Cell Migration I:
Neural Crest Cell Migration

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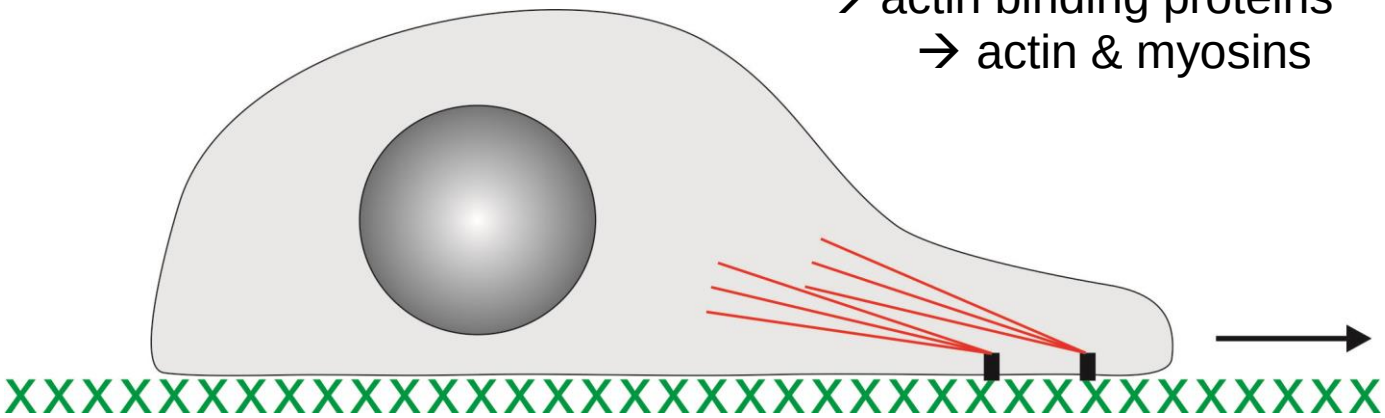
Types of Cell Movement

- passive: cell sheets
  flow
- active: cilia or flagella
  ameboid
  adhesion dependent crawling
  (nuclear translocation)

Three Steps in Adhesion Dependent Cell Movement

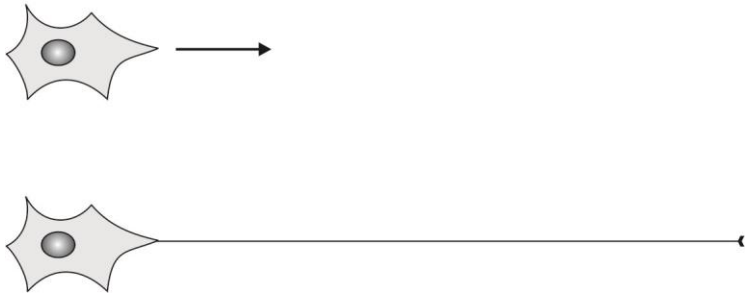
- extension: lamelipodia or filopodia
- attachment: receptor mediated
  CAM or ECM molecule substrate
  adhesion plaques
- contraction: ligand
  → receptor
  → actin binding proteins
  → actin & myosins



Cell Migration vrs. Axon Growth

Cells of the CNS and PNS migrate via adhesion dependent crawling from their site of division to where they differentiate.

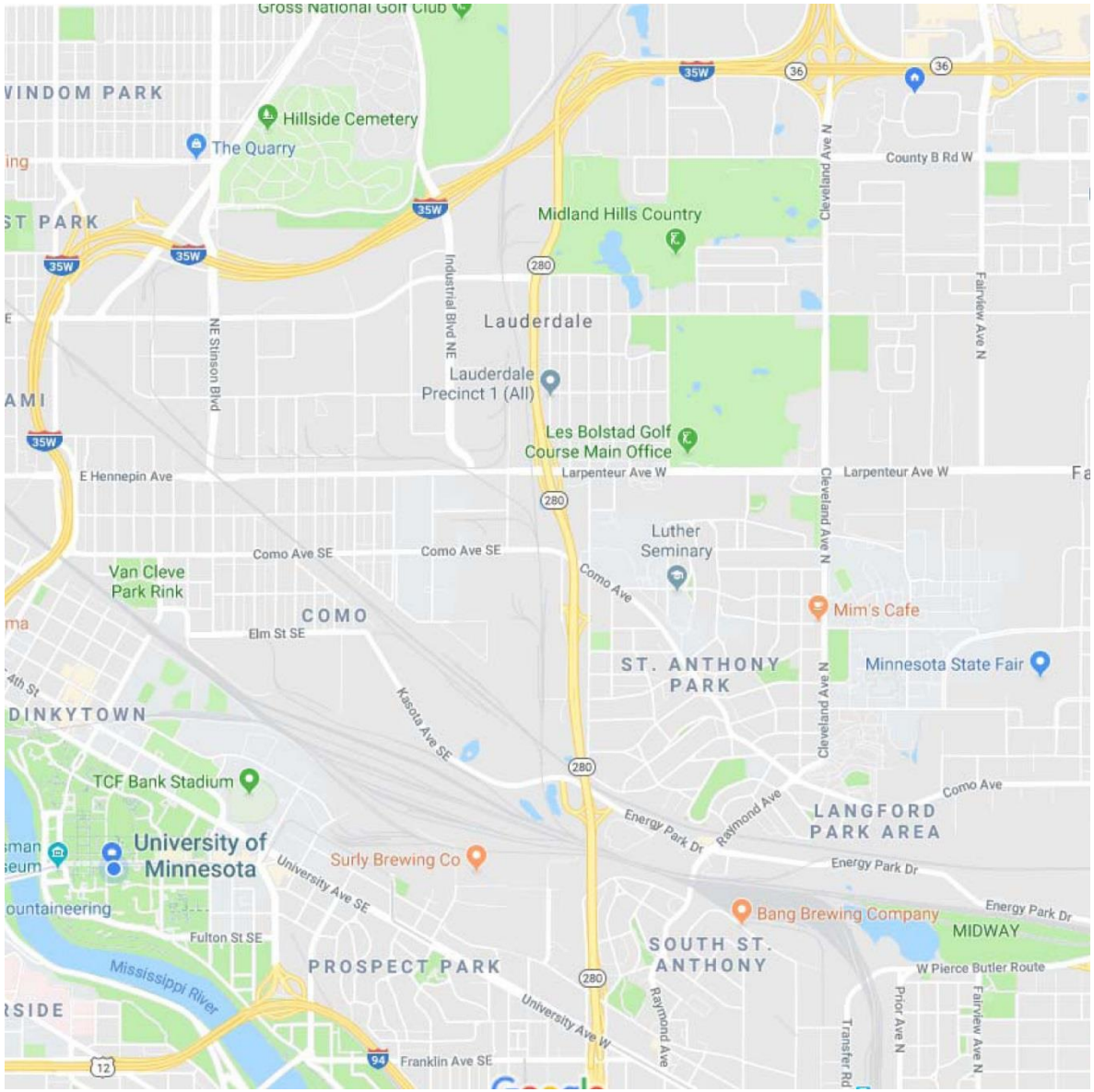
Axon growth is a similar process except that the cell body is not pulled into the extending cell process.



Issues to Discuss Relative to Cell Migration & Axon Growth

- What are the adhesive substrates that cells use to move?
- What guides cell movements?
- What are the motors that propel cells?

Issues to Discuss Relative to Cell Migration & Axon Growth



Let’s start by reviewing some molecules.

Extracellular Matrix (ECM)

ECM = immobilized extracellular molecules not covalently bound to a cell surface

Major components of the ECM:

- Collagens
- Non-collagenous glycoproteins
- Glycosaminoglycans
- Proteoglycans

Review of the Major Components of the ECM

- collagens:

  - major structural component of the ECM, particularly in connective tissue

  - fibrous proteins composed of three polypeptides wound around one another

  - 45 genes encode for 28 unique collagen types (homo- or heterotrimeric)

  - many other ECM molecules interact with collagens

  - cells anchor to collagen indirectly or sometimes directly via receptors

  - e.g. collagen type I – V are most abundant

Review of the Major Components of the ECM

- non-collagenous glycoproteins:

  - major ECM substrate for cell migration

  - typically have binding sites for collagen, GAGs, and cell membrane receptors

  - e.g. fibronectin, laminin

Review of the Major Components of the ECM

- glycosaminoglycans (GAGs):
  - unbranched polysacharid chain of repeating disaccharide units, often sulfated
  - most are covalently bound to a protein core making it part of a proteoglycan
  - hyaluronic acid is an exception (i.e. not a proteoglycan)
  - polyanionic nature of GAGs holds counter ions increasing the hydration and thus the cell free space of a tissue
  - e.g. heparin sulfate GAG, chondroitin sulfate GAG

Review of the Major Components of the ECM

- proteoglycans:
  - GAGs (few or many) bound to a protein core
  - may be several GAG types, but often named by the most common GAG type
  - can bind to cells and other ECM components
  - can block cell-cell and cell-ECM interactions due to the hydrated sphere associated with these very large highly charged molecules
  - many bind growth factors
  - e.g. heparan sulfate pg., chondroitin sulfate pg.

