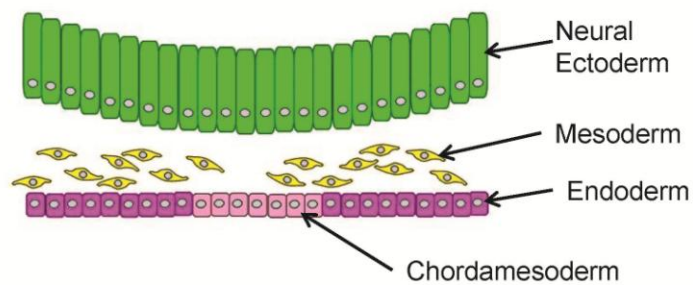


Neurulation: Making the Brain and Spinal Cord

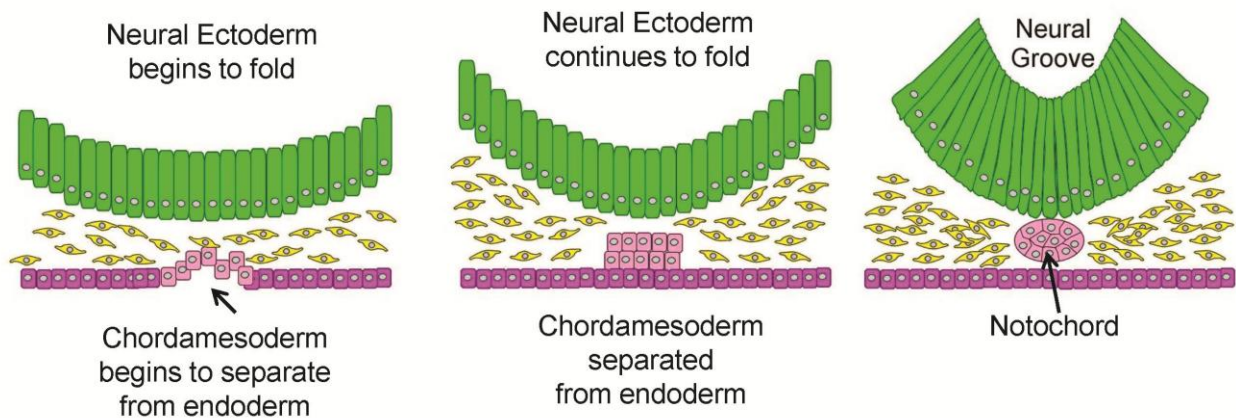
The Primary Germ Layers

The following diagram shows the general arrangement of tissues in a cross-section of the human embryo after gastrulation. During gastrulation the chordamesoderm projected through the primitive node by convergent extension as the notochordal process. After gastrulation, three germ layers are evident: Ectoderm, mesoderm, endoderm. After forming, the notochordal process then embeds within the endoderm as shown in the following figure. As neurulation progresses, the chordamesoderm will again separate out as the presumptive notochord.



Notochord Formation

At this point the presumptive notochordal tissue will separate from the endoderm and re-organize as the notochord below the neural ectoderm as the neural ectoderm begins folding to form the neural tube.



