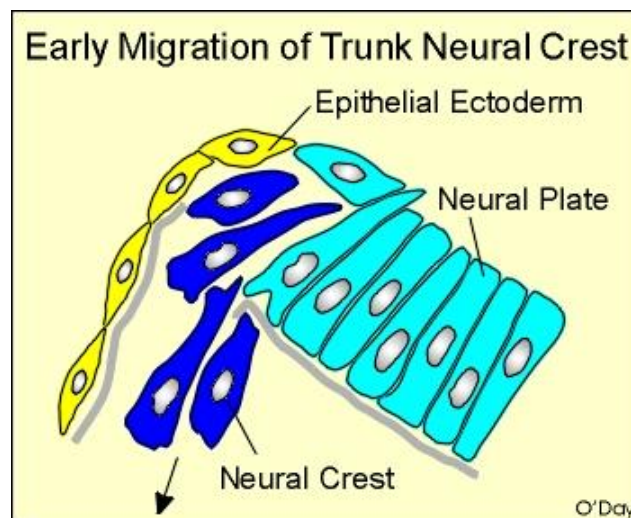


## The Neural Crest: From Pigmentation to Craniofacial Defects

### Formation of Neural Crest: An Epithelial-Mesenchymal Transformation

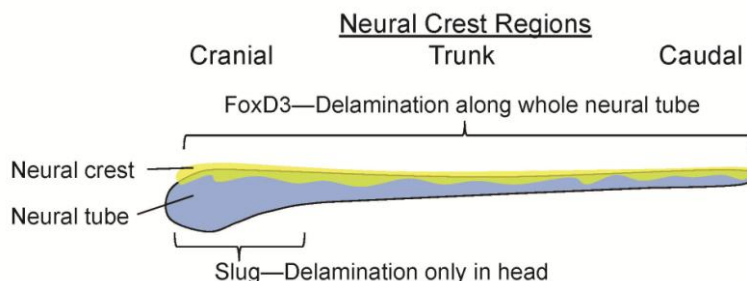
Because they play such an important part in embryonic development and because they contribute so many different cell types to the developing embryo, the neural crest is considered by some to be the fourth germ layer. The neural crest cells are interesting because they will form many critical cell & tissue types and certain cancers and other problems are associated with them. While little is known about the migration of neural crest in humans, the situation has been well studied in frogs, birds and more recently in mammals.



Prior to their departure from the neural tube neural crest cells exist as part of an epithelium. First they must digest the extracellular basal lamina (shown in grey), a process that involves matrix metallo-proteinases (MMPs). After leaving they have taken on a mesenchymal organization. Thus neural crest formation involves an epithelial-mesenchymal transformation.

### Transcription Factors Mediate Neural Crest Delamination

The actual separation of the neural crest cells from the neural plate and tube is an example of delamination. At least three genes are involved. These include the transcription factors *FoxD3* and *Slug* as well as bone morphogenetic protein 2/4. Over-expression mutations in mice have shown that *FoxD3* can cause delamination all along the neural tube while over-expression of *Slug* only leads to delamination of head neural crest.



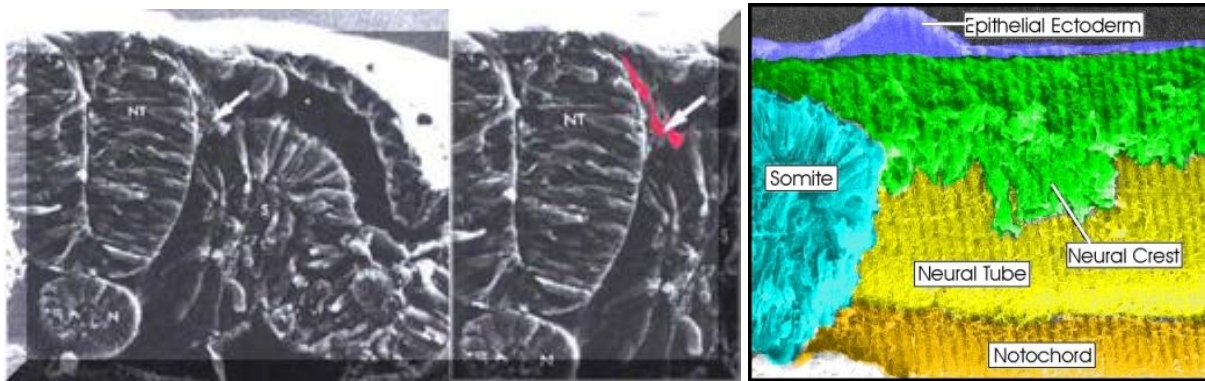
# The Neural Crest: From Pigmentation to Craniofacial Defects

