Neural Development

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Neural development

- 1) Generation and cell-type specification neurons
- 2) Neuronal connectivity
- 3) Cell death and survival

- **No assigned text**
- **For additional reference suggest:**
  - **Fundamental Neuroscience: Squire et al., 2nd edition, Academic Press**
- **Each class: lecture, break, paper presentation**

1) Generation and cell-type specification neurons

- **Lecture 1:**
  - **Induction: setting up portions of the embryo that will give rise to neural tissue**
- **Lecture 2:**
  - **Regionalization: Patterning neural tissue along Anterior/Posterior and Dorsal/Ventral axes**
- **Lecture 3:**
  - **Neurogenesis and migration: cortical development, neuronal migration, neuronal stem cells**
- **Lecture 4:**
  - **Determination and differentiation: strategies for generating specific types of neurons**

2) Neuronal connectivity

- **Lecture 5:**
  - **Axon guidance: guidance cues and receptors that control axon and dendrite navigation**
- **Lecture 6:**
  - **Target selection: formation of neural maps and recognition of synaptic partners**
  - **Cytoskeletal signaling events: cell biology of growth cone motility**

3) Cell death and survival

- **Lecture 7:**
  - **Neurotrophins: Neurotrophic hypothesis, survival factors and receptors**
- **Lecture 8:**
  - **Cell death signaling pathways: Molecular machinery of cell death, regulation by neurotrophic signaling**

Neural development

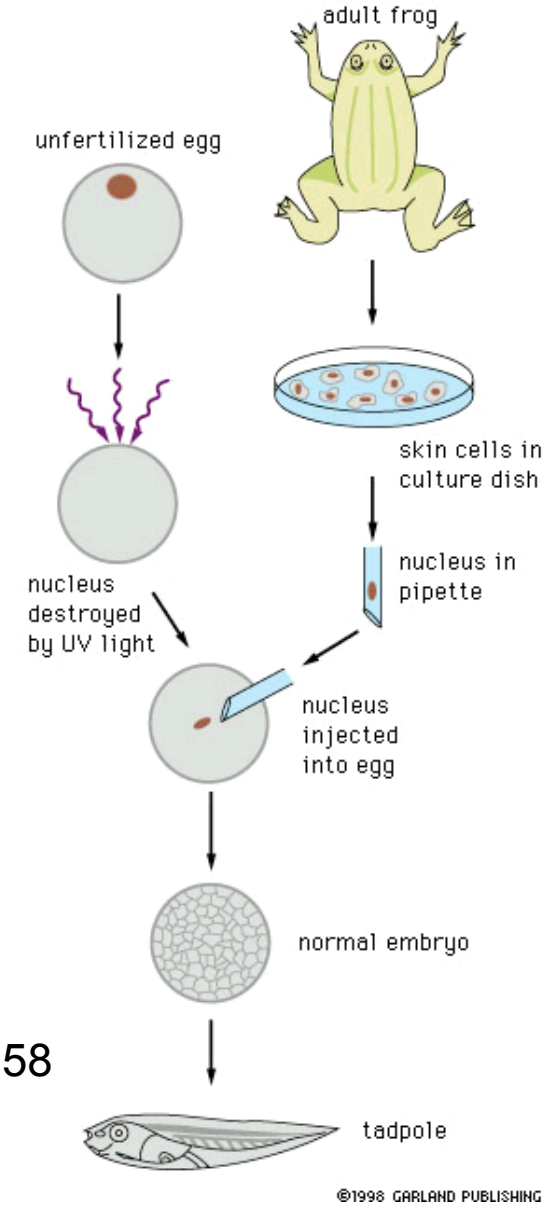
- Strategies for the generation of diverse cell types during development
- Introduction to key model systems
- Molecular mechanisms of neural induction in vertebrate embryos

Neural development

- Key question in developmental biology:
  - How does a fertilized egg generate so many different cell types?
  - In neural development: how generate so many different kinds of neurons?
- Cell differentiation depends on differential gene activity:
  - Estimated ≈20,000-30,000 protein-coding genes in the human genome, plus ≈250 regulatory small RNAs --- any one cell expresses 10,000-20,000 genes
  - How is this achieved?

Establishing differential gene activity

- **Different cell types could inherit different genes:**
  - **Cloning experiments show that most cells have essentially the same genetic material**



John Gurdon, 1958

Establishing differential gene activity

- Cell differentiation is largely achieved through differential gene activation
- Basic strategies for setting up patterns of differential gene activation during development:
  - asymmetric cell division
  - cell:cell signaling

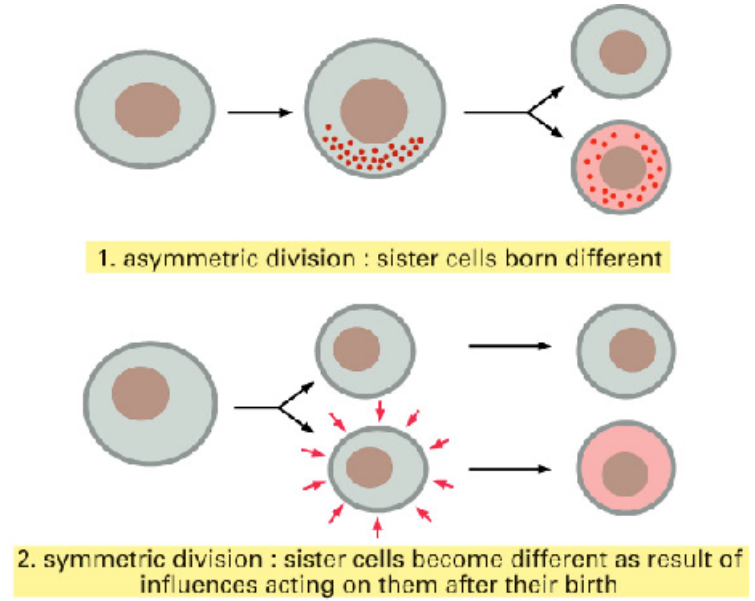


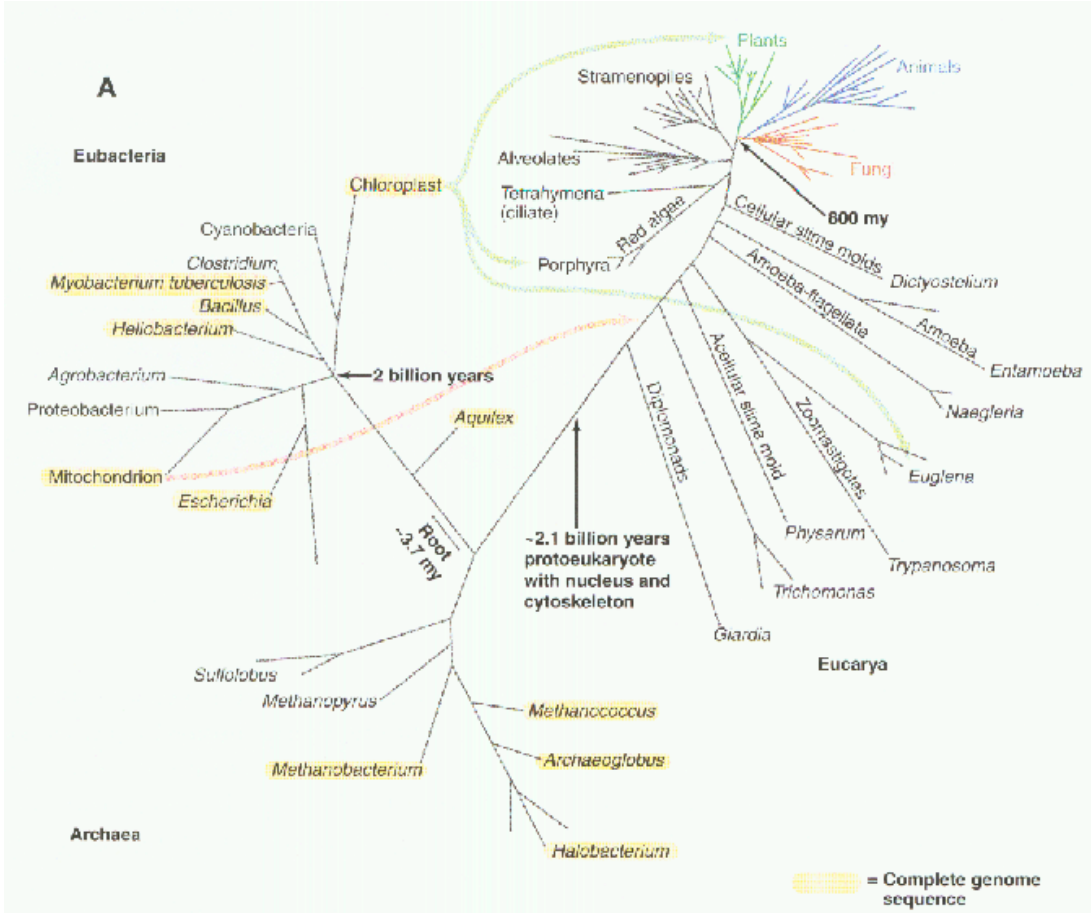
Figure 21–10. Molecular Biology of the Cell, 4th Edition.