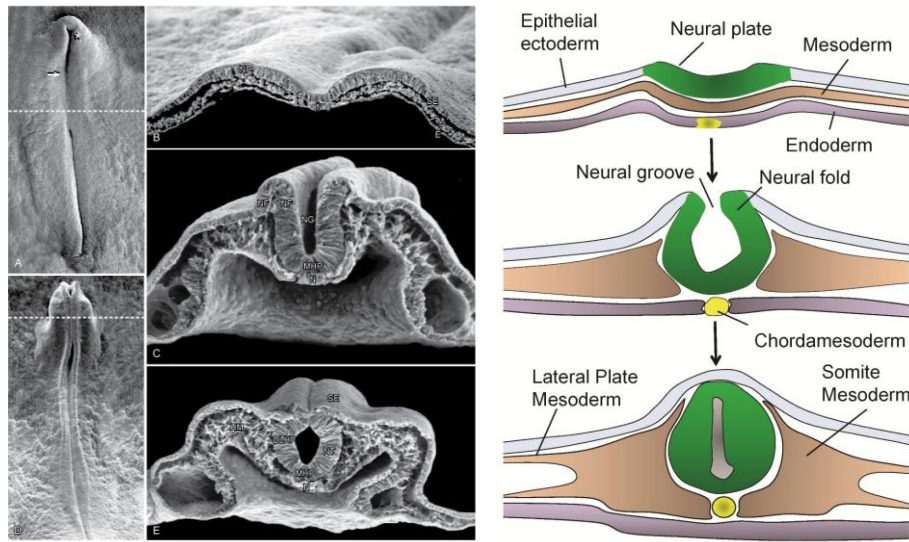
- Folding of the neural ectoderm results in the neural tube
- The neural tube is the precursor of the brain & spinal cord

The Events of Neurulation

- Neural ectoderm thickens
- Neural folds form producing Neural Groove
- Neural folds contact starting from centre of embryo towards head (cranial) and tail (caudal) regions
- Cells rearrange forming Neural Tube with overlying Ectoderm

Neurulation: Making the Brain and Spinal Cord

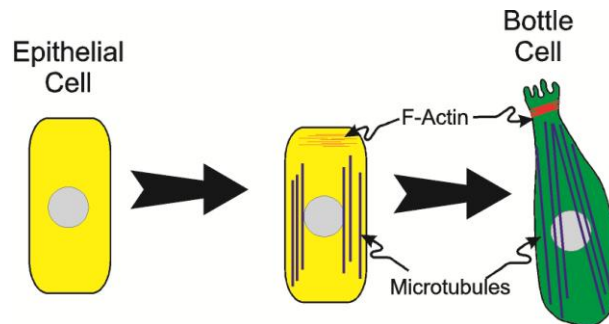
These events have been well characterized as seen in the following scanning electron micrographs:



Left panels: from *Larsen's Human Embryology*. The key regions are the neural plate that thickens and folds to produce the neural folds and neural groove before closing to form the neural tube. The status of the notochord can also be observed as well. Right panel, diagram of these events.

While the neural tube is forming, other embryonic tissues are undergoing changes too as the embryo itself is elongating and undergoing torsion (bending).

Bottle Cells



- Bottle cells appear at time of neurulation
- Change in shape of epithelial to bottle cell is one of driving forces for event
- Cytoskeletal changes lead to bottle cell shape
- Microfilaments (F-actin) at apex
- Microtubules down long axis of cell

F-Actin (Microfilaments)

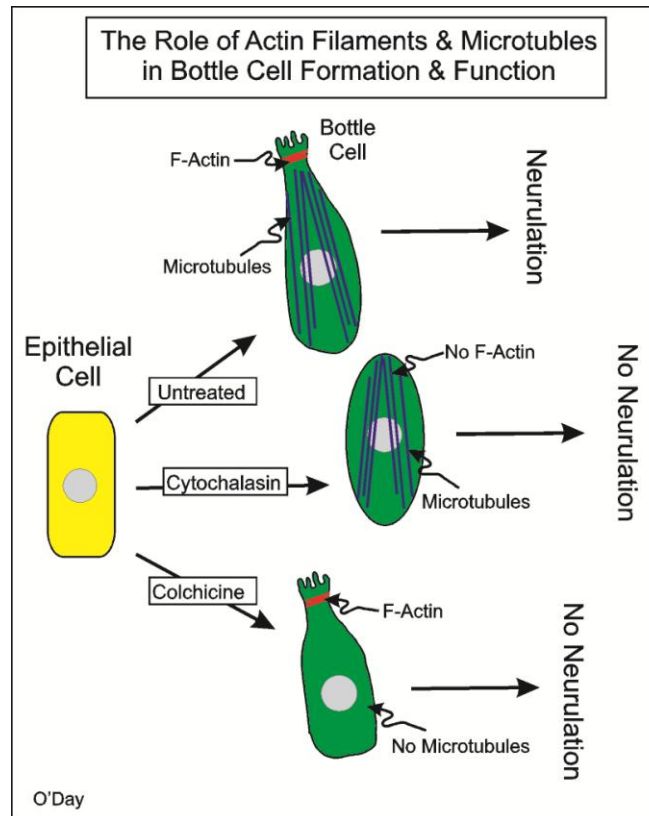
- Long polymers of G-actin: appear like two twisted strands of pearls
- Contractile filaments that work with myosin for contraction
- Cytochalasins (B, C, D) inhibit F-actin formation & thus their function
- Phalloidin binds to intact F-actin filaments
- Detected using anti-actin antibodies or rhodamine-phalloidin

