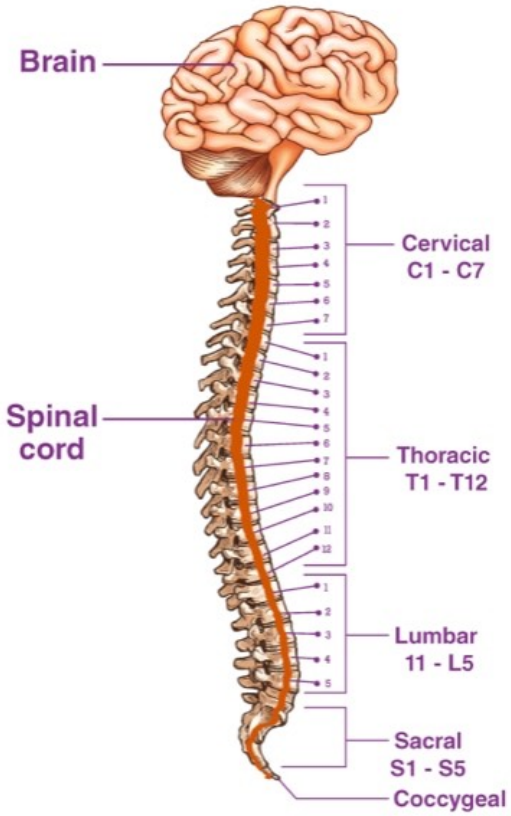
Embryology 3: Segmentation and Formation of Gut Tube

Genes and Development

Segmentation

Many organisms show clear patterns of segmentation in their body plan. Worms and centipedes show a clearly observed repeated pattern of segments along the length of their bodies. In human, segmentation is less clear from outside the body, but inside the body, segmentation can been seen in the spinal cord, which contains a series of thirty-three distinct vertebræ along its length.

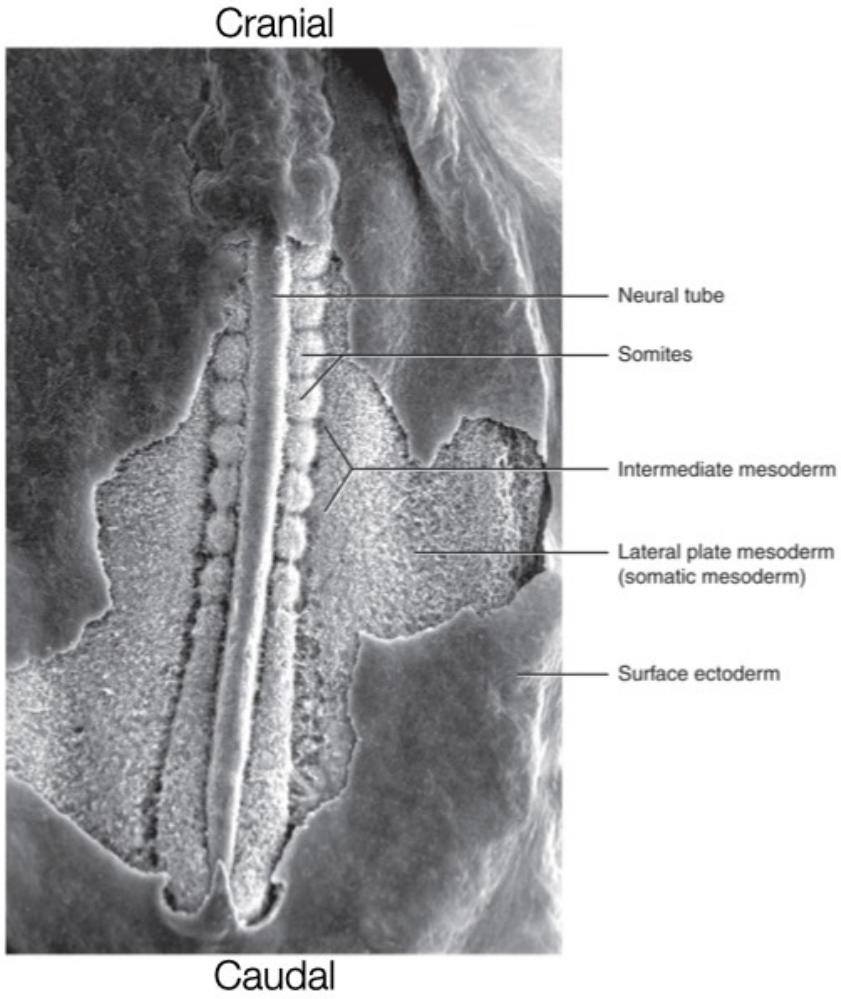
Although vertebræ are appear structurally similar, they have functional differences. The seven vertebræ in the neck provide structure support and facilitate movement of the head. The next twelve vertebræ connect to the ribs. The following five in the lumbar region have evolved for weight bearing. The musculature which attaches to the vertebræ also differs along the spinal cord.



The spinal cord is segmented into a series of vertebrae.

Somites

Segmentation along the caudal - cranial axis is generated through the formation of blocks of tissues called somites. Somites are transient and repeated structures which give rise to vertebræ, ribs, skeletal muscles, and dermis which is a layer of connective tissue underneath the epidermis in skin. After gastrulation, somites form from mesoderm on both sides of the neural tube. Somites form in a directional order starting from the cranial end of the embryo and extending toward the caudal end. Somites develop in pairs on opposite sides of the neural tube. Pairs develop one-by-one down the neural tube. In humans, somites develop at a rate of one pair every six hours. The regular rate of somite formation during embryogenesis allows one to measure the age of the embryo based on the number of somites.

