphorylated type I receptors activate transcription factors (Smad1, 5, 8 or ”R-Smads”) by phosphorylating them.

Phosphorylated R-Smads move to the nucleus.

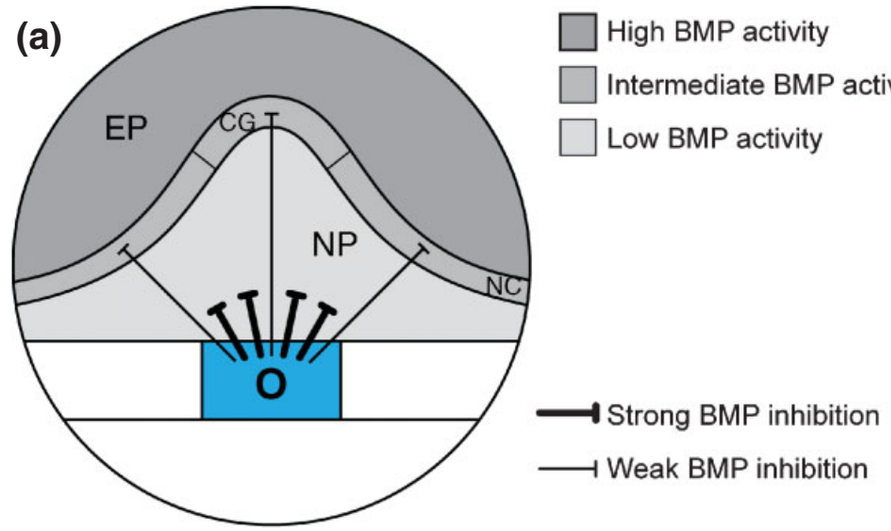
Together with co-Smad (Smad4), R-Smads activate the transcription of various epidermal genes.

Inhibiting BMP signaling induces the neural tissue

animal cap assay (in vitro)



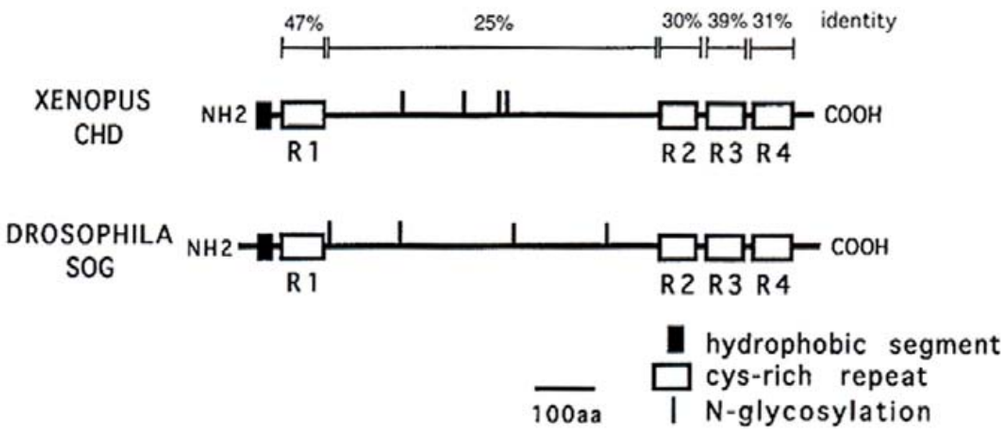
in vivo model

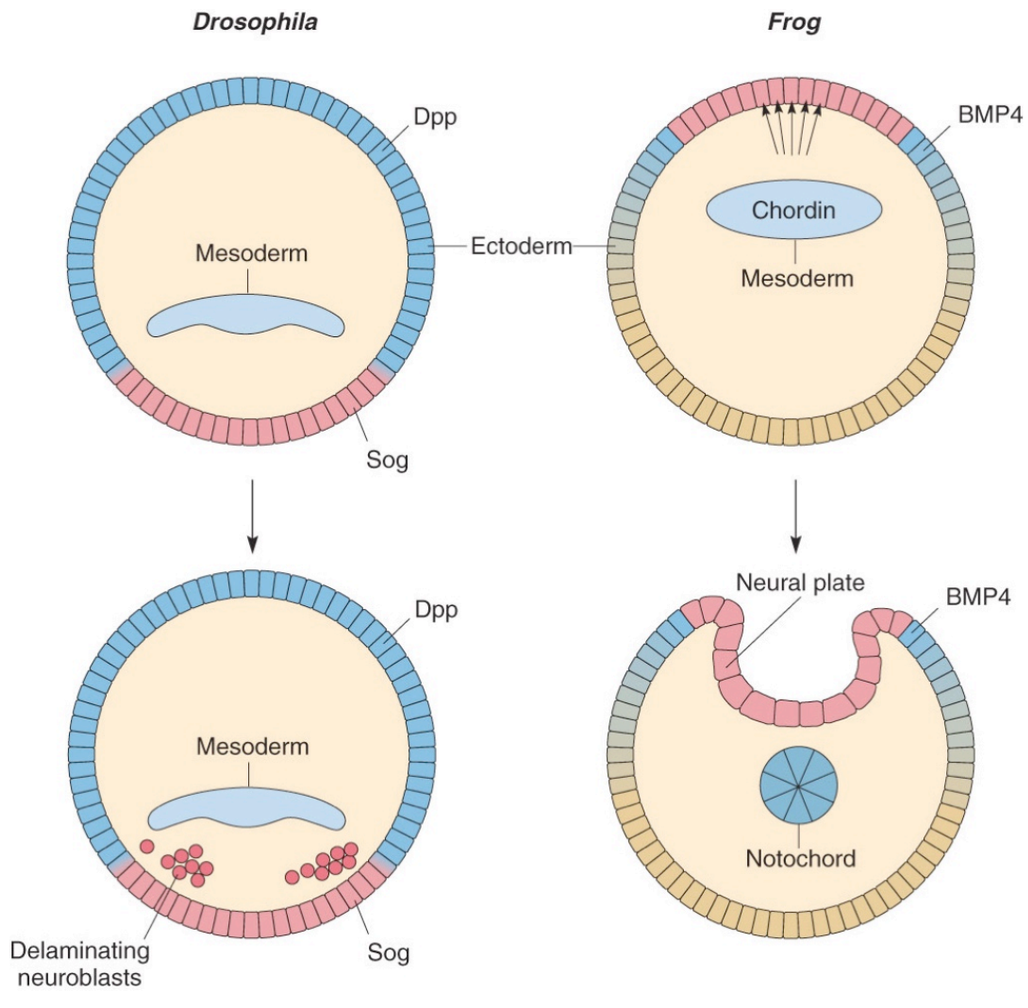


[Default model of neural induction]
Cells within the ectoderm layer of the frog gastrula have an autonomous tendency to differentiate into neural tissue, which is inhibited by BMPs.

Conserved mechanisms of neural induction

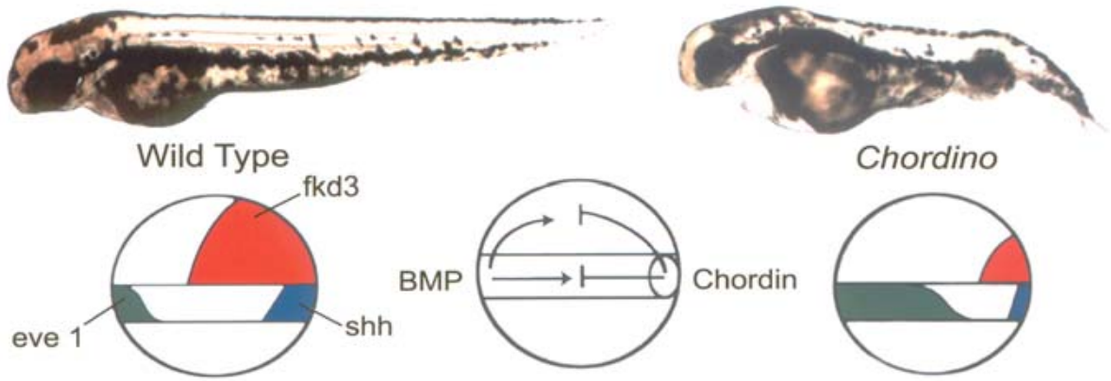
Embryonic axis is flipped in arthropods (epidermis forms in dorsal ectoderm and neural tissues arises from the ventral ectoderm), but antagonism between BMP (=Dpp in flies) and Chordin (=Sog in flies) is the same in neural induction.



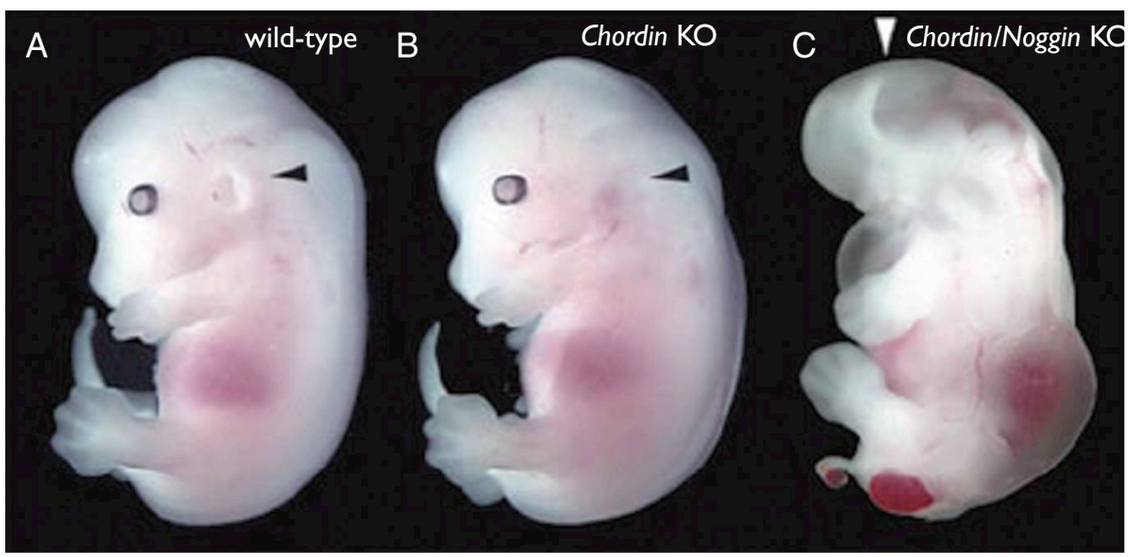


Molecular redundancy in neural induction

Neural induction in vivo depends on multiple ligands and inhibitors.
Chordin mutants in zebrafish still have the neural plate, although it was smaller. When all three neural inducers are knocked down, a complete loss of neural tissue is observed.