Neurulation: Making the Brain and Spinal Cord

Microtubules

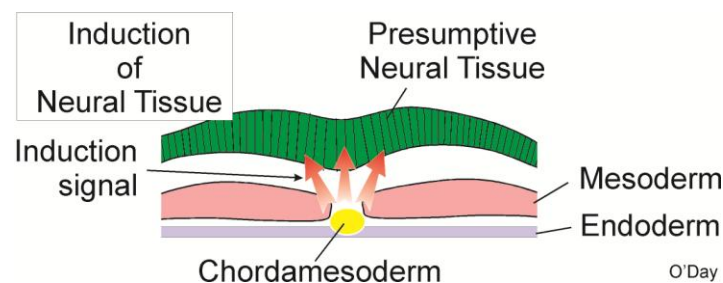
- Polymers of tubulin
- Detect with anti-tubulin antibodies
- Define cell shape, chromosome separation, roles in motility, intracellular movements
- Inhibited by colchicine
- Stabilized by Taxol

Cytoskeleton of Bottle Cells & Neurulation



- Inhibition of either F-Actin or microtubules inhibits neurulation
- F-Actin acts like purse strings to pull apex of cell
- Microtubules elongate cell to give it polarity for movement

Induction of Neural Tissue by Chordamesoderm

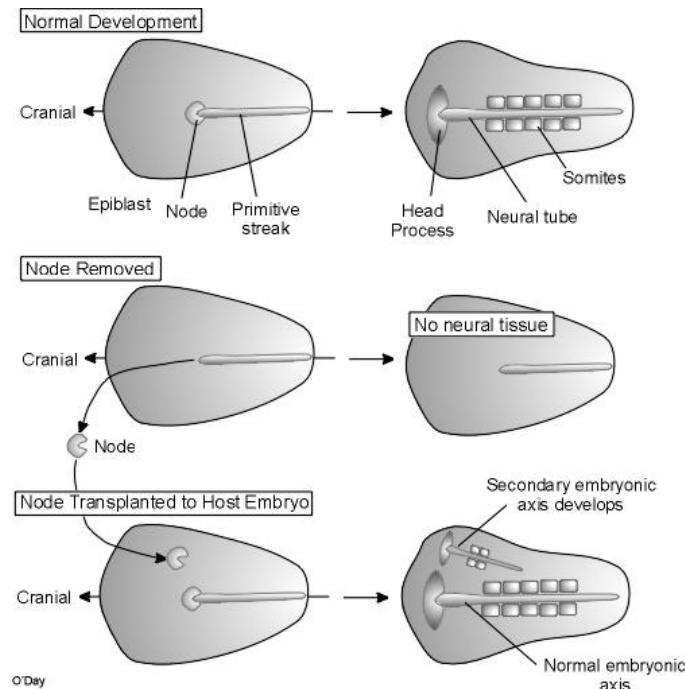


Neurulation: Making the Brain and Spinal Cord

- During gastrulation special region of mesoderm underlies overlying ectoderm
- Chordamesoderm is future notochord
- Signals emitted by chordamesoderm have not been identified

Hensen's Node Induces Neural Axis

In the previous lecture on gastrulation, the role of the node (Hensen's Node) in organizing the overall body plan was discussed briefly. Historically, this special region of tissue has been shown to establish the vertebrate body plan in everything from frogs to zebra fish to mice. Experimentally this organizing ability is shown by the ability of transplanted nodal tissue to induce a second embryonic axis as revealed in the next diagram.