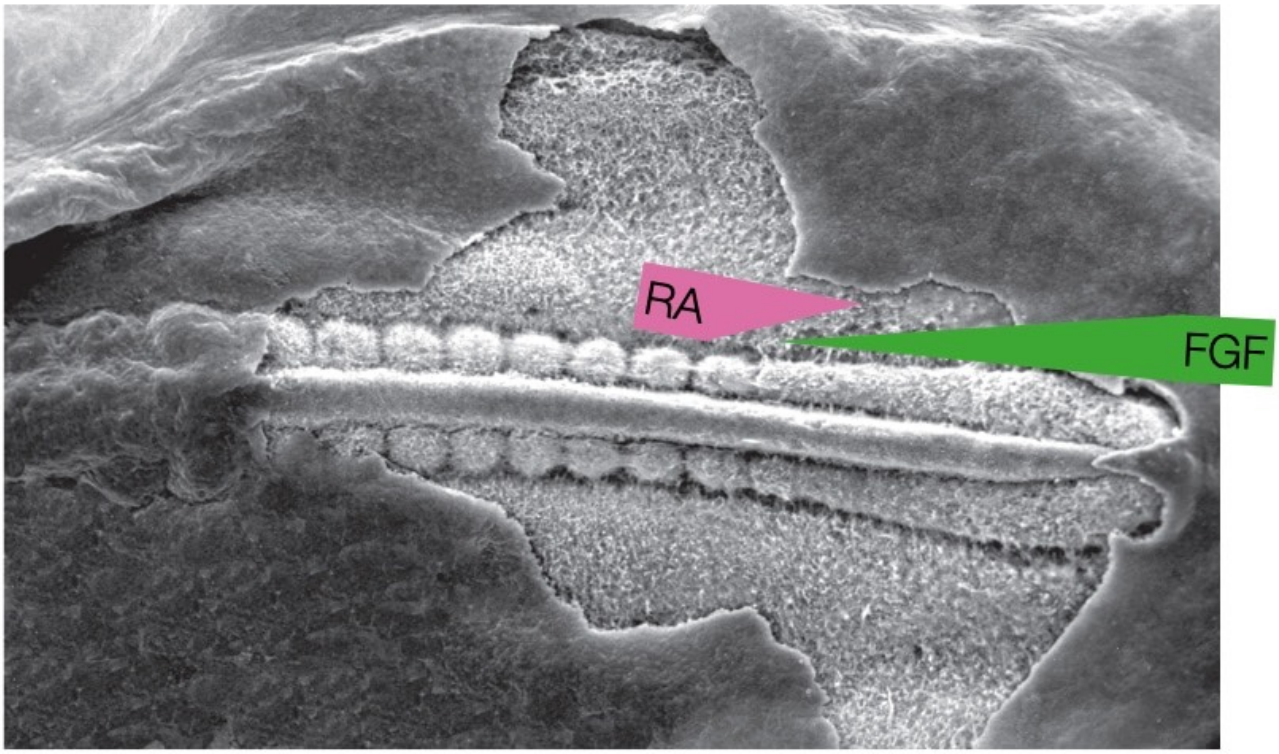


Somites are a mesoderm-derived structure that generates segmentation along the cranial-caudal axis.

A challenged faced by the embryo is how to generate repeating, regularly spaced somites from a disorganized collection of cells in the mesoderm. One widely accepted model involves gradients of two signaling molecules and a timer.

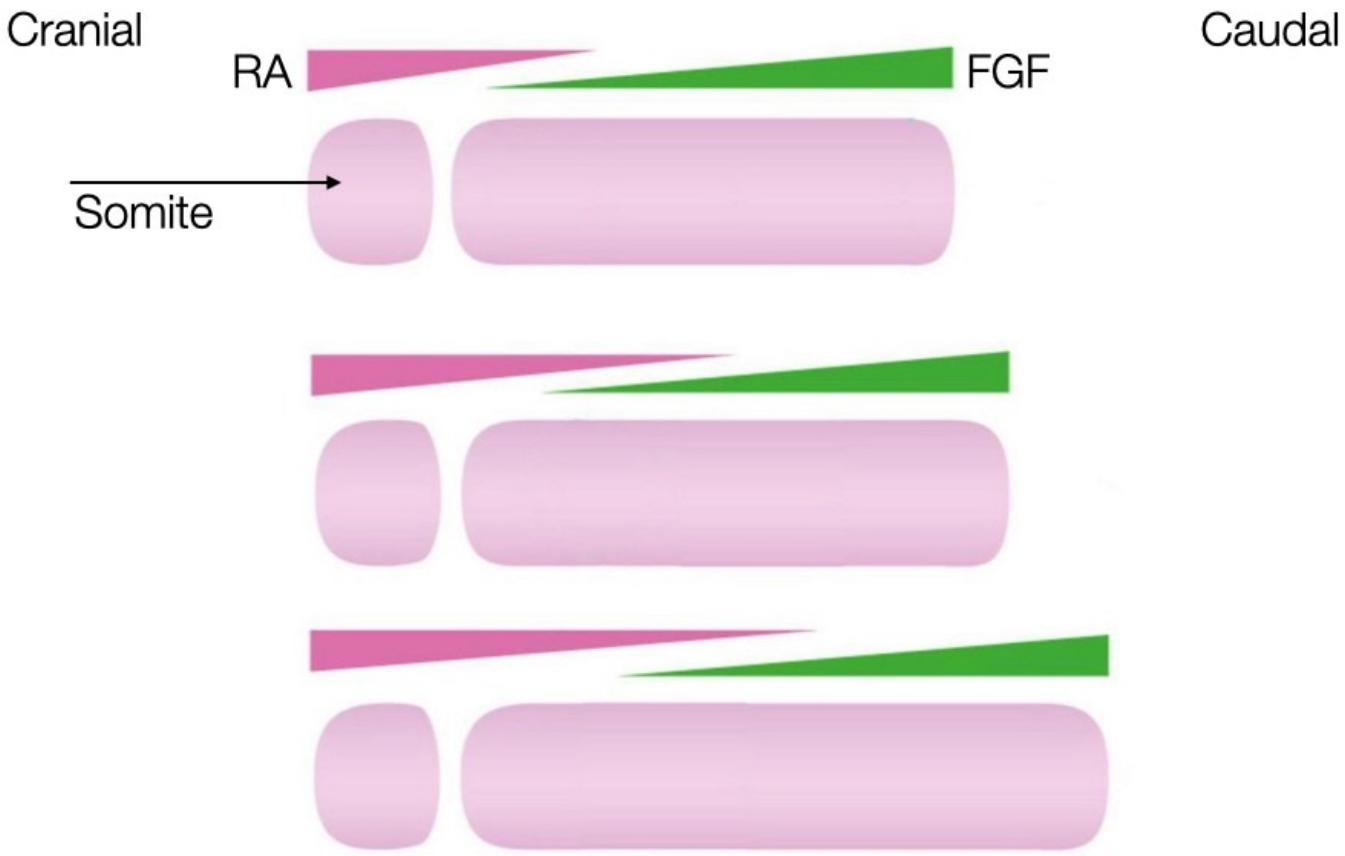
The first gradient starts from the caudal end of the embryo and involves release of the signaling molecule fibroblast growth factor (FGF). The concentration of FGF deceases as one moves toward the cranial end of the embryo. FGF keeps cells in the mesoderm in an undifferentiated state and therefore prevents cells in the mesoderm from forming somites.