Evolution and development

- Mechanisms for responding to the environment are ancient
- Simple multicellular organisms contain networks of neurons
  - eg., Cnidarians (jellyfish, hydra) -- last common ancestor with humans estimated to be >500 Mya
  - Neurons in these organisms share basic properties with those in “higher” organisms -- eg., V-gated ion channels and proteins involved in synaptic transmission

Evolution and development

- **New forms are built upon structure of biological predecessors.**



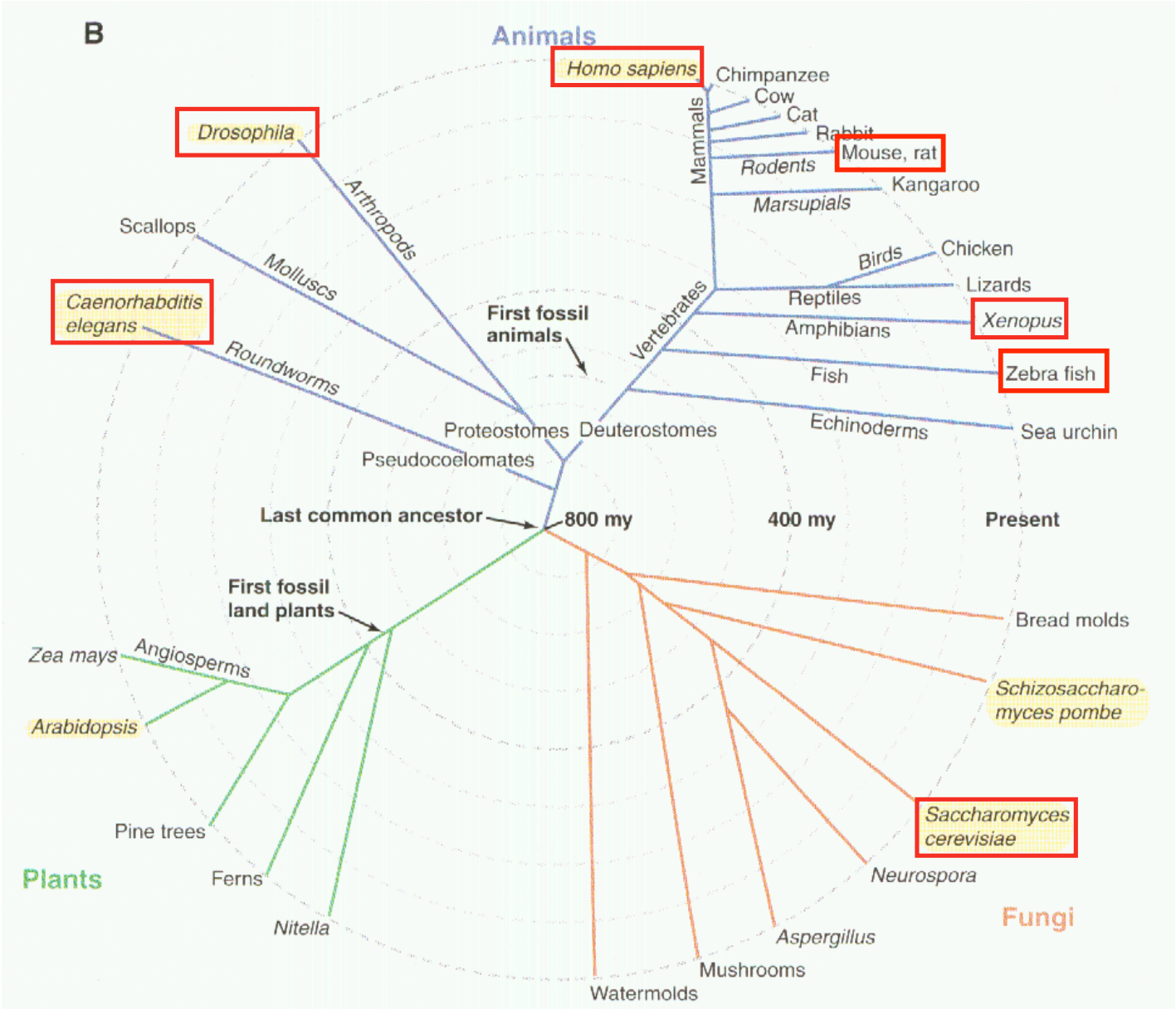
Evolution and neural development

- **Recurring themes:**
  - **Different animals use different combinations of same basic molecular mechanisms to construct the nervous system**
  - **Neural development relies on mechanisms similar to those underlying development of other parts of the body**

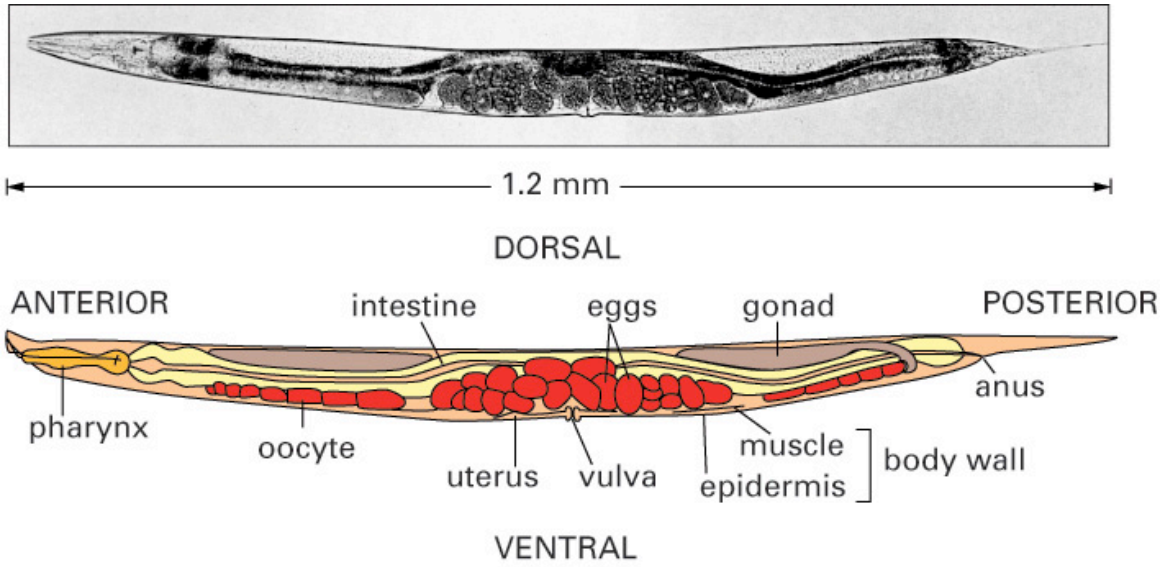
Key model systems

- ***Caenorhabditis elegans*** **(nematode)**
- ***Drosophila melanogaster*** **(fruit fly)**
- ***Danio rerio*** **(zebrafish)**
- ***Xenopus laevis*** **(frog)**
- ***Mus musculus*** **(mouse)**
- ***Homo sapiens***

How are model systems related?