Head is almost absent in double mutants of Chordin and Noggin in mice, but mutating only one of the two genes does not cause severe defects.



At least three of the four BMPs (BMP2/4/7) need to be disrupted in Xenopus embryos to expand the neural plate.

FGF signaling and neural induction

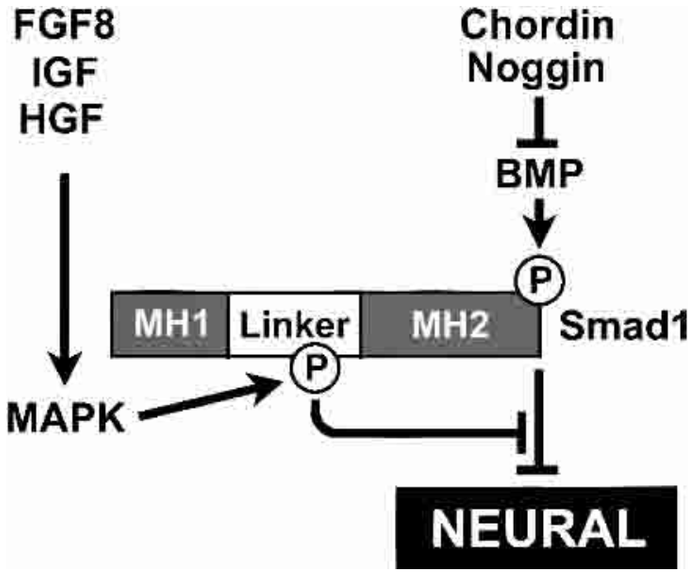
Factors other than BMP inhibitors could play a role to alter the competence of the ectoderm to become neural.

-Blocking FGF (fibroblast growth factor) signaling prevented BMP antagonists to induce neural tissues in embryos.

-In chick, FGF can induce a neural tissue with a caudal character.

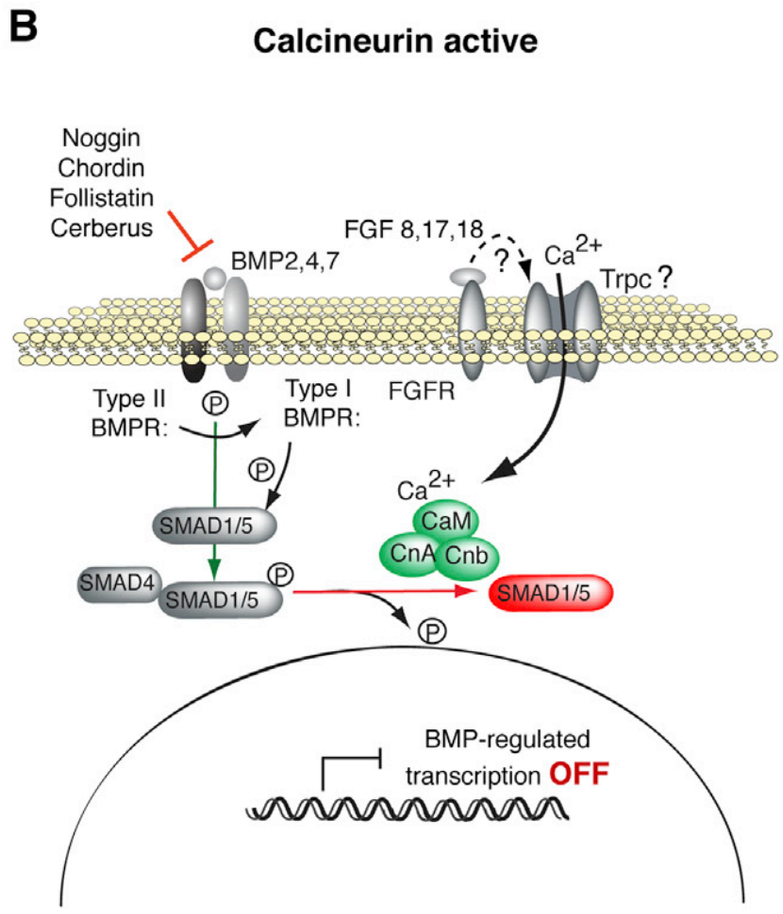
<u>Possible molecular interactions between FGF and BMP pathways:</u>

(1) MAPK phosphorylates linker domain of RSmads (receptor-regulated Smads, Smad1/5/8) and inhibit their activity.



Pera et al., 2003

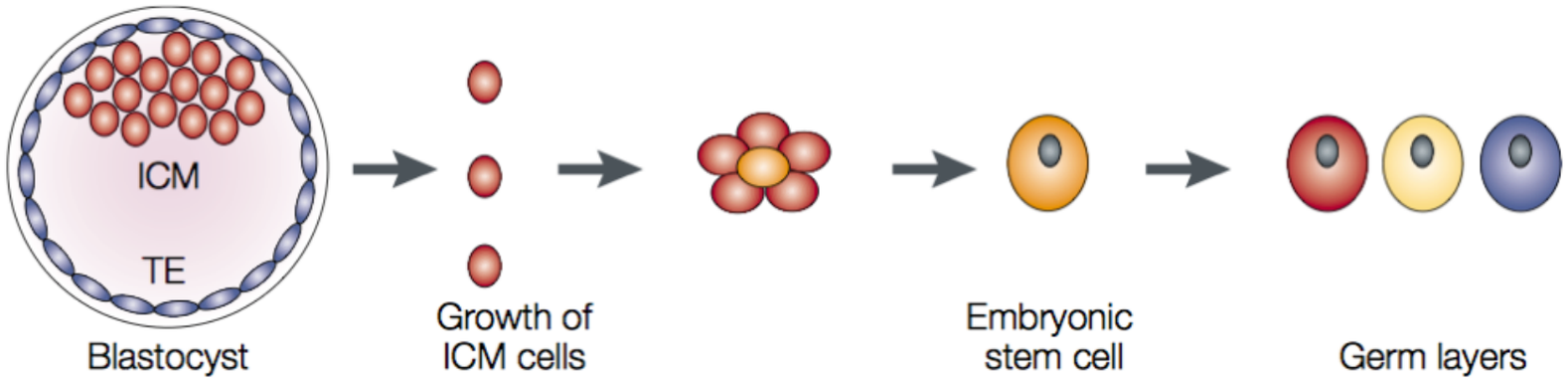
(2) FGF signaling regulates Ca^{2+} entry, which activates the serine-threonine phosphatase Calcineurin (CN)



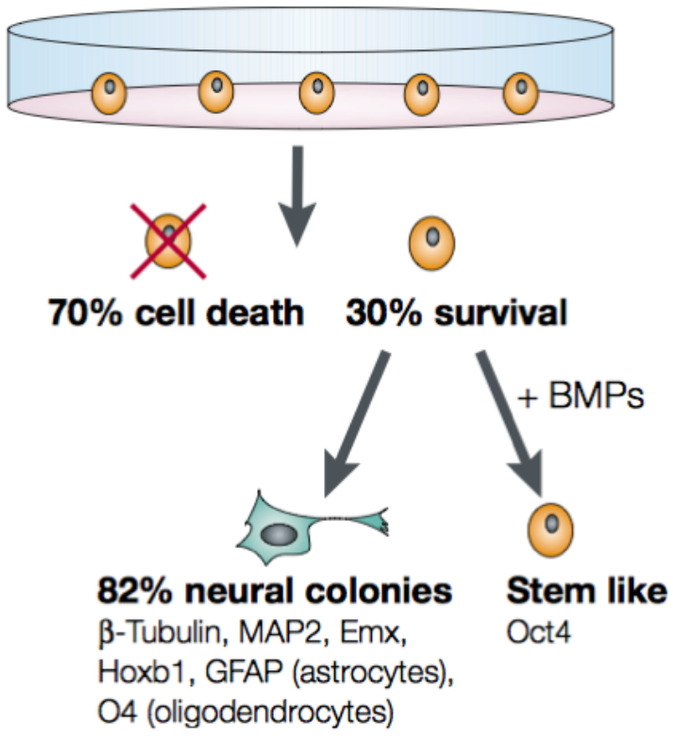
Cho et al. 2014

Neural induction in mouse ES cells

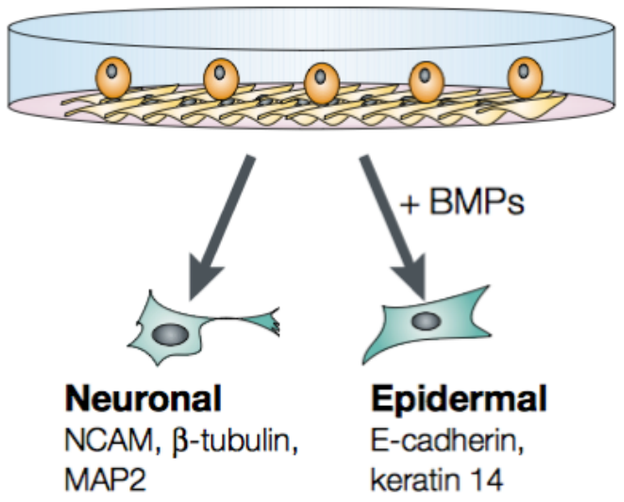
Xenopus animal caps and mouse ESCs share signaling mechanisms for neural induction.