    Brevican, versican and neurocan are CSPGs abundant in the developing nervous system.

Cell Adhesion Molecules (CAMs)

- major classes:
  - Immunoglobulin-like CAMs (Ca^{2+} independent)
    - e.g. N-CAM, L1, Ng-CAM, DSCAMs, nectins
  - Ca^{2+} dependent CAMs (cadherins & protocadherins)
    - e.g. N-cadherin
  - Selectins (bind sugar moieties)
  - Integrins (bind CAMs or ECM molecules)

- binding:
  - homophilic
  - heterophilic

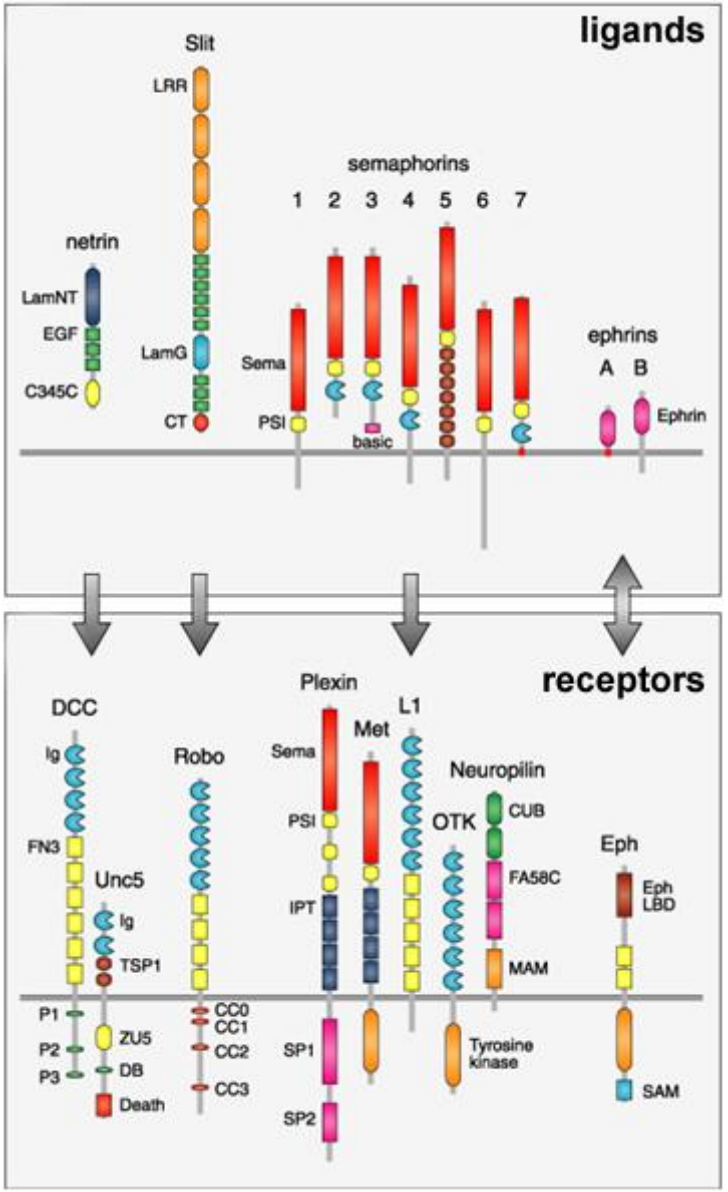
- examples:
  - N-CAM
    - broadly distributed
    - variable intracellular domain
    - variable adhesivity
  - TAG-1 (axonin-1)
    - restricted distribution

Modifiers of Cell Adhesion

- Gangliosides (phospholipids)

Guidance Molecule Families (ligands & receptors)

(can attract or repel)



Guidance Molecule Families (ligands & receptors)

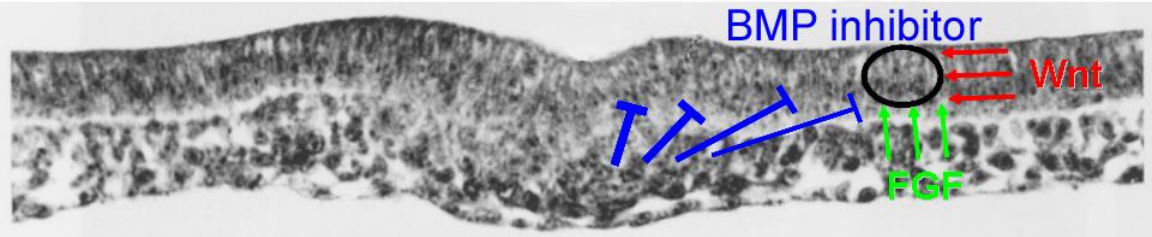
Other families of guidance molecules include:

- chemokines (e.g. SDF-1)
- neurotrophins (e.g. N-CAM)

Let’s look at the embryology of the neural crest.

Embryology of Neural Crest

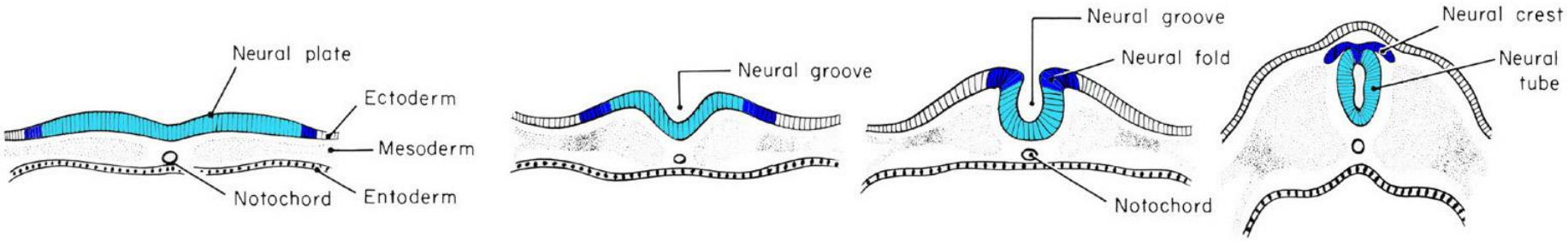
- Cells at the border of the neural plate are induced to be neural crest by signals from mesoderm and surrounding ectoderm.



- Cells induced to be neural crest express a certain set of transcription factors that includes Snail (snail 1) and Slug (snail 2).

Embryology of Neural Crest

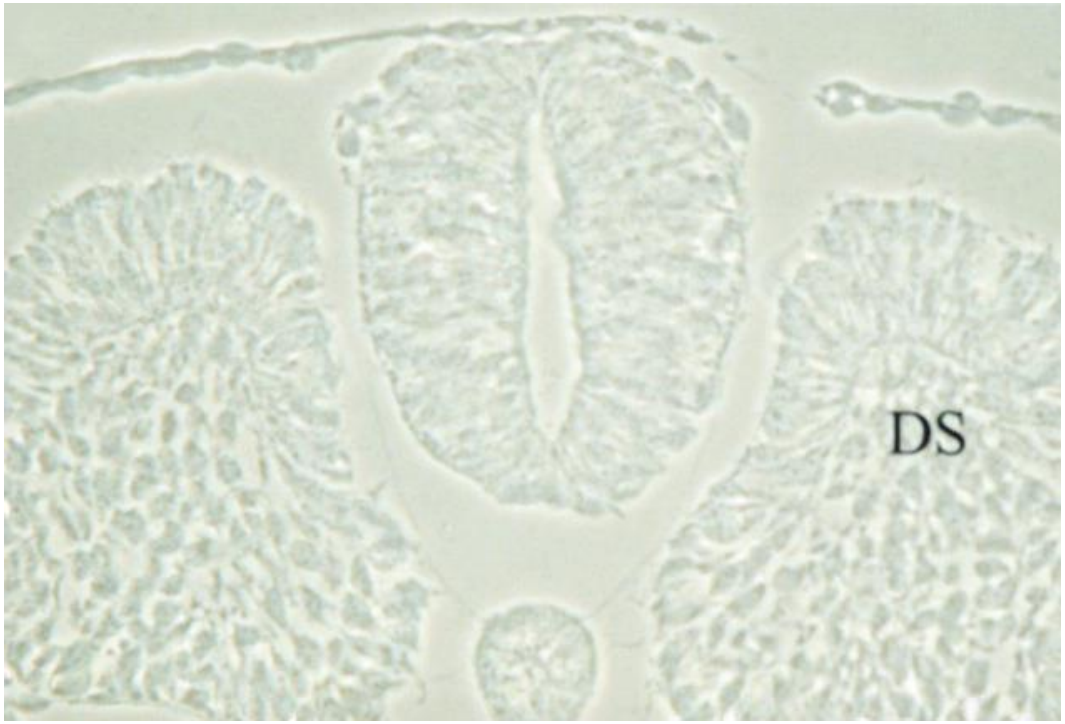
- Crest cells initially form the margin of the neural plate, then the elevated neural folds, and then the dorsal portion of the early neural tube.