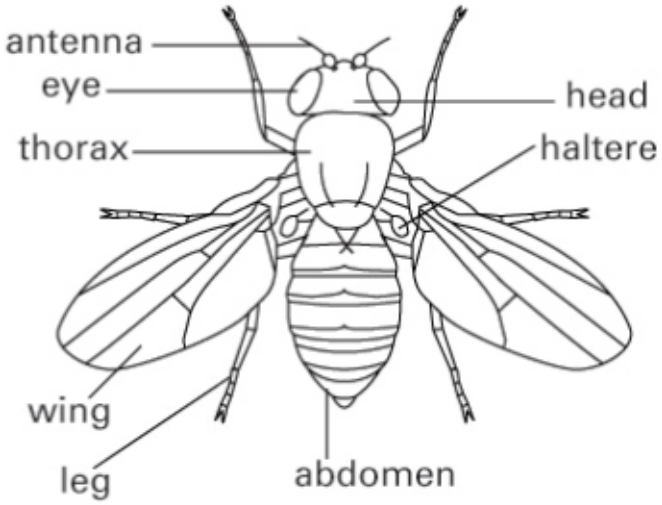


Caenorhabditis elegans (nematode)



- **Rapid generation time (3 days); easy to grow in lab**
- **Powerful classical and molecular genetics: genomic sequence is known**
- **Transparent: readily visualize individual neurons and even synapses in intact animals using Green Fluorescent Protein**
- **Invariant development: 959 somatic cells: 302 neurons, 56 glia; complete cell lineage and neuronal wiring diagram is known**
- **Disadvantages:**
  - **difficult to specifically knock-out/modify genes**
  - **electrophysiology tough**

Drosophila melanogaster (fruit fly)

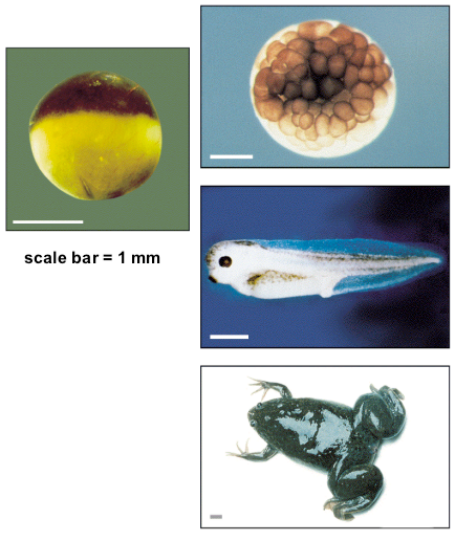


- Short generation time (10 days); easy to grow in lab
- Powerful classical and molecular genetics; wealth of molecular genetic tools; genomic sequence is known
- Can specifically knock-out/modify genes
- Even though invertebrate, has sophisticated brain (>150,000 neurons)
- Electrophysiology increasingly routine
- Disadvantages:
  - limited transparency
  - slower genetics than *C. elegans*

Danio rerio (zebrafish)

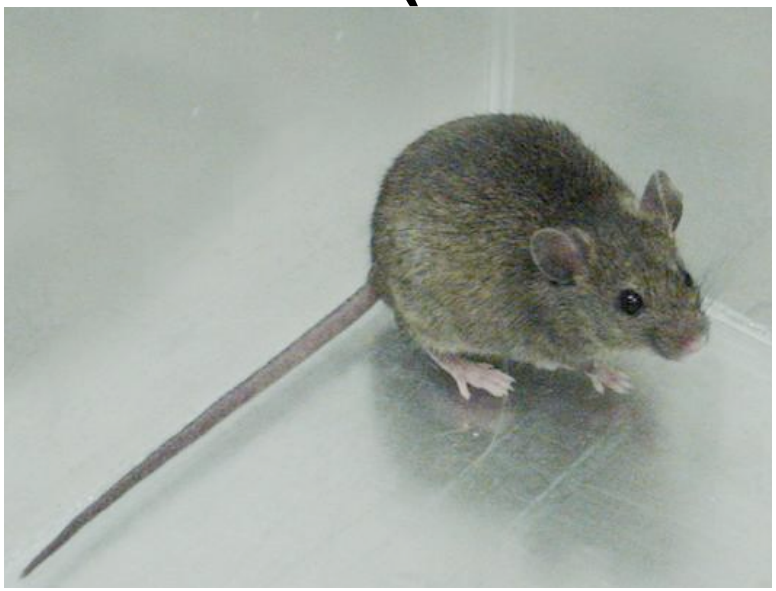
- Long generation time (≈90 days)
- Genetically amenable; morpholinos allow rapid reverse genetics
- Transparent and hardy: highly suited for live imaging of neural development
- Sophisticated vertebrate nervous system and behaviors
- Electrophysiologically accessible
- Disadvantages:
  - molecular genetics less powerful than other genetic systems
    - fewer tools (newer system, smaller community of scientists)
    - long generation time
  - genome sequence in progress, not yet completed

Xenopus laevis (African claw-toed frog)



- Very long generation time (1-2 years)
- Intractable classical genetics; morpholinos allow reverse genetics
- Excellent for experimental embryology: large eggs, embryos develop externally and rapidly, hardy, good for transplantation and biochemistry
- Disadvantages:
  - no forward genetics
  - limited molecular genetic tools
  - genome not sequenced; tetraploid
- *Xenopus tropicalis* : smaller, but more genetically tractable
  - diploid
  - shorter generation time: 4 months

Mus musculus (house mouse)



- Generation time ≈60 days
- Genetically amenable; genome is sequenced;
- Highly sophisticated molecular genetic tools for genetic alteration
- More closely related to humans than other molecular genetic model systems
- Electrophysiologically accessible
- Disadvantages:
  - Not transparent and embryo not readily accessible for manipulation (develops inside mother)
  - Expensive to house and care for --- impediment to many for large-scale experimentation

Early development of *Xenopus laevis*

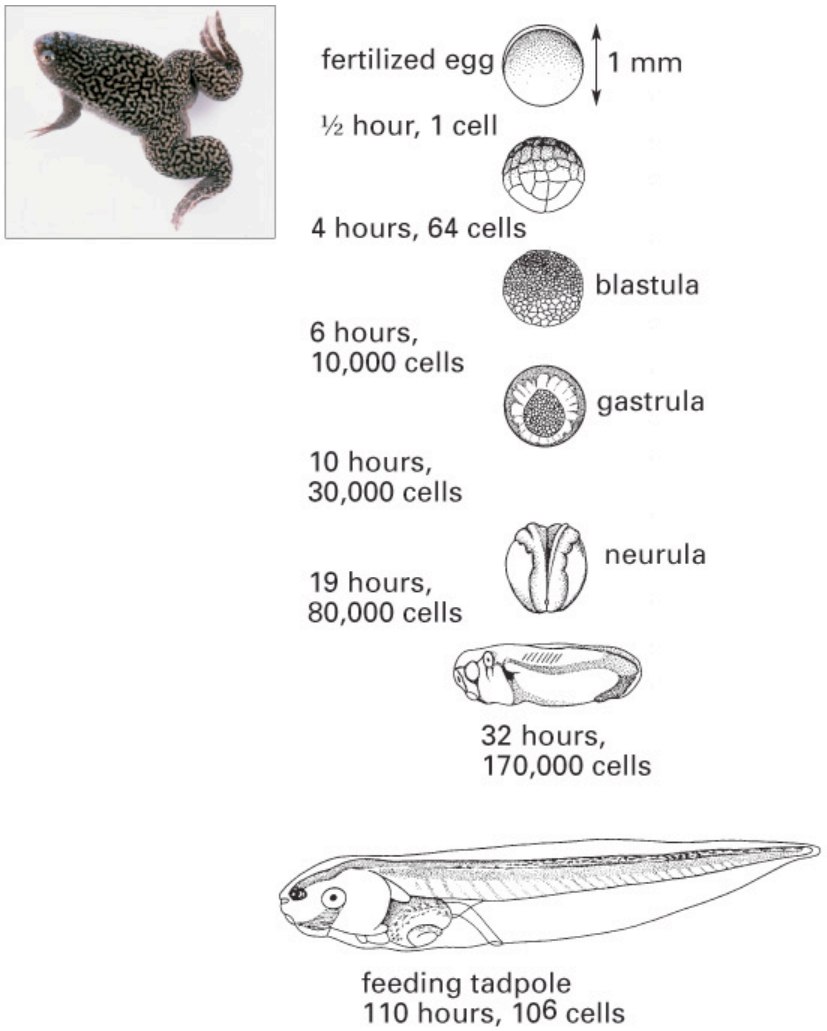
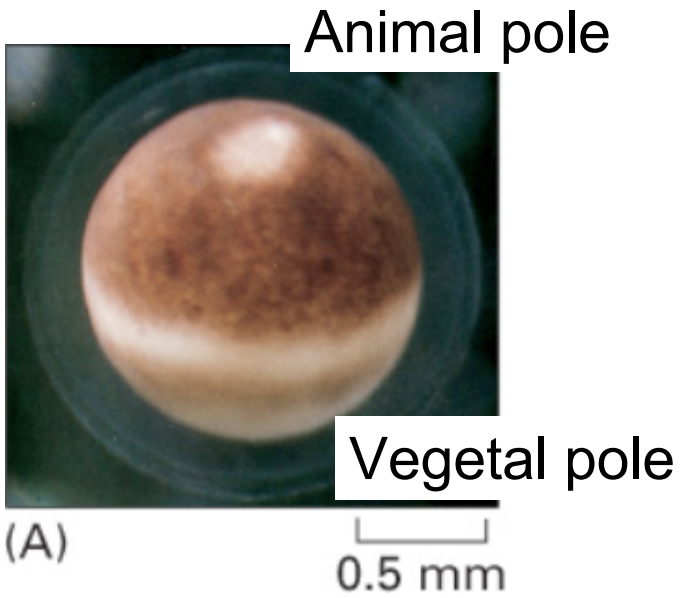


Figure 21–65. Molecular Biology of the Cell, 4th Edition.

