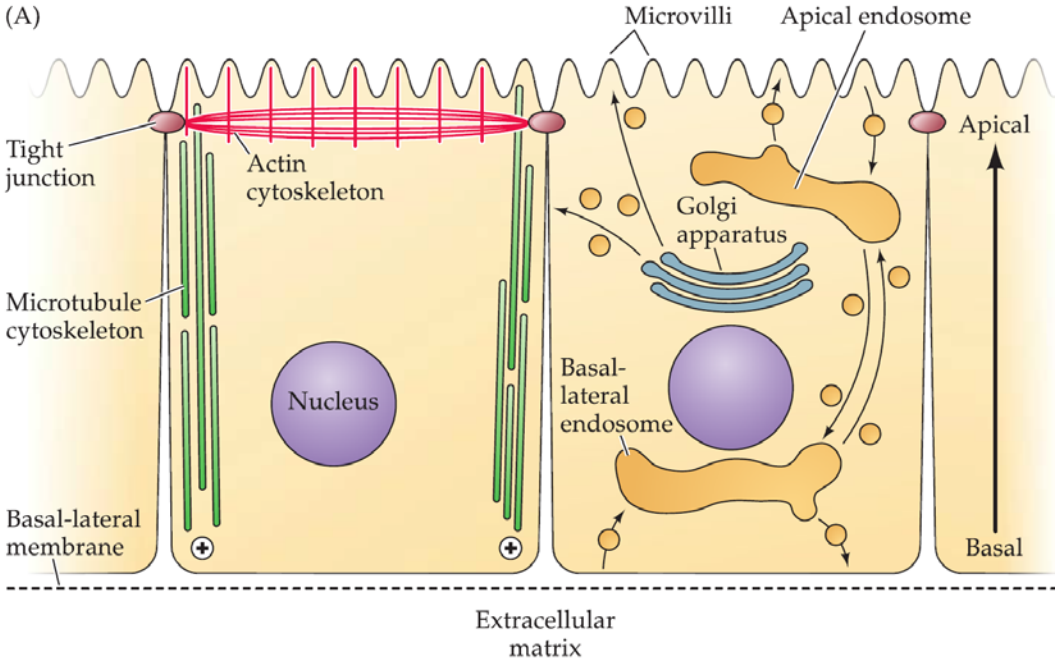
Part 2, Nervous System Development and Diseases

2.2. Construction of neural circuits

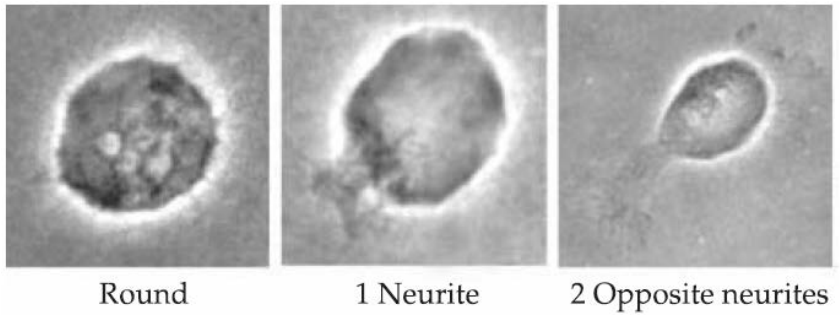
Cell polarity

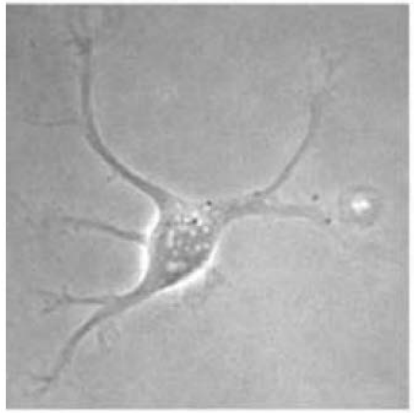
- Neurons are especially elaborate examples of **polarized epithelial cells,** a fundamental cell class found in most tissues.
  - The **apical domain** has a distinctive actin cytoskeleton, and membrane extensions (villi) that increase the surface area for taking in and releasing specific molecules
    - There are tight junctions.
    - The Golgi apparatus is oriented toward the apical membrane.
  - The **basolateral domain** makes contact with the extracellular matrix.
    - It has specialized adhesion contacts.
    - The plus ends of microtubules are oriented toward the basal membrane.
    - Its surface is specialized for intercellular communication.

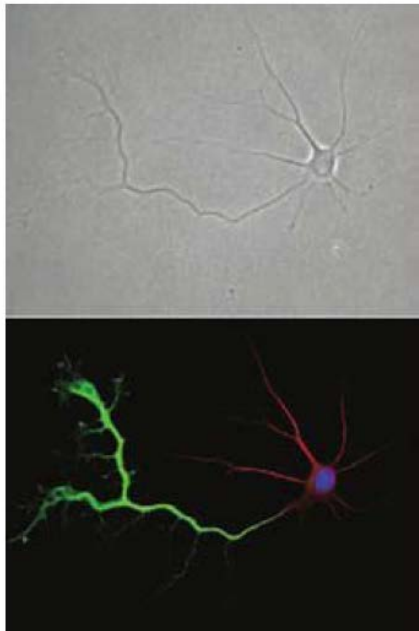


Neuronal polarization: the first step in neural circuit formation

- Distinguishing the **apical domain** (the eventual **axon**, adapted for secretion) from the **basal domain** (the **dendrites**, which will eventually become specialized for receiving signals).
  - Once neurogenesis is complete, outgrowth of neuronal processes begins.
  - Initially, a number of apparently equivalent small extensions (referred to as ***neurites,*** since at first they have neither axonal nor dendritic identities) protrude from the immature neuron.
  - Microtubule and actin components of the cytoskeleton as well as other proteins are redistributed among the neurites so that a single process is identified as the **axon** (the apical domain).
  - The remaining processes become **dendrites** (the basal domain).

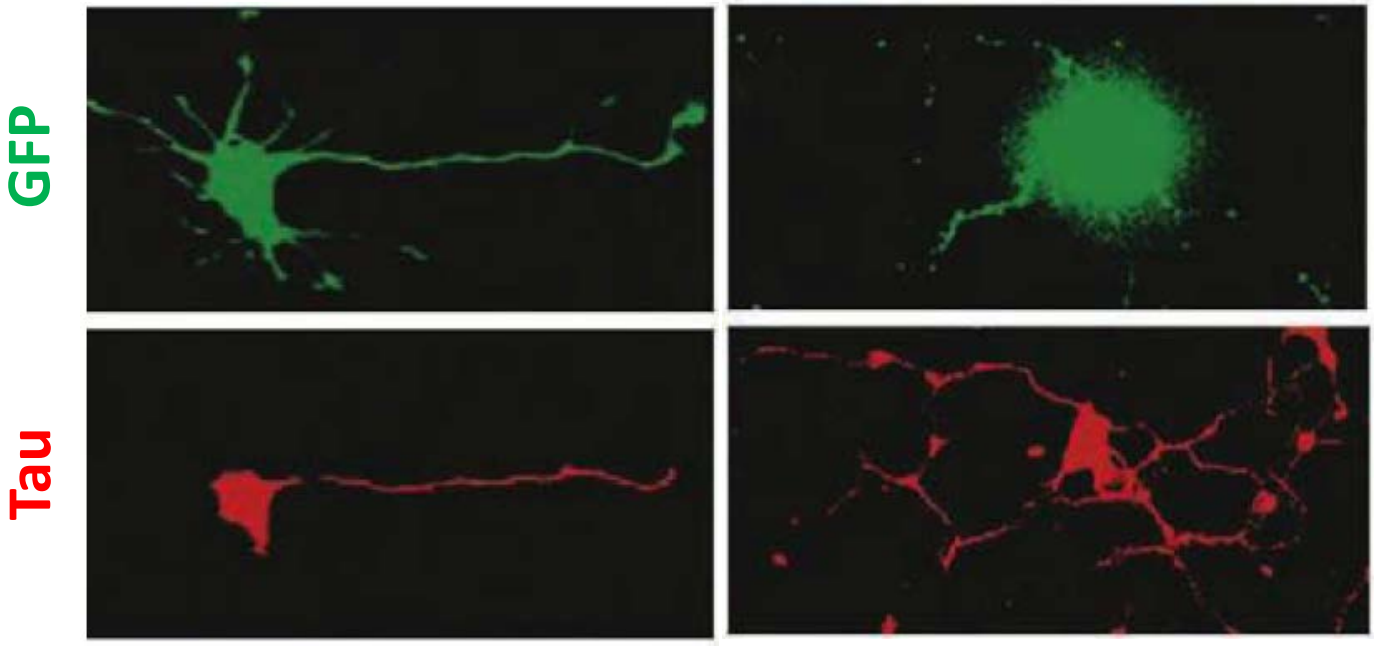






Regulation of cell polarity

- Members of the **PAR** family, are distributed preferentially in the nascent axon.
  - **PAR proteins** interact with cytoskeletal elements and signaling molecules, including Rho and other protein kinases; signal transduction molecules activated by secreted Wnts; neurotrophins; and cell surface-bound cell adhesion molecules.
  - When the function of PAR proteins or related signaling molecules is disrupted, the specification of a single axon does not occur.