Mouse ES cells in limiting-dilution; no feeder cells, no serum; + LIF and FGF2.
Tropepe *et al*., 2001



Mouse ES cells in stromal feeder layer; + SDIA produced by the feeder layer.
Kawasaki *et al*., 2000



Munoz-Sanjuan and Brivanlou (2008)

Summary 1 (up to neural induction)

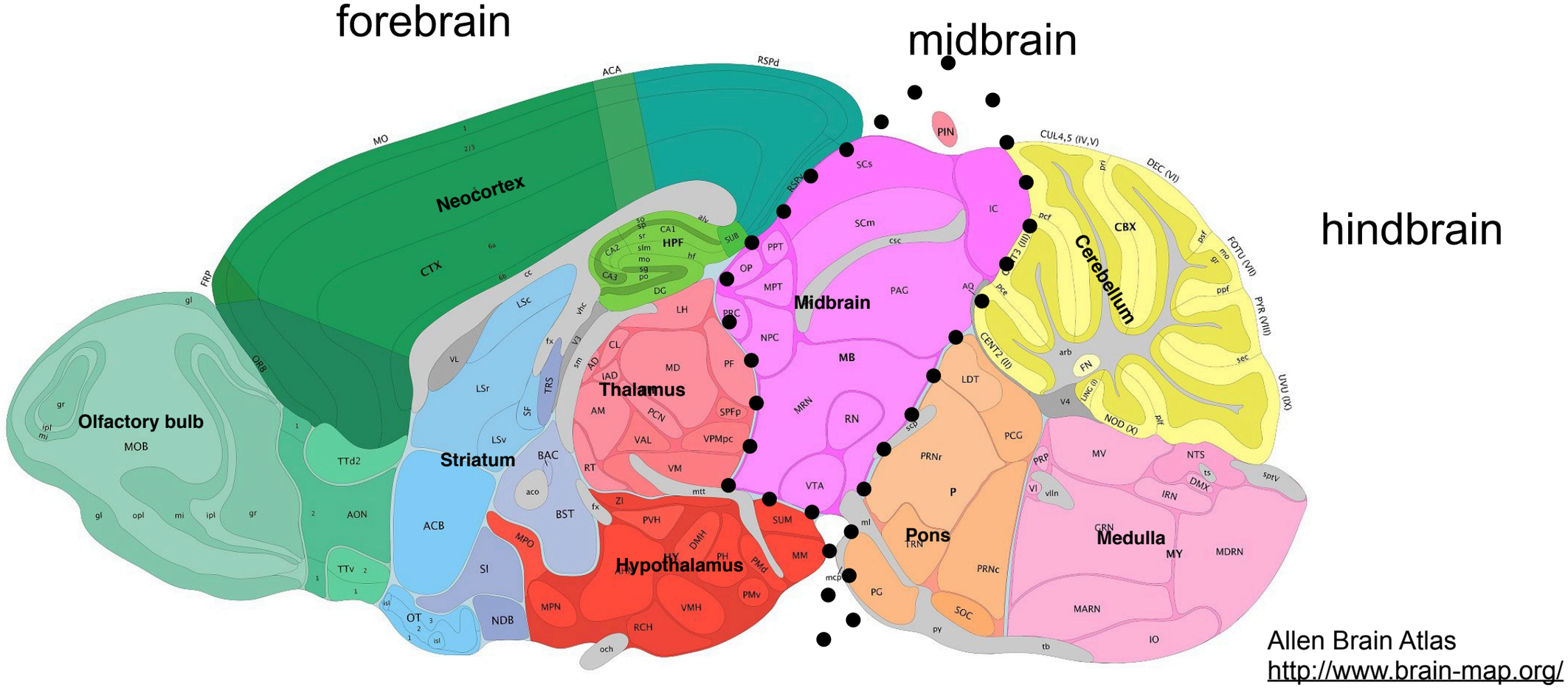
-first steps of embryonic development
  fertilization, cleavage, gastrulation

-Gastrulation causes formation of three germ layers.
-Neural induction causes ectoderm to form the neural tissue.
  mesoderm-derived organizer tissue produces BMP inhibitors.

-Neurulation results in the formation of the neural tube.

-Overall, these early processes are conserved across many species.
  Even the Drosophila uses BMP inhibition for neural induction.

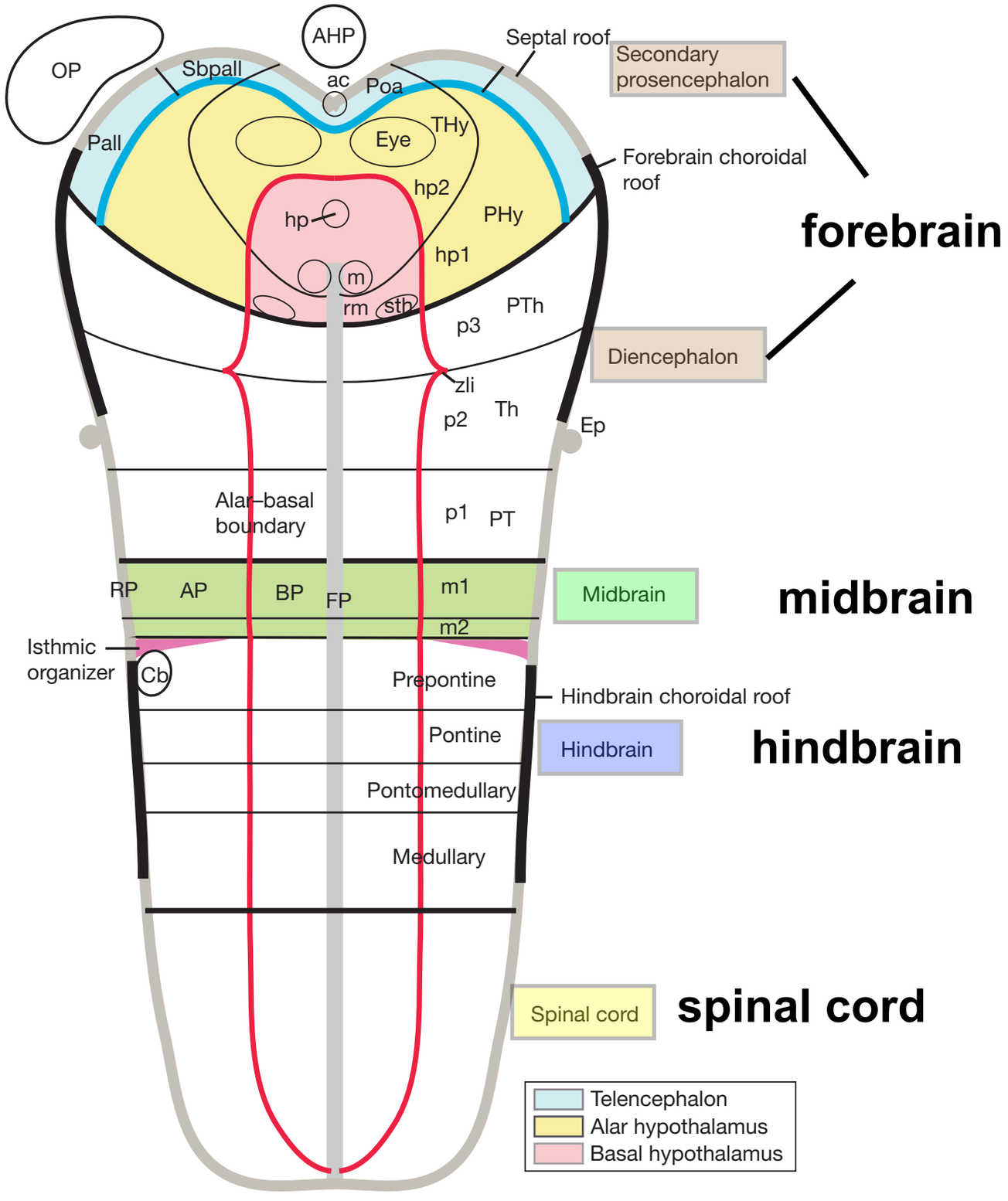
Regional organization of the adult brain



Allen Brain Atlas
http://www.brain-map.org/

How is the regional difference of neural tissue established?

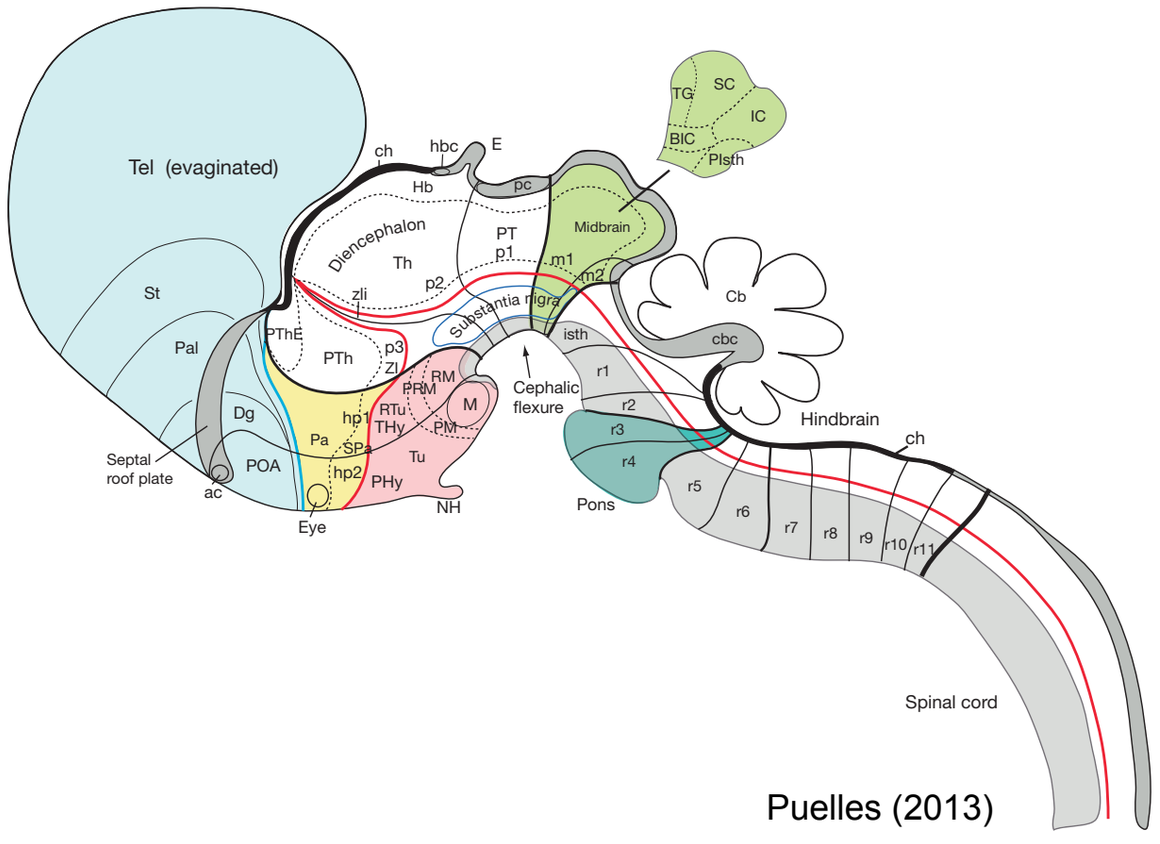
Position of early neural cells determines their later fate



-Fate mapping studies (mainly on chick embryos) generated the regional correspondence between neural plate and the mature brain.