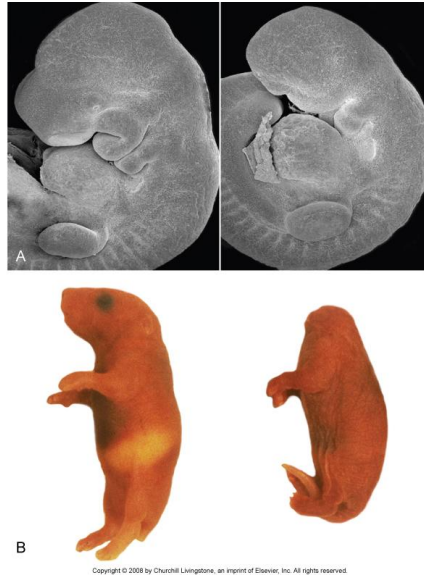


- These classic experiments were done in chicken embryos
- Remove HN: No neural tissue development
- Transplant HN to host embryo: 2nd Neural Axis induced
- Primary Induction: Induces primary embryonic axis
- These experiments have also been done in the mouse showing that the node is an embryonic organizer in mammals.
- More recent studies have shown however, that the mammalian node is not a classic organizer because it can't act alone to induce the embryonic axis but requires other anterior germ tissues to be fully effective
- Progressive cellular interactions are more important rather than a single inductive event that characterizes the organizer activity of the Hensen's Node region in lower vertebrates.

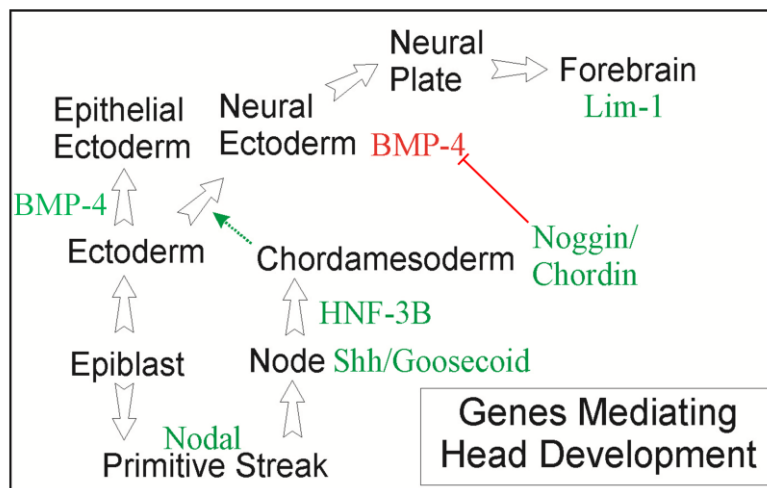
Genes and Head Development

The node secretes over one dozen different molecules. The node expresses genes called Noggin and Chordin that are not expressed by the anterior visceral endoderm. On the other hand, the anterior visceral endoderm expresses specific genes such as Lim-1, which are essential for head development. Lim-1 is a homeobox gene. The picture below shows the effects of knocking out the Lim-1 gene in mice. The forebrain and associated structures don't form when Lim-1 is knocked-out.