## Losing Contact and Finding Their Way Home

Since they will leave their original location to move to other sites, the neural crest cells must lose their original cell-cell adhesivity as they digest an escape route through the underlying basement membrane (extracellular matrix). What changes in cell-cell adhesivity occur and how do the cells remove the extracellular matrix? The cells must then exit into the surrounding embryonic tissues where they will follow specific routes to their final destinations. How do they know where to go and, more importantly, how do they avoid going to the wrong places? Once the cells reach their new destination, they will form new cell-cell associations and differentiate into the appropriate cell & tissue types. How do they know they have arrived at the right place? How do they know what to differentiate into? What happens if they end up in the wrong place? These are all topics that we will discuss in this lecture. Let's begin by learning a bit more about these special cells.

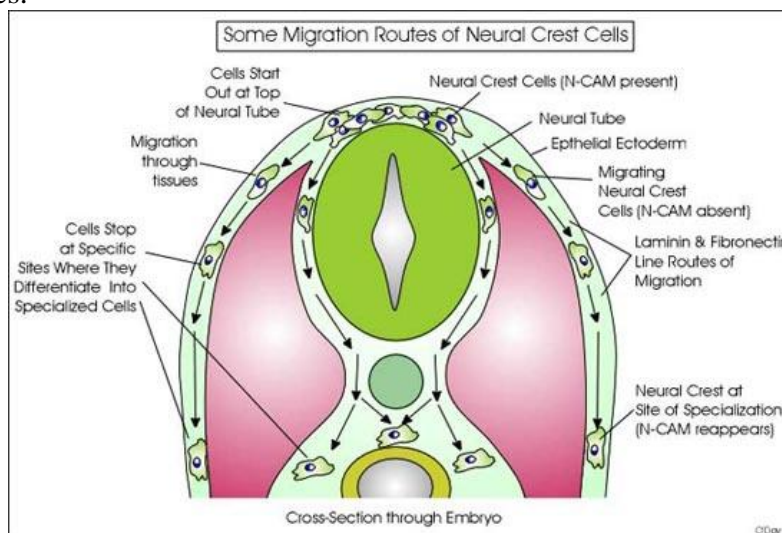
## SEM of Neural Crest

Migrating neural crest cells can be observed in cross-sections of early embryos using the scanning electron microscope (SEM). The cells have been coloured red in the right hand panel so you can identify the unstained cells in the left-hand panel (arrow).