-Cells in early neural tissue acquire identity that is appropriate for their location ("positional identity"). This process is called regionalization.

-Positional identity contributes to the generation of different types of neurons.

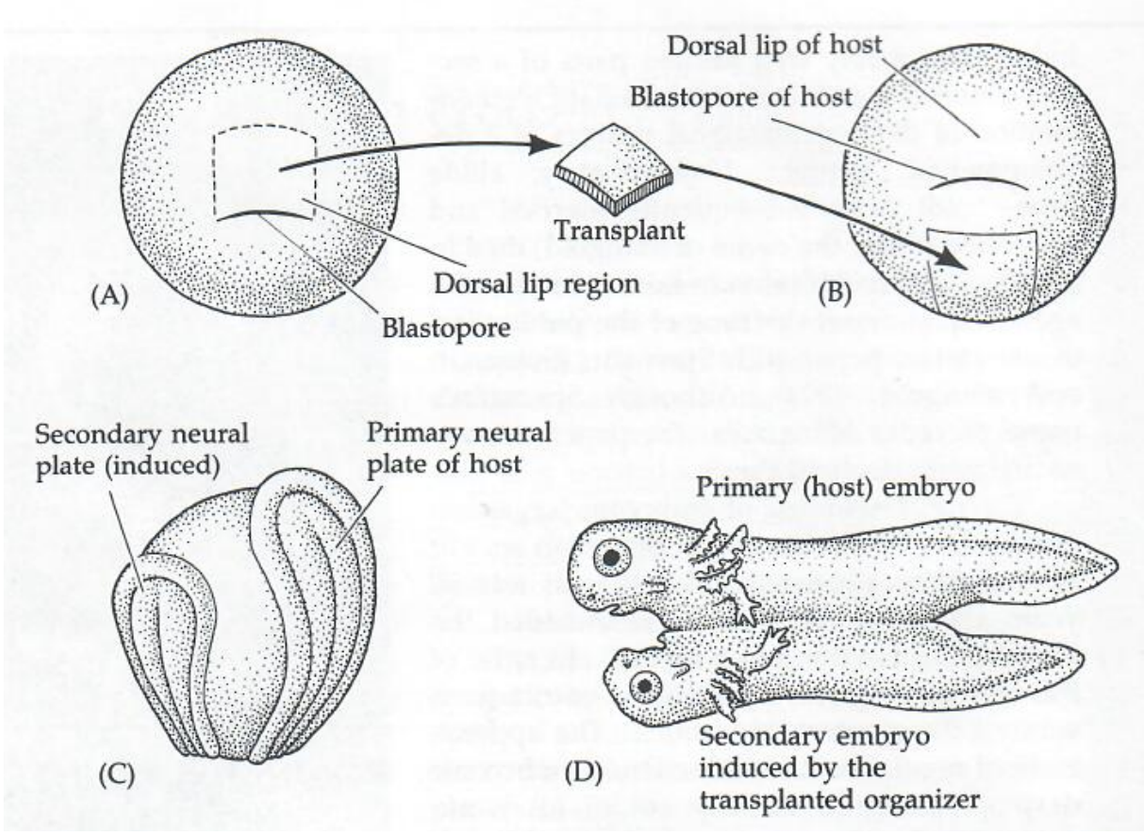


Puelles (2013)

Linking neural induction with regionalization

The transplanted organizer induced ectopic nervous system from cells not fated to form a neural plate.

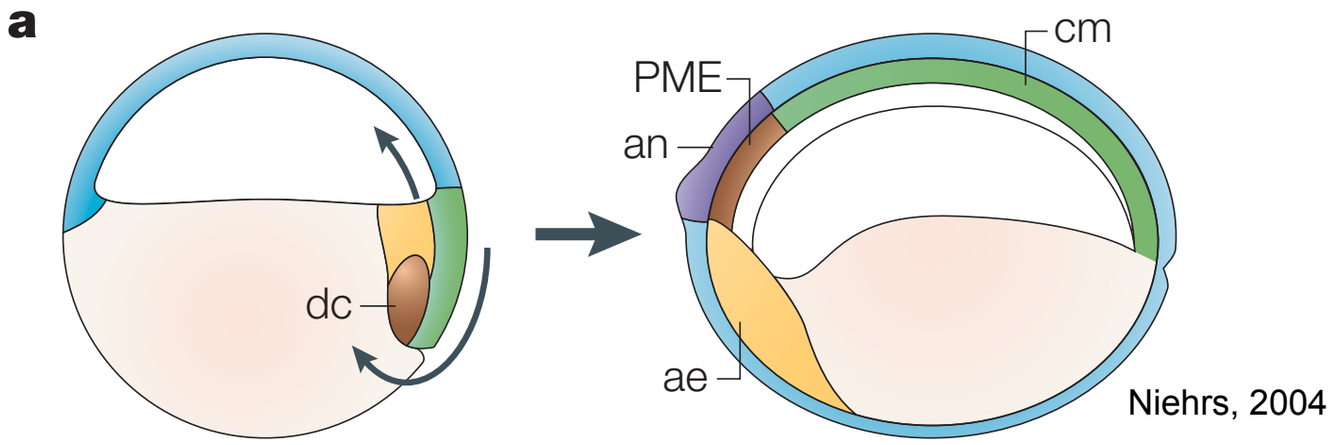
The induced nervous system was appropriately patterned along its rostro-caudal (anterior-posterior) and dorso-ventral axes.



-Does BMP inhibition not only induce the neural tissue but also pattern the induced neural tissue?

-Are other signaling mechanisms involved in neural patterning?

The dorsal lip later becomes distinct axial mesodermal tissues



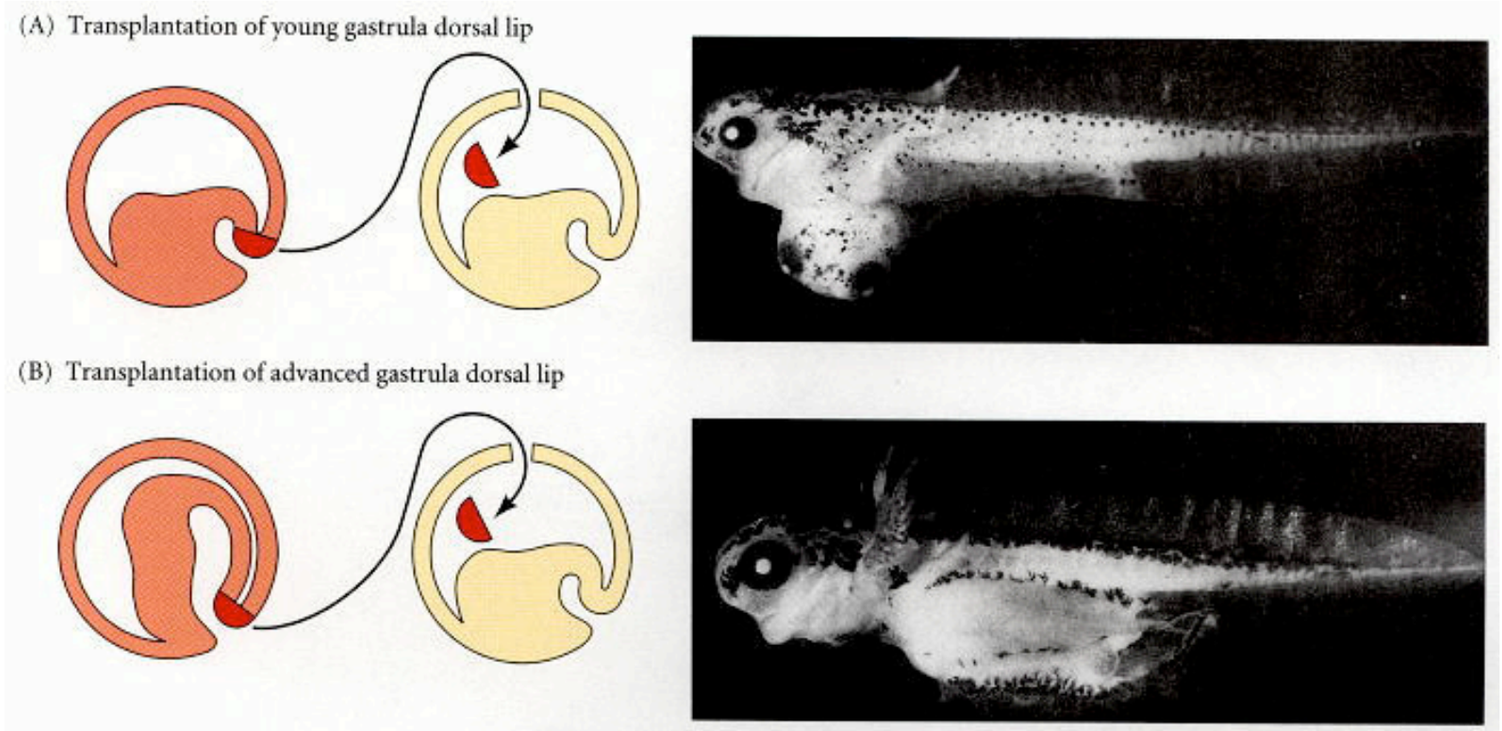
upon gastrulation,
-Early dorsal lip (dc: deep cells of the organizer) becomes prechordal mesoderm (PME) that underlies the anterior neural plate (an)
-Late dorsal lip becomes the notochord (cm: chordamesoderm) that underlies the posterior neural plate
(prechordal mesoderm and notochord are collectively called axial mesoderm because they are located in the midline)

-"Leading edge cells" (shown in yellow) becomes the anterior endoderm (ae))

Like the dorsal lip itself, both prechordal mesoderm and notochord can also induce the neural tissue.

If neural induction occurs largely via vertical signaling between the axial mesoderm and the overlying ectoderm, prechordal mesoderm and notochord may differentially induce rostral vs caudal neural tissue.