The second gradient starts from the cranial end the embryo and involves release of retinoid acid (RA) from the most recently formed somite. RA concentration decreases as one moves toward the caudal end of the embryo. Retinoic acid can induce cells in the mesoderm to differentiate into somite but only where FGF concentrations are low. Thus, there is a region adjacent to the most recently formed somite that contains sufficient RA and low FGF to favor formation of a somite.



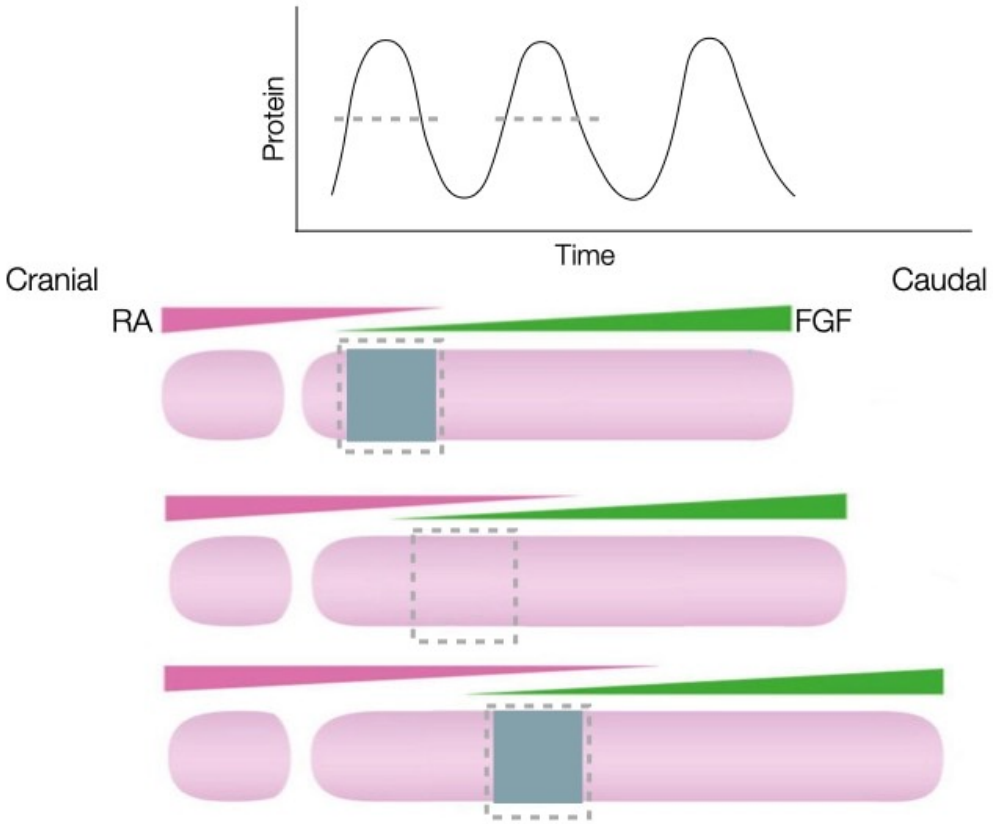
Opposing gradients of retinoid acid and FGF exists along cranial-caudal axis.

As the embryo grows in length caudally, the source of FGF moves caudally which moves the zone favorable to forming a somite gradually toward the caudal end of the embryo. The gradients of FGF and RA are sufficient to induce differentiation of mesoderm from a cranial to caudal direction but cannot generate blocks of a somites. That is because the zone moves gradually and continuously toward the caudal end, so the gradients alone would generate one long somite and not a series of individual somites.



Gradients move in a caudal direction as embryo grows.

To generate individual somites, the embryo needs a timer that when on allows a region of mesoderm to become a somite when the zone of high RA/low FGF passes through it, but when off, prevents mesoderm from becoming somite even when exposed to high RA and low FGF. The timer in the embryo is an oscillation in the concentration of several key proteins in the mesoderm cells. When the concentration of these proteins is above a certain threshold, the cells can differentiate if exposed to high RA and low FGF. When the concentrations of the proteins is below the threshold, then the mesoderm cells remain as mesoderm even if exposed to high RA and low FGF.