## Neural Crest Cells Migrate Through the Embryo

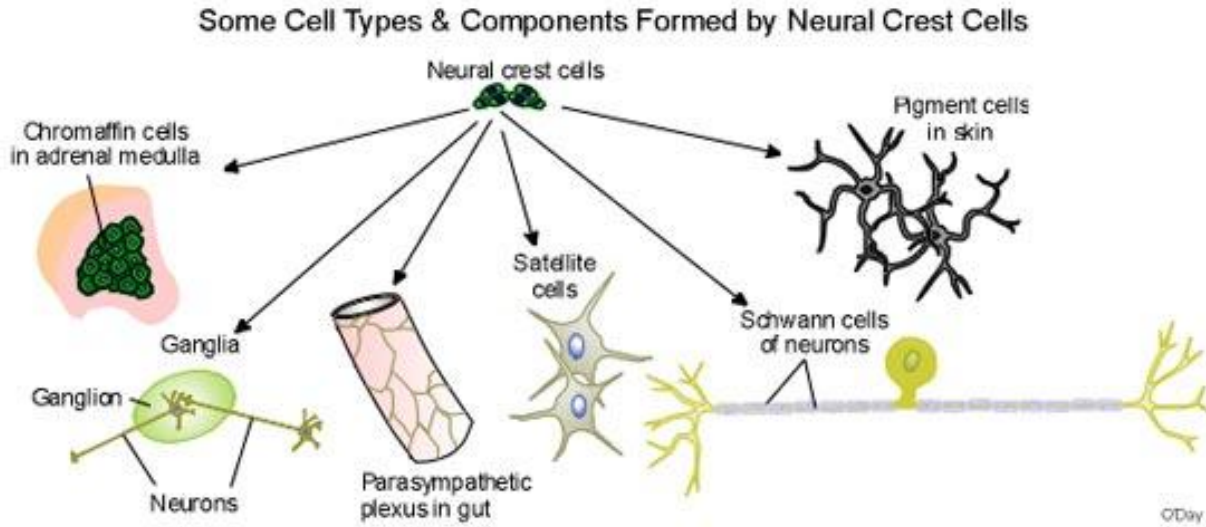
Neural crest cells will contribute to diverse types of cells throughout the body. As a result they must leave their place of origin and migrate to other regions in the embryo. This stylized cartoon gives a general picture of these migration routes.



## The Neural Crest: From Pigmentation to Craniofacial Defects

### Major Derivatives of the Neural Crest

Many different kinds of cells are derived from the neural crest as indicated in the table below and the following diagrammatic representation.



Overall, the neural crest can be divided into four major groups: Cranial, Trunk, Vaga & Sacral and Cardiac Neural Crest. These functional groups overlap. For simplicity we will simply compare some of the cell types in two regions. Later we will look at the regulation of cell differentiation of cells from one of these two regions. In the end a good understanding of neural crest cell functions, importance and regulation should be provided.

The following three tables show some of the major cell and tissue types that are derived primarily from the neural crest. This is not the full list but will give some idea of the critical role of the neural crest in human development. The components that are derived from neural crest in the body region (Trunk Crest) and in the head region (Cranial Crest) are separated to provide a further understanding that different regions of neural crest differentiate into unique components.

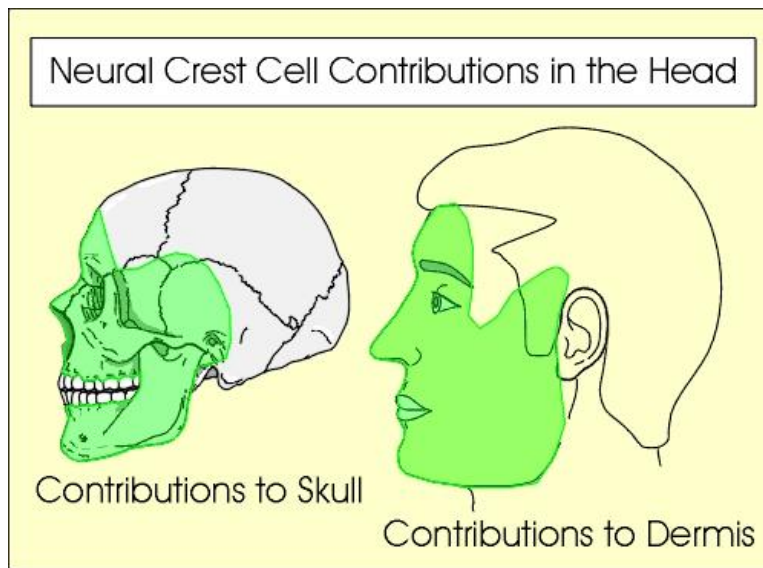
NERVOUS SYSTEM	TRUNK CREST	CRANIAL CREST
Autonomic Nervous System	Parasympathetic Ganglia (e.g., pelvic plexus)	Parasympathetic Ganglia (ciliary, submandibular, visceral)
Sensory Nervous System	Spinal Ganglia	Ganglia of Trigeminal (V), Facial (VII) and Vagus Nerve, etc.