Temporal specificity of neural induction

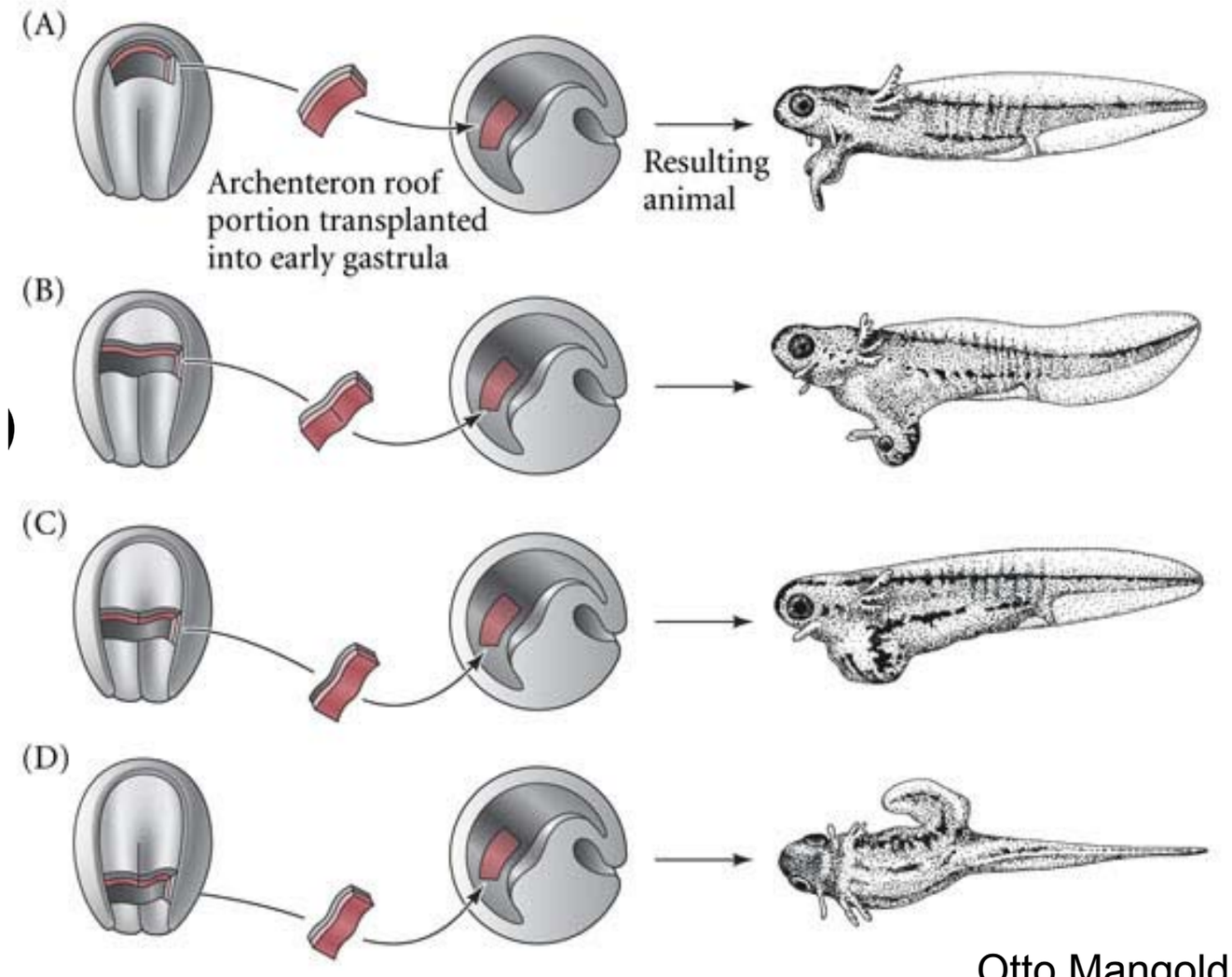


Young (early) dorsal lip induces a secondary head.

Older (late) dorsal lip induces a secondary trunk.

Regional specificity of neural induction

transplantation of different rostral-caudal parts of the archenteron roof (future axial and paraxial mesoderm) into early gastrula

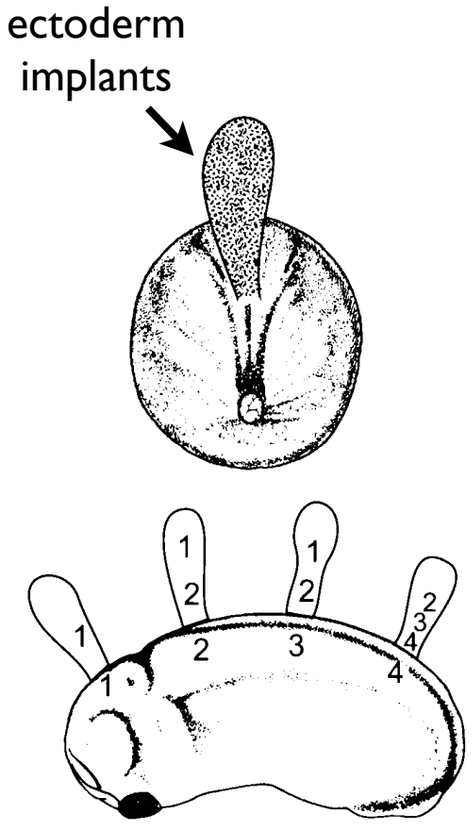


induced tissue	
balancers and oral apparatus	
head structures (forebrain and midbrain)	
hindbrain	
spinal cord	

Otto Mangold (1933)

Mangold proposed that distinct organizers induce different regions of the neural tissue separately (“head-trunk-tail organizer model”).

Niewkoop's activation-transformation model



Niewkoop (1952)

Grafts of early ectodermal tissues were transplanted into different parts of the future neural tissue.

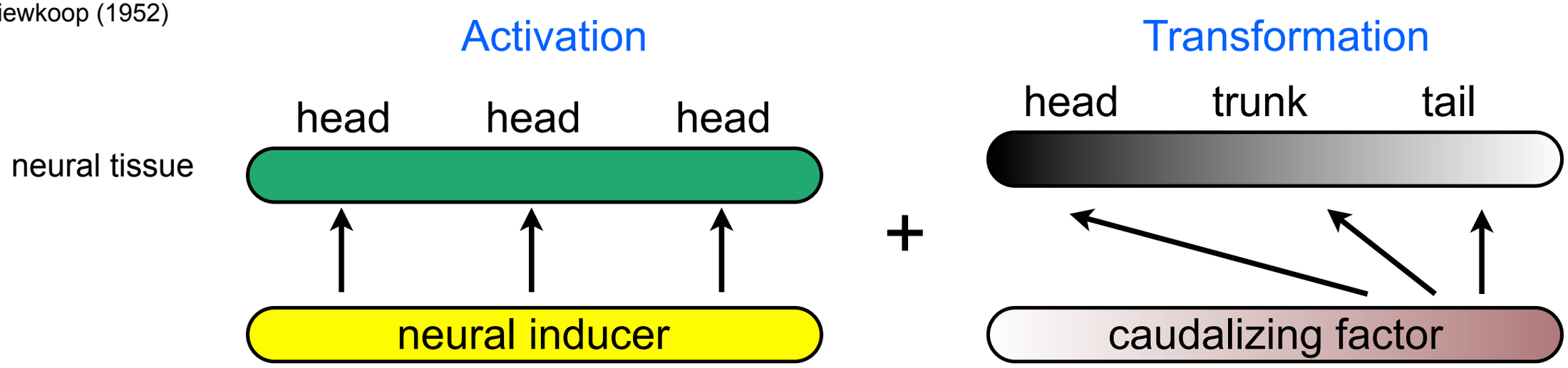
-The proximal part of the graft included neural tissue, whereas the distal part included non-neural tissue

-Within the induced neural tissue, the more distal part was always rostral whereas the more proximal part was always caudal (1 is the most rostral, 4 is the most caudal)

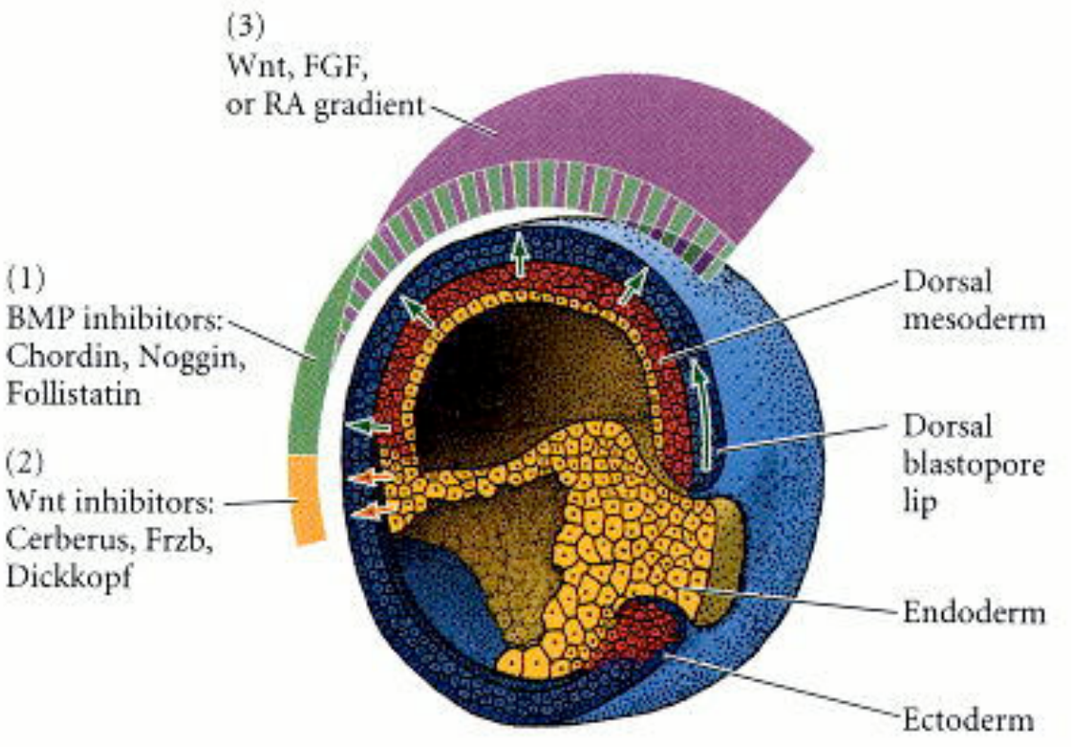
The level of the graft in the host always determined the regional character of the most caudal neural tissue in the graft.