EMT

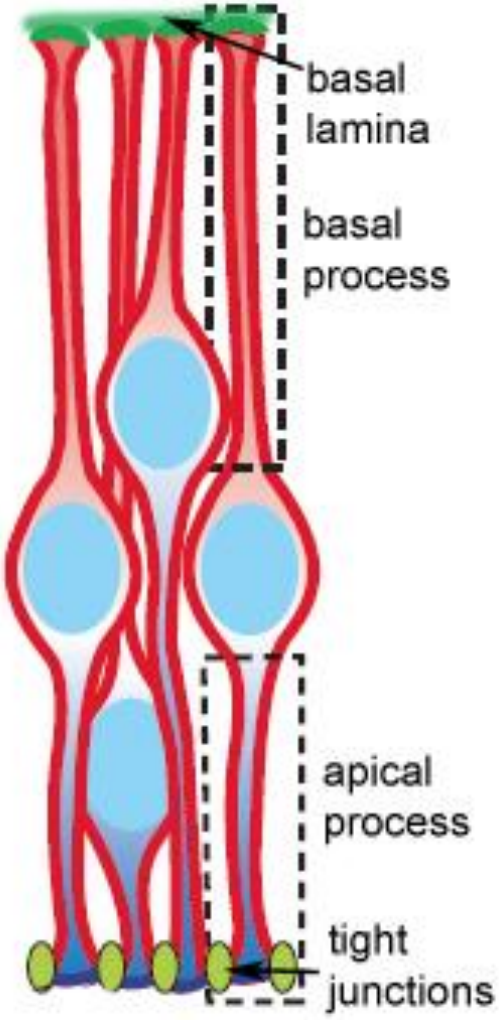
- During and immediately after neural tube closure, crest cells delaminate from the neural tube and accumulate dorsal-lateral to the tube as the neural crest.

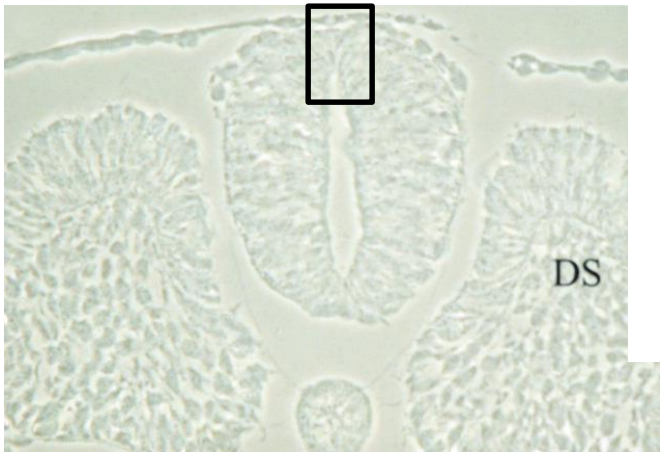
- Delamination is described as an epithelial-to-mesenchymal transformation (EMT).



EMT

- Cells of the neuroepithelium (i.e. neural tube) are anchored together by tight junctions.
- These tight junctions include at least three cadherins: N-cadherin, E-cadherin and cadherin-6B.





EMT

- EMT is a complex process that requires up or down regulation of expression of many genes:
    - Snail & Snail2 (Slug) repress expression of the cadherins and promote expression of integrins.
    - ADAM metalloproteinases and γ-secretase cleave cell adhesion molecules releasing the crest cells from the neural epithelium.
    - Crest cells increase expression of molecules needed for motility.

Hey!

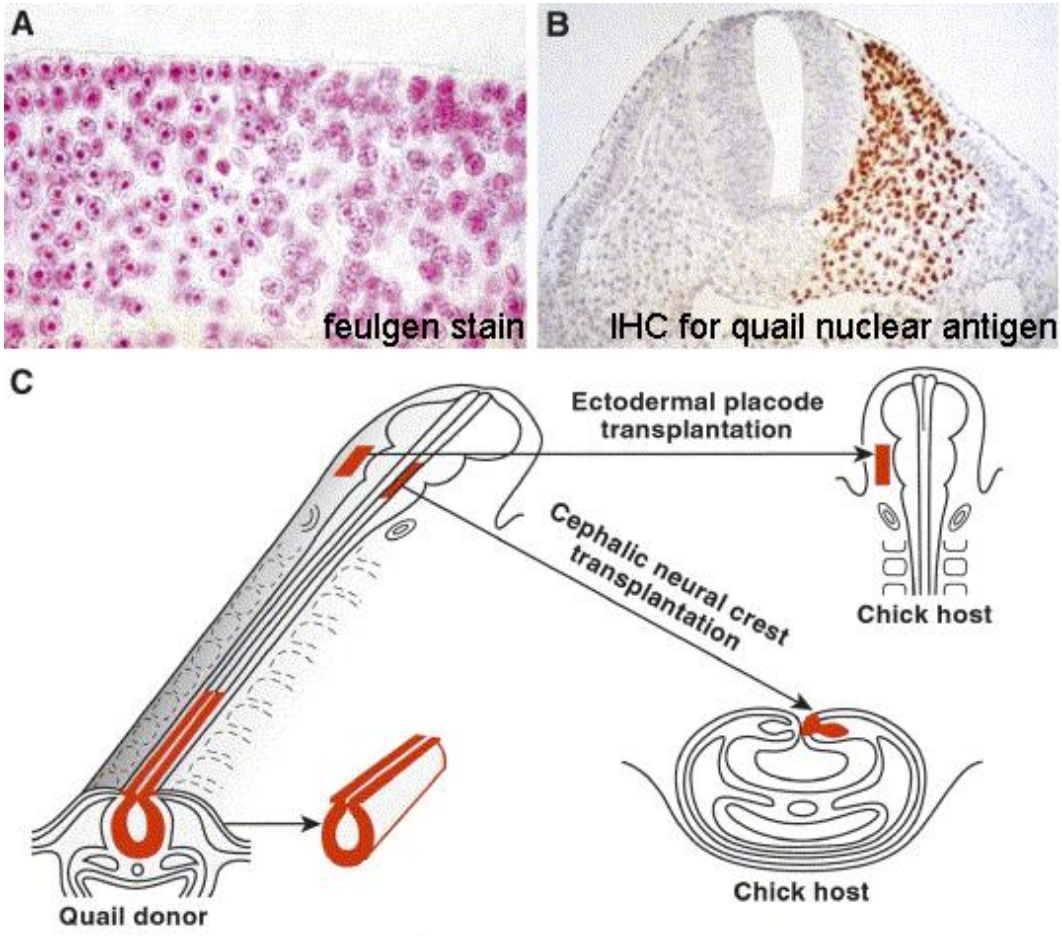
Cells often need to change
their adhesive properties in order to migrate!

Neural Crest Cell Migration

- Neural crest cells migrate throughout the body.

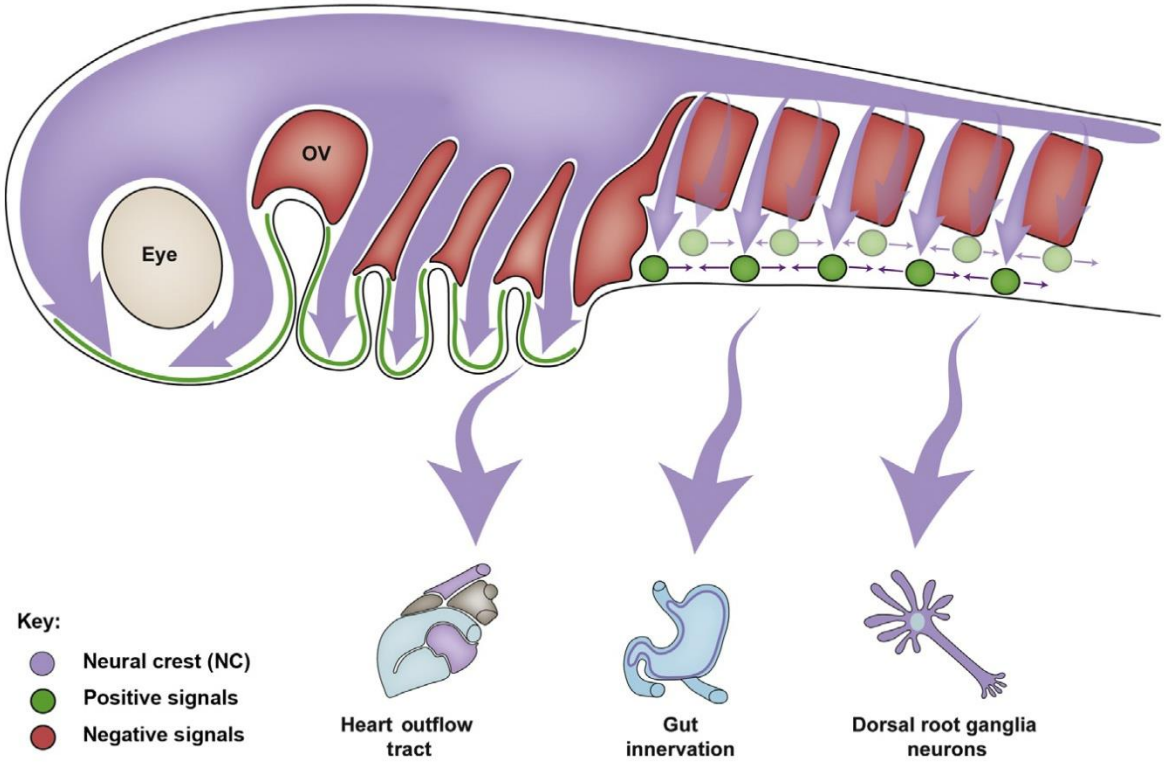
Neural Crest Cell Migration

- Homotypic transplants of quail neural crest to chick revealed the route of migration and the derivatives of neural crest.



