I want to tell you a story, a story of an incredible journey which ends with the creation of one's face, or at least the skeleton underlying it as well as the connections of the outside world to the central nervous system. And even the color of one's skin, whether scales, feathers or fur. This is the story of neural crest cell development. Imagine a cell being an integral part of the early neural plate, seemingly identical to its neural epithelial neighbors, and then it changes from an epithelial morphology to a modal mesenchymal cell, and it actually leaves the developing neural tube. Leaves the CNS, to go where? To migrate away into the periphery of the embryo giving rise to a multitude of different cell fates like those of the skeletal and connective tissues of the head. The neurons and glia of the sensory and sympathetic and parasympathetic nervous systems. Cells of the adrenal gland, and pigments cells of the skin.

I have to admit, the neural crest cell is probably my favorite cell. Even though they are a transient cell type not found in the adult, their presence in the early embryo has played a pivotal role in vertebrate evolution. The two most remarkable features of the neural crest cell are that it functions as a multipotent stem cell, and that it undergoes a significant migration to its final destination. And it's specific factors in the environment during this journey which influence both its literal trajectory and the trajectory of its cell fate. To quote the famous poet Robert Frost. "Two roads diverged in a wood, and I, "I took the one less traveled by "and that has made all the difference." The path a neural crest cell takes makes all the difference in the fate it adopts.

Let's take a moment to first to find some of the contextual anatomy of where neural crest cells are born and the paths they take. Neural crest cells are first specified in the neural plate at the presumptive boundary between the neural ectoderm and the non-neural ectoderm. While riding the wave of neural plate folding, these bilaterally-positioned pre-migratory neural crest cells ultimately come together at the dorsal mid-line upon neural tube closure. At this time, neural crest cells will delaminate from the dorsal neural tube by undergoing an epithelial-to-mesenchymal transition, or EMT. These, now migratory crest cells, leave the dorsal neural tube and enter the peripheral environment of the embryo. As visualized with a blue florescence color in this immune

labeling of a cross-section of the mouse neural tube, you can see how the blue neural crest cells are all along the dorsal part of the neural tube and extending downward into the paraxial mesoderm. The axial level, along the anterior-to-posterior axis where neural crest cells are born will present very different environments for them to invade.

As it pertains to neural crest, we can generally divide the embryo up into three different regions, the head, the heart and the trunk neural crest. In the head region, cranial neural crest cells, as they are called, migrate ventrally to contribute not only to the neurons' glia and pigment of the head, but also the cartilage and bone of the face, jaw and middle ear. Cranial neural crest cells largely migrate in collective streams, as seen in this movie of the chick hindbrain captured by the Fraser Lab. Many of the cranial neural crest cells populate transient embryonic structures called the pharyngeal arches. Cranial neural crest cells from different arches will support the development of specific skeletal elements as seen here in this color-coded illustration of the human craniofacial skeleton.

If we move a bit posterior to the neck region, where the heart will develop, which is around the axial level of somite two in amniotes, then we will be at the position of cardiac neural crest. Cardiac neural crest cells enter through some of the more caudal pharyngeal arches and give rise to the portions of the aortic arteries, the septum between the aorta and pulmonary arteries, the outflow tract, as well as even some non-cardiac tissues like the thymus and thyroid. The remaining posterior segments give rise to the trunk neural crest. And although, over the course of evolution, it appears trunk neural crest have lost the natural ability to form cartilage and bone, they do still give rise to a diversity of cell types, again, dependent on their axial level.

For instance, among other contributing regions, the most anterior domain of the trunk neural crest known as vagal crest, significantly contributes to the enteric nervous system of the gut. In addition, the trunk neural crest cells is probably best-known for its generation of the dorsal root ganglion. Those neurons in glia that relay sensory stimuli to the CNS. Even more posteriorly-restricted trunk neural crest cells will give rise to the

adrenal medula. While throughout the full length of the trunk, more superficially migrating neural crest will interact with the epidermis to develop into melanocytes. The cell diversity derived from neural crest and the trunk can also be somewhat divided based on the pathway neural crest cells take throughout the paraxial mesoderm.