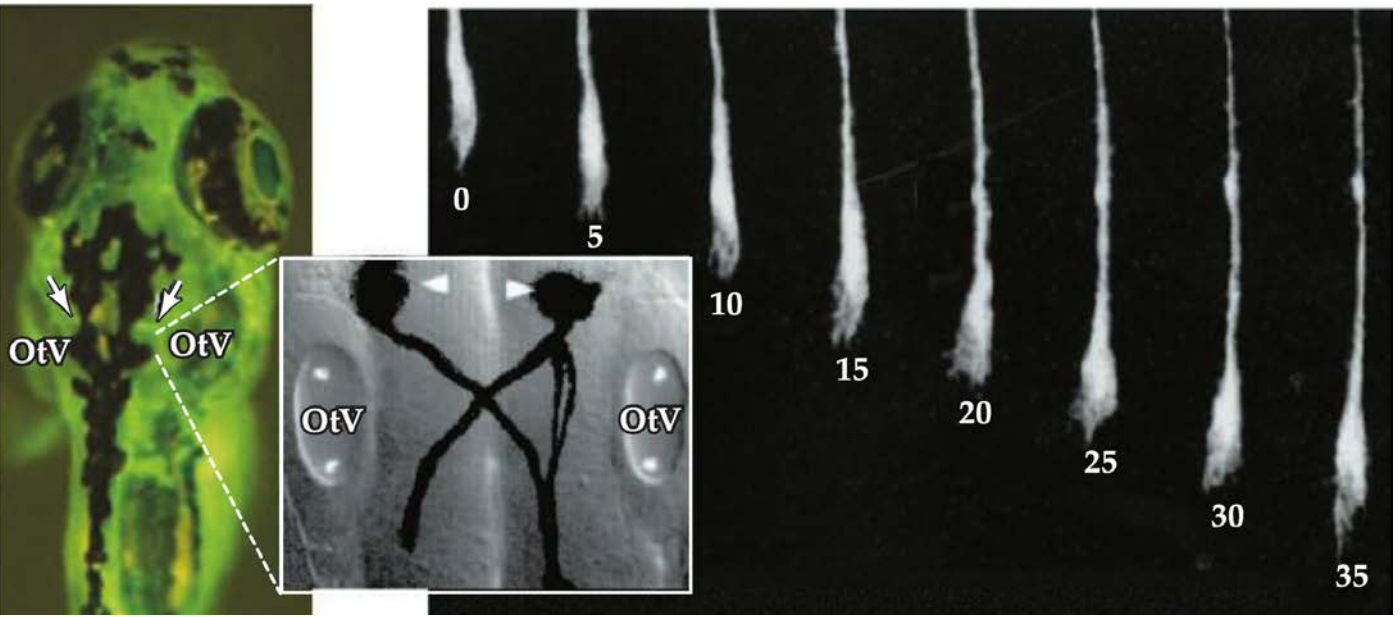


Axon

- Once the **axon** has been specified, it navigates over millimeters or even centimeters, through complex embryonic terrain, to find appropriate synaptic partners.
- "The growing fibers are clearly endowed with considerable energy and have the power to make their way through the solid or semi-solid protoplasm of the cells of the neural tube. But we are at present in the dark with regard to the conditions which guide them to specific points."

  --Ross G. Harrison, 1910

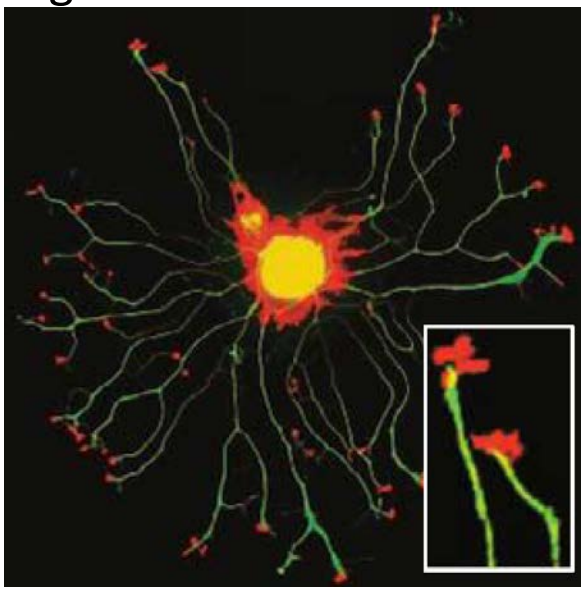
  - Harrison got his BA and PhD from Johns Hopkins.
  - He was considered for a Nobel prize for his work on nerve-cell outgrowth.

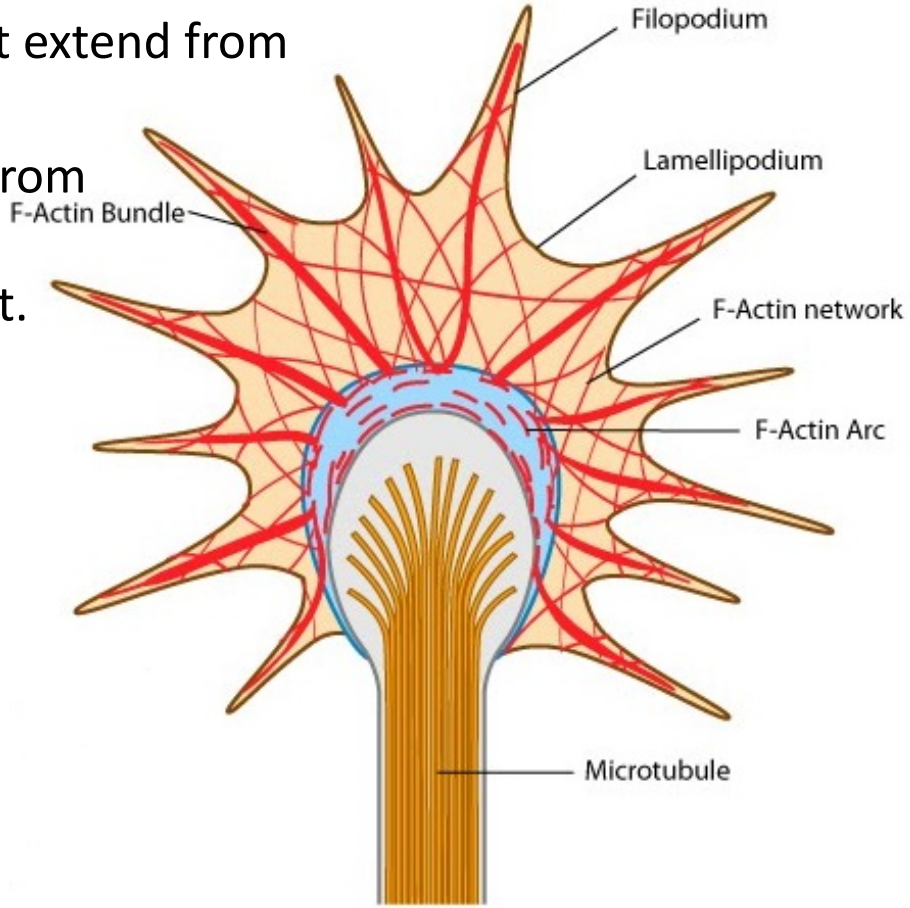


Over 35 minutes, the zebrafish Mauthner neuron axon advances approximately 50 μm.

Axon growth cones

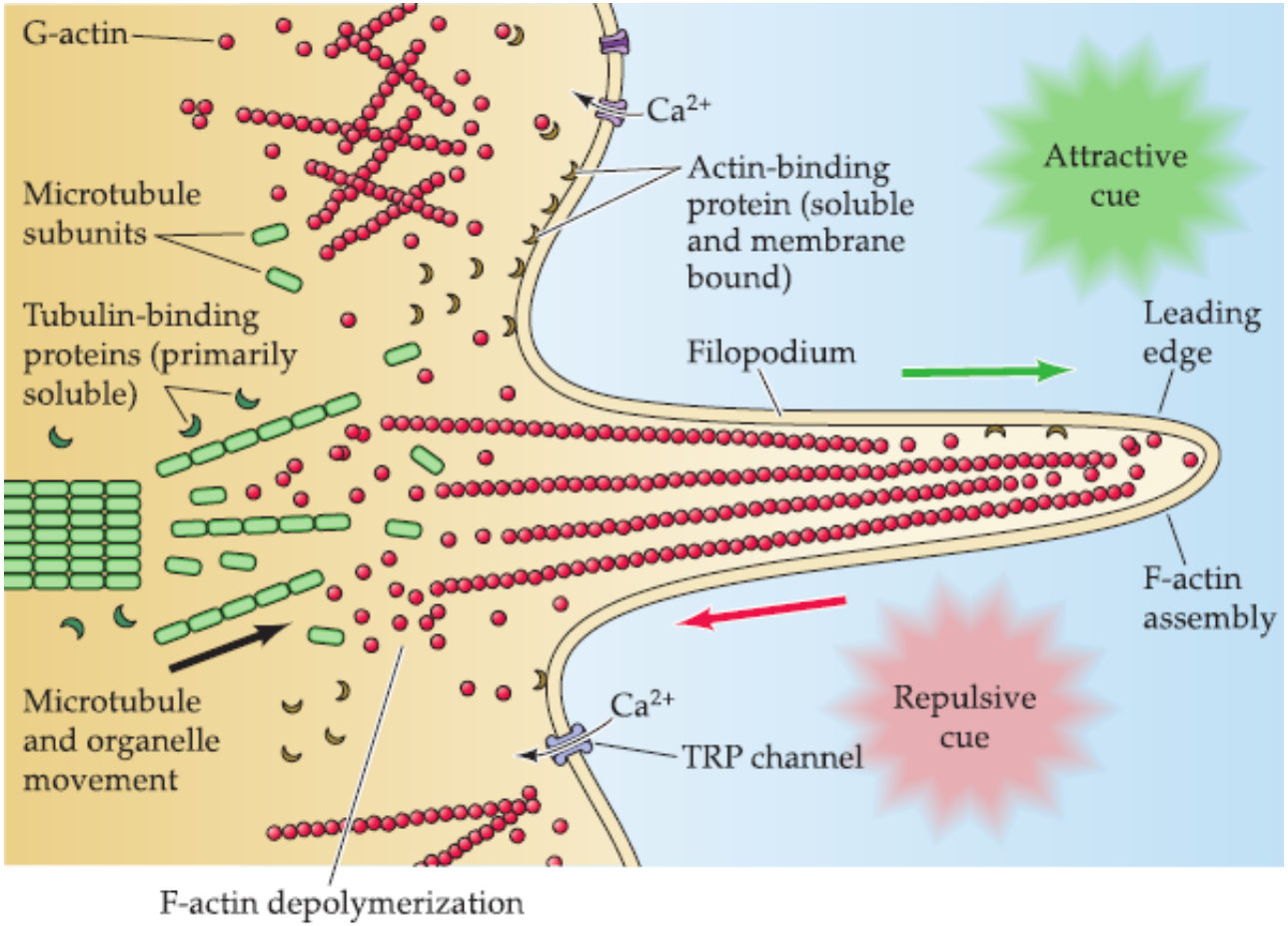
- Axon growth cone: a specialized structure at tip of extending axon.
  - Growth cones are highly motile.
  - They explore the extracellular environment, determine the direction of growth, and then guide the extension of the axon in that direction.
  - **Lamellipodium**: a sheetlike expansion of the growing axon at its tip.
  - **Filopodia**: numerous fine processes that extend from each lamellipodium.
    - Filopodia rapidly form and disappear from the terminal expansion, like fingers reaching out to sense the environment.