NON-NEURAL CELLS	TRUNK CREST	CRANIAL CREST
Various Cell Types	Satellite Cells of Sensory Ganglia; Schwann Cells of PNS, Enteric Glia	Satellite Cells of Sensory Ganglia; Schwann Cells of PNS, others
Pigment Cells	Melanocytes	Melanocytes
Endocrine/Parendocrine	Adrenal Medulla, Neurosecretory Cells of Heart & Lungs	Carotid Body (Type I Cells), Parafollicular Cells of Thyroid

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**Mesectoderm:** Mesenchyme derived from ectoderm, esp. neural crest

MESECTODERM	TRUNK CREST	CRANIAL CREST
Skeletal Components	None	Cranial Vault, Nasal & Orbital, Otic Capsule, Visceral Cartilages, External Ear Cartilage & more
Muscle	None	Ciliary Muscles, Dermal Smooth Muscles, Vascular Smooth Muscle, & more
Connective Tissue	None	Dermis & Fat of Skin, Cornea of Eye, Dental Papilla, Connective tissue of Stroma of Thyroid, Parathyroid, Thymus, Salivary & Lachrymal Glands, etc.

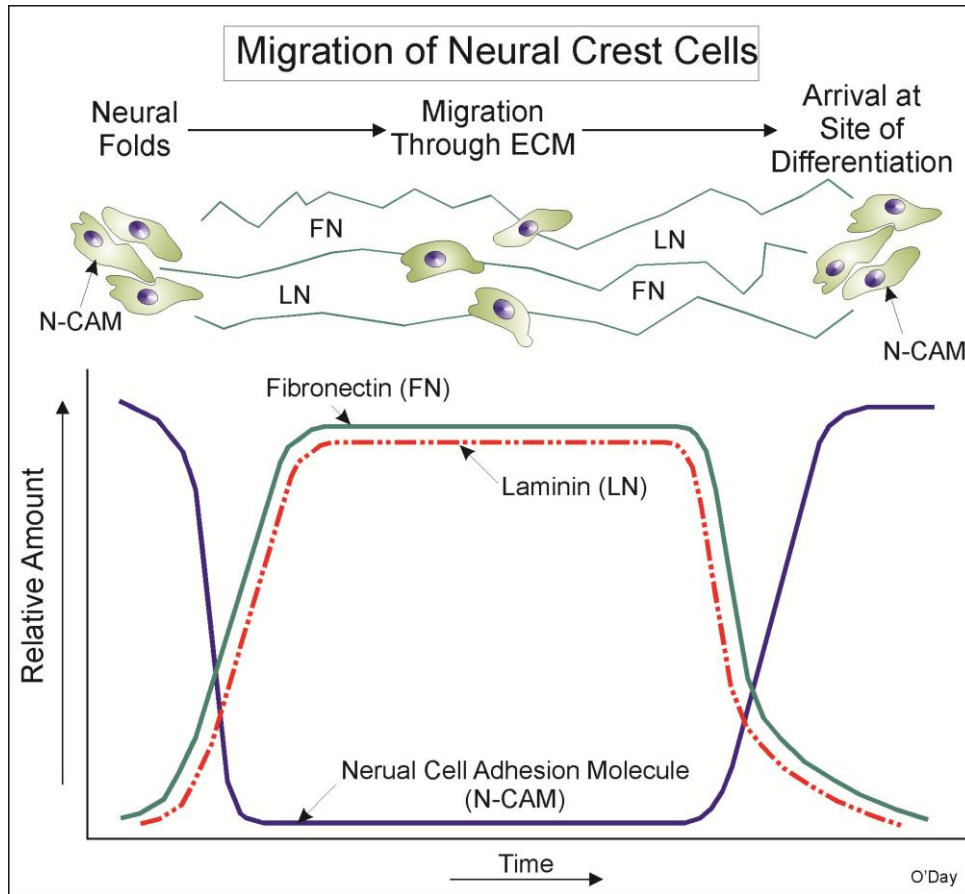


### Migration of the Neural Crest

The following graphic shows a cartoon of neural crest cells at the various stages of neural crest migration with a graph below depicting the levels of N-CAM (green) expressed at the surface of the neural crest cells at each stage. The graph also reveals the levels of laminin (LN) and fibronectin (FN) that are detected in the extracellular matrix that line the migration routes taken by the neural crest.

