Whether it's the nerve cord of the larval sea squirt or the highly folded cerebral cortex of the elephant's brain, formation of the central nervous system is arguably one of the most fascinating and important events during embryonic development. The central nervous system, including the brain and spinal cord, is the first major organ type to be created in the embryo, which forms during a process called neurulation. In this tutorial, I will cover the fundamental principle of neurulation, which mainly pertains to the establishment of an ectodermal tube that extends from the most anterior to the most posterior regions of the organism. And the most anterior region, the head, this tube gives rise to the brain, of course, whereas the spinal cord is made in the remaining posterior portions of this tube.

Let's contemplate this for a moment. The most basic form of our own central nervous system is that of a tube, particularly during its initial period of construction. The central cavity, or lumen, of this tube is called the ventricle, and the outer walls of this tube made up of all the neuronal and glial cells that give this system its connected function. How is this tube made? For instance, with the right forces, even water can form tubes. Obviously, such wave tubes are not sustainable, but ironically, there are forces in the embryo that can drive waves of cell movement in common directions to fuel neural tube formation.

To answer this fundamental question of how to form a tube, we need to understand what the starting material may be. It is, of course, a tube of cells that will create the CNS, and from this tube comes the differentiation of a huge diversity of neuronal and glial cell types which establish highly stereotypical connections to wire up the nervous system. Additionally derived from the neural tube are neural crest cells that leave the CNS and migrate off into the periphery giving rise to a multitude of different cell types. This tube also undergoes targeted regions of expansion and morphogenesis to create the folded structures of the brain. We will discuss the varied mechanisms of neural tube patterning in future dev tutorials. Here, we will solely focus on the events surrounding initial neural tube formation. Let's begin with some cells. What could happen to make this pile turn into a tube? Take a moment and draw your own pile of cells. Now how

would you move these cells around to create an outer layer with an inner cavity? Perhaps you thought of simply establishing the cavity in the middle of these cells. If you did, then you would be describing the process of secondary neurulation, which occurs in the posterior of some organisms when a mix neuro-mesodermal cells, mesenchyme, come together to form a rod after which a lumen is established at its center. However, what if this pile of cells first flattens into more of a sheet of cells or a plate? Then how would such a tube form?

Please indulge and take another moment to spatially play with this neural plate and turn it into a tube. What has to happen in order to make this possible? I'm sure at some point you contemplated the need for this plate of cells to physically bend, but where, and how? How does a sheet of cells bend, or rather fold? The answer is localized cell shape changes from box-shape epithelial cells to more trapezoidal cells that have a smaller apical surface as compared to its basal surface. If enough cells at a focal point change this way then the whole tissue will fold at that point, much like a hinge. This tissue folding behavior of the neural plate is called primary neurulation.

Neurulation is the process by which the three ectodermal regions of the surface ectoderm, the neural crest, and the neural tube, are made physically and functionally distinct from one another. Let's watch a frog embryo undergo neurulation. This movie begins with gastrulation in which you can see the movement of the ectoderm both into the embryo initiating at the dorsal blastopore lip, as well as enveloping the vegetally positioned endoderm. Then neurulation begins as the neural folds converge upon the midline and then zipper up anteriorly to close the neural group. Let's see that one more time, because such awesomeness is hard to take in all at once.

The movements of gastrulation cover the embryo with ectoderm. Then convergent movements upon the midline, folding of the neural plate, and subsequent closure. Simply amazing, isn't it? How does this all happen? Before we can delve into the molecular mechanisms governing neurulation, let's take a step back and define some of the as well as identify the key anatomical features of the developing neural tube. The

image you see here is a generalized cross section of the late neurula after the neural tube has been formed. The most dorsal structure is the surface ectoderm that will develop into the epidermis, skin. Immediately under the surface ectoderm and located at the midline of the embryo lies the neural tube with its centralized lumen or ventricle. Bilaterally adjacent to the neural tube is the paraxial mesoderm which will give rise to the muscle and bone. Of particular relevance, the spinal cord is the bone that will, of course, build the vertebrae. Also at the midline but ventral to the neural tube is the notochord. The notochord is the defining feature of all chordates, and you will come to learn that the notochord plays major roles in patterning all surrounding tissues.

Let's put this neural tube in a bit more context. Here's an illustration depicting a top down view, also known as dorsal, of a chick neurula just prior to neural tube formation. Although the neural tube is not fully formed yet, its initial stages of development can be seen here at different axial levels. Beginning most posterior and also seen in cross section view, the flatter neural plate is evident. As our gaze moves up, the neural plate is folding first at the midline, just above the notochord, establishes the neural groove. Additionally, the dorsalmost extents of the buckled neural plate seen lining either side of the midline are termed the neural folds.