Gradients and a timer are needed to generate distinct somites.

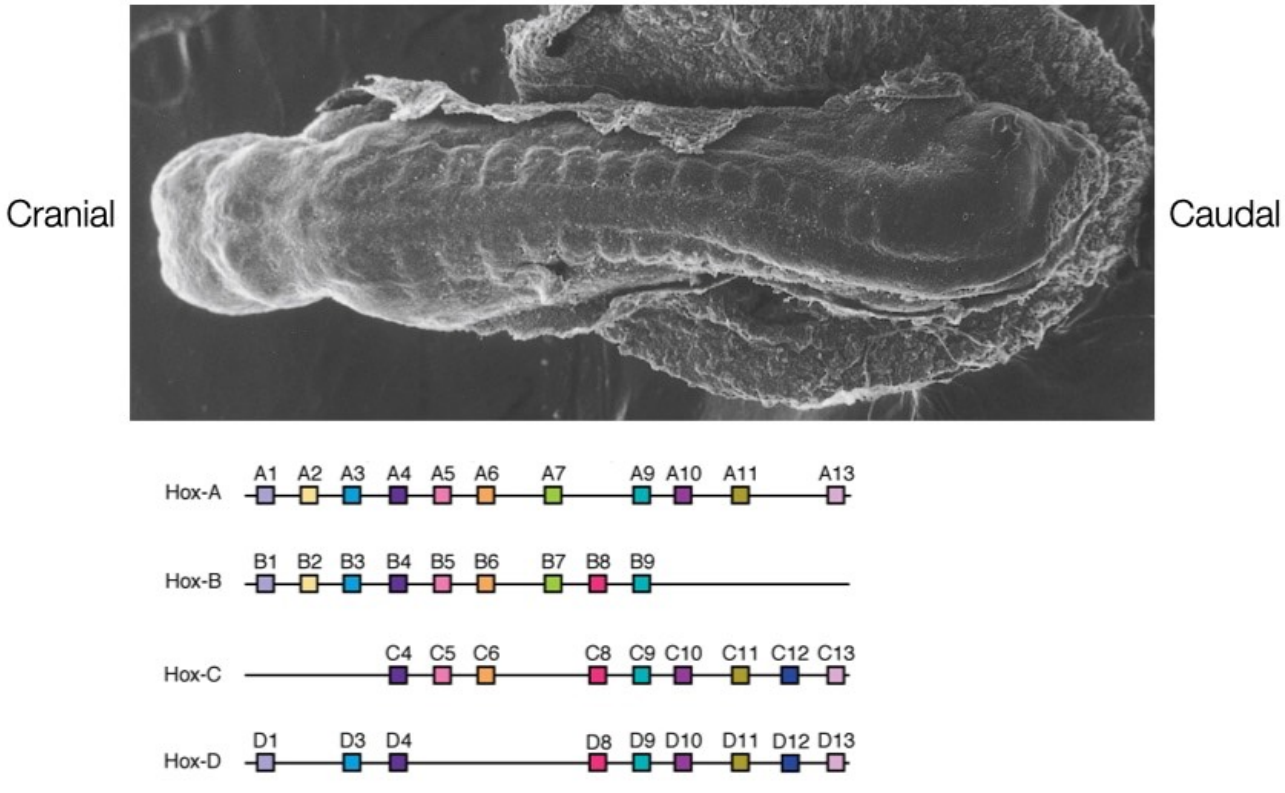
Negative feedback in gene expression generates the oscillating patter of the timer. For example, a protein involved in making cells ready to commit to a somite fate reduces its own production by turning off transcription of its gene. When levels of the protein are low, transcription from the gene is active and the protein is synthesized. The protein levels increase to a point which makes the cells amenable to differentiate into somites if the cells are in a high RA and low FGF zone. The high levels of protein also turn off transcription, allowing degradation of the protein and its mRNA to reduce the concentration of the protein to a level at which cells no longer differentiate into somites even if they are in a high RA and low FGF zone.

This two gradient and timer system has been conserved throughout evolution because a few small adjustments to the oscillating pattern can alter the size and number of somites in the embryo. For example, if the oscillating pattern is increased in frequency, then the embryo forms many, smaller somites. Research has found that the frequency of the oscillator is much higher in organisms such as snakes compared to mammals.

Patterning Somites

The gradient and timer are sufficient to generate a sufficient individual somites, but the somites differ cranially to caudally based on the structures their cells will generate (recall the difference in vertebræ along the spinal cord).

Another conserved mechanism generates a pattern of differentiation along the series of somites. The mechanism involves a large set of genes called homeobox genes (HOX). There are four families of homeobox genes (A - D) that are located on separate chromosomes. Each family member contains up to 13 individual genes (not all family members contain 13 genes). Related genes have the same order within the families (i.e., A1, B1 and D1 encode related proteins). The genes appear to be activated in the order that they reside in the chromosomes from 1 to 13 with lower number genes being activated first. Activation of the Hox genes also depends on when the mesoderm cells descended through the primitive streak. Cells which descend first form the most cranial mesoderm and those that descend later form more caudal mesoderm. Thus, cells that descend first and occupy cranial spaces will express the lowered number Hox genes. Cells that descend later and occupy more caudal spaces in the mesoderm will express higher numbered Hox genes.



Differential expression of Hox genes generate pattern difference between somites.