Early development of *Xenopus laevis*

- **After fertilization a series of cleavage divisions divides the embryo**
- **Establishes distinct embryonic cell populations: micromeres near animal pole; macromeres near vegetal pole**



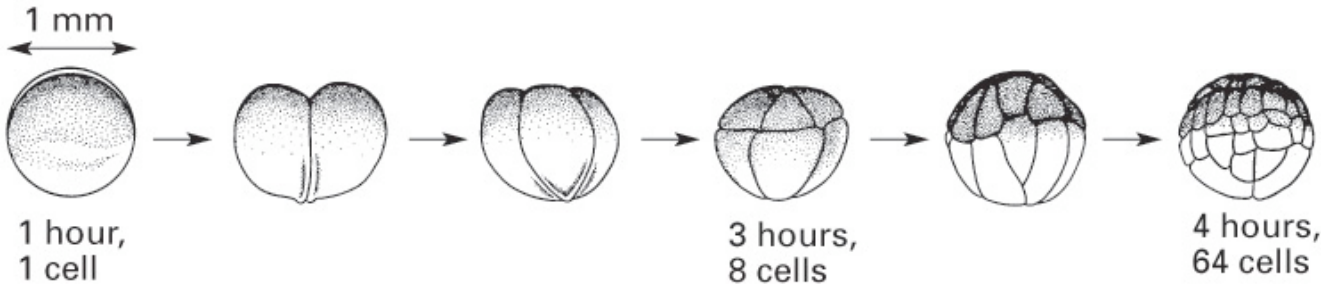


Figure 21–67. Molecular Biology of the Cell, 4th Edition.

http://www.welc.cam.ac.uk/~smithlab/movies/cleavmov.qt

Xenopus laevis gastrulation

- By the 128 cell stage the embryo is a hollow ball of cells-- blastula
- Undergoes gastrulation: cell movements that position the germ layers:
  - prospective endoderm is brought inside the embryo
  - prospective ectoderm covers the surface of the embryos
  - prospective mesoderm is positioned between these two layers

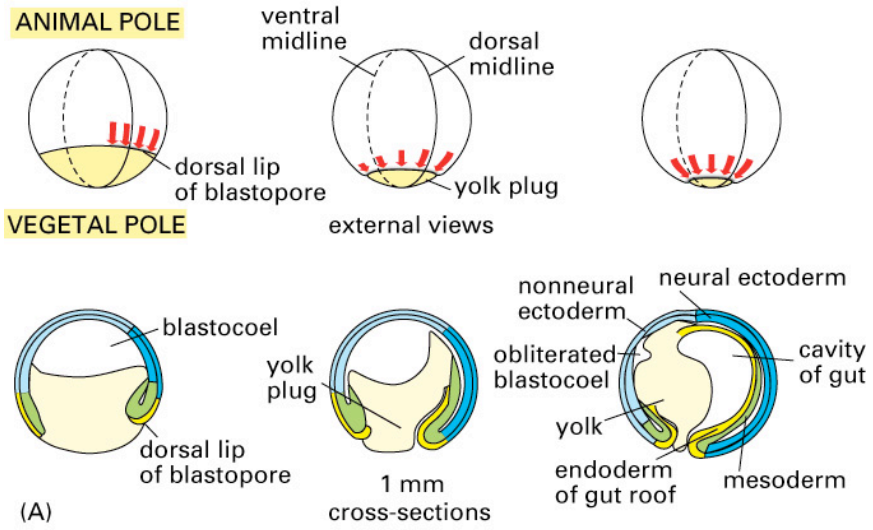


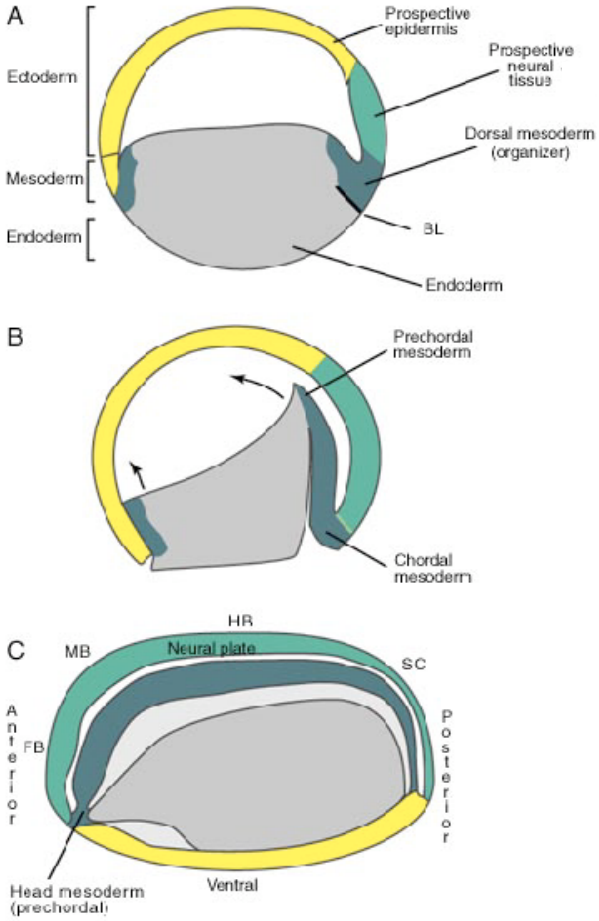
Figure 21–71 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

http://www.welc.cam.ac.uk/~smithlab/movies/gastmov.qt

http://worms.zoology.wisc.edu/frogs/gastxen/wholegas.mov

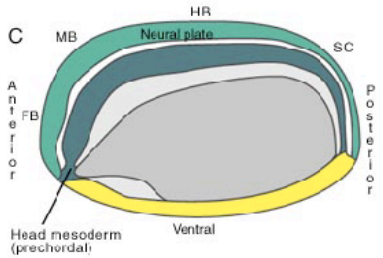
Neural tissue is derived from ectoderm

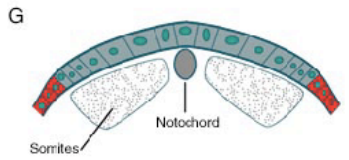
- Different tissues generated from ectoderm depending on AP and DV position
  - requires the action of the "organizer"
- Dorsal ectoderm forms neural plate
  - generates the neural tube and CNS

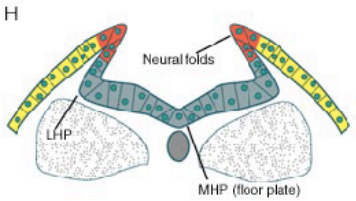


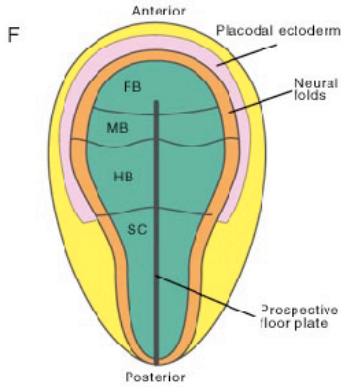
Ectodermal patterning along the dorsal/ventral axis

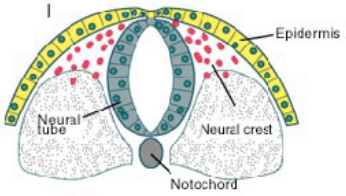
- Neural plate closes to generate neural tube (CNS)
- Neural folds give rise to migratory neural crest cells (PNS)
- Placodal ectoderm: cranial sensory ganglia and sensory structures (eg., eyes)
- Ventral ectoderm: epidermis