Neural Crest Cell Migration

- Neural crest (NC) cells from different rostral-caudal levels give rise to different tissues.



Neural Crest Cell Migration

- Neural crest (NC) cells from different rostral-caudal levels give rise to different tissues:
    - Cranial NC cells (from diencephalon to 3rd somite) give rise to many tissues of the head including neurons and glia.
    - Cardiac NC cells (from hindbrain to 4th somite) give rise to the septa of the heart.
    - Enteric NC cells (from 1st to 7th somites) give rise to the entire enteric nervous system.
    - Trunk NC cells (from 4th to 37th somites) give rise to most sensory and autonomic neurons, glia of the PNS, endocrine cells of the adrenal gland and melanocytes.

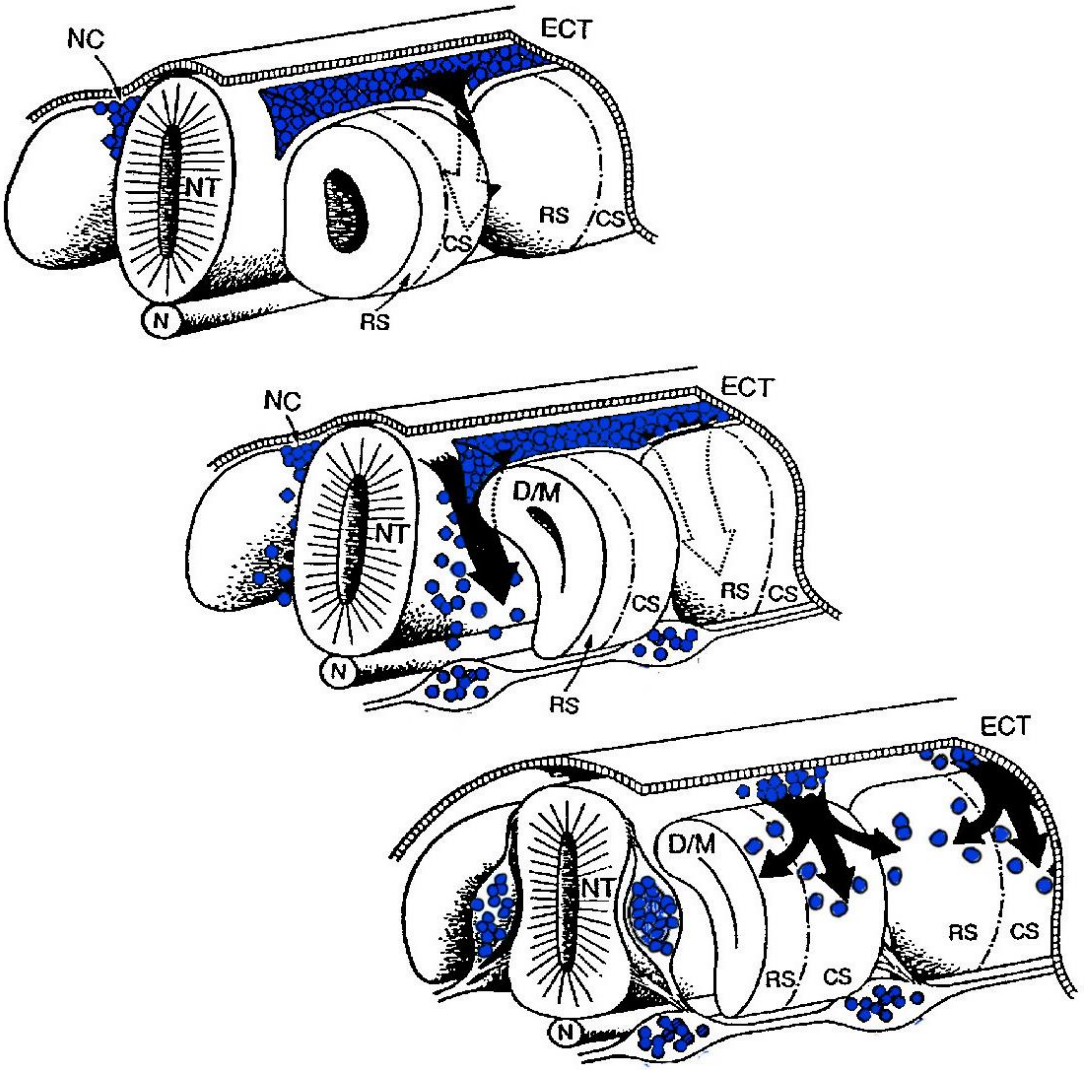
(The 1st somite is at the level of the hindbrain.)

Neural Crest Cell Migration

- Derivatives of neural crest include:
  - neurons:
    - most cranial nerve sensory ganglia
    - dorsal root ganglia
    - sympathetic ganglia
    - parasympathetic ganglia
  - neurosecretory cells:
    - thyroid calcitonin (C) cells
    - adrenal medulla cells
  - glia:
    - schwann cells of nerves
    - satellite cells of ganglia
  - melanocytes
  - skeleton, connective tissue and muscles of head & face
  - mesenchyme of thyroid & salivary glands

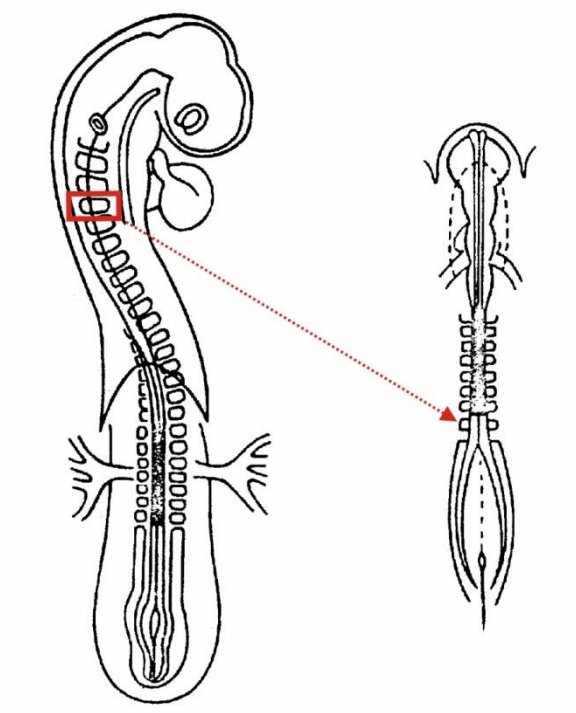
Neural Crest Cell Migration

- Neural crest cells in the trunk region migrate in three waves.



Neural Crest Cell Migration

- Heterotypic quail-chick transplants suggested that elements in the environment directs the migration route.

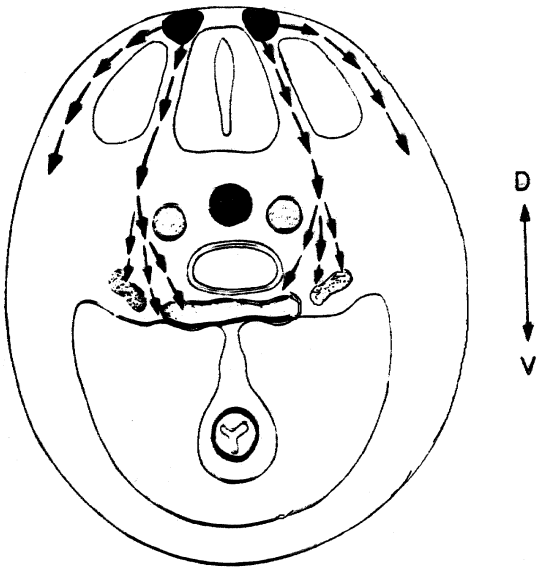


Crest cells migrate by adhesion dependent crawling.