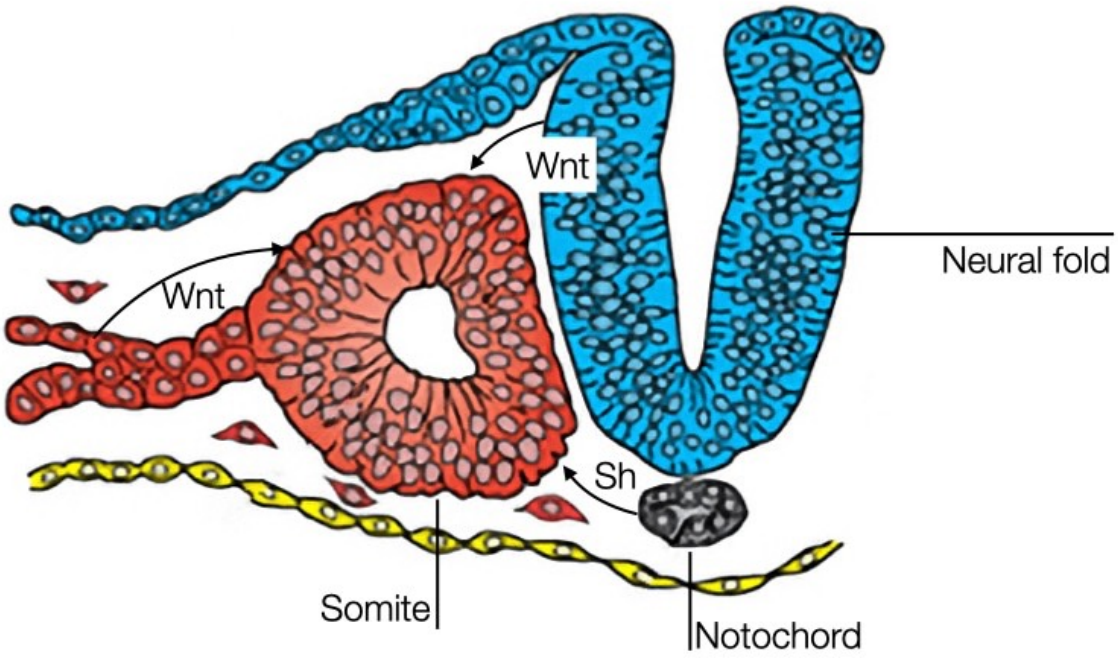
As a result, the expression of Hox genes varies along the length of embryo with cranial somites expressing low numbered Hox genes and caudal somites expressing higher numbered Hox genes.

This difference in gene expression determines different patterns of tissue formation along the length of the somites.

Differentiation of Somites

Cells in somites give rise to several different structures and tissue types, including vertebræ, muscle and dermis in the skin. Different regions of the somite develop into these structures based on their exposure to different signaling molecules. Signaling molecules released from surrounding structures will determine the fates of cells in somites.

When somites first form, the cells resemble fibroblasts and form a ball of interacting cells. These cells transition into epithelial-like cells and assemble into a structure with a central cavity. Under the influence of different signaling molecules, the epithelial cells will transition again into cells with different fates.

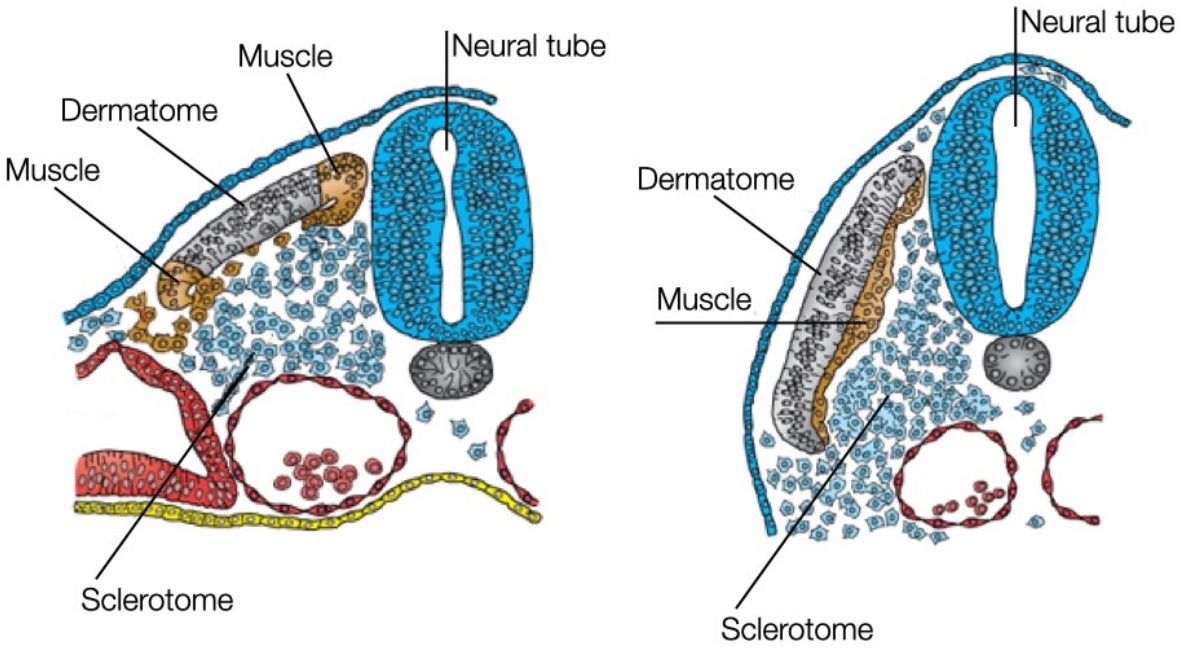


Signaling molecules from surrounding tissues affect fate of cells in somites.

