

Pattern formation in the nervous system: neuronal identity and axon guidance

Reading: Chp 13: 424-440

Learning Goals Be able to:

Describe the general mechanisms by which axons find their targets, and give examples of kinds of molecules involved.

Explain how both inhibitory and excitatory cues can determine axonal pathfinding.

Interpret and design experiments that would demonstrate the importance of certain molecules in pathfinding.

Both developing neurons (neural crest, cortical neurons, etc) and their axons migrate to their final positions via some of the same mechanisms. Migration along the basal lamina involves interactions between extracellular matrix (ECM) components and surface receptors on either cells or axons. At the tip of the growing process is a growth cone, which is both the engine and the steering apparatus for outgrowth. It is a highly mobile lamellipodium, which appears to be feeling its way along as the axon grows.

Some of the molecules involved in migrations along basal lamina, as for non-neuronal migrations, are fibronectin and laminins in the ECM and integrins on cell surfaces. These molecules are believed to often offer relatively non-specific or short-range guidance since they are widely expressed in the developing nervous system, though there may well be exceptions.

Most growth cones follow paths pioneered by other neurons, so that bundles of axons called fascicles are formed as additional axons grow out toward a particular target. Thus, pathfinding by later axons can be mediated by homotypic interactions between the cells, involving N-CAM, specific cadherins, and fasciclins. However, the axon of the first neuron to follow a new pathway, called a pioneer neuron, cannot use this mechanism. Note that the problem faced by a pioneer axon, sometimes involving traversing a large distance and multiple different cellular environments is significant.

Many of the above molecules are widely expressed in the developing nervous system and are probably generic axon growth promoters, with little instructive information about pathfinding (but create a permissive region for axons to grow in). However, specific members of these gene families, or alternate splice forms, modifications, etc. may be important in guidance. What molecules are instructive in axon guidance?

Mechanisms of axon pathfinding

Chemotaxis (long range): the growth cone responds to an increasing concentration of a diffusible molecule along a certain pathway – this can be both attractive or repulsive.

Haptotaxis (short range): substrates (either cells or ECM) have differential adhesive specificity to receptors in the growth cone that allows for contact dependent movement.

What is the molecular nature of these specific cues? In all instances, the ligand is in the environment, while the receptor for that ligand is on the growth cone. In some cases though, the distinction between receptor and ligand seems to have been blurred (see Ephrins, below). In the schematic of axon guidance molecules, note that a number of domains are used widely in cellular recognition processes- EGF, Ig, Fibronectin type III, Semaphorin.

A little more detail on some of the molecules:

a) Netrins: can either attract or repel. Netrins are secreted ligands, bound by DCC/UNC5 receptors on the growth cone. Netrins act as chemoattractants in guiding commissural axons to the

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floor plate. In the brainstem, netrins act as chemorepellent to direct trochlear neurons away from the floor plate. (13.23.24.25)

b) Ephrins (transmembrane and lipid-anchored)- bound by Eph receptors. These receptors are members of the protein tyrosine kinase (RPTK) family: both ligands and receptors are membrane bound. (13.20)

The signal transduction cascades for these axon guidance systems are largely unknown (aside from the Ephs), but are being actively dissected at this time. In some way, these systems must regulate cytoskeletal and adhesive behavior to control the behavior of the growth cone. Overall, a combination of spatially restricted expression of guidance cues and their receptors permits selective axonal pathfinding.

Connections between the retina and the brain: an excellent example of axonal pathfinding

One of the best understood examples of how ordered connections to a specific brain region are established and refined comes from studies of how axons from vertebrate retinal neurons project to the brain. They must find their way to and synapse with the correct cells in the optic tectum (equivalent to the superior colliculus in mammals) of the visual cortex to produce an accurate visual map in the brain. It was demonstrated 50 years ago that these axons appear to "know" their destinations based on relative positions of cells in the retina, independently of where at the surface of the brain they start from. This was shown in a wonderful experiment by Roger Sperry in the 1960s in which the eye of a frog was removed (optic nerve, which contains axons from the eye to the tectum), and then replaced, but rotated 180 degrees. The frog's axons re-grew, so that it could still respond to visual input, but its perception was flipped 180 degrees to normal! We now know some of the factors that contribute to forming and refining these connections.

Both attractive and repulsive forces combine to enable the axons to specifically innervate some regions and not others. Axons are guided along pathways of molecules, and they grow better on some than others. In addition, many axons are guided by repellent rather than attractive substances. In the innervation of the tectum, there is a stereotyped pattern of connections between the retina and the tectum: Nasal retina—posterior tectum