Transplanted dorsal lip of blastopore can induce secondary embryo

- **Hans Spemann and Hilde Mangold (1924)**
  - **Transplanted dorsal lip of blastopore (BL) from one embryo to the ventral side (presumptive belly) of another embryo**
  - **Transplanted tissue initiated gastrulation and patterned surrounding cells**
  - **Got conjoined twin embryos!**

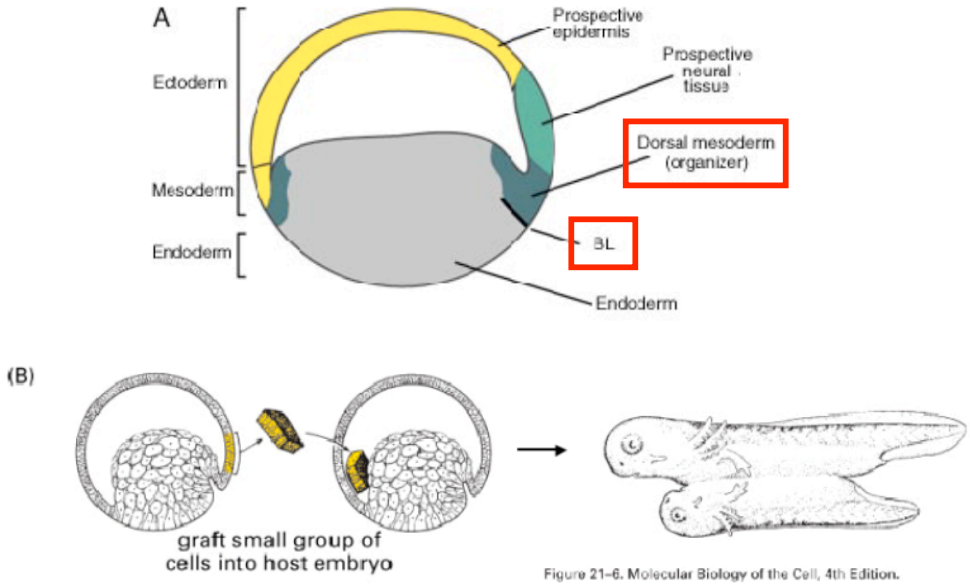
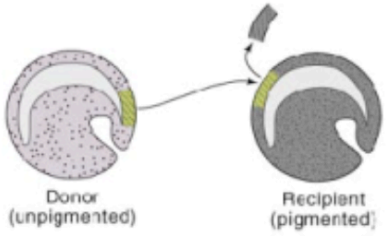
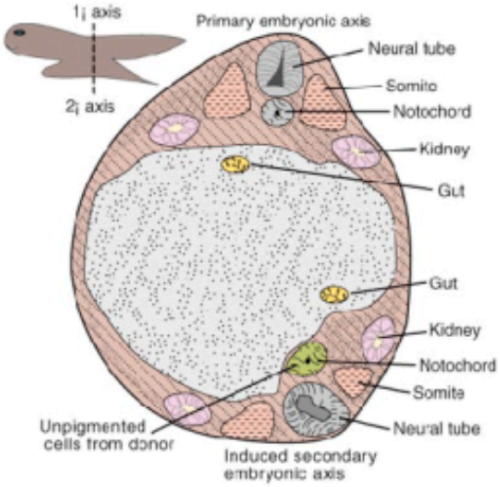


Figure 21–6. Molecular Biology of the Cell, 4th Edition.

Transplanted dorsal lip induces host tissue to form neural plate

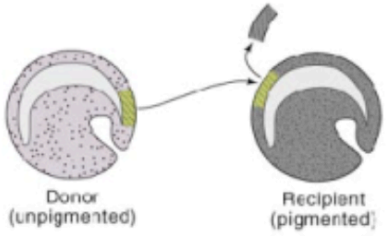
- Spemann and Mangold performed the transplant between embryos with different pigmentations:
  - could track fates of donor and host cells
- Transplanted tissue
  - initiated gastrulation
  - gave rise to notochord, prechordal mesoderm and other mesodermal structures (normal fate of dorsal lip)
- Host tissue
  - recruited to form much of secondary embryo including neural plate: Neural induction

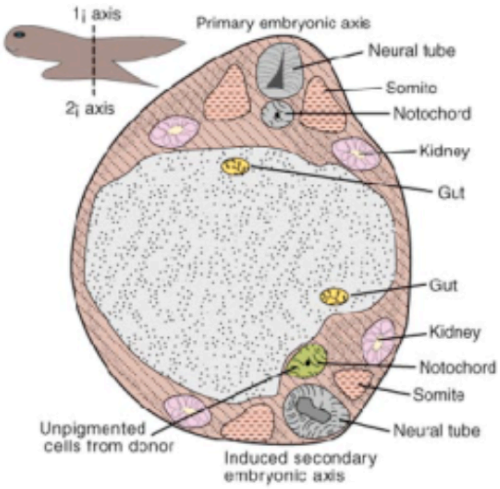




The Organizer

- **Dorsal lip and its derivatives called the Organizer:**
  - **Induced neural plate formation**
  - **Organized host and donor tissue to form secondary embryo**
- **Chick and mammalian embryos also contain an organizer region (Henson's node)**



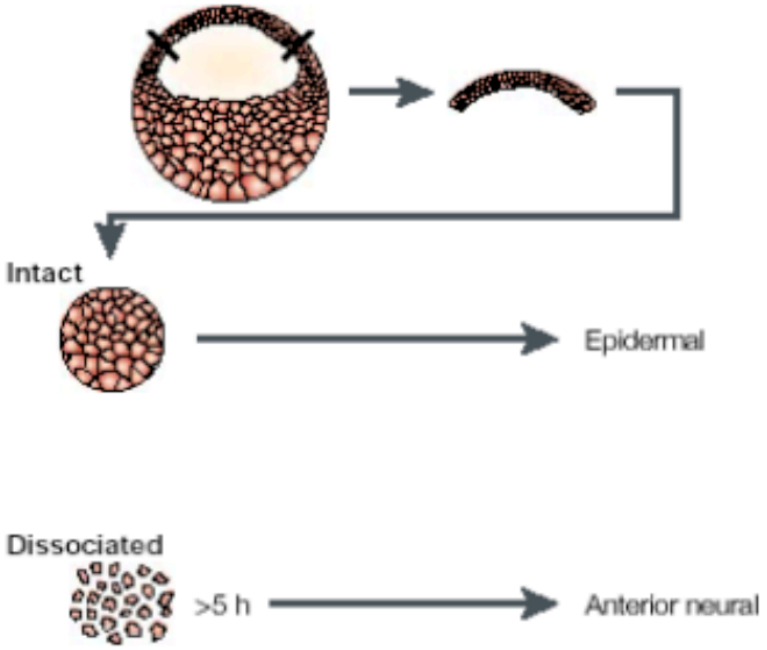


The molecular basis of neural induction

- **Classical embryological terms:**
- **Induction: An interaction between two tissues, as a result of which the responding cells change their fate.**
- **Competence: responsive capacity**
- **Neural induction:**
- **Over 50 years until substantial progress toward understanding molecular basis of Organizer activity**
- **Consensus view: secreted factors from the Organizer induced nervous system formation**

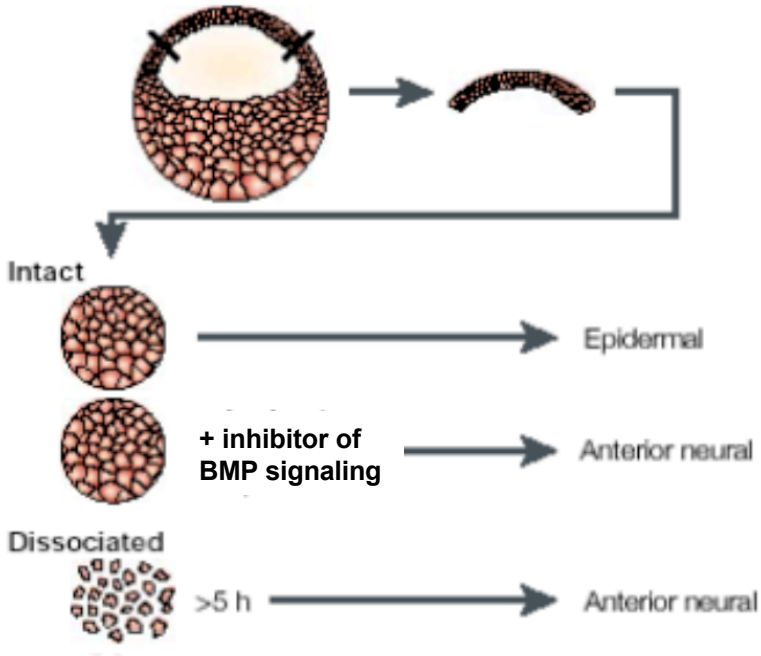
Is neural fate the “default” state of ectoderm?