One signaling molecule is sonic hedgehog which is released by cells in the notochord and at the ventral side of the neural tube. Sonic hedgehog diffuses and impacts cells in the somite closest to the notochord and neural tube. Sonic hedgehog causes these cells to dedifferentiate into mesenchymal cells and dissociate from the somite. Eventually, the cells will form the vertebræ and ribs.

The remaining cells in the somite will be influenced by Wnt to develop into muscle tissue. Cells in the dorsal side of the neural tube secrete Wnt which affects cells in the somite closest to the dorsal

region of the neural tube. In addition, mesoderm located laterally to somite also secrete Wnt which affects the cells on the lateral side of the somite. Between these two regions in the somite is an area of cells that is exposed to low concentrations of Wnt. These cells will differentiate into a structure called the dermatome which will form a connective tissue layer in the skin called dermis.

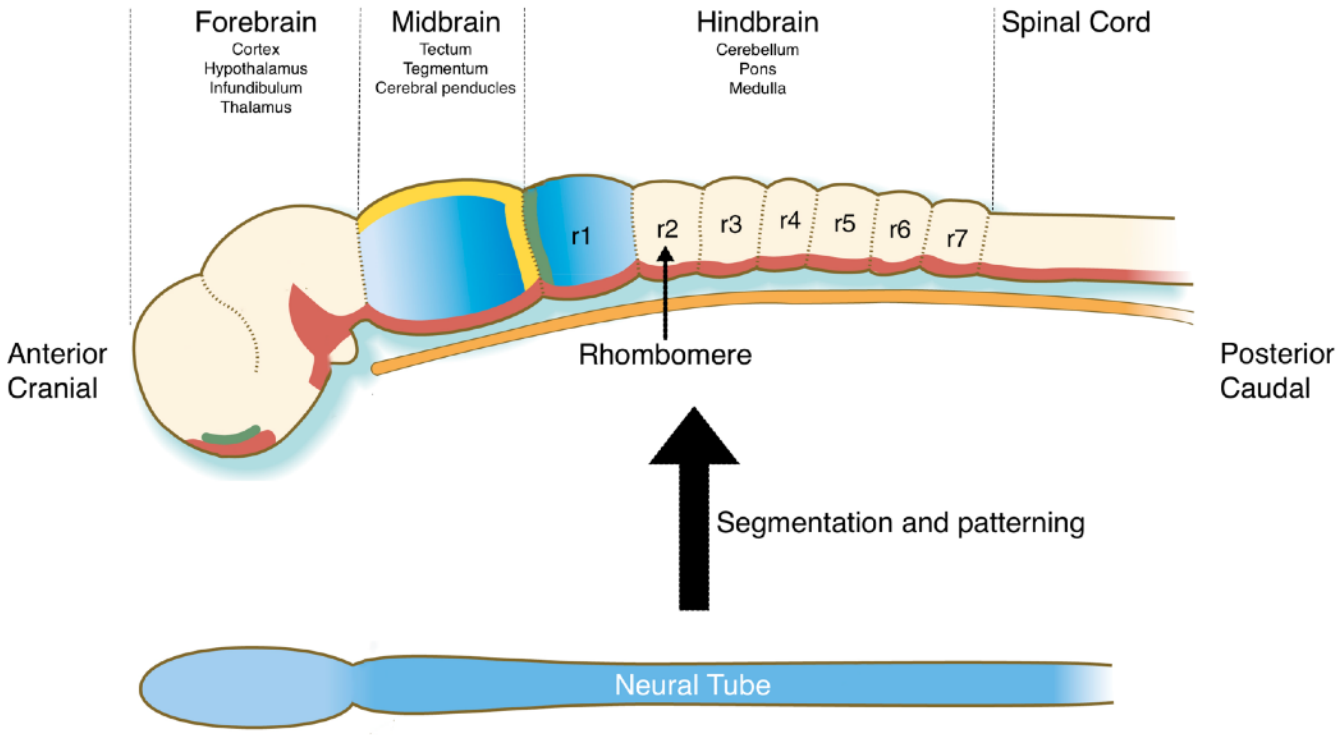


Cells in somites differentiate into bone, muscle and dermis.