Previously I mentioned that neurulation can occur in two different ways, called primary and secondary neurulation. Primary neurulation specifically refers to anterior neural tube formation through the folding and pinching off of the neural plate into a hollow tube. Secondary neurulation creates a similar hollowed neural tube through the aggregation of mesenchyme into a solid cord that then subsequently forms a cavity at its core.

It is important to know that although the mechanisms of neurulation are largely conserved, some variation among different vertebrate species does exist, particularly as it pertains to the timing and ways in which the epidermis and neural tube fuse into their own separate structures. For instance, in amniotes, separation will occur at the moment the neural folds contact. However, the surface ectoderm of the frog neurula will fuse prior to neural tube closure, and further different is how the zebra fish neural plates

coalesce into a neural keel prior to the separation of surface ectoderm. At this point, I would like to focus a bit on the cellular mechanisms associated with primary neurulation in amniotes. Primary neurulation can be described as four separable yet temporally overlapping events. During the first stage, neuroepithelial cells continue to proliferate and expand the neural plate.

Isn't this an absolutely gorgeous scanning electron micrograph of the chick neural plate completed by Kathryn Tosney? It was fractured perfectly to see the neuroepithelial cells spanning its full apical-basal axis. Importantly, the more laterally positioned cells are already specified to become the presumptive neural crest and surface ectoderm. The defining feature of stage one occurs when the neural plate begins to bend at the midline at a location known as the medial hinge point. During stage two, this medial folding event leads to highly elevated neural folds, the peaks of which converge in toward the midline. Similar to the medial hinge point, dorsolateral hinge points form during stage three at about two thirds of the way up the divided neural plate. These dorsolateral hinge points enable the two neural folds to bend inward toward one another. Finally, the folds make contact in stage four, during which the neural crests delaminate and migrate away, and the opposing edges of the surface ectoderm fuse together and pull away from the neural plates. Simultaneously, the opposing edges of the neural plates also fuse to close the neural tube.

Recently, the Niswander Laboratory has used in toto imaging to observe these stages of neurulation in a live mouse embryo. The following movie shows a dorsal view of the mouse neurula, and you can see the neural folds come together and fuse at the midline along the anterior to posterior axis. This data can be processed into optical cross section projections which reveal all the successive stages from neural plate elevation to dorsolateral hinge point formation and near contacting of the neural folds. Throughout these stages, two of the most critical events were the formation of the hinge points. Recall earlier in this tutorial, we hypothesized that in order to fold a sheet of cells it would require localized cell shape changes. Let's look a little bit closer at these shape changes. Here's our starting neural plate made of neuroepithelial cells. Cells of the

presumptive medial hinge point undergo an asymmetric shape change such that their apical surface gets smaller than its basal surface, a cell behavior called apical constriction. This is a repeated approach in development used to bend tissue, which you may have come across when studying, say, gastrulation for instance.

Recall the formation of bottle cells by apical constriction to initiate invagination in sea urchins or establish the dorsal blastopore lip in Xenopus. Therefore, localized apical constriction builds trapezoidal cell shapes that force a buckling of the neural plate so it in fact bends. As seen in these schematics, apical constriction occurs in both the medial hinge point and dorsolateral hinge point locations. What mechanisms are controlling apical constriction specifically in the locations of the medial and dorsolateral hinge points?

Let's apply our developmental biology mantra, find it, lose it, move it. Find it. Noggin is a gene also affiliated with the dorsal blastopore lip during gastrulation and is also uniquely expressed in the neural folds. Lose it. Loss of noggin results in the failure of the neural tube to in fact close. How must noggin function then? Well from this experiment, we can conclude that noggin is required to positively promote neural tube closure, but how? Presuming you have, in fact, already studied gastrulation and early axis formation, do you remember anything about how noggin functions? Well, noggin is a secreted protein that functions to directly bind to and inhibit another secreted protein called bone morphogenic protein. BMPs are critically important morphogens. They regulate the expression of downstream targets through the activation of the SMAD family of transcription factors.

Now noggin binds BMPs directly and prevents BMP's ability to bind to its own receptor. What would these data suggest about the role BMP signaling plays in hinge point formation? Is it that BMP signaling in fact promotes hinge point formation? Or BMP signaling is inhibiting hinge point formation? Perhaps some happy medium of BMP signaling is required for hinge point formation. Now when you're contemplating these things, if you happen to choose B or C, you would be right, both. Let's move it to prove

it. By ectopically expressing a constitutively active BMP receptor, researchers showed that too much BMP signaling can completely abolish formation of the hinge points. In contrast, ectopic expression of a dominant negative BMP receptor which would repress the function of the normal endogenous receptors resulted in an earlier and exaggerated medial hinge point. So it appears that hinge point formation follows the fabled porridge analogy. BMP cannot be too hot, not too cold. It needs to be just right.