- Evidence complicating the consensus view
- When cultured in isolation, animal cap cells form epidermis
- If they are dissociated, however, they form neural tissue
- Is neural fate the “default” state?



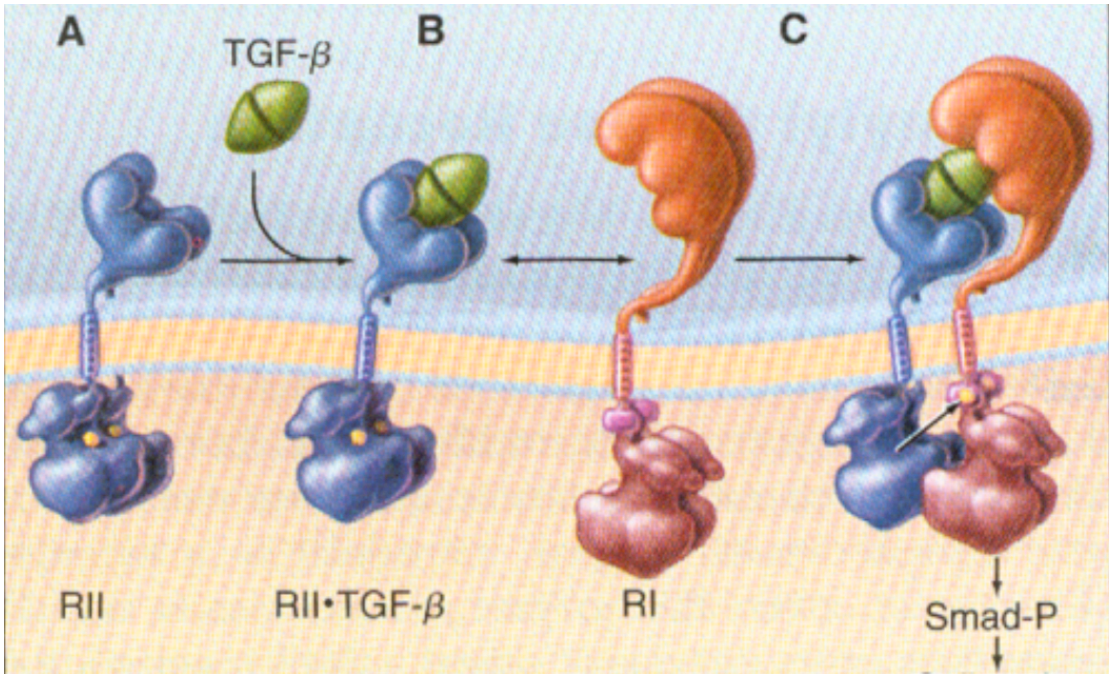
Inhibition of BMP signaling can induce neural tissue

- Evidence complicating the consensus view
- When cultured in isolation, animal cap cells form epidermis
- If they are dissociated, however, they form neural tissue
- Achieve similar effect by inhibiting BMP signaling

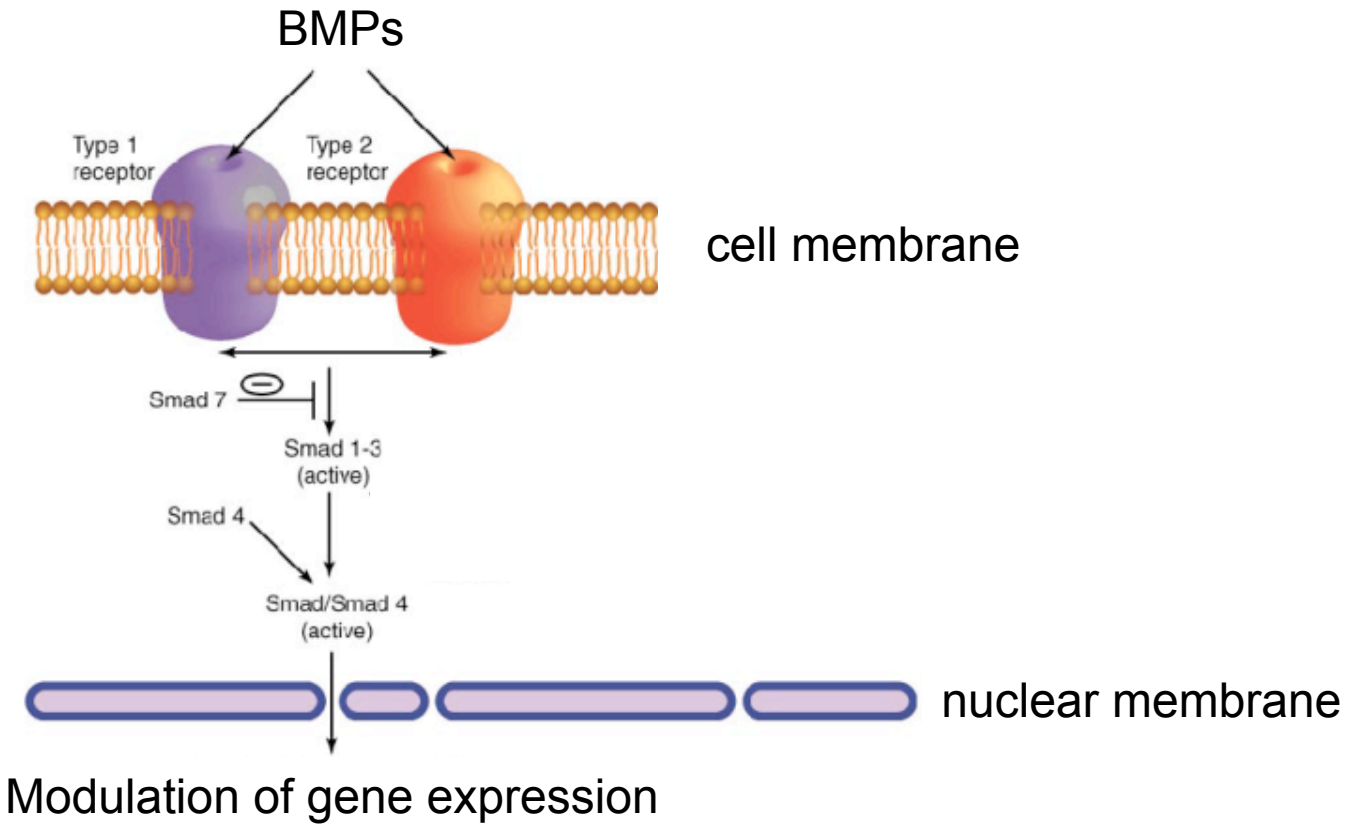


BMPs are secreted signaling molecules

- BMP (Bone Morphogenic Protein) family includes:
  - TGF-Beta, Activin, Decapentaplegic (Dpp)
- BMPs act through transmembrane serine/threonine kinase receptors