Patterning the Neural Tube

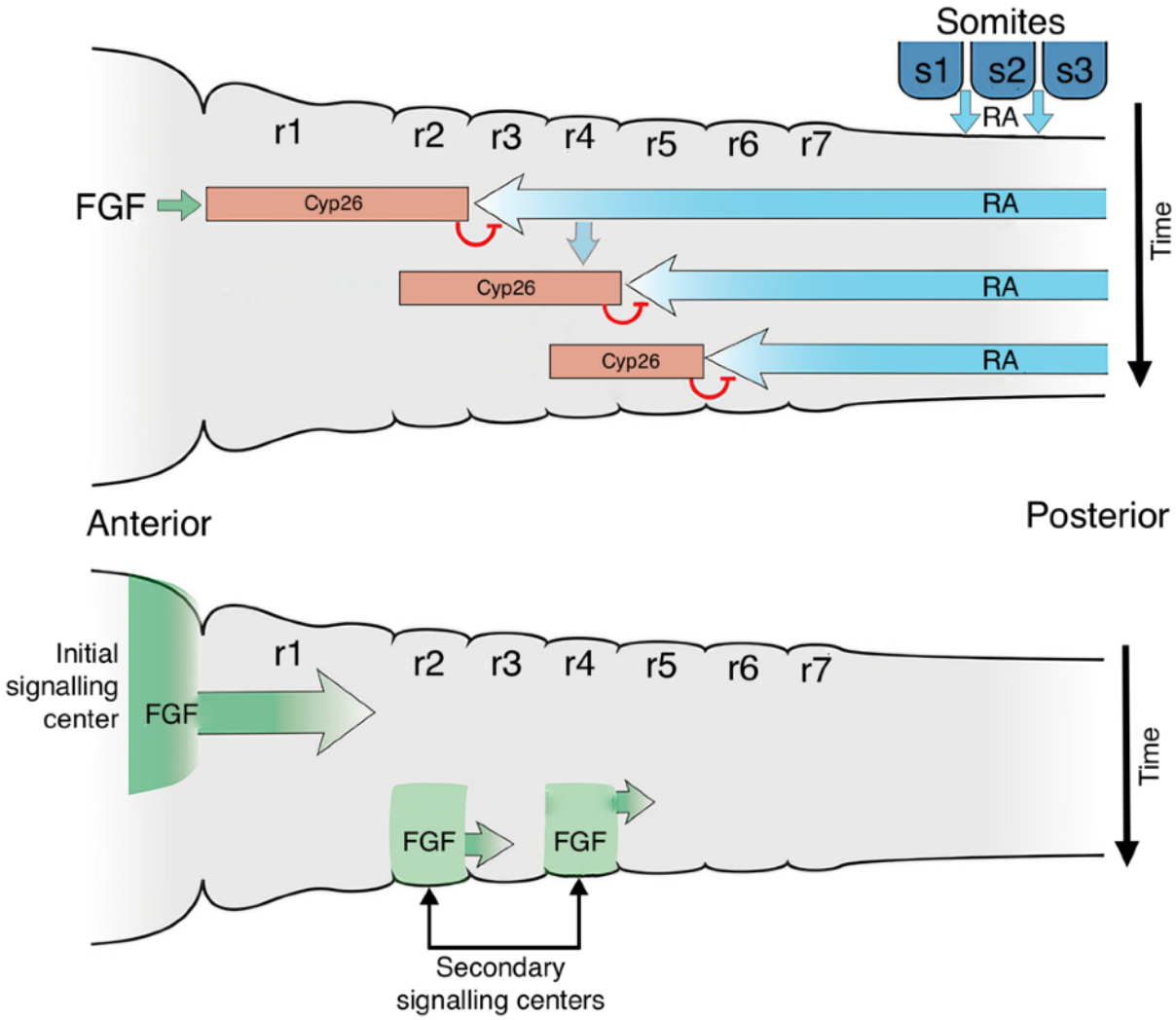
Recall that the neural tube forms from ectoderm and runs from the cranial end of the embryo to near the caudal end. Cells in the neural tube will develop into all the neurons and support cells in the central nervous system, including the brain and spinal cord. Because different regions of the brain and spinal cord perform different functions, the neural tube is segmented into different regions during development. The major regions that initially form are the forebrain (prosencephalon), midbrain (mesencephalon), hindbrain (rhombencephalon) and spinal cord.

Here, we'll focus on patterning of the hindbrain. The hindbrain is not only essential for the development of the future cerebellum, pons and medulla in the brain, but it also produces neural crest cells that will help form craniofacial structures, such as the jaw.. Mutations or teratogens that affect patterning of the hindbrain or production of neural crest cells can lead to malformations in structures in the face. During development, the hindbrain regions of the neural tube is patterned into a series of blocks tissue called rhombomeres. Each rhombomere is considered a distinct developmental unit which will give rise to neurons with different functions and neural crest cells that generate different structures in the head and neck.



The neural tube is segmented and patterned into different structural and functional regions.

The patterning of the hindbrain uses a similar mechanism as segmentation of mesoderm into somites. Gradients of FGF and retinoid acid (RA) will establish an anterior-posterior axis along the hindbrain and somites have a key role in generating this gradient. The anterior most somites, which are located toward the caudal (posterior) end of the hindbrain region of the neural tube, produce RA. RA diffuses both caudally where it induces formation of somites and cranially (anterior) where it keeps cells in the neural tube in an undifferentiated state. FGF is produced by cells at the border between the midbrain and hindbrain and diffuses caudally. Where FGF concentrations are high, cells produce an inhibitor of RA called Cyp26. Thus, the region of hindbrain closest to the midbrain is exposed to low concentrations of RA and begins to differentiate into rhombomeres. Once formed, the rhombomeres produce FGF, which decreases RA concentrations in the adjacent caudal region of the neural tube, allowing it to form rhombomeres. This process proceeds down the hindbrain to form seven rhombomeres. Each rhombomere will develop cells with different functions based on expression of different Hox genes.