Growth cone motility

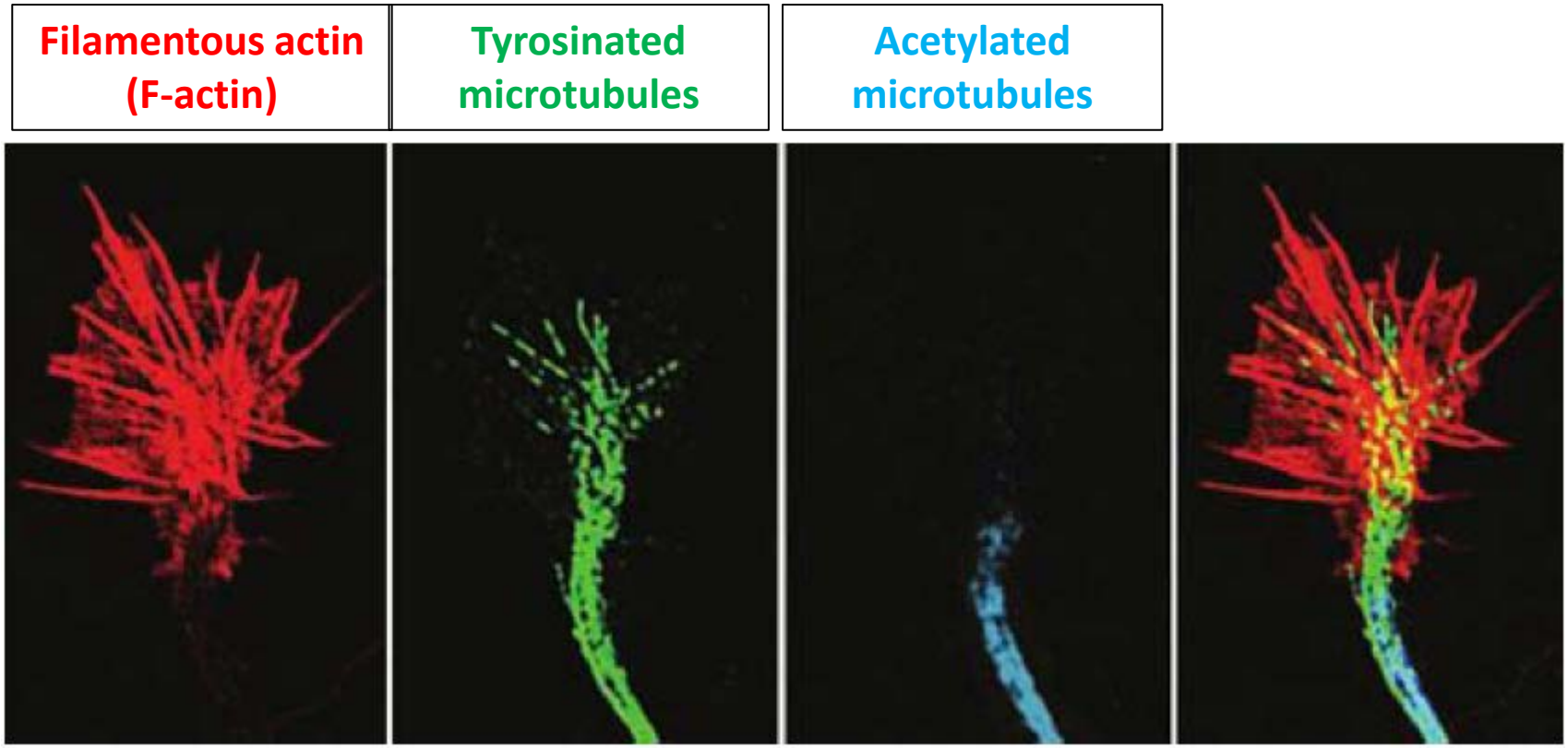
- Growth cone motility reflects rapid, controlled rearrangement of the cytoskeleton.
- The force to move the axon is generated by ATP-dependent modification of the **actin** and **microtubule** cytoskeletons.



- **actin cytoskeleton**: regulating changes in lamellipodial and filopodial shape for directed growth.
- **microtubule cytoskeleton**: responsible for the elongation of the axon itself.

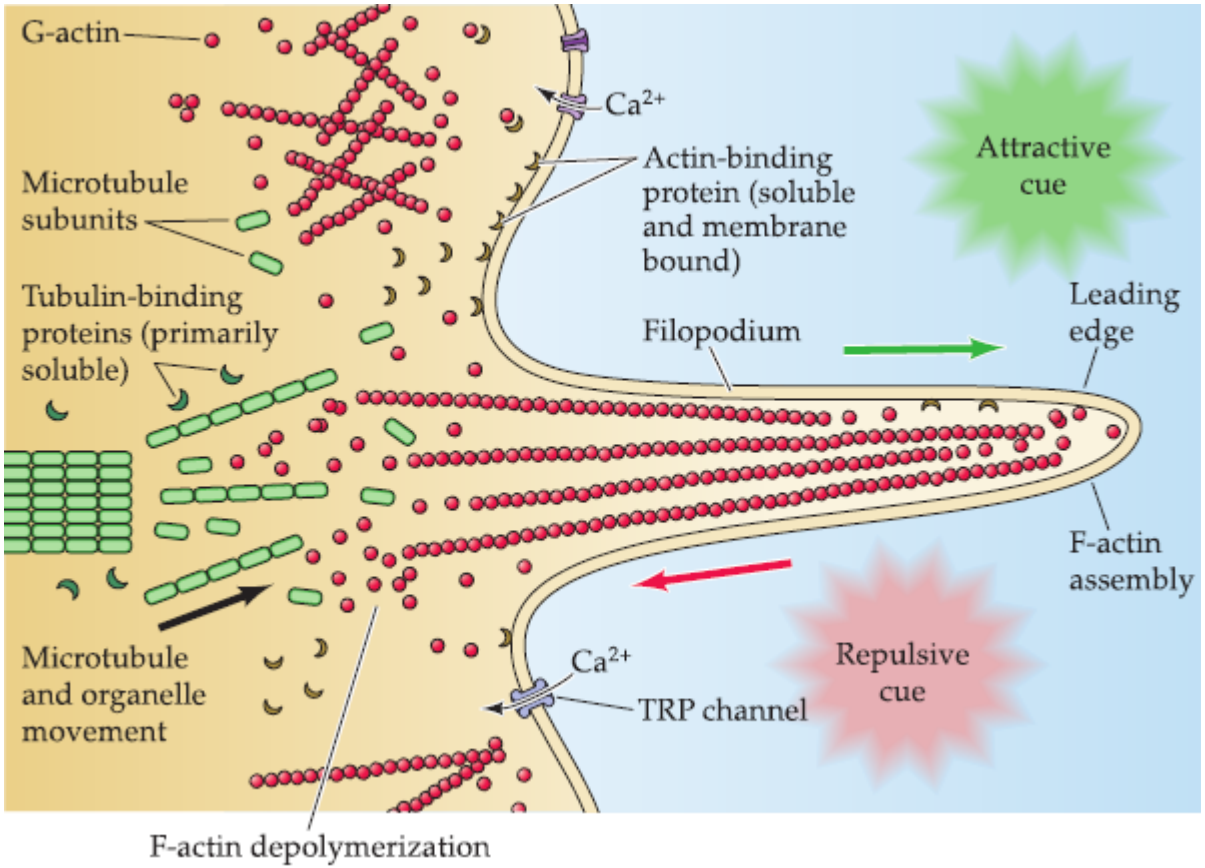
Molecular basis of growth cone motility

- The molecular composition of both the **actin** and **microtubule** cytoskeletons changes in distinct regions of the growing axon, suggesting a great deal of ***dynamism*** within growing neural processes.