Activation-transformation model:

Activation: induction of anterior neural tissue
Transformation: dose-dependent caudalization of the neural tissue



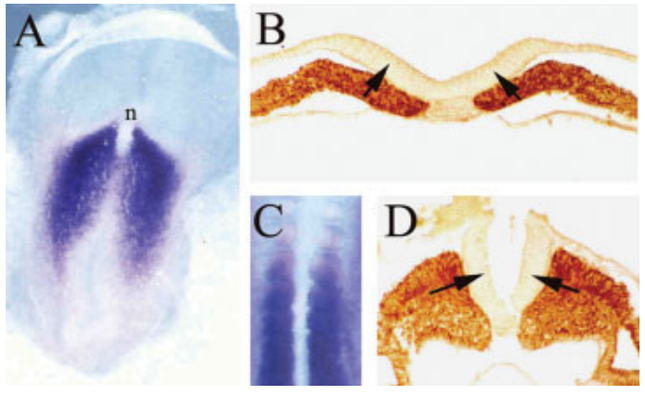
Molecular basis for the activation-transformation model



Gilbert, 2002

Activation (neural induction):
BMP antagonists
-produced by the organizer and its descendants

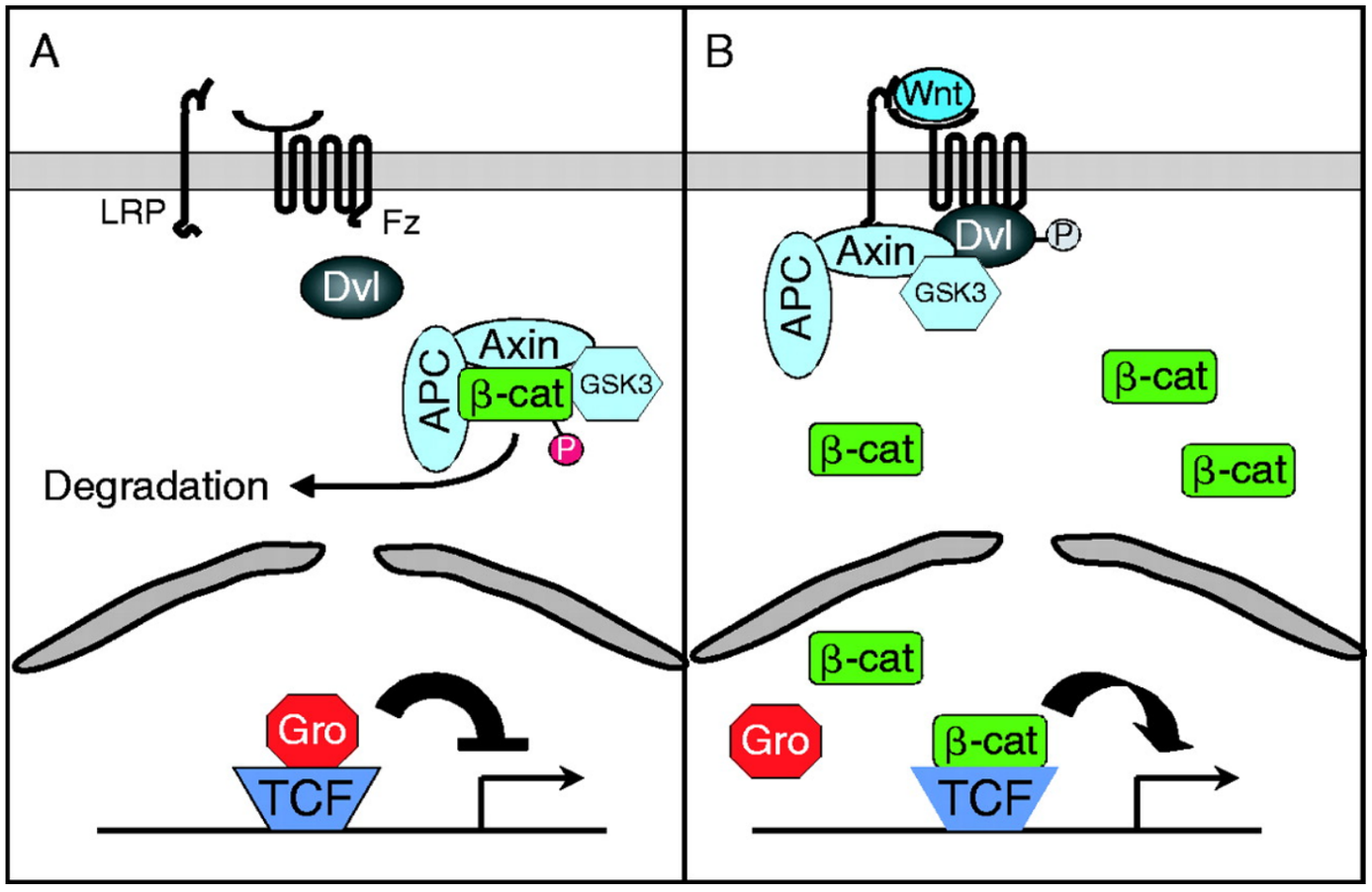
Transformation (caudalization)
Wnt, fibroblast growth factors (FGFs) and retinoic acid (RA)
-produced by the paraxial mesoderm (somites), not by the axial mesoderm (descendants of the organizer).



The RA-synthesizing enzyme, ALDH1A2, is expressed in somites.

Wnt signaling pathways

-evolutionarily conserved secreted proteins
-19 Wnt genes in human genome
-multiple signaling pathways depending on the cellular context and receptors
-More than 15 Wnt receptors or co-receptors (including Frizzled (Fz) and LRP5/6 for β-catenin-dependent pathway)
-In the absence of Wnt binding, β-catenin is phosphorylated by the kinase GSK3, and undergoes degradation.
-With Wnt binding to Fz/LRP co-receptors, the destruction complex is inactivated and β-catenin is free to be transported to the nucleus, where it binds to TCF/LEF transcription factors and activates transcription of downstream genes.
-Wnt signaling is also inhibited by extracellular antagonists such as Dkk1.

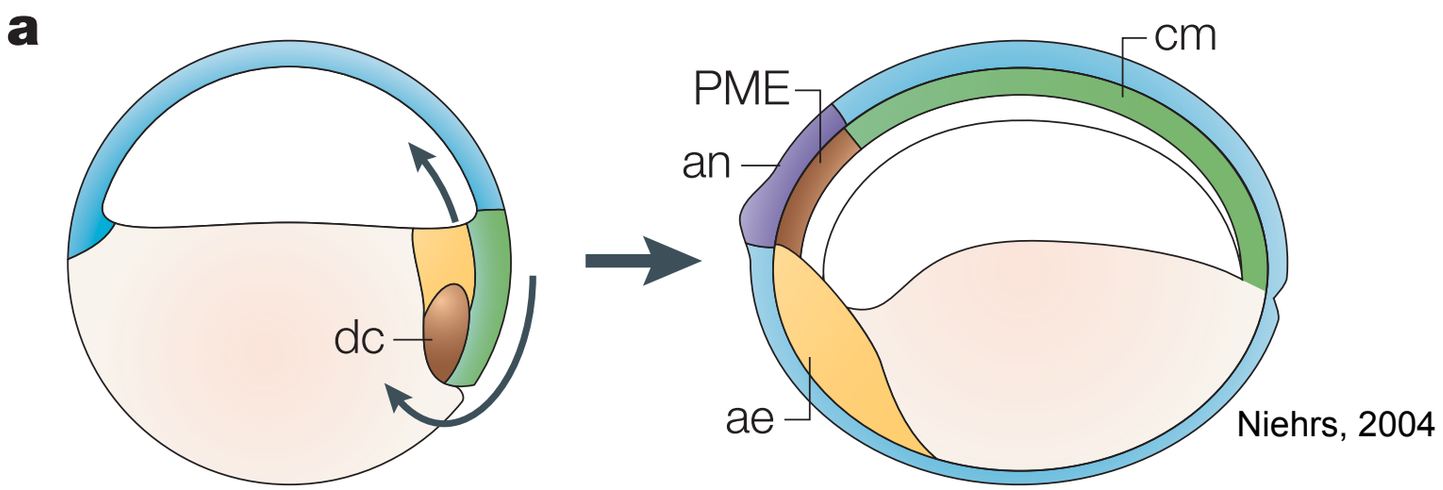


Cadigan and Liu, 2006

Transforming Wnt activity is antagonized in the rostral neural tissue

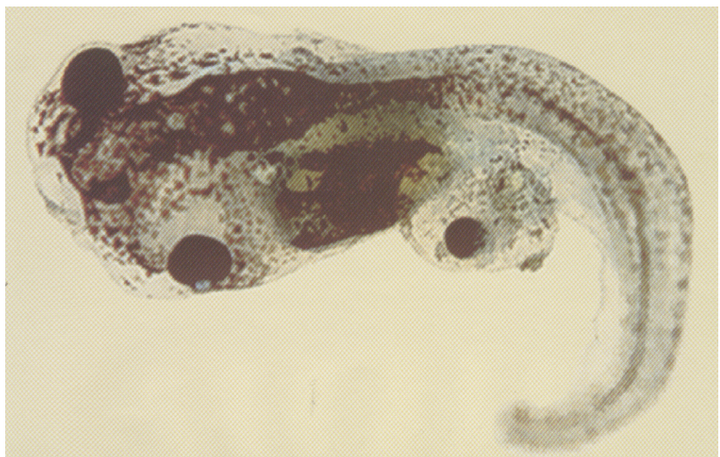
Wnt antagonists (Cerberus, Frzb, Dickkopf, etc.) prevent the neural tissue from becoming caudal

-These antagonists are expressed in the rostral, non-neural tissue (anterior endoderm; ae) and prechordal mesoderm (PME).