So, what is the substrate to which migrating crest cells adhere?

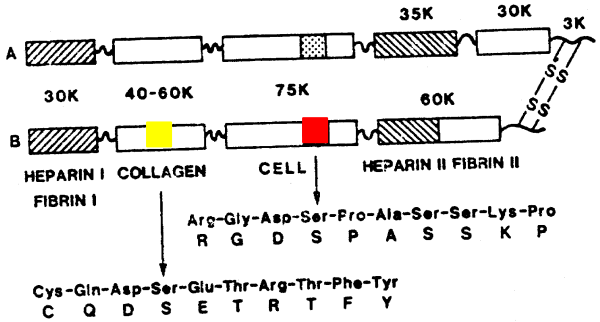
Fibronectin provides an adhesive substrate for migrating crest cells.

- Fibronectin is expressed in the path of crest cell migration.
- In culture, crest cells adhere to and move on a substrate of fibronectin.



Fibronectin provides an adhesive substrate for migrating crest cells.

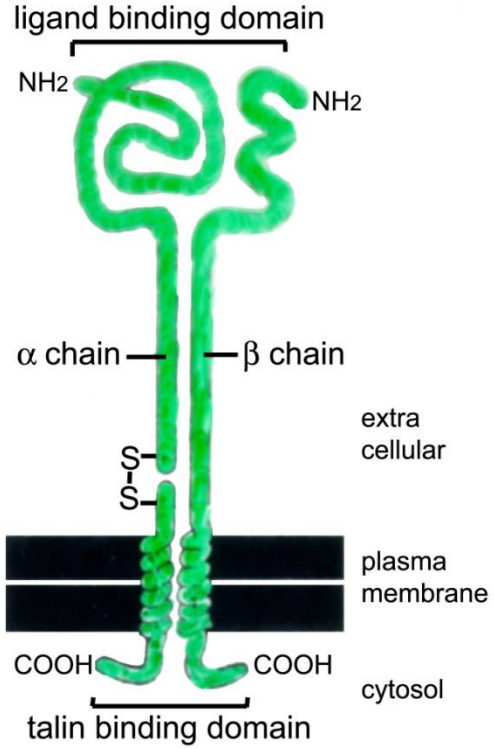
- Injection of the cell binding fragment of fibronectin into the region of crest cell migration in the embryo blocked further migration; other fragments had no effect.



- Crest cells failed to migrate in transgenic mice with a knockout of the fibronectin gene.

Integrins

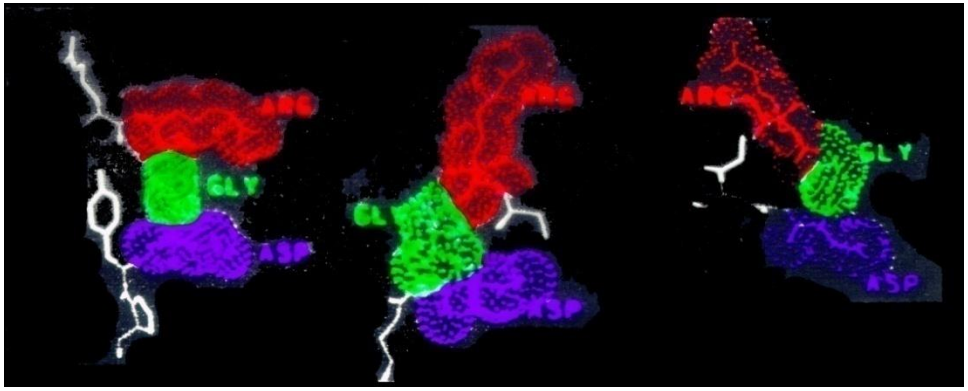
- family of cell surface receptors
- two subunits, α (140kD) and β (160kD), non-covalently associated
- at least 18 α and 8 β chains combine into 24 known heterodimers



Integrins

- many integrins bind the RGD (Arg-Gly-Asp) peptide sequence in their ligands, but none bind all RGD containing proteins

- sequences flanking the RGD amino acids determine the tertiary structure of the ligand



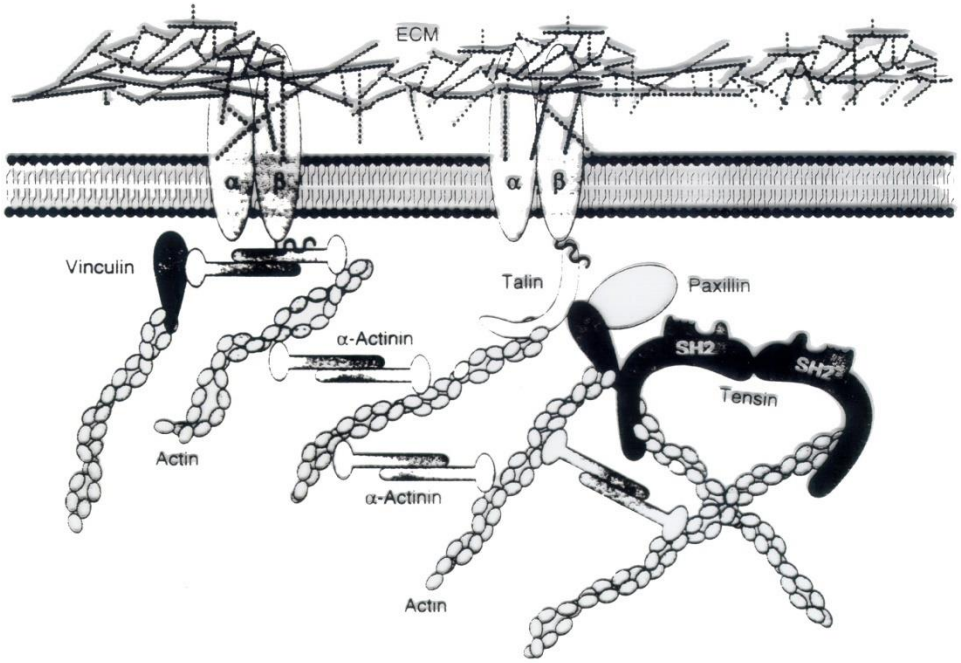
Integrins

- binding specificity of the integrin is determined by its heterodimer combination

β chain	***α chain***	***ligand***
β_1	α_1	laminin, collagen
	α_2	laminin, collagen
	α_3	laminin, fibronectin
	α_4	fibronectin, V-CAM
	α_5	fibronectin
	α_6	laminin
	α_7	laminin
	α_{VN}	fibronectin, vitronectin
β_2	α_{LFA}	I-CAM1, I-CAM2
	α_{Mac1}	fibrinogen
	α_{p150}	?
β_3	α_{1b}	fibronectin, vitronectin, fibrinogen
	α_{VN}	vitronectin, thrombospondin, fibrinogen
β_4	α_6	?
β_5	α_{VN}	vitronectin, fibrinogen
β_P	α_4	?

Integrins

- both subunits are transmembrane and interact with the actin cytoskeleton via actin binding proteins (e.g. talin and vinculin)



Integrins and Crest Cell Migration

- Migrating crest cells express at least 7 integrins, all of which recognize fibronectin and/or laminin:

  $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$ and β_8

- Murine sacoma cells, transfected with $\alpha_4\beta_1$, integrin and transplanted to the crest region in an embryo migrated appropriately.

- Injection of a cell line secreting an antibody to β_1 integrin into the region of crest cell migration blocked further migration.

- Trangenic knockouts of each α or β_1 integrin subunit individually in mice had minor effects on migration of specific crest cell populations.

Hey!

Migrating crest cells adhere to fibronectin (and other molecules) in the ECM using integrins as the receptor!

Fibronectin is more broadly distributed than the pathway used by migrating crest cells, and fibronectin does not appear to convey directional information.