Neurulation: Making the Brain and Spinal Cord



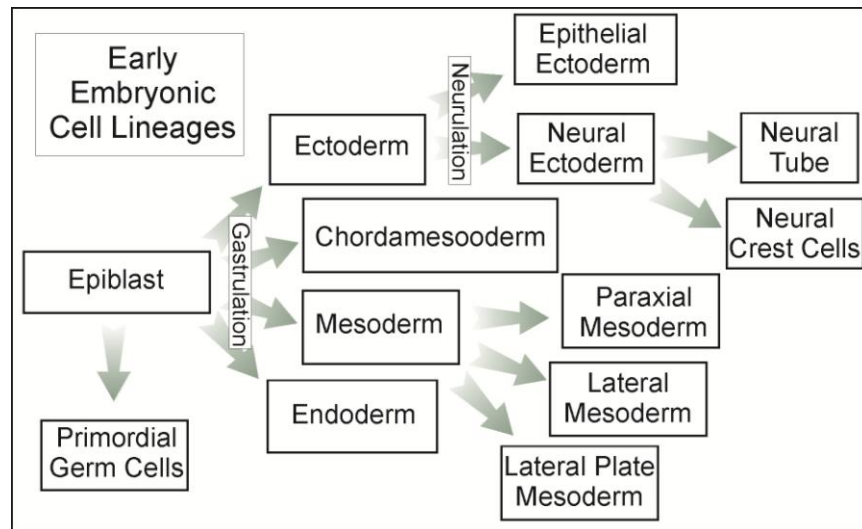
Normal mouse embryos (SEM, top left panel; lower left panel) and Lim-1 KO embryos (SEM, top right panel; lower right panel) are shown. Lim-1-deficient mice are essentially headless with the most anterior structure observed being the pinnae of the ears (Shawlot & Behringer, 1995. Nature 374: 425-430; Figure 4-4 from *Larsen's Human Embryology*). The expression of Lim-1 is regulated by a number of prior events some of which are shown in the next figure.



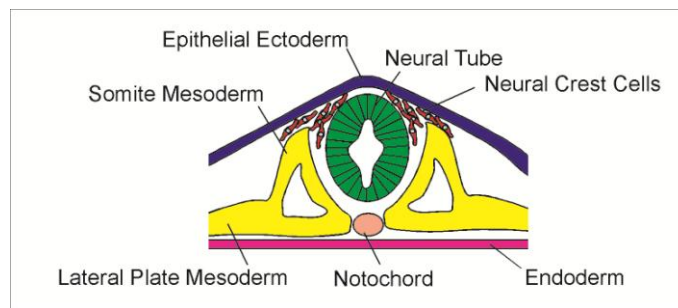
- The epiblast gives rise to the primitive streak where the expression of Nodal drives the expression of Sonic Hedgehog (Shh) and Goosecoid.
- Shh and Goosecoid cause the expression of the transcription factor Hepatocyte Nuclear factor-3B (HNF-3B).
- This results in the expression of Noggin and Chordin, which bind to bone morphogenetic protein-4 (BMP).
- Noggin and Chordin bind to BMP-4 to prevent its function in the neural ectoderm.
- In the absence of BMP-4 function, Lim-1 is expressed leading to forebrain development.
- As seen on the left side of the figure, when BMP-4 is not inhibited, epithelial ectoderm forms from ectoderm.
- The importance of both Goosecoid and Noggin in brain development was shown by the fact that expression of either protein in non-node tissues can induce secondary axes.

Neurulation: Making the Brain and Spinal Cord

Cell Lineages at the Neurula Stage



Late Neurula



- Neural Tube: One layer of cells surrounding a lumen & covered by external limiting membrane
- Neural Crest have separated out--begun to migrate
- Neural Tube thickens & folds to form brain
- New layers of nerve cells will appear in brain & spinal cord (neurogenesis)
- Embryo is now set up for development of all tissues and organs

References

O'Rahilly and Muller, 2008. Significant features in the early prenatal development of the human brain. *Annals of Anatomy* 190: 105-118.