As seen in the following graphic representation, prior to cell migration, the neural crest cells express N-CAM (green) at their cell surfaces when they are still present in the neural folds. N-CAM is a known cell adhesion molecule first isolated from neural tissues. N-CAM is a member of the immunoglobulin-like CAMs as seen in the figure below. The neural crest cells lose their N-CAM and their cell-adhesivity prior to migrating and continue to express these low levels throughout the migration process. The neural crest cells also lose cadherins from their surfaces and tight junctions disappear further emphasizing that several changes in cell-cell adhesion are occurring. Enzymes secreted by the emigrating neural crest likely digest a pathway through the basal lamina.

## The Neural Crest: From Pigmentation to Craniofacial Defects



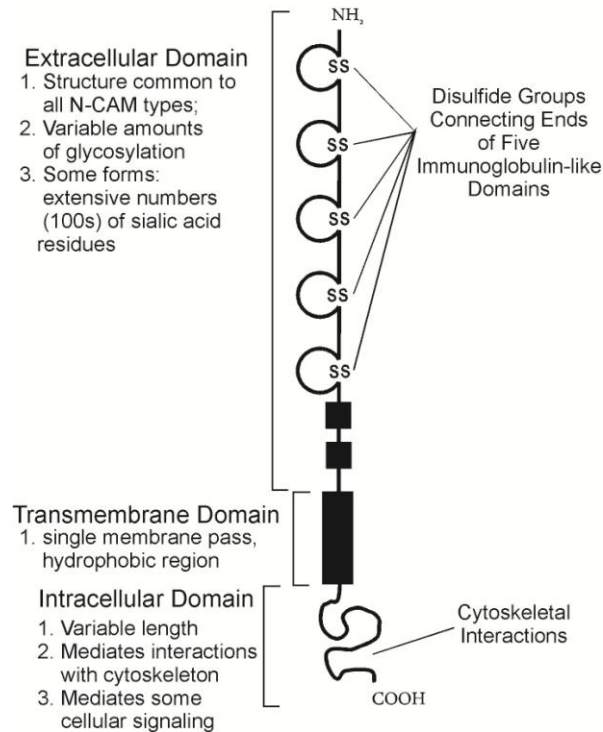
Once they have escaped, the neural crest cells get many signals to keep them on the proper migration routes. First extracellular matrix protein such as fibronectin (FN), laminin (LN) and tenascin plus some collagen and proteoglycan molecules are involved. As seen in the figure below FN & LN levels are highest in the tissues that surround the migrating neural crest cells and lowest where the cells stop moving. Once they have arrived at their site of differentiation, the neural crest cells again express N-CAM and other cell adhesion molecules.

The situation is far from this simple. While FN, LN and other molecules keep the neural crest on the straight and narrow, many other molecules are involved as well as outlined below.

### **N-CAM: Neural Cell Adhesion Molecule**

- N-CAM is a glycoprotein: it contains 30% sialic acid (neuraminic acid) by weight
- Originally isolated from neural retina where it functions developmentally
- It is a member of the Immunoglobulin Superfamily
- It is one of more than 20 CAMs: for example: L-CAM is Liver CAM
- Due to splicing, a single N-CAM can produce 100 different forms of N-CAM
- Mediates  $\text{Ca}^{2+}$ -independent cell adhesion

## A Schematic Diagram of N-CAM



It should be noted that other cell adhesion molecules, such as cadherins, also are lost as neural crest cells migrate away from the top of the neural tube and then reappear once the neural crest cells localize to their new locales.

### Factors That Guide Neural Crest Cells

The neural crest cells are guided to their final destinations by components of the extracellular matrix, other directional molecules and chemotactic signals.