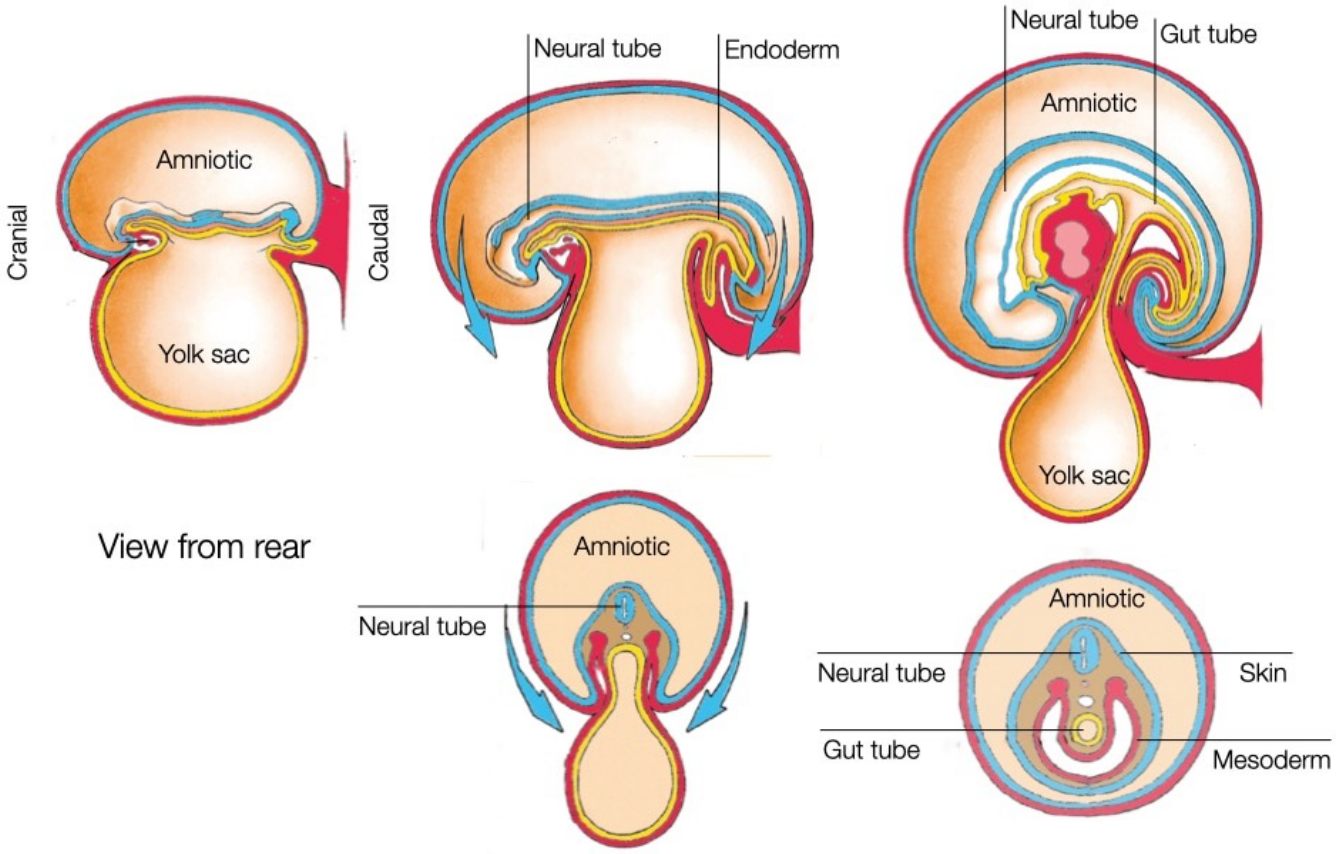
Gradients of retinoid acid and FGF pattern the hindbrain into rhombomeres

Formation of the Gut Tube

As the embryo develops, it lengthens in the cranial-caudal direction due to convergent extension and cell division (see the second embryology lecture for details). While the embryo lengthens, the yolk sac underneath the embryo dœsn't increase in size. As a result, the embryo begins to fold inward at its cranial and caudal ends and grow toward the center to accommodate its larger size. At the same time, the embryo also begins to fold inward laterally. The folding along the four edges of the embryo brings the ectoderm, mesoderm and endoderm layers inward on the ventral side (facing the yolk sac) of the embryo. As those layers are drawn inward, the connection between the embryo and yolk sac is pinched together until all that remains is a narrow duct that connects the embryo to the yolk sac. This structure will eventually develop into the umbilicus.

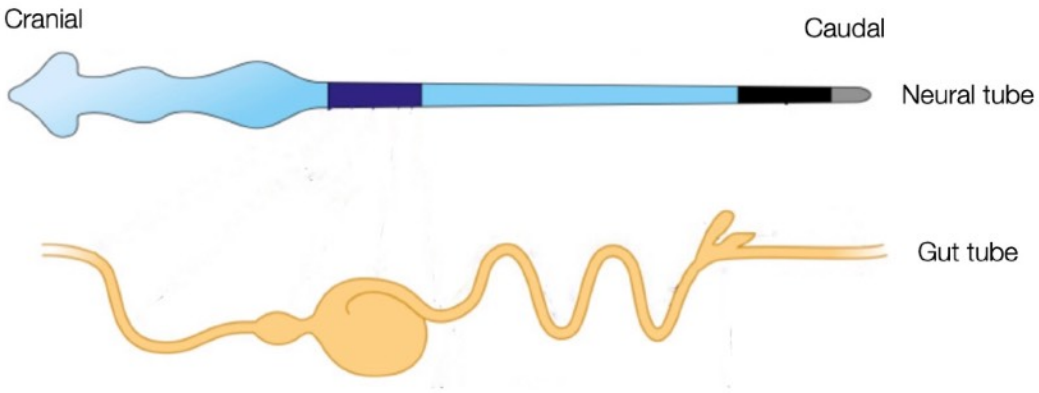
The folding generates a tube within the embryo that is surrounded by endoderm. This structure is called the gut tube and will develop into all of the structures of the GI tract, including esophagus, stomach and intestine.

After the folding complete, the embryo is surrounded by a layer of ectoderm (future skin) everywhere except at the site of the future umbilicus. Internally the embryos contains two tubes that run cranial to caudal: neural tube and gut tube.



Inward folding of embryo generates gut tube from endoderm.

The neural tube and gut tube run parallel to each other along the cranial-caudal axis of the embryo. The close proximity of the tubes is important because cells generate during closing of the neural tube, neural crest cells, migrate through the mesoderm to reach the gut tube. Once there, the cells will form ganglion of neurons along the wall of the gastrointestinal tract to control peristalsis and the secretion of acid and other factors.



Neural and gut tubes run in parallel from cranial to caudal in the embryo.