There are two distinct pathways. One called the ventral pathway, and the other called the dorsal lateral pathway. Now remember, all neural crest cells are originating from the same location at the dorsal neural tube, yet they choose different pathways to migrate upon. Choosing the dorsal lateral pathway is critical for melanocyte differentiation. Whereas, migration along the ventral pathway leads to the development of sensory ganglia and enteric neurogenesis. Even more interesting is how ventrally migrating trunk neural crest completely avoid the posterior half of somites. How do neural crest cells know which pathway to take? In fact, what's it even mean to know where to go?

Before we can address this question, let's ponder how neural crest cells delaminate in the first place. This illustration depicts the moment just after the neural tube and surface ectoderm have fused into separate structures. At the dorsal-most extent of the neural tube, pre-migratory cells are seen in the process of delamination, that of changing to a motile mesenchymal cell morphology. How is this possible? Take a moment to consider how is this localized EMT occurring? In other words, what are the types of molecular and cellular changes that have to occur to enable this shape-change? Also, what is the trigger to this EMT, and where might it be originating from? Well, what did you come up with?

For a cell to change from an epithelial morphology to a migratory cell, it would certainly require significant regulatory changes to the cytoskeleton. OK, so we have to rearrange the cytoskeleton. That primarily means microtubules and actin filaments as seen here as green and red respectively in this cell and culture. And any time you're going to do that, you should immediately think Rho GTPases who are the master-building enzymes of the cytoskeleton. But wait, these premigratory neural crest cells were tightly associated with the neuroepithelial cells of the neural tube. What must have changed?

As we have seen numerous times before, a critical feature of cell-to-cell interactions is adhesion, and more specifically cadherin-mediated differential adhesion can cause cells to separate, or in this case, delaminate. Leave a layer. In fact, neural tube cells express N-cadherin. Surface ectoderm expresses E-cadherin, and pre-migratory neural crest express cadherin-6B. This differential adhesion establishes three different populations of ectodermal cells. However, for these neural crest cells to become migratory, and leave the neural tube, their adhesion to one another needs to be relaxed. Therefore, what mechanism might reduce cadherin-6B to enable delamination?

It has been known that the gradient signals of bone morphogenic protein and Wnts from the surface ectoderm specify the neural crest cell fate. As a result of these signals, an EMT gene-regulatory network is initiated in the pre-migratory cells influenced heavily by the transcription factor SNAI2. SNAI2 leads to the down-regulation of cadherin-6B, low enough to foster detachment from the neural tube and migration. Interestingly, research is suggesting that low levels of cadherin-6 asymmetrically localized to the apical half of premigratory cells is required for the initiation of a similar polar activation of different Rho GTPases in these neural crest cells. Altogether, these changes in adhesion properties and asymmetric regulation of Rho GTPase activity promote migration.

As neural crest cells delaminate, our next question might be how are they bilaterally split down the sides of the neural tube? It appears that a critical cell-to-cell interaction governs this migratory behavior. It is called contact inhibition of locomotion. Here, you see neural crest cells in the live zebra fish embryo demonstrating this contact inhibition. As two migrating neural crest cells make contact with one another, then they respond by sending new protrusions in the opposite direction. This seemingly simple behavioral response drives the overall directional migration away from the source of neural crest generation.

Developmental biologists have demonstrated through cell lineage analysis that neural crest cells are, in fact, multipotent stem cells. And work by Dr. Nicole Lai-Derone and others have strongly supported a model of the gradual maturation of neural crest

progenitor cells over the course of their migratory journey. Many of the growth factors critical for specific cell fates have been identified such neuregulin for glia and endothelin-3 for pigment. But, this forces us to raise one of our previous questions again. How do neural crest cells know which pathway to take?

Recall that I mentioned trunk neural crest cells within the ventral pathway completely avoid the posterior half of somites and only migrate through the anterior half. The ventral somite of amniotes is made of a mesodermal cell-type called sclerotome which will eventually give rise to the skeletal elements of the vertebrate among others. Let's do what we do. Let's find it and move it. In contrast to the anterior half of the somite, the posterior sclerotome expresses a ligand called ephrin. Could ephrin function as a sort of repellant to neural crest cell migration?