Actin and tubulin

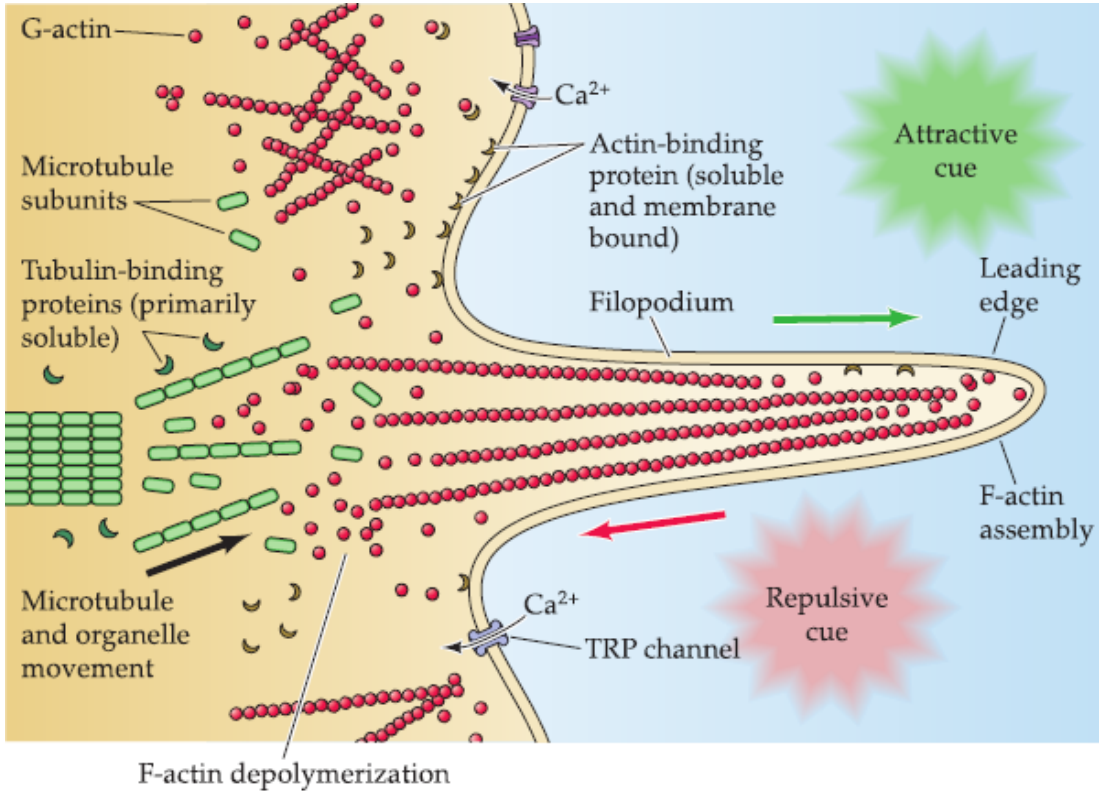
- **Actin** is the primary molecular constituent of a network of cellular filaments found in the lamellipodia and filopodia of growth cones.
- **Tubulin** is the primary molecular constituent of the microtubules that run parallel to the axis of the axon and give it both structural integrity and a means for transporting proteins from the nerve cell body to the axon terminal.



- Actin and tubulin are found in two forms in the growth cone and axon:
  - freely soluble **monomers** in the cytoplasm.
  - **polymers** that form filaments (actin) or microtubules (tubulin).

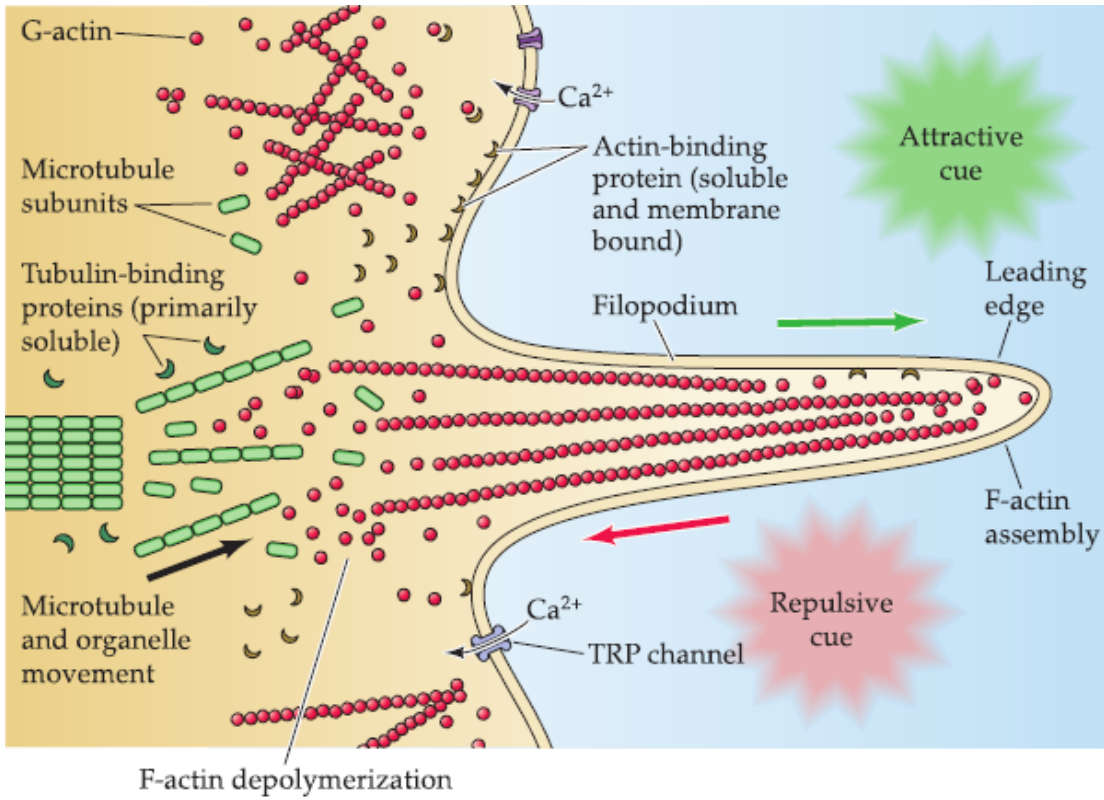
Polymerization and depolymerization

- The dynamic *polymerization* and *depolymerization* of **actin** at the membrane of the lamellipodiurn, as well as within the filopodium sets the direction of growth cone movement, in part by generating local forces that orient the growth cone toward or away from substrates.
- The *polymerization* and *depolymerization* of **tubulin** into microtubules consolidates the direction of movement of the growth cone by stabilizing the axon shaft.
- The interface between the actin and microtubule cytoskeletons regulates the balance of active growth *versus* stability.



Proteins regulating polymerization and depolymerization

- Binding to these molecules and catalyzing posttranslational modifications, or by recruiting enzymes that modify the primary molecular elements of the cytoskeleton.
  - **Actin-binding proteins**:
    - Found throughout the growth cone cytoplasm.
    - Either bind actin directly, or modify actin monomers by phosphorylation and other posttranslational modifications.
  - **Microtubule-binding proteins**:
    - More concentrated in the axon shaft.
    - Modulate posttranslational modifications of monomeric and polymerized tubulin.
    - Other microtubule-binding proteins ("**motors**") are essential for moving molecules and organelles ("cargo") up and down neuronal processes.



Mechanisms regulating polymerization and depolymerization

Adhesion molecules and diffusible signals

75 years: “in dark”
30 years: brighter

↓

voltage- regulated Ca^{2+} channels

transient receptor potential (TRP) channels activated by second messengers

Second messenger pathways that mobilize intracellular Ca^{2+} stores

↓

regulation of intracellular levels for Ca^{2+}, an intracellular messenger

↓

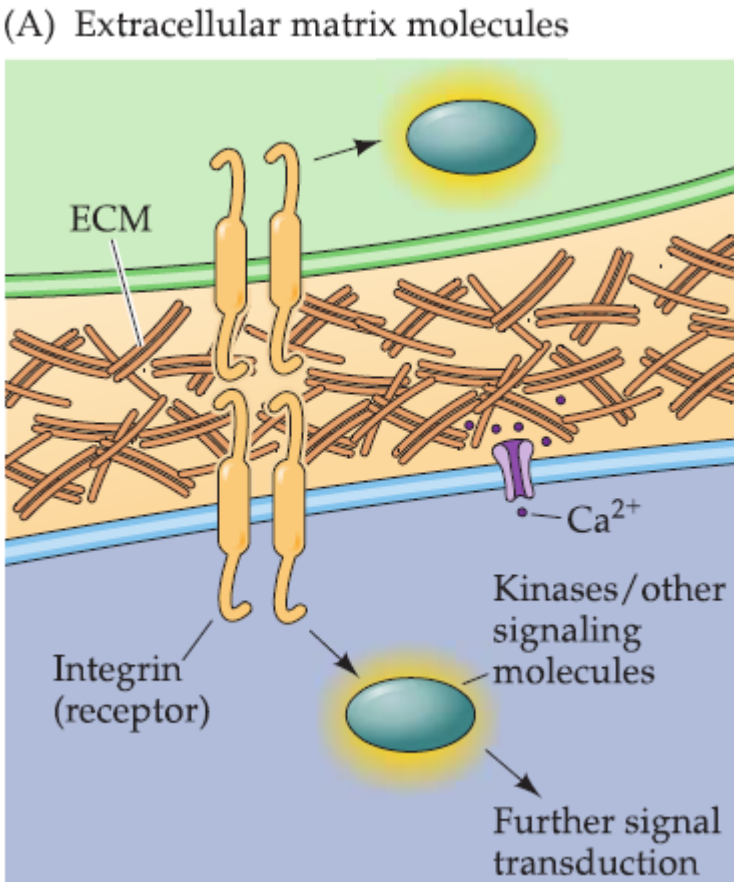
The constant flux between monomeric actin and tubulin *versus* the polymerized actin filaments and microtubules

Non-diffusible signals for axon guidance

- the extracellular matrix molecules and their integrin receptors
- the Ca^{2+}-independent cell adhesion molecules (CAMs)
- the Ca^{2+}-dependent cell adhesion molecules, or cadherins
- the ephrins and Eph receptors