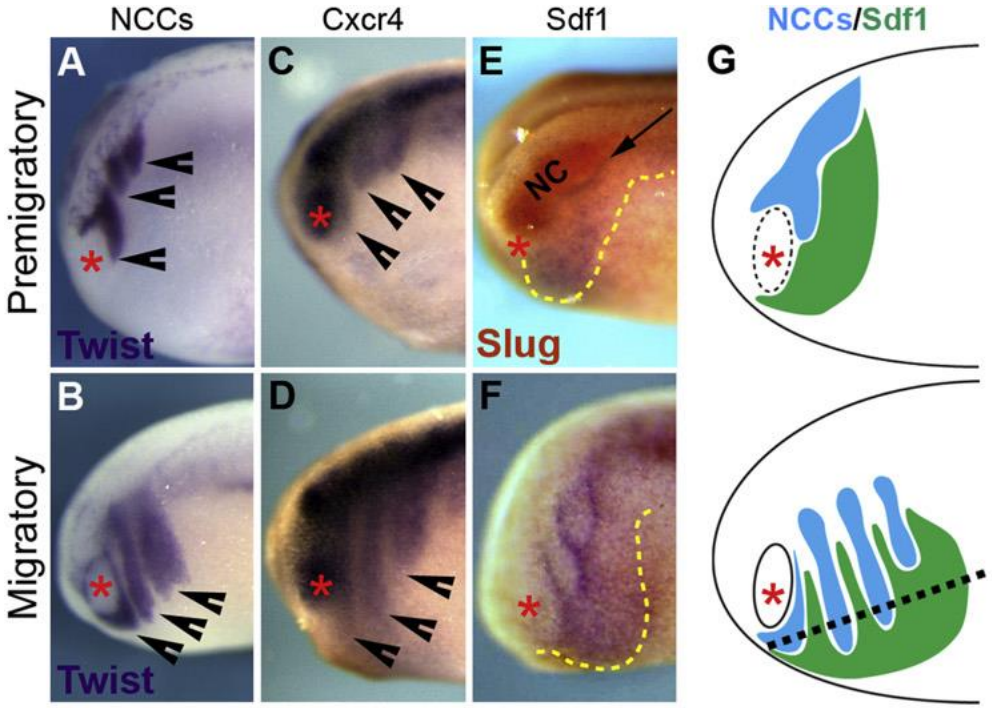
So, what directs migrating crest cells?

What gives migrating crest cells direction?

- Chemotaxis – cells follow a concentration gradient of a bound adhesive molecule:
  - Crest cells migrate from regions of low to high fibronectin concentration in culture.
  - However, no concentration gradient of fibronectin has been demonstrated in vivo.

What gives migrating crest cells direction?

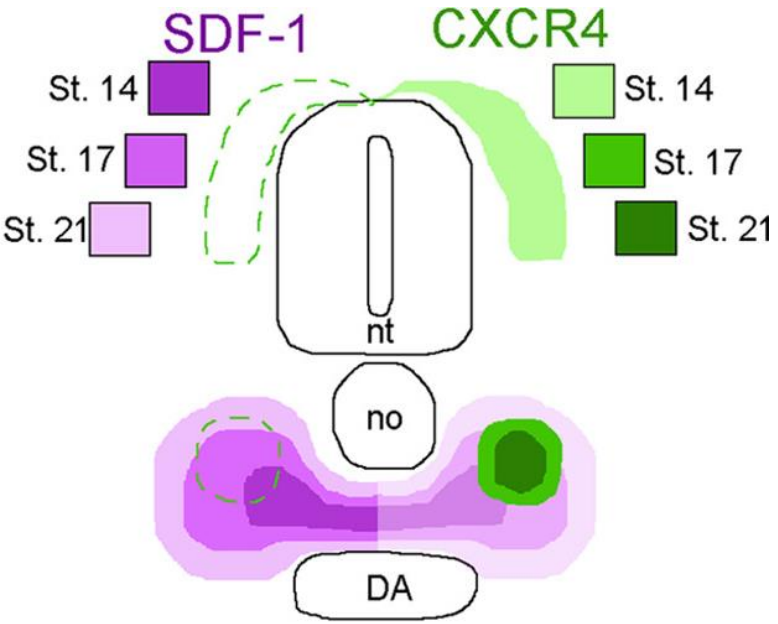
- Chemotropism – cells follow a concentration gradient of a soluble molecule in their environment:
  - Many cranial crest cells express Cxcr4, a receptor for Stromal cell-derived factor 1 (Sdf1).
  - Cranial crest cells that express Cxcr4 migrate towards a source of Sdf1.



What gives migrating crest cells direction?

- Trunk neural crest cells destined to form sympathetic ganglia express Cxcr4.
- Crest cells destined to form dorsal root ganglia do not express Cxcr4.
- Sdf-1 is expressed by the mesenchyme where the ganglia form.
- Cxcr4^{+} crest cells in culture migrate towards a source of Sdf-1.

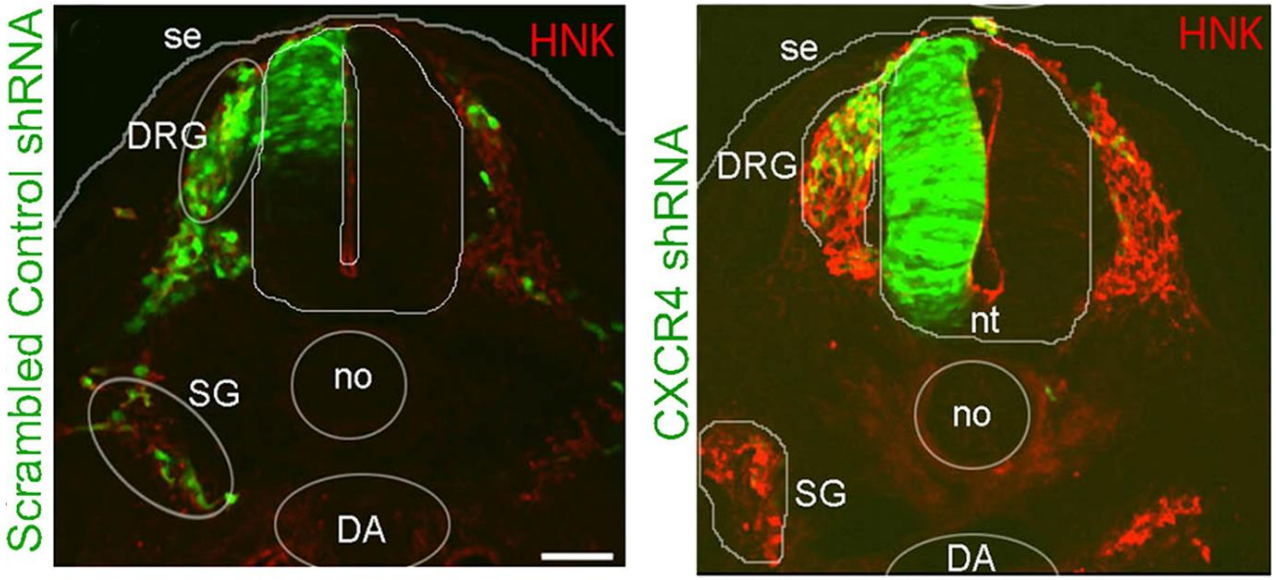
(Kasemeier-Kulesa JC, et al., 2010, *J. Neurosci*. 30:13088)



What gives migrating crest cells direction?

- Knockdown of Cxcr4 eliminated crest cell migration to the sympathetic ganglion.

(Kasemeier-Kulesa JC, et al., 2010, *J. Neurosci*. 30:13088)



What gives migrating crest cells direction?

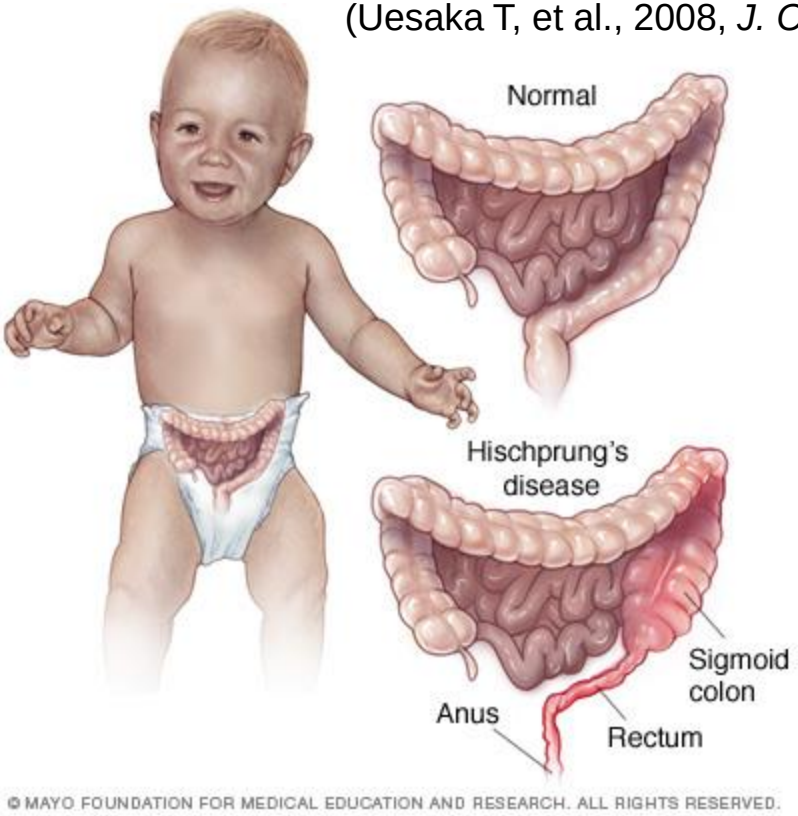
- Enteric crest cells express Ret, the receptor for GDNF, and are attracted to the gut by GDNF released by the gut mesenchyme.