#### Proteins known to guide neural crest cells:

**Fibronectin/Laminin:** proteins of extracellular matrix (ECM)

**Neuregulins:** proteins of the NGF family

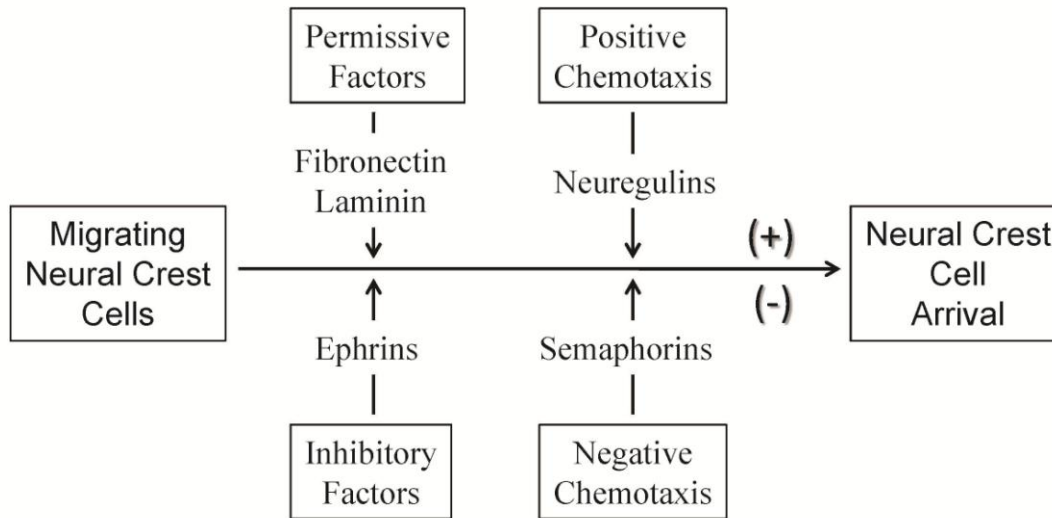
**Ephrins:** protein growth factors; bind to Ephrin Receptors (receptor tyrosine kinases)

**Semaphorins:** cell surface and secreted proteins; act over short distances

Permissive signals direct the cells to where they should go. Fibronectin and laminin in the ECM serve as permissive factors, lining the extracellular roadways along which the cells will move (see below). Near their destination, positive chemotaxis occurs under the direction of molecules such as neuregulins. In contrast, inhibitory factors ensure the cells won't go to sites where they shouldn't go. Ephrins are main inhibitory factors. Ephrin is a protein that is localized in regions where the neural crest cells should not go (e.g., at the posterior region of each sclerotome). Neural crest cells contain ephrin receptors and when they contact the ephrin molecules their movement is inhibited. The ephrin receptor is a receptor tyrosine kinase and it is believed that binding to ephrin leads to the activation of its activity followed by phosphorylation of cytoskeletal elements involved in cell movement. Semaphorins also keep cells away from sites by directing

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negative chemotaxis. Other inhibitors also appear to regulate the migration of neural crest while some chemoattractants may guide neural crest cells into certain regions (e.g., stem cell factor; not shown).



There are a number of different positive and negative factors that direct neural crest cells and the aforementioned are major examples. However, different factors play a role for neural crest cells with different developmental fates. Also some of the above may function differently in different situations. For example, melanocytes respond positively to Ephrins. Mutations in any of these directional molecules can lead to developmental defects. Thus mutations in Ephrins have led to craniofacial malformations in humans as well as defects in hair, teeth and skin, among other things.