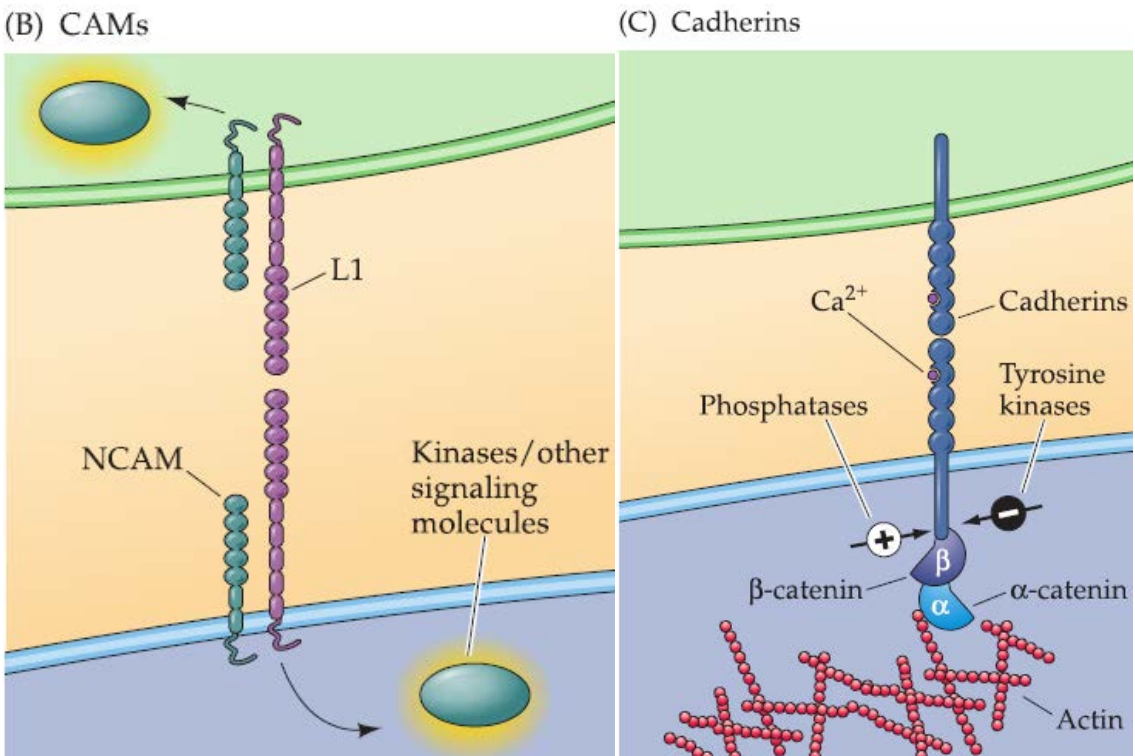
Extracellular matrix cell adhesion molecules

- the first to be associated with axon growth
- **laminins**, **collagens**, and **fibronectin**
  - Secreted by the cell itself or by its neighbors
  - Forming polymers and creating a durable local extracellular substance, rather than diffusing away from the cell after secretion.
- Cell surface **receptors**: **integrins**
  - Integrins do not have kinase activity or any other direct signaling capacity.
  - Triggers a cascade of events-perhaps via interactions between the cytoplasmic domains of integrins with kinases and other signaling molecules, as well as Ca2+ channels-that can stimulate axon growth and elongation.



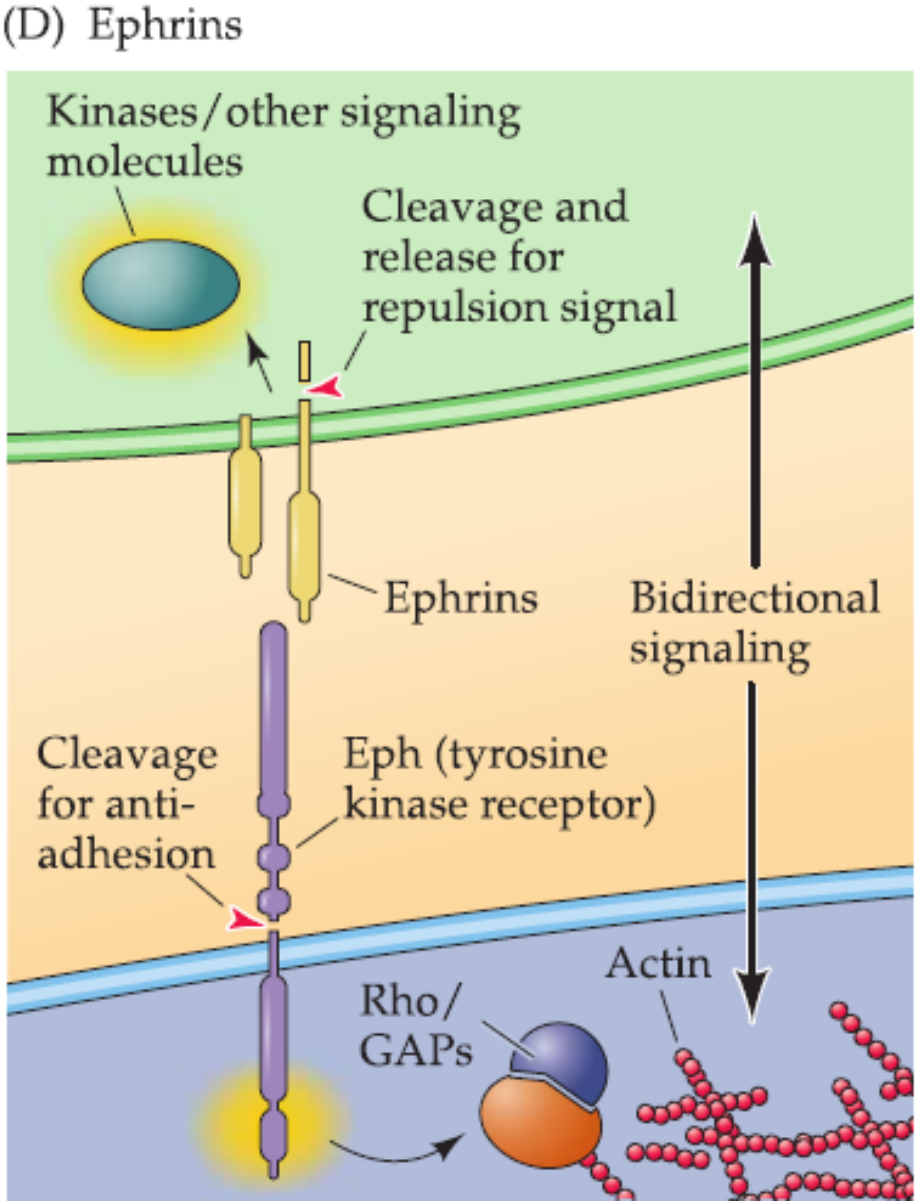
CAMs and cadherins

- Found on growing axons and growth cones as well as on surrounding cells and targets.
- Both CAMs and cadherins have dual functions as ligands and receptors, usually via homophilic ("like with like") binding.
  - **CAMs**, especially the **L1** CAM: bundling, or *fasciculation,* of groups of axons.
  - **Cadherins**: important determinants of final target selection in transition from growing axon to synapse.
- Both CAMs and cadherins rely on a somewhat indirect route of signal transduction.
  - Ca^{2+}-independent CAMs interact with cytoplasmic kinases to initiate cellular response.
  - Ca^{2+}-dependent cadherins engage the APC/β-catenin pathway.



ephrins

- **Ephrin** ligands and their tyrosine kinas receptors (**Eph receptors**, or **Ephs**).
- Bidirectional signaling.
- Ephrins and Ephs activate a variety of signaling pathways and can be either growth-promoting or growth-limiting.

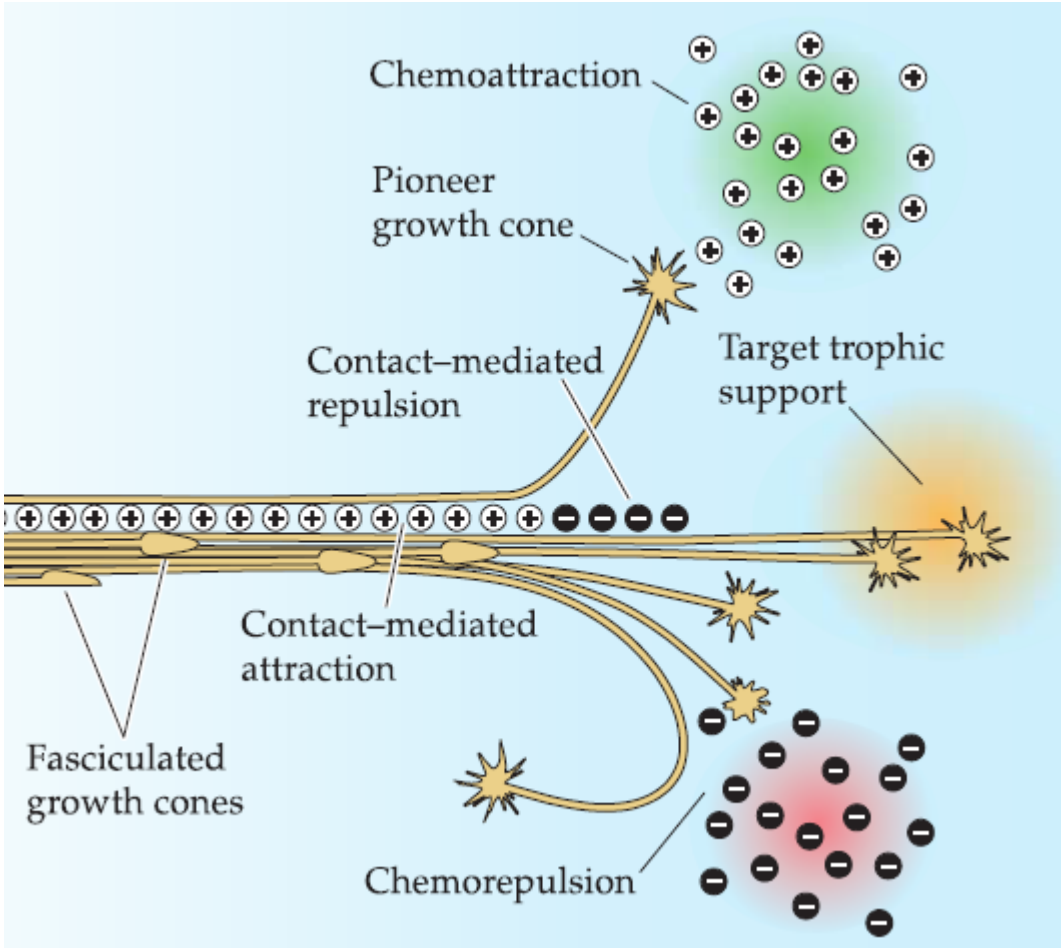


Human developmental or neurological disorders related to adhesive interactions and signal transduction

- X-linked hydrocephalus.
- MASA (an acronym for *m*ental retardation, *a*phasia, *s*huffling gait, and *a*dducted thumbs).
- Kallman's syndrome (which compromises reproductive and chemosensory function).
- X-linked spastic paraplegia.