(Young HM, et al., 2001, *Dev. Biol*. 229:503)

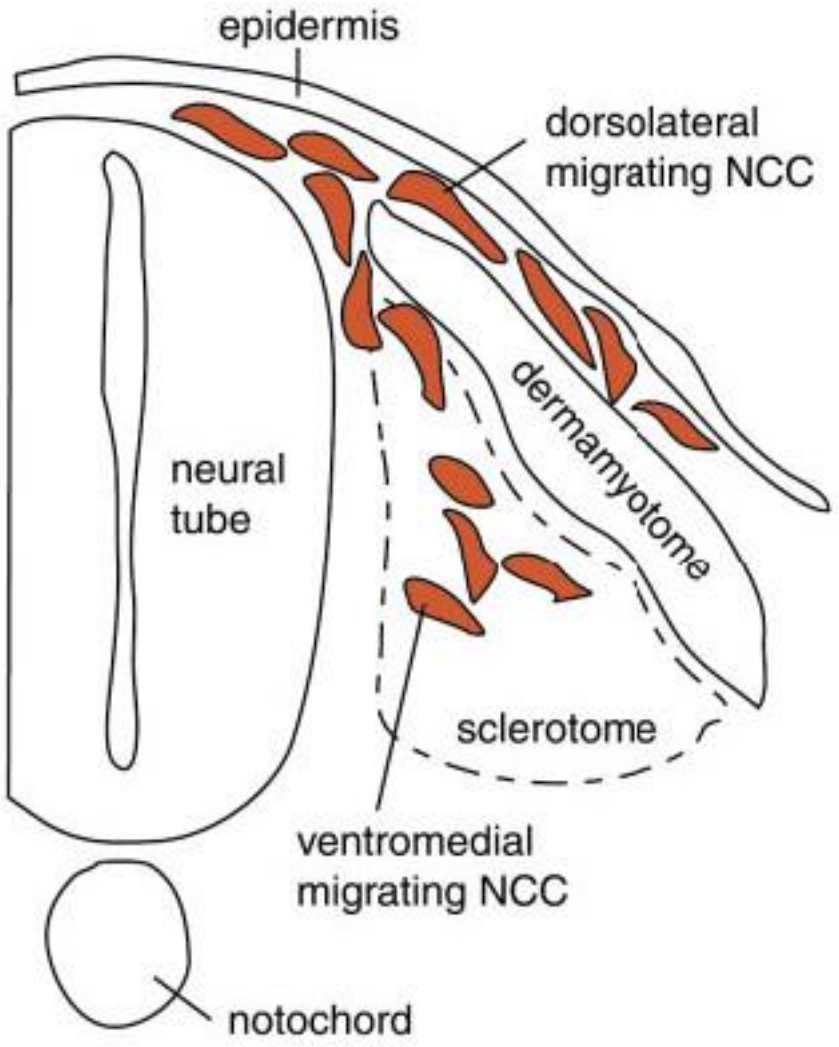
What gives migrating crest cells direction?

- Mutations in the Ret gene, as well as other mutations, cause Hirschsprung disease or aganglionic megacolon.
- ~1 per 5000 live births.

(Uesaka T, et al., 2008, *J. Clin. Invest*. 118:1890)



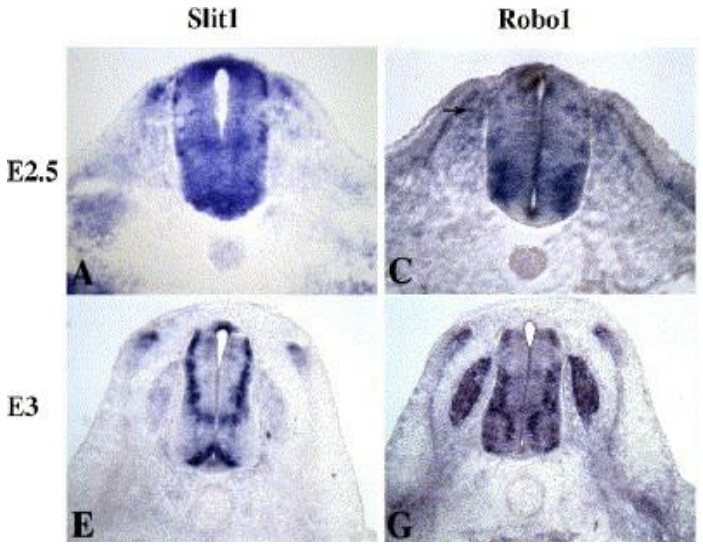
Why do early migrating cells not go dorsolaterally?



Why do early migrating cells not go dorsolaterally?

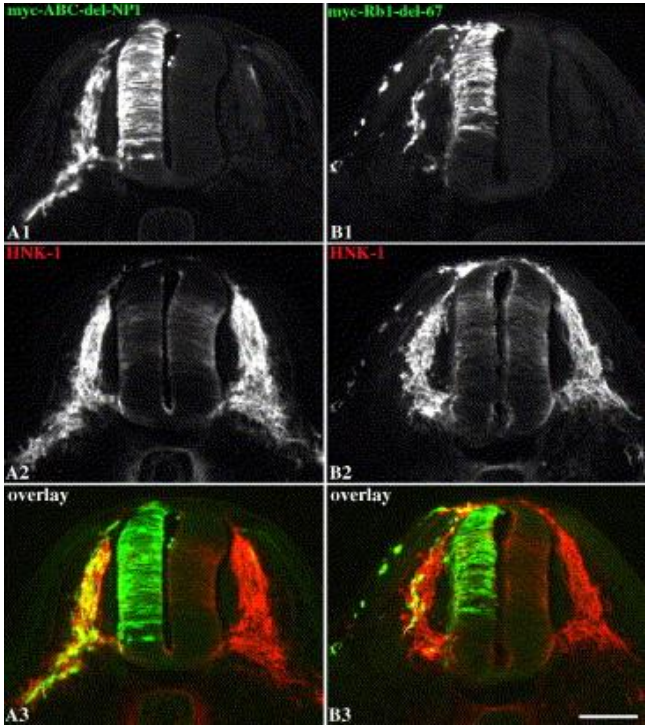
- Unlike late migrating cells, early migrating cells express Robo1/2.

- Slit2 is expressed early in the dermamyotome, and Slit2 repels early crest cells in culture.



Why do early migrating cells not go dorsolaterally?

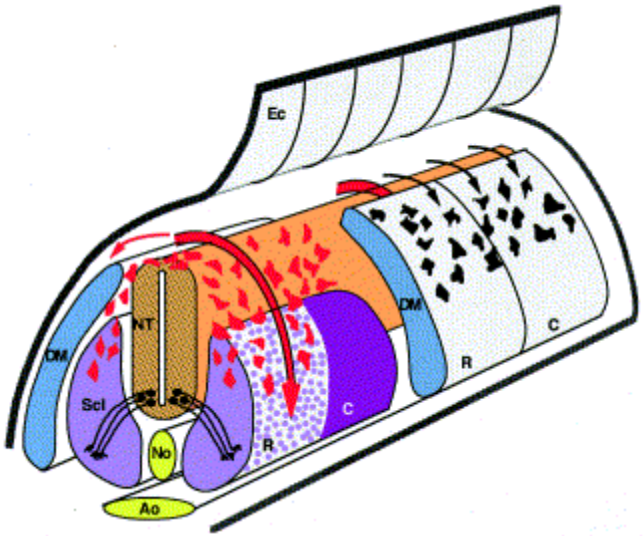
- Transfecting crest cells with a dominant negative Robo allowed early migrating cells to go dorsolaterally.



Thus:

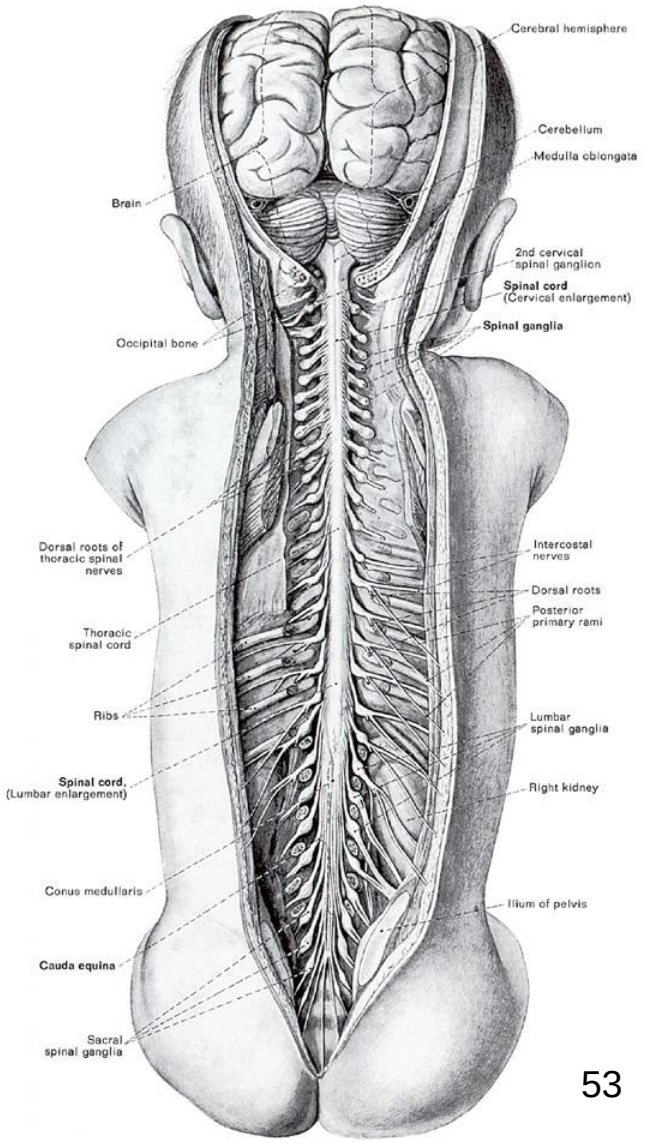
- Slit-Robo signaling blocks early migrating cells from going dorsolaterally.