There are a lot of different factors in the developing somite. So, in this case, researchers chose to test this question more directly using an in vitro-culture system. Neural crest cells were cultured on a plate coded with alternating stripes of ephrin protein. Sure enough, neural crest cells preferentially grew only on the stripes devoid of any ephrin. This represents but one example of many different neural crest guidance cues.

Neural crest cells expressing the corresponding receptors in their plasma membrane are able to sense specific cues strategically laden in the environment. In this way, neural crest cells can be attracted and/or repelled from different regions of the embryo and ultimately guided to their final destinations for differentiation. I have discussed the importance of cell adhesion, of contact inhibition and the role of guidance cues. I wish to bring all these mechanisms together to help describe how cranial neural crest cells stream in a collective group as a final example.

As in amniotes, this xenopus embryo exhibits migratory streams of cranial neural crest cells as revealed in this in situ hybridization for two neural crest specific genes. This form of neural crest migration is called collective migration. There are many

mechanisms controlling this collective migration. Firstly, this group of cranial neural crest cells is held together modestly with a low level of N-cadherin adhesive action which is illustrated as a light blue receptor connecting cells in this drawing. Similar to the posterior sclerotome, the region segmenting these migratory paths express chemorepellants. That prevents mixing of the different streams of cranial neural crest cells. Moreover, these cranial neural crest cells actually secrete a self-attracting chemokine called complement 3A. This low-level adhesion, local attractions and the leading edge of cells exhibiting contact inhibition of locomotion altogether promote a collective migration behavior. But, where should this stream migrate?

Interestingly, positioned just ventral to the leading edge of the group of cranial neural crest cells lies a cluster of placodal cells. These placodal cells are also migratory with a bit of help from the cranial neural crest. It appears the cranial neural crest follow these placodal cells. In fact, Dr. Roberto Mayor's lab conducted live imaging of explant cultures of xenopus cranial neural crest with and without these placodal cells. As seen in these single-control cultures, cranial neural crest cells quickly disperse. Whereas, the placodal cells stay clustered together with little movement. Although, when both cranial neural crest and placodal cells were co-cultured, a remarkable following behavior was seen. Watch this movie again. Each of these three separate replicates show a maintained group of neural crest consistently following the placodes now directional movement.

This is just a really cool set of experiments. But, what sort of interaction is occurring between these two groups of cells to promote this shared migration? Could the placode be secreting some sort of come-hither signal? Could cranial neural crest, in turn, be pushing the placode? Indeed, the answer to both these questions is yes. The placode expresses the stromal-derived factor-1, or SDF-1, and the cranial neural crest express the SDF-1 receptor CXCR-4. A gradient of SDF-1 from the placode provides a directional chemoattractant for the collective migration of the cranial neural crest toward the placode. In support of this knockdown of the CXCR-4 receptor in neural crest, abolishes their following behavior in these explant co-cultures. Importantly though, when

the leading crest cells contact the placode, it triggers the contact inhibition of locomotion which pauses the cranial neural crest, but also propels the placode further. Subsequently, the SDF-1 gradient will reinvigorate the attraction of neural crest such that they chase down the placode yet again. The Mayor Lab has aptly called this the chase-and-run mechanism of collective migration.

My own laboratory is interested in the development of cranial neural crest cells as well, and in particular, their establishment of the pharyngeal arches to build the craniofacial elements of the zebra fish larva. In the background of this tutorial, you have seen disrupted pharyngeal arches caused by this embryo being exposed to a common pollutant found in fossil fuels. The most posterior arch fails to separate which results in reduced craniofacial elements. What is the teratogenic mechanism behind these phenotypes? Is this an example of the environment impacting cranial neural crest specification? Or perhaps their migration? But wait, if their migration is impacted, won't there their specification be altered too? Neural crest development is quite complex, but it is that complexity that has offered so many unique vertebrate adaptations from changes in the shape and strength of jaws to alterations in color patterns of fur coats and many more. I have only scratched the surface of neural crest development in this tutorial, and hope you will use this as a supportive push for your own journey to reveal new insight into neural crest development. Thanks for listening and happy developing.