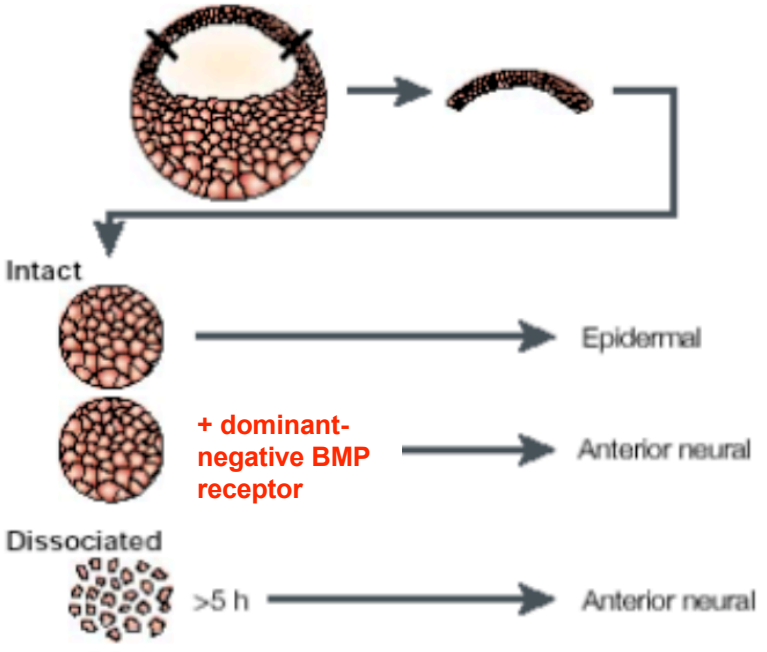


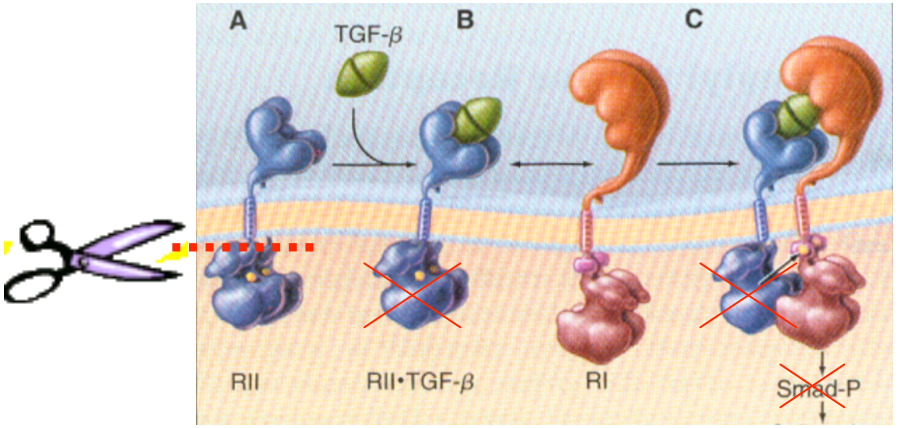
BMPs activate a signaling pathway that can alter patterns of transcription



Inhibition of BMP signaling promotes neural induction in animal cap assay

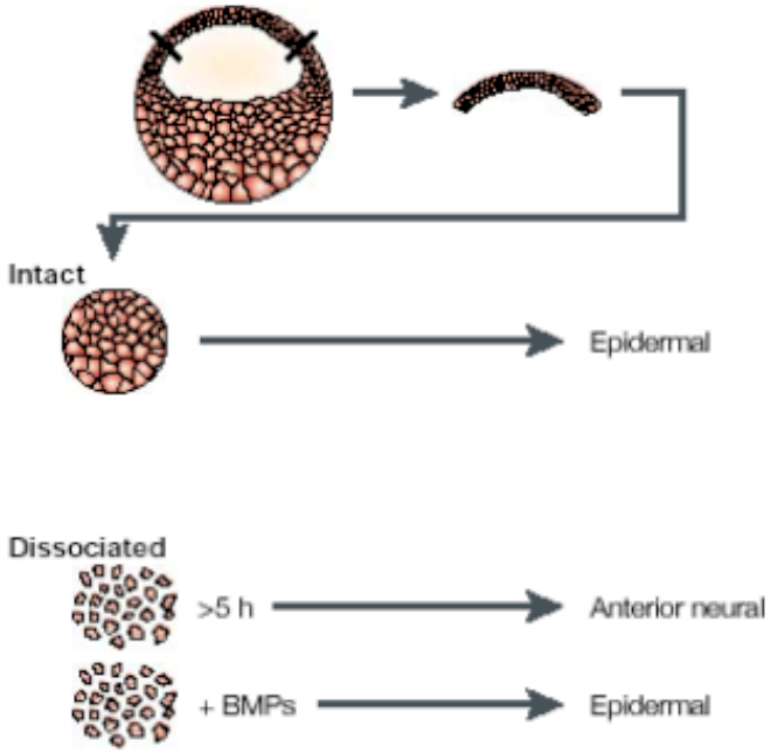
- When cultured in isolation, animal cap cells form epidermis
- If they are dissociated they form neural tissue
- Achieve similar effect by expressing dominant-negative activin (BMP) receptor





Addition of BMP can block neural induction in animal cap assay

- When cultured in isolation, animal cap cells form epidermis
- If they are dissociated they form neural tissue
- When treated with BMPs dissociated cells once again form epidermis



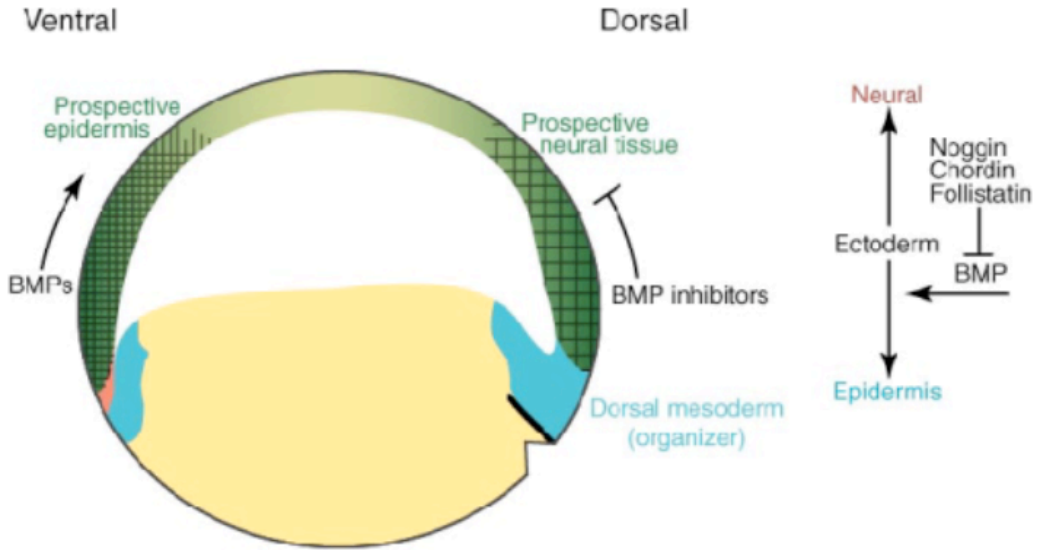
New model of Organizer activity

- Neural fate may be “default” state
- Neural induction influenced by BMPs
  - inhibited by activating BMP signaling
  - promoted by blocking BMP signaling
- Organizer may induce nervous system formation by secreting inhibitors of BMP activity

The Organizer produces multiple inhibitors of BMP signaling

- Organizer produces:
  - chordin
  - cereberus
  - follistatin
  - noggin
- These are secreted proteins that directly bind to BMP family proteins and prevent them from activating BMP receptors