Chemoattraction and chemorepulsion

- A growing axon must eventually find an appropriate target while avoiding inappropriate ones.
  - **Ramon Cajal** proposed that target-derived signals, most likely released by the target cells themselves, selectively attract growth cones to useful destinations---**chemoattraction**.
  - **chemorepulsion**.



Difficulties in identifying chemoattractive and chemorepulsive signals

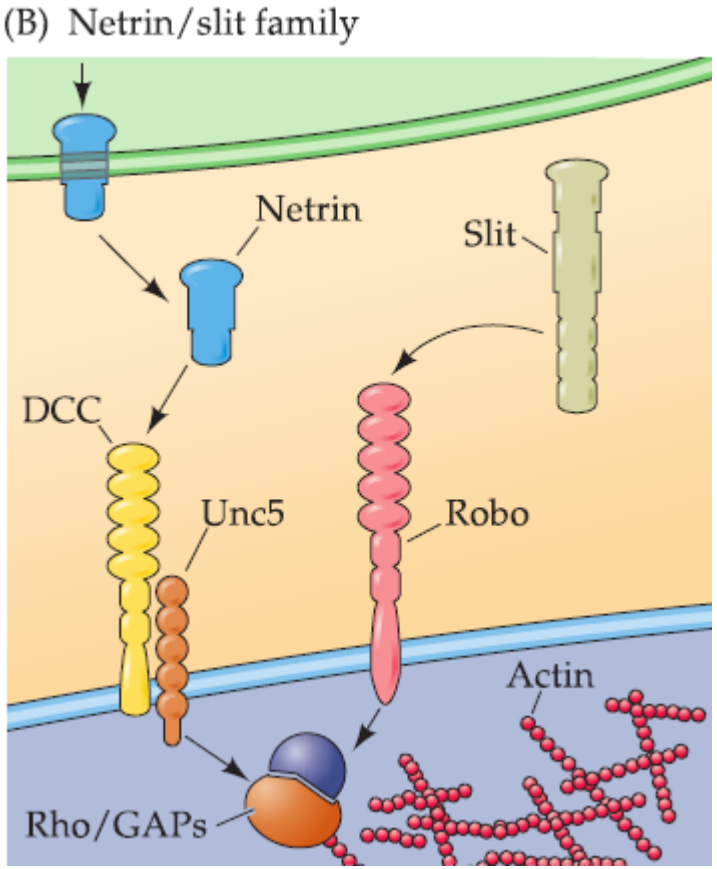
- Small amounts of such factors expressed in the developing embryo.

- Difficulties in distinguishing *tropic* molecules (which *guide* growing axons toward a source) from *trophic* molecules (which *support* the survival and growth of neurons and their processes once an appropriate target has been contacted).

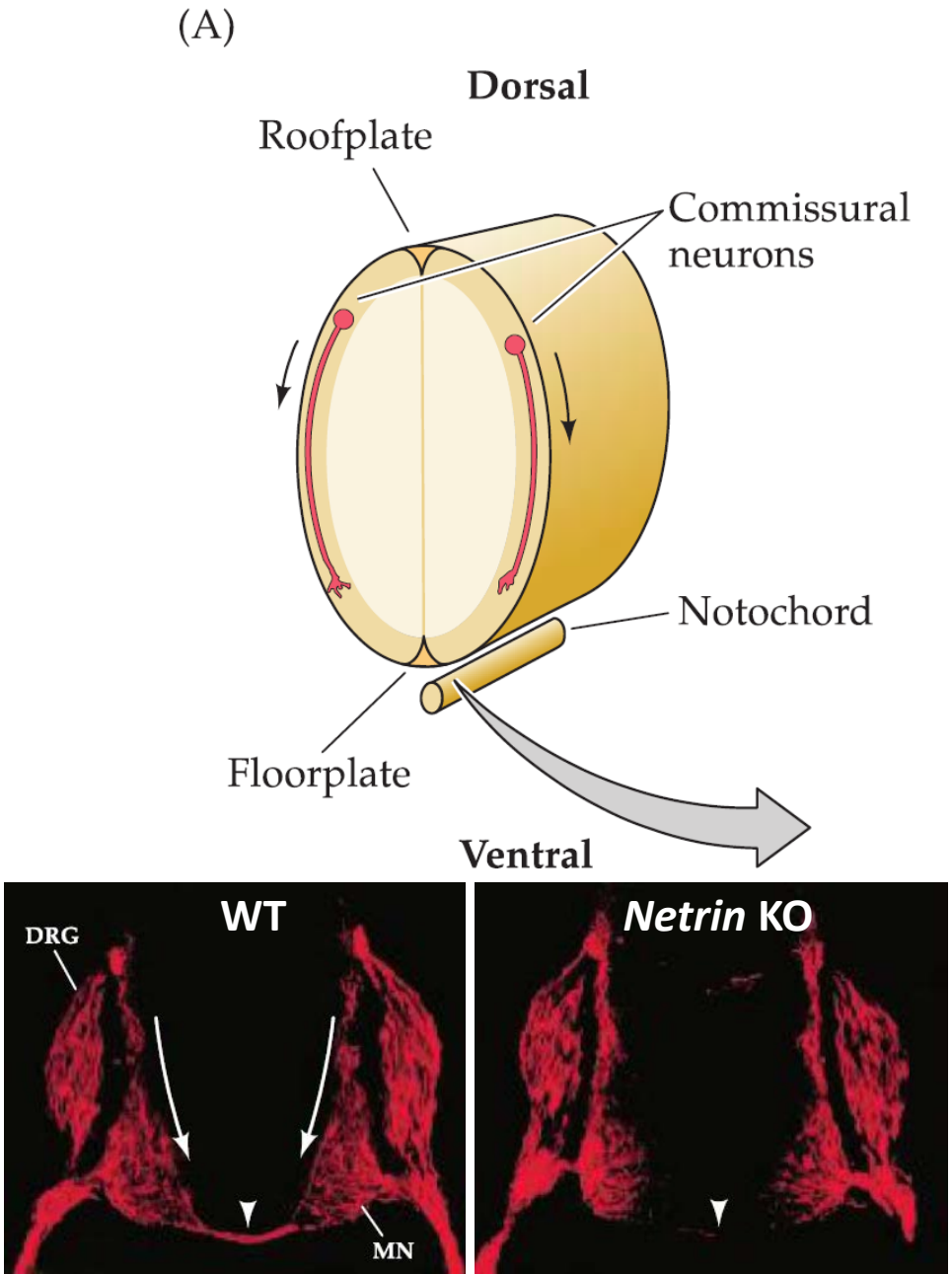
- Solved by laborious biochemical purification and analysis of attractive or repulsive activities from vertebrate (chick) embryos and genetic analysis of axon growth in both *Drosophila* and C. *elegans*.

Netrins & Slits

- Sanskrit, "to guide“: One of the first families of chemoattractant molecules to be identified.
  - **Chick**: proteins with chemoattractant activity following biochemical purification.
  - **C. *elegans***: influenced axon growth and guidance.
    - ***Unc***: "uncoordinated,“ which describes the behavioral phenotype of the mutant worms.
- **DCC** (*d*eleted in *c*olorectal *c*ancer) receptor mediates netrin-dependent chemoattraction.
- **Unc5** receptor mediates netrin –dependent chemorepulsion.
  - Netrin receptors have **repeated amino acid motifs** in their extracellular domains; a **transmembrane domain**; and an **intracellular domain** with no known enzymatic activity.
  - The **Rho/GAP** family of signaling proteins, all of which modulate second messenger-mediated cytoskeletal modification, provide a final step in netrin signaling.
- **Slit** and its receptor **robo**: chemorepulsion.