These and other data have led to a model whereby BMP signaling promotes apical junctions that stabilize equivalent neuroepithelial morphology between the apical and basal extents. However, reduced BMP signaling leads to less contacts, less apical contacts, that is, and consequent constriction in that location. Moreover, the amount and spatial restriction of noggin expression is itself negatively regulated by yet another player that is ventrally secreted, which is the sonic hedgehog morphogen. Thus in this way of double negative gates, the regions of the neural plate that experience just the right amount of BMP signaling will respond with apical constriction and hinge point folding, as seen here in this time lapse movie of dorsolateral hinge point formation in the mouse neural plate.

How does the neural tube achieve its final closure? The process of neural tube closure has been compared quite aptly to the zippering close of a coat. Like the teeth of the zipper coming together, non-neural ectodermal cells at the leading edge of the neural folds send long filopodial bridges that make contact with the opposing neural fold. Time lapse imaging of these filopodial protrusions do suggest the potential for an important role in pulling the juxtaposed sides together. However, the functional significance of these extensions is currently unknown.

To gain further insight into the mechanisms of neural tube closure, researchers have turned to the highly conserved larval Ciona model system. Prior to taking on the sedentary adult life of a tunicate, the free swimming Ciona larva possesses a notochord as well as a nerve cord, and this forms according to processes very similar to that of primary neurulation, as seen in these whole mounts and cross sectional views of the

Ciona neurula. Fusion of the neural folds in Ciona zippers in a posterior to anterior direction. With the easy accessibility and time lapse microscopy amenable with this tunicate larva, we can even watch the fold process of the neural tube zipping closed.

What are the forces driving neural tube zipper advance in Ciona? The Monroe Lab has recently looked very closely at the membrane junctions of cells undergoing neural tube fusion in Ciona. They discovered that the epidermal cells immediately ahead of the zipper activate actomyosin contraction with their anteriorly adjacent neuroectodermal cells. This creates a scenario in which a pulling force is generated just anterior to the last formed zipper junction, although equally critical to the anterior contraction to zipper advance is the reduction of posterior resistance. This is achieved by the subsequent release of the most posterior attachments. In support of this model, when the Monroe Lab exposed these tunicate embryos to a drug that inhibits myosin function, they failed to close their neural tubes.

The final question in this process of neural tube closure is what promotes and sustains the separation of the surface ectoderm and the neural tube? Here's the neural tube, and separated and above it, the surface ectoderm. What is so unique about these two tissues that allows them to be separated at this developmental time when only a brief period earlier they were one whole, continuous sheet of cells? Have you come across any developmental mechanisms that serve to keep one type of cell together while also preventing interactions with a different cell type?

Consider pausing the tutorial for a moment and really think about what mechanism could facilitate such a cell behavior. If you thought about cell to cell adhesion as your mechanism, you would be certainly warm. If you went so far as to devise a hypothesis about differential adhesion, then you would be on fire right. One such family of adhesion molecules that is known to regulate differential adhesion is the cadherin family of CAMs, cell adhesion molecules. Cadherins facilitate hemophilic binding between cells that serve to keep cells expressing the same cadherin type and amount adhere together. In fact, you may recall from Chapter Four in the 11th edition of Developmental Biology that

different tissues exhibit different properties of surface tension based on the different amounts and types of cadherin molecules they are expressing. So in the context of neural tube formation, our hypothesis might be that the neural plate and presumptive surface ectoderm begin to express different cadherin molecules. This is indeed the case.

Here is a schematic of the expression patterns of two different CAMs, N-cadherin found in the neural plate, whereas E-cadherin found in the presumptive epidermis. Researchers have tested the role of N-cadherin in this process by losing it and moving it. Loss of N-cadherin in zebra fish prevents neural tube closure. Mis-expression of N-cadherin in the surface ectoderm only on one hemisphere of the Xenopus embryo also resulted in the failure of neural fold to close and separately fuse with the opposing surface ectoderm. According to the CDC, just under 3,000 births annually have neural tube defects. Understanding the mechanisms regulating neural tube development can have real impacts on our prevention of such defects.

For instance, when it was discovered that deficiencies in folic acid are associated with a failure in proper neural tube closure, then the US Food and Drug Administration mandated that all grain products be enriched with folic acid, a policy that has helped reduce the rates of such birth defects. Current research on the mechanisms by which folic acid influences neural tube development is still ongoing, but exciting evidence is suggesting its biological active metabolites from folic acid may regulate the epigenetic states of genes important in neural tube closure.

I often teach a summer science course at Smith College for high school students. Some of my past students treated zebra fish embryos with alcohol to model fetal alcohol syndrome, and to my surprise, they produced embryos with neural tube defects just like the one you have been seeing in the background of this tutorial. Can you tell what seems odd about this neural tube? I call it the flux capacitor phenotype after one of my favorite movies. You see a bifurcation of the ventricle and a lack of complete neural tube closure. I have no idea how this occurred, and request that you use the principles

of neurulation we have covered in this tutorial to generate hypotheses for how this morphology could've come to be. Good luck, and happy developing.