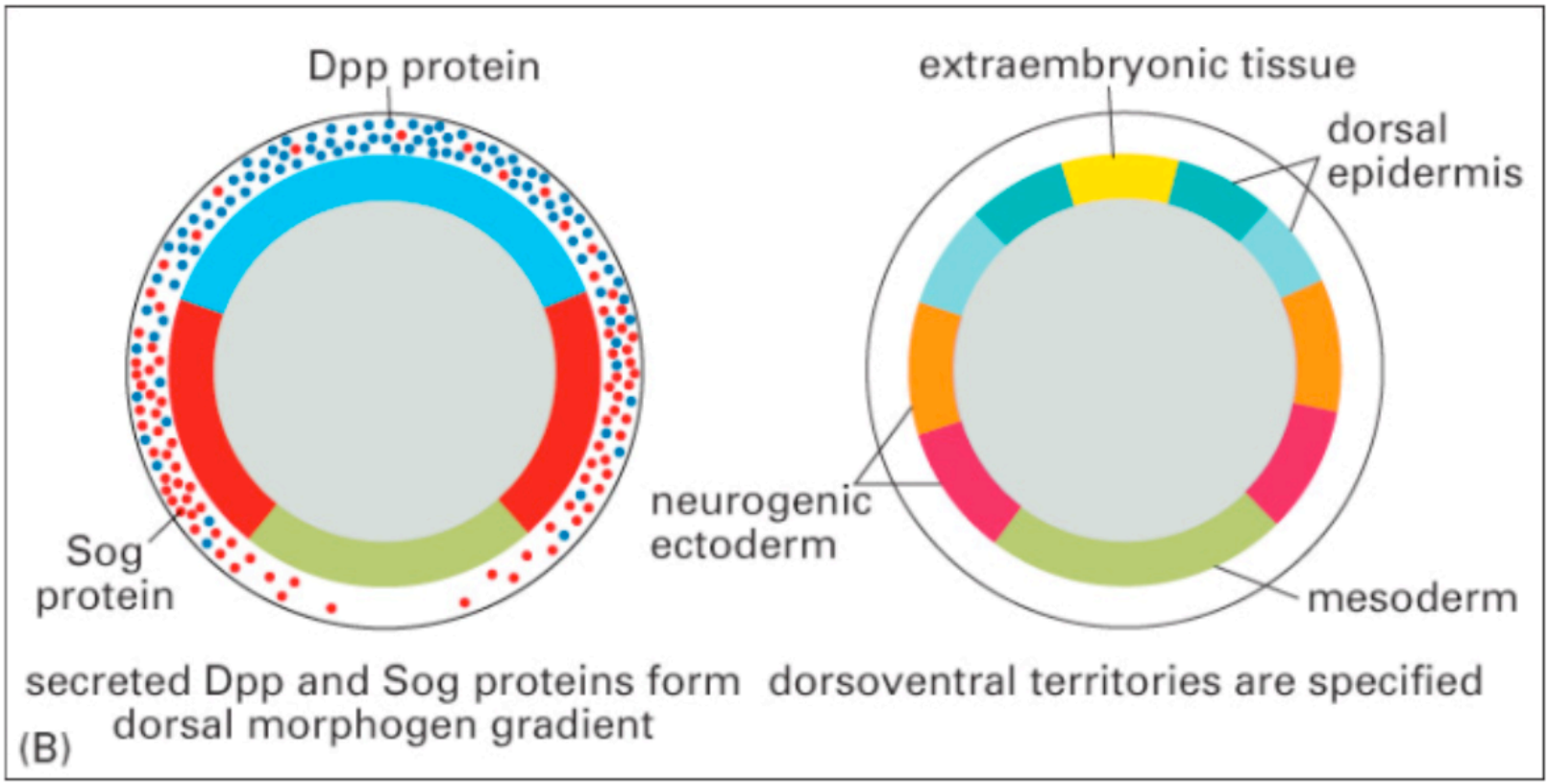
The default model of neural induction



Is the default model correct?

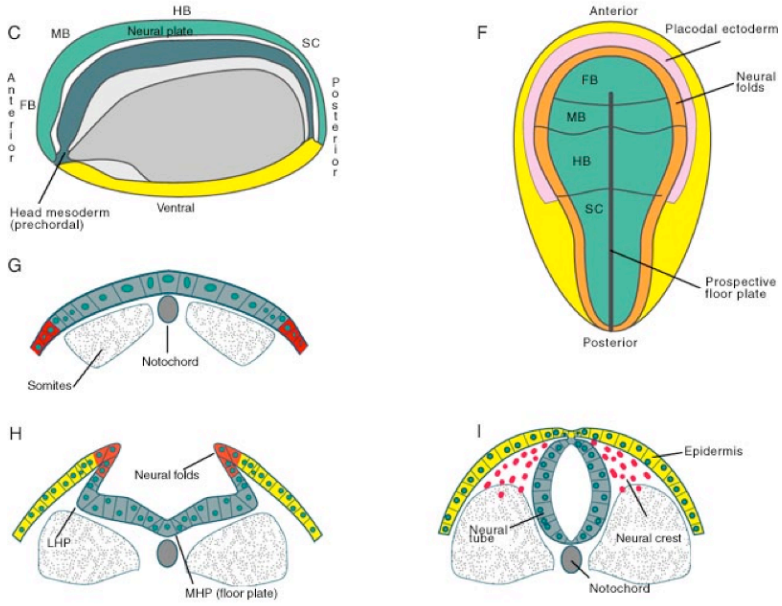
- Loss-of-function genetic evidence:
- Mice:
  - noggin, chordin, follistatin and cereberus mutant mice: no defects in neural induction in single mutants
  - chordin/noggin double mutant: loss of prosencephalon
- Zebrafish:
  - chordino mutant: decrease in size of neural plate
  - bmp2 and bmp7 mutants: increase in amount of neural tissue
  - bmp2/bmp7 double mutant resemble single mutants --- act together?

Inhibition of BMP signaling is also involved in neural induction in invertebrates



Neurulation

- Bending of neural plate
  - Accompanied by convergence-extension along AP axis
- Dorsal edges fuse to form neural tube

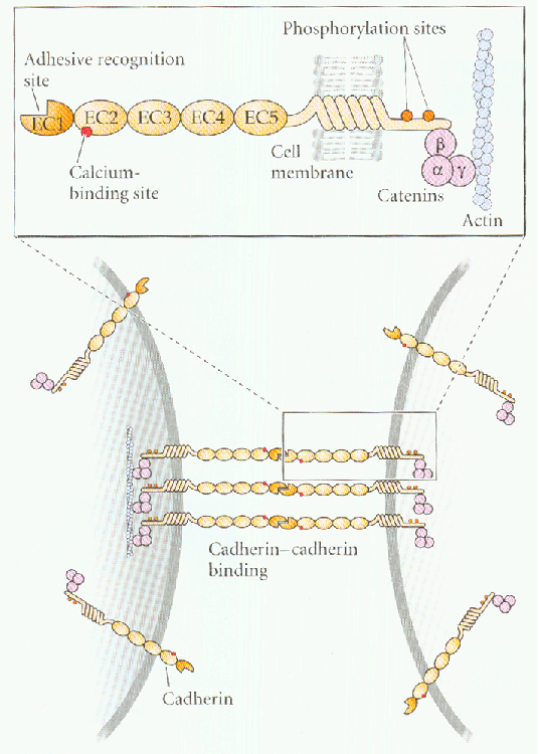


Cell:cell recognition in neurulation

- **Boundaries between tissues can be created and maintained through the action of cell adhesion molecules**
  - **Different tissues: different types of cell adhesion molecules**
  - **Different tissues: different levels of cell adhesion molecules**
    - **Homophilic: interact with the same protein on adjacent cell**
    - **Heterophilic: interact with different protein on adjacent cell**

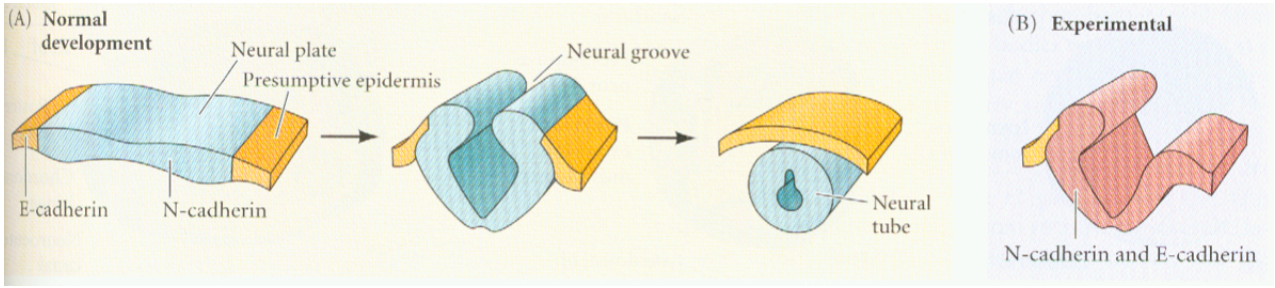
Cadherins:

- **Cadherins: Calcium-dependent cell adhesion molecules**
  - **Interact with cadherins on adjacent cells**
  - **Anchored to actin cytoskeleton via catenin complex**
  - **Multiple types: E-cadherin, P-cadherin, N-cadherin, EP-cadherin etc…**
  - **Homophilic: E-cadherin binds E-cadherin best, N-cadherin binds N-cadherin best, etc…**



Cadherin function in neurulation

- Epidermal ectoderm express E-cadherin
- Neural tube cells express N-cadherin
  - Inject N-cadherin mRNA into one side of embryo
    epidermis on one side now expresses N-cadherin
    that side of neural tube will not separate from epidermis



  - Inject mRNA encoding extracellularly truncated N-cadherin into one side of embryo
    - » truncated N-cadherin disrupts N-Cadherin function on one side
    - » the neural tube on that side will not separate from epidermis

Next time: regionalization

- Patterning along:
- Rostral/Caudal (AP) axis
- Dorsal/Ventral (DV) axis
  - Start with DV axial patterning in Spinal Cord