Why is crest cell migration restricted to the rostral half of the sclerotome?



Segmental Organization of the Peripheral Nervous System

Restricting migration results in separation of ganglia between the segments of the body.



Why is crest cell migration restricted to the rostral half of the sclerotome?

- Surgical rotation of the somites showed that pathway restriction is inherent in the nature of the somites.

Why is crest cell migration restricted to the rostral half of the sclerotome?

- Molecules that promote migration are not restricted to the rostral sclerotome but are broadly distributed.

- Certain molecules that can repel or inhibit crest cell migration are restricted to the caudal sclerotome.

SCLEROTOME	
ROSTRAL	CAUDAL
fibronectin	fibronectin
laminin	laminin
collagen type IV	collagen type IV
thrombospondin	T-cadherin
	versican (CSPG)
	collagen type IX (CSPG)
	F-spondin
	ephrin-B2 (B1 in chick)
	semaphorin 3F

Why is crest cell migration restricted to the rostral half of the sclerotome?

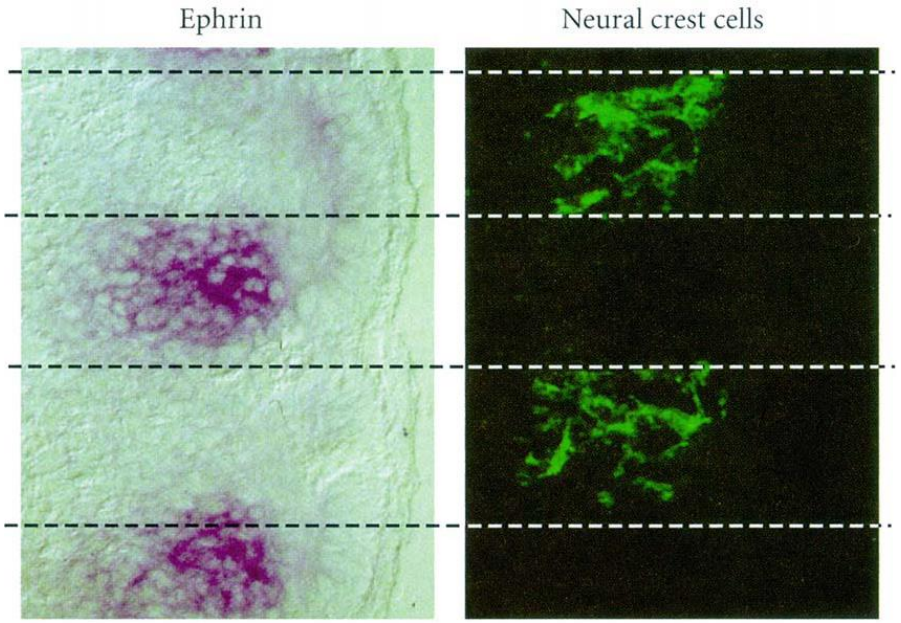
- Collagen type IX strongly inhibits migration in culture.

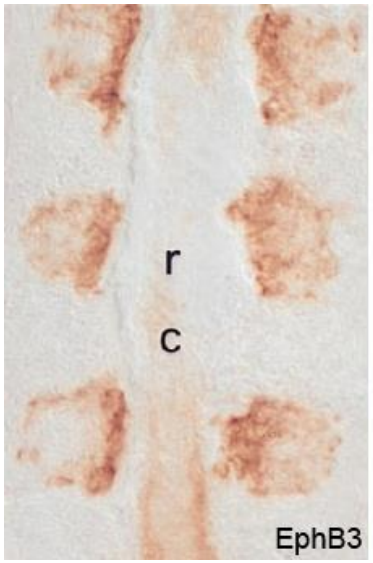
- Versican is over expressed in ectopic sites in splotch mutant mice; these mice lack DRGs, have few Schwann cells and reduced pigmentation.

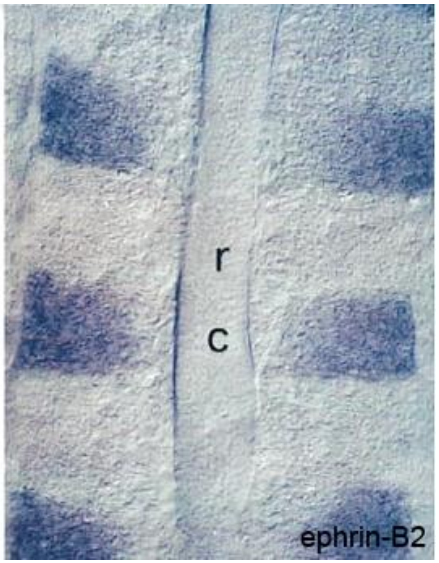
- Migrating crest cells express neuropilin2, the receptor for sema3F; crest cells in Npn2 or Sema3F k.o. mice migrate through caudal sclerotome but still form segmentally appropriate DRGs.

Why is crest cell migration restricted to the rostral half of the sclerotome?

- Migrating crest cells express EphB's, receptors for ephrin-B's.
- In culture, ephrin-B2 repels migrating crest cells.

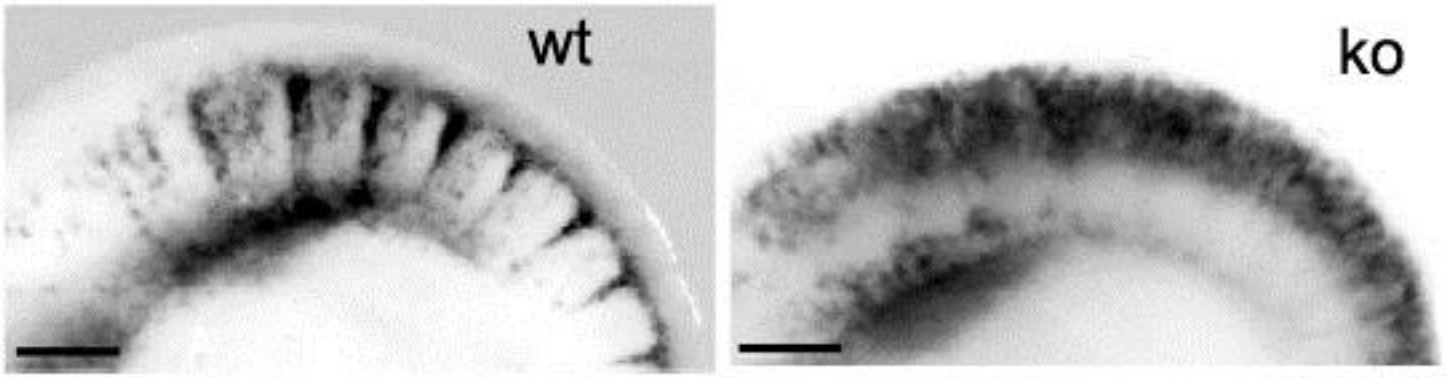






Why is crest cell migration restricted to the rostral half of the sclerotome?

- The peripheral nervous system failed to segment in ephrin-B2 knockouts.



Why is crest cell migration restricted to the rostral half of the sclerotome?

Therefore:

- Migrating cells appear to be repelled from caudal sclerotome.
- Multiple factors appear to be involved.

Why do crest cells stop migrating?

- It is not intrinsic to the cell; crest cells that have finished migrating when transplanted back to the crest will migrate again.

- Crest cells may lose receptors for substrate molecules in response to some guidepost cue and increase adhesiveness for other cells.

  Crest cells increase expression of N-CAM and cadherin as they aggregate into ganglia at the end of migration.

Hey!

So, what guides migrating crest cells?

- **Attractive molecules (often in gradients)**
- **Repellant molecules**
- **Guidepost molecules (that initiate changes in the cell)**