### Regulation of Neural Crest Cells Fate

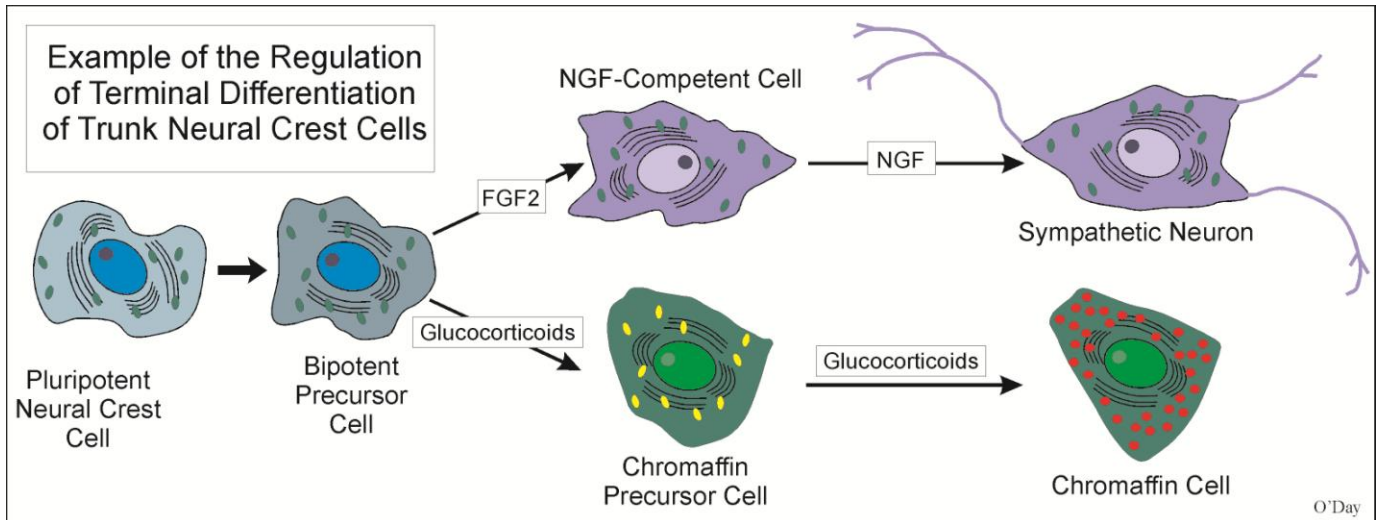
The neural crest cells are considered to be pluripotent which means that a single neural crest cell has the ability to form many (but not all) different cell types. Their differentiation is influenced by extracellular molecules they encounter and cell-cell interactions. It should be noted that some of the neural crest cells are committed very early to a specific pathway of differentiation. For example, this is revealed by the early expression of neurogenin, a transcription factor that regulates the activity of specific genes, in sensory neurons. Other neuron types don't express neurogenin but, instead, express other neuron-specific transcription factors. On the other hand, many other populations of neural crest cells seem to be specified by the environments in which they find themselves.

Once these neural crest cells have stopped migrating they are localized to new environments with new cell-cell associations and different components in the extracellular environment. These interact with each type of neural crest cell to produce specific cell types ensuring that the proper structures form in the appropriate places. Thus the presence of the brain ensures surrounding neural crest cells will differentiate into bones of the cranial vault while interactions with the developing ear (otic vesicle) will influence others to develop into the otic capsule. In the mouth, the oral ectoderm directs neural crest to become the dentine of teeth while the spinal cord and somites will direct the formation of sympathetic neurons. The extracellular matrix that the neural crest cells encounter on their travels will mediate their development into melanocytes (pigment cells).

### Differentiation of Trunk Neural Crest Cells

The differentiation of trunk neural crest cells into sympathetic neurons and chromaffin cells serves as an example of how factors progressively determine a cell's fate and its final differentiation.

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Trunk neural crest cells begin as pluripotent cells which become limited in their capabilities when they enter the tissues destined to become the adrenal medulla. At this point they are only able to differentiate into one of two cell types: sympathetic neurons or chromaffin cells. Thus they are restricted in their potency, they are now bipotent and no longer pluripotent. The action of fibroblast growth factor 2 (FGF2) first primes the cells so they can respond to nerve growth factor (NGF) which then induces terminal differentiation of the sympathetic neuron. In contrast, sequential treatment of the bipotent precursor cells with glucocorticoids induces chromaffin cell differentiation. Clearly, neural crest cells have a great developmental potential and their developmental fate is regulated by their environment. Sometimes, neural crest cells don't make it to their proper places in the embryo and as a result they don't get the proper signals to direct the appropriate differentiation. Genetic anomalies can also affect the way the neural crest cells behave. Let's have a look at these issues.

### Neural Crest & Abnormal Development

- Defects in migration: e.g., Waardenburg Syndrome--Unusual patterns of pigmentation (e.g., white stripe in hair); cleft lip & palate
- Neural Crest Tumours: e.g., Neurofibromatosis--common genetic disease; peripheral nerve tumours; Pheochromocytoma--tumour of adrenal medulla; tiny but secretion of lethal amounts of hormone
- Other: albinism (lack of melanocyte differentiation)

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