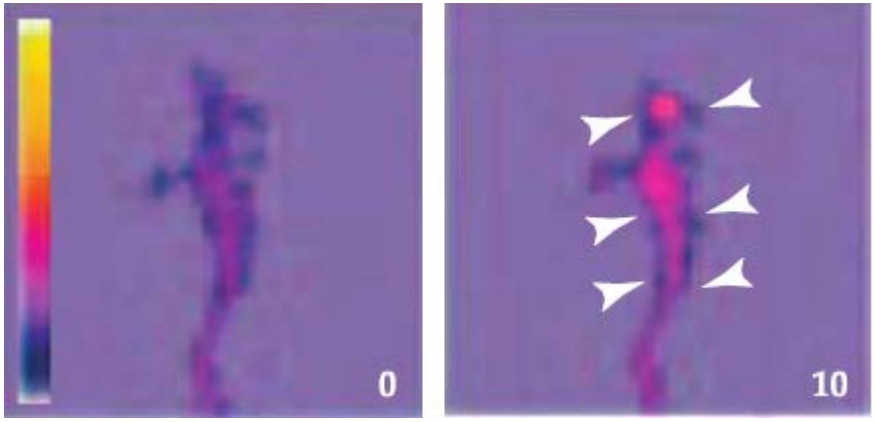


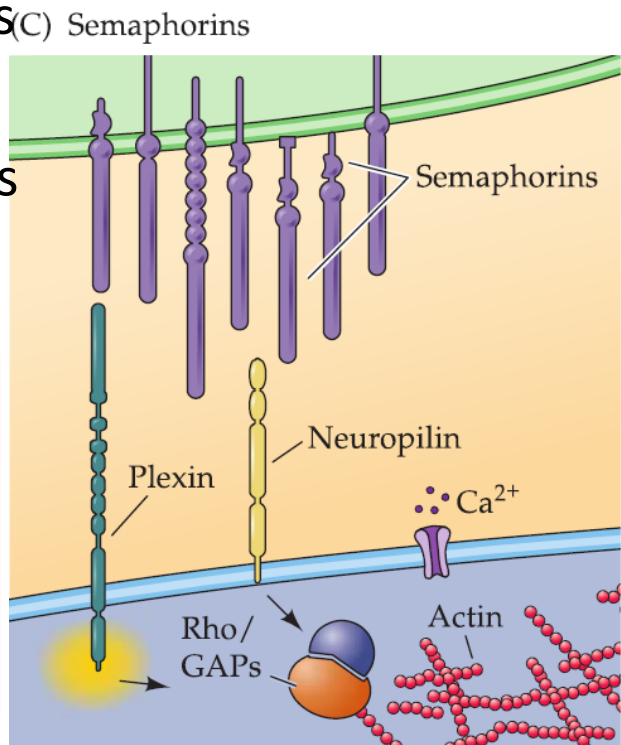
Commissural axon guidance



Semaphorins

- Semaphorins are bound to cell surfaces or to the extracellular matrix, where they prevent the extension of nearby axons.
- Their receptors are transmembrane proteins (including the **plexins** and a protein called **neuropilin**) whose cytoplasmic domains have no known catalytic activity.
- Semaphorin signaling leads to changes in Ca^{2+} concentration that presumably activate intercellular kinases and other signaling molecules to modify the growth cone cytoskeleton.
- Semaphorin treatment of cultured neurons results in **growth cone collapse**, which is Ca^{2+} signaling-dependent.





Selective synapse formation

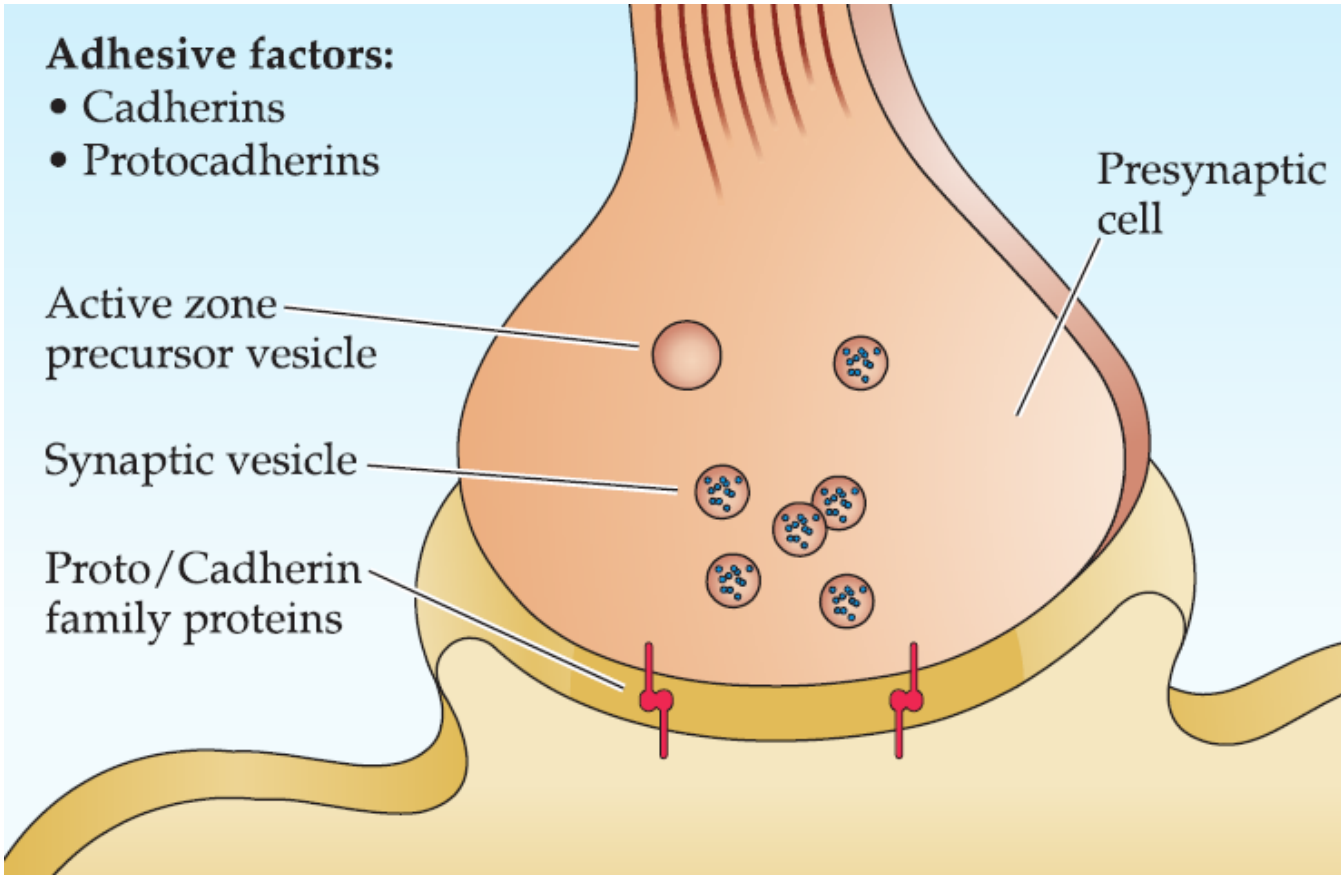
- Once an axon reaches its target region, additional cell-cell interactions dictate which target cells to innervate from among a variety of potential synaptic partners.

- Stages of synapse formation:

Molecular mechanisms involved in synapse formation

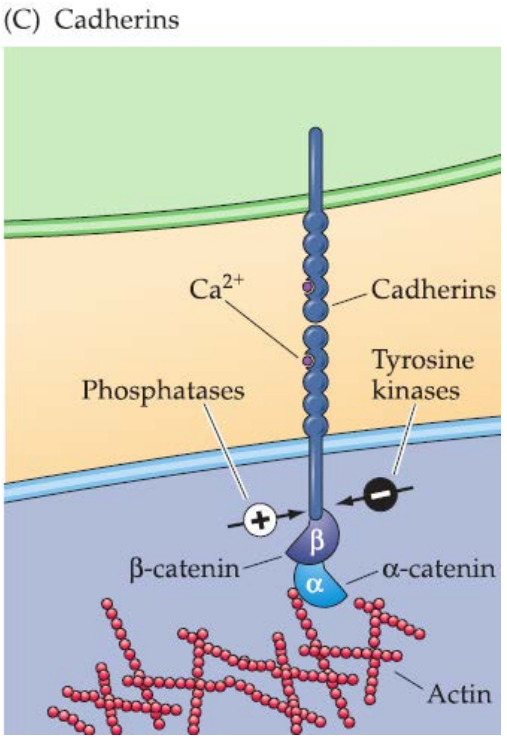
1. **Initiation**: local regulation between the presumptive pre- and postsynaptic membranes mediated by members of the **cadherin** and **protocadherin** family of Ca^{2+} cell adhesion molecules.

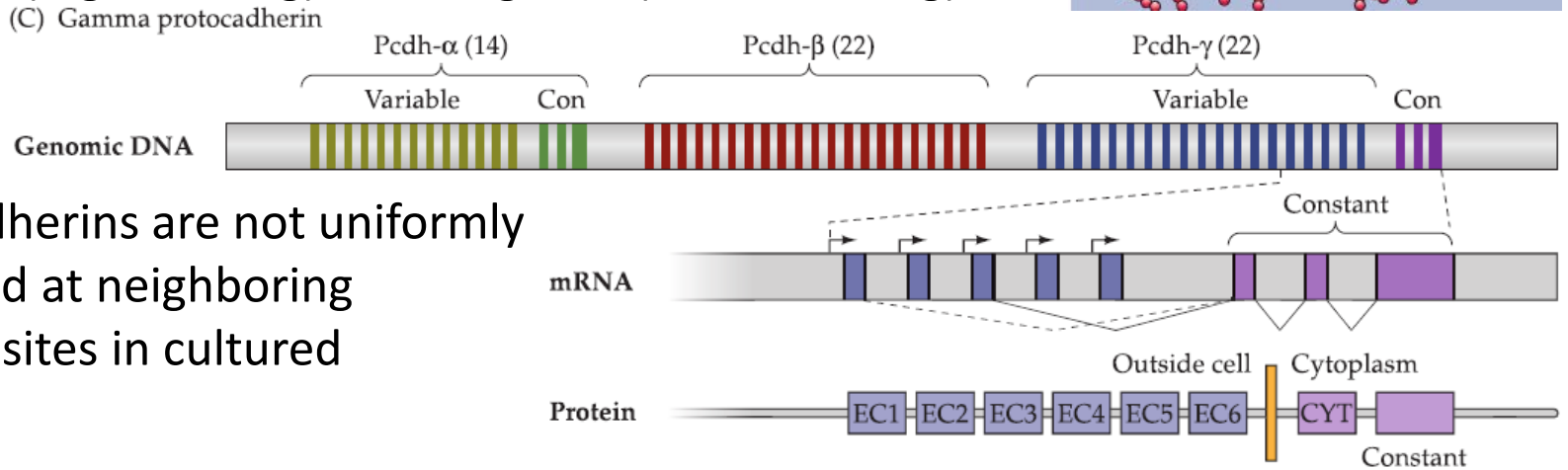
   - Initial accumulation of synaptic vesicles as well as transport vesicles that contain molecular components that contribute to the presynaptic active zone.