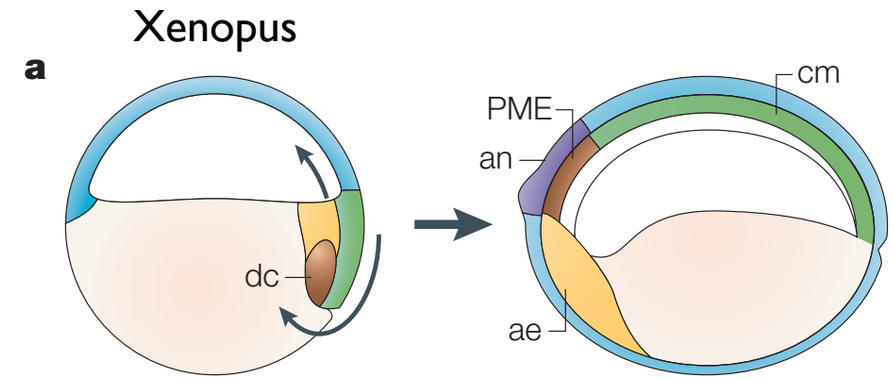


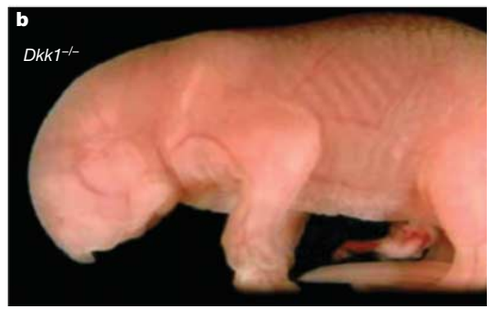
Niehrs, 2004

Over-expression of Cerberus generates an ectopic head.

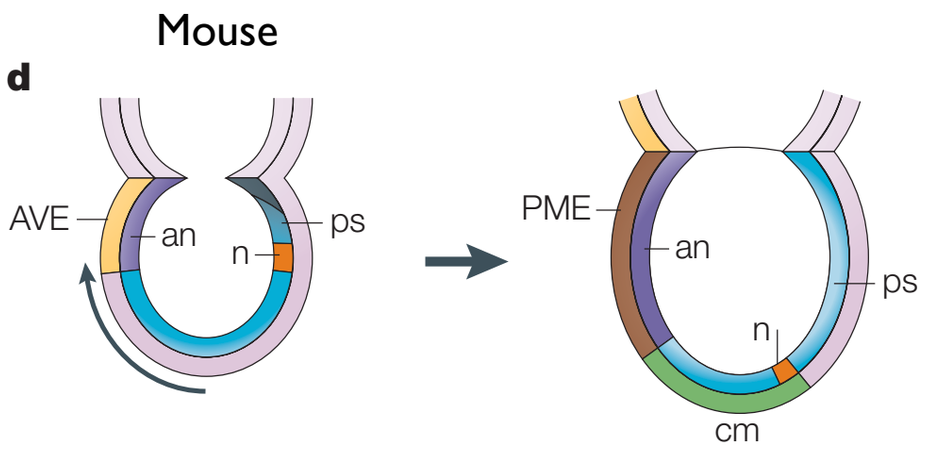


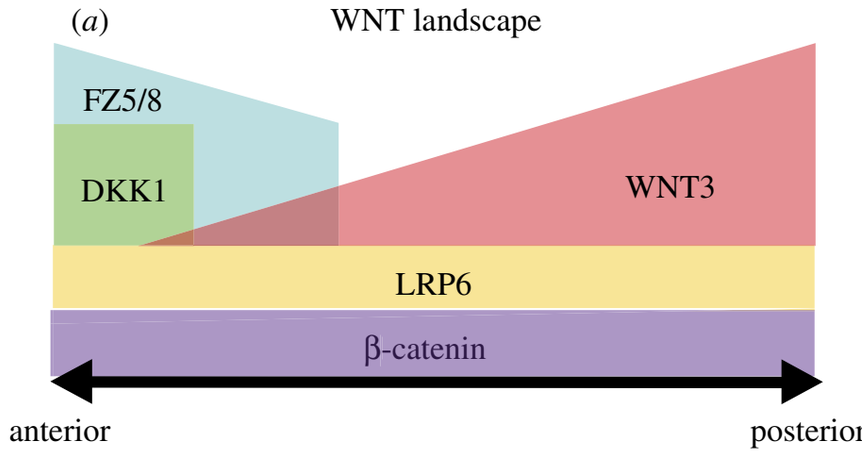
Activation-transformation model in mammalian embryos





Dkk1 (mouse homolog of Dickkopf) mutant mice lack the head.





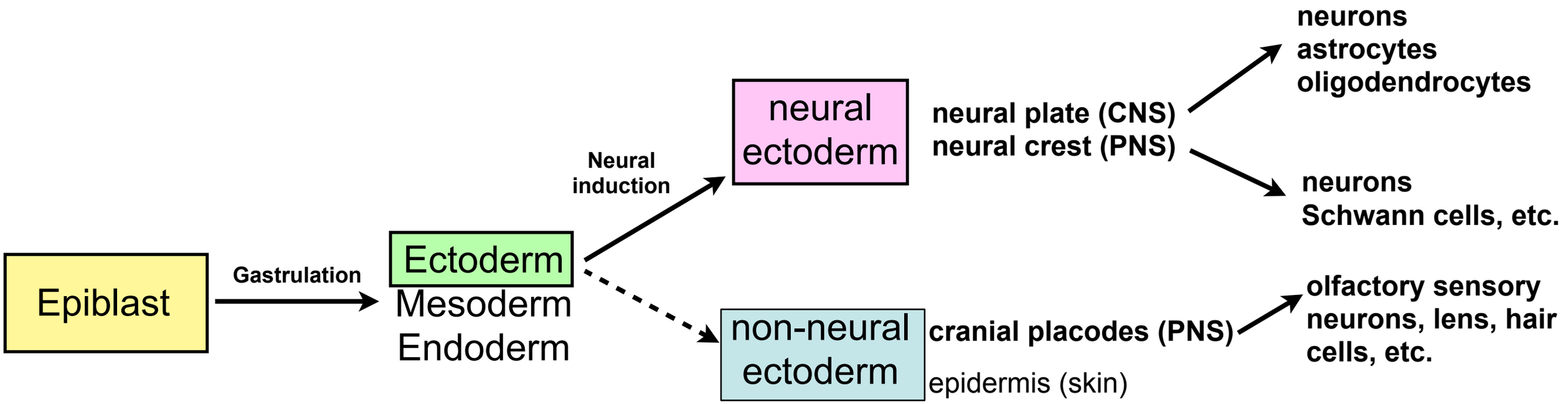
Arkel and Tam, 2012

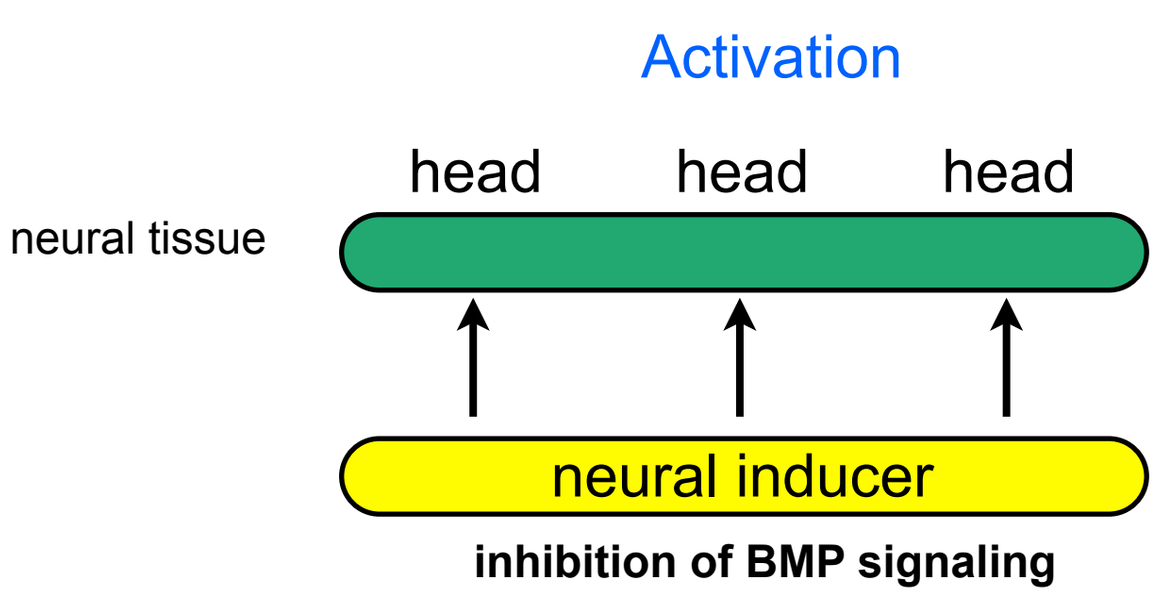
Cylinder-shaped mouse embryo becomes asymmetric by the formation of anterior visceral endoderm (AVE). Like the amphibian anterior endoderm, AVE produces Cerberus, a Wnt inhibitor.

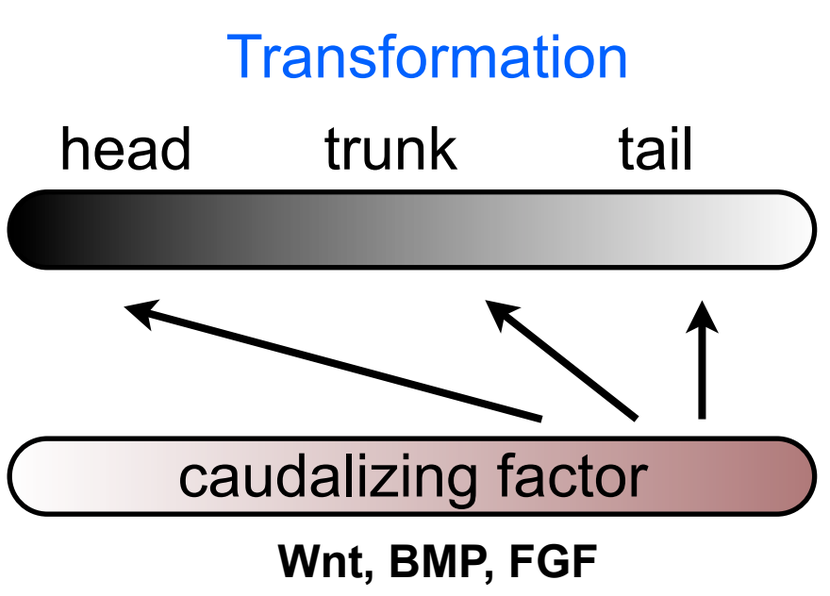
Formation of AVE triggers the formation of primitive streak (ps) on the opposite side. ps is equivalent to the amphibian blastopore, and produces Fgf and Wnts.

Node (or Hansen's node) is formed at the anterior end of ps. Node is equivalent to the amphibian organizer, and produces chordin, a BMP inhibitor. Derivatives of the node (cm and PME) also produce BMP inhibitors.