Protocadherins

- **protocadherins**: resemble the general cadherin family of cell adhesion molecules.
  - single gene encodes a large number of protocadherins.
  - three regions (α, β, γ) consisting of multiple alternatively spliced exons that encode the extracellular and transmembrane domains of individual protocadherin variants (there are total 58).
  - a conserved domain encoding the intracellular portion of all protocadherin isoforms.
  - Protocadherin isoforms on opposing cells bind to each other with varying affinity based upon their degree of similarity (high binding) or divergence (lower binding).

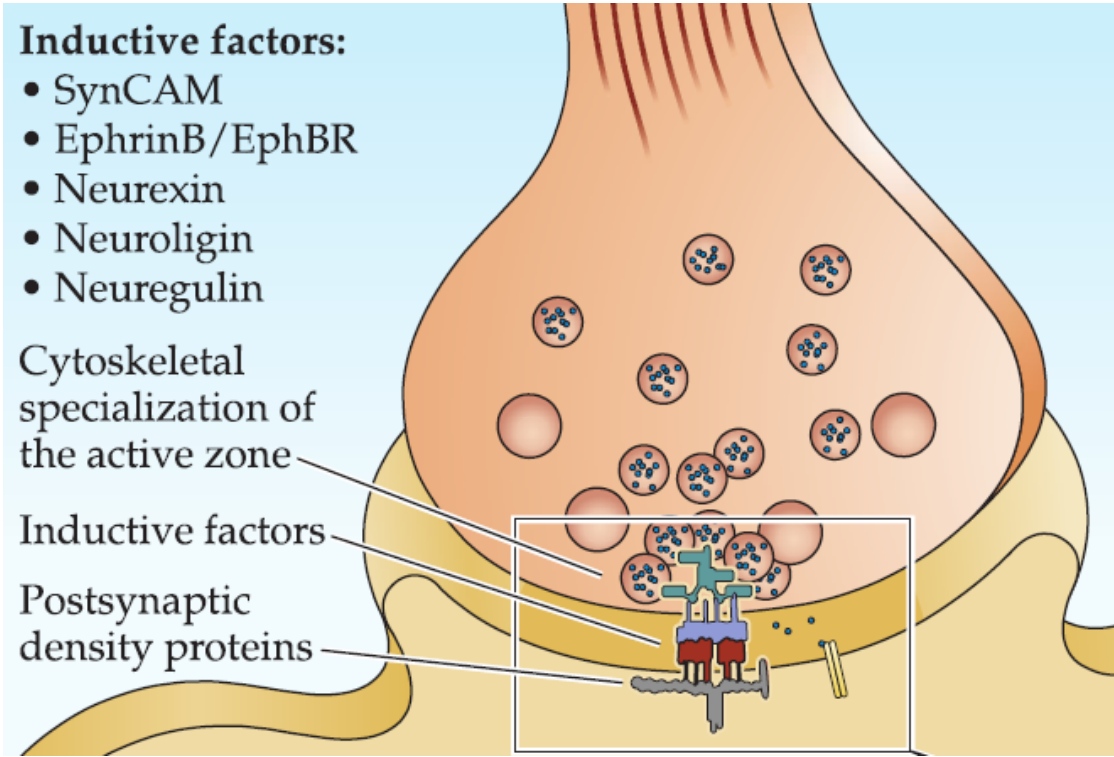




- protocadherins are not uniformly expressed at neighboring synaptic sites in cultured neurons.

Molecular mechanisms involved in synapse formation

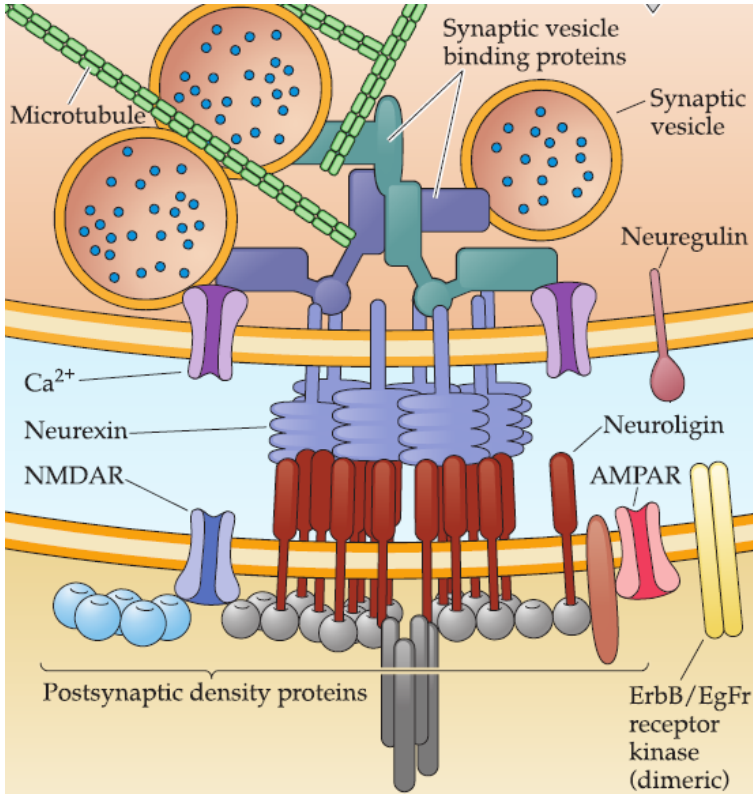
2. Additional adhesion molecules such as SynCAM, neurexin and neuroligin, ephrinB and EphB, are recruited. Adhesive signaling between these molecules initiates differentiation of the presynaptic active zone and the postsynaptic density.
   - The presynaptic terminal also releases molecules (e.g., neuregulin) that influence the expression and clustering postsynaptic receptors and associated proteins.



Molecular mechanisms involved in synapse formation

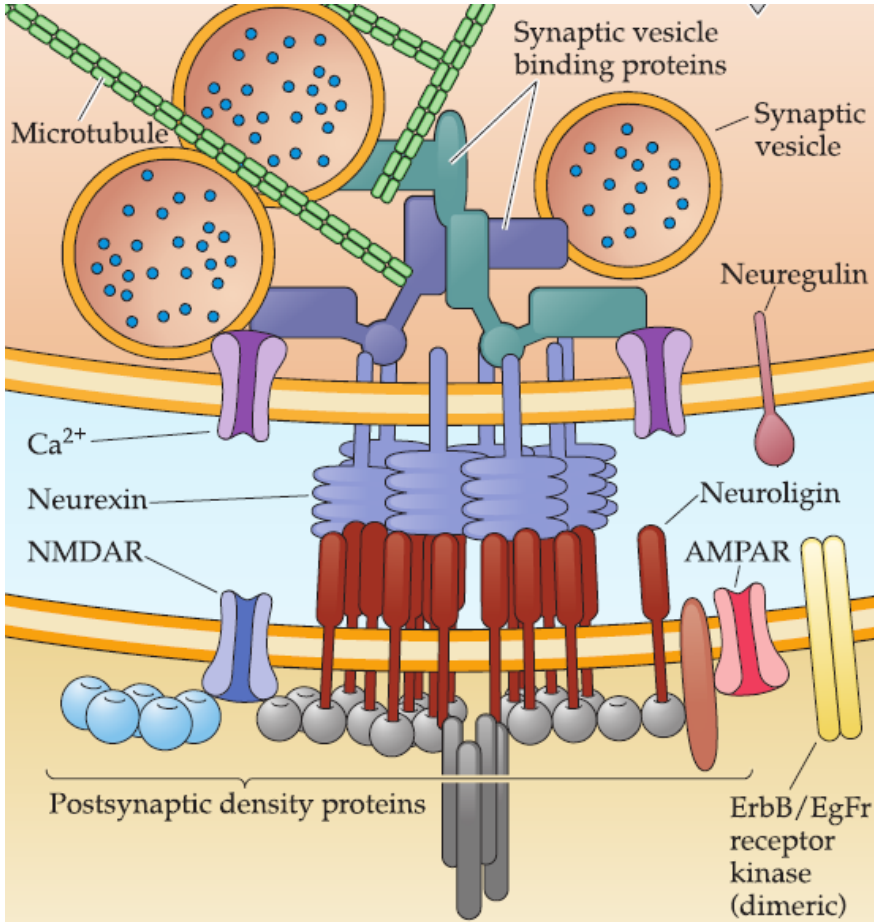
3. The interaction of **neurexin** (a presynaptic transmembrane adhesion protein) with **neuroligin** (a postsynaptic adhesion protein) is central for recruiting and retaining cytoskeletal elements that localize synaptic vesicles to the presynaptic terminal and mediate their fusion.

   - **Neurexin** have a specialized transmembrane domain that helps localize synaptic vesicles, docking proteins, and fusion molecules contributed by active zone vesicles in the presynaptic terminal.
   - **Neuroligin**, upon binding neurexin, is essential for localizing neurotransmitter receptors and postsynaptic proteins to the postsynaptic specialization.



Neuregulin1 (Nrg1)

- **Nrg1** is a transmembrane protein usually made in presynaptic cells and released following proteolytic cleavage of the ectodomain (outside portion) of the protein.
- Neuregulin is released via local proteolytic cleavage and binds to dimeric **ErbB receptor** kinases or to dimeric **ErbB/EGF**(epidermal growth factor) receptor kinases.
- Nrg1 signaling is thought to elicit increased synthesis and insertion of neurotransmitter receptors at a nascent postsynaptic site.



DSCAM1 in fly

- **DSCAM1:** named for its similarity to the mammalian *down* syndrome *c*ell *a*dhesion *m*olecule.
- **DSCAM1** has 38,000 isoforms based on the numbers of exons in the gene and predicted splicing.