AVE cannot induce neural tissue by itself, but inhibits caudalization of the neural tissue by blocking the Wnt pathway.







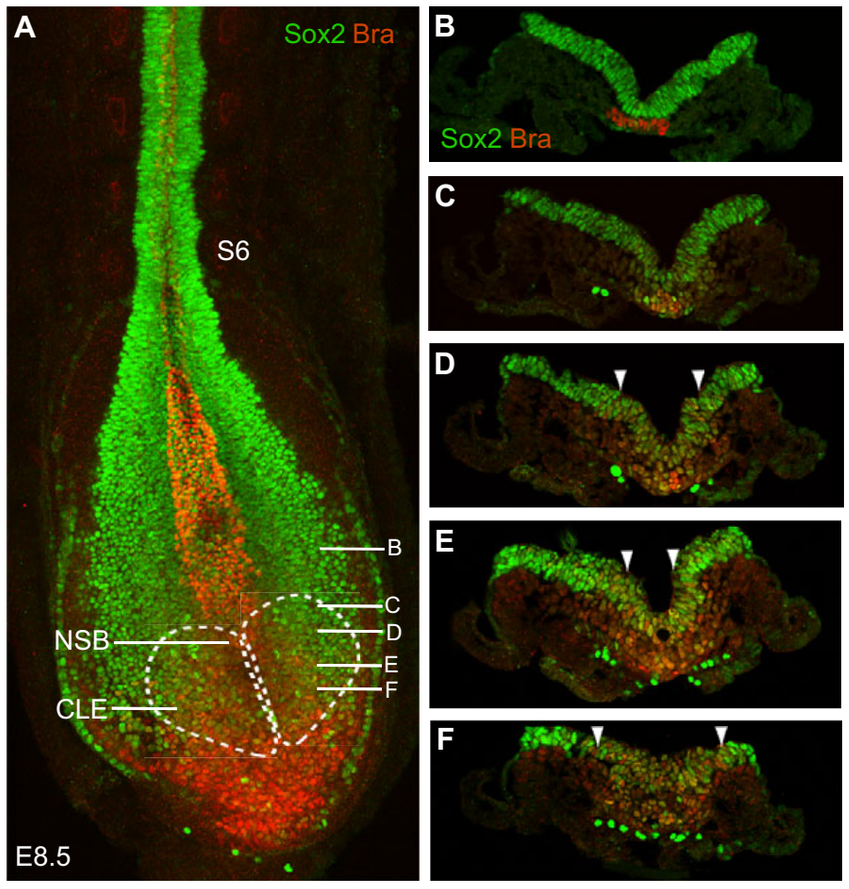
Does this simple scheme apply to the entire nervous system including the spinal cord?

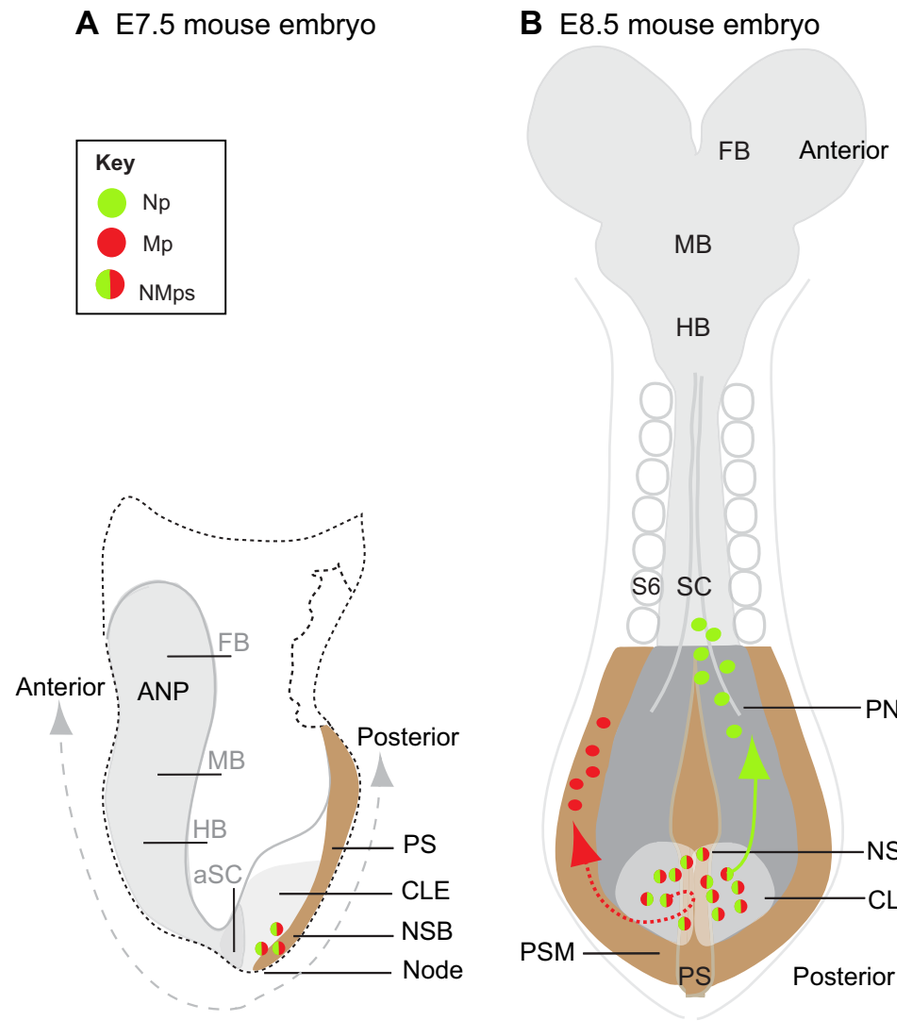
Distinct developmental origins of posterior and anterior neural tissues

-Clonal analysis of cell lineage indicated that many spinal cord cells have a close lineage relationship to somite-forming paraxial mesoderm.

-Cells expressing both mesodermal (Brachyury or Bra) and neural (Sox2) markers (neuromesodermal progenitor cells (NMPs)) persist near the primitive streak long after the gastrulation is completed.

-NMPs give rise to both the spinal cord and presomitic mesoderm (source of somites).

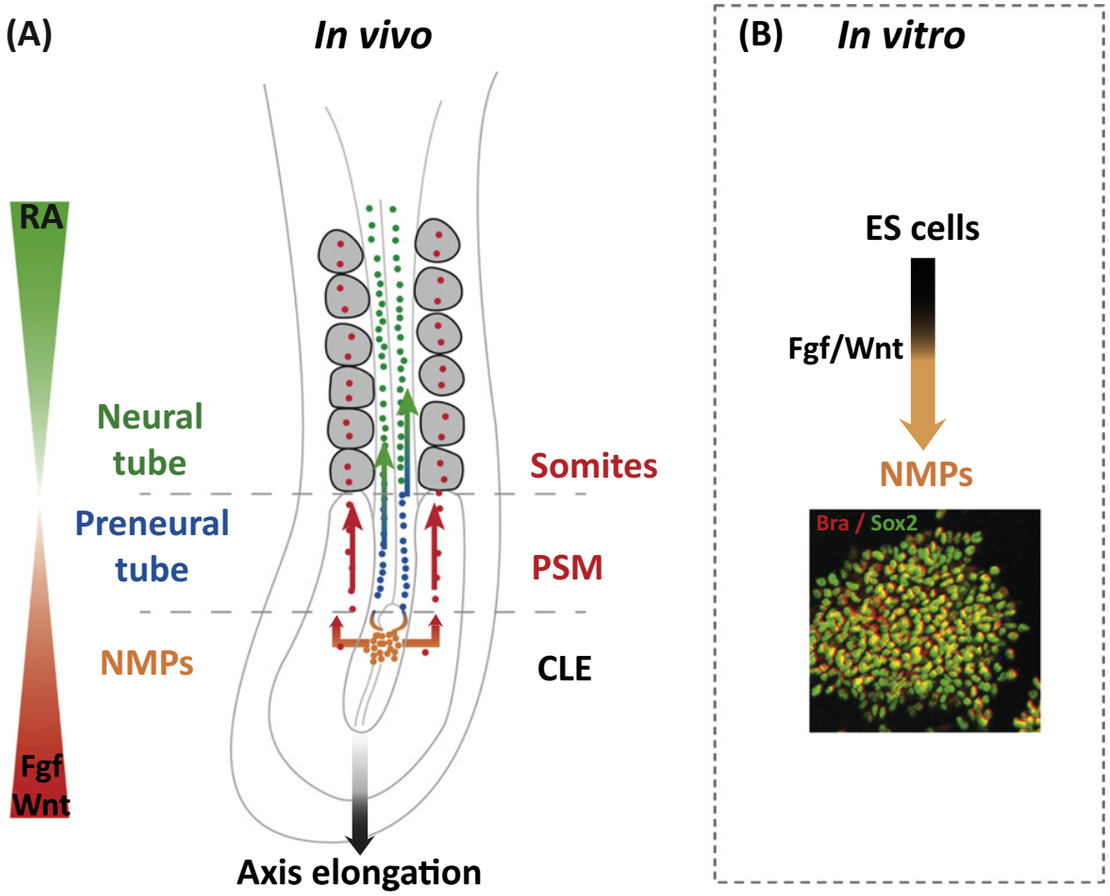




SC: spinal cord
PNT: preneural tube
NSB: node-streak border
CLE: caudal lateral epiblast
PS: primitive streak
PSM: presomitic mesoderm

Henrique et al., 2015

Proposed mechanisms for the generation of neural cells in the spinal cord



Gouti et al., 2015

-The posterior gradients of FGF and Wnts oppose the activity of RA (retinoic acid).

-FGF induces formation of preneural tube and RA causes them to transition into neural progenitor cells.

-Unlike the brain, much of the spinal cord appears to be generated without going through the “ectoderm-anterior neural” path.

Summary (early rostro-caudal patterning)

-Activation-transformation model appears to be conserved in different animal species from Xenopus to chick and mouse.

activation: neural induction by inhibition of BMP signaling

transformation: caudalization by Wnts, RA, FGF

-The above model appears to apply only to the rostral (anterior) nervous system.

-For most of the spinal cord, neural cells are derived from neuromesodermal progenitor cells (NMPs). Sequential actions of FGF and RA cause NMPs to become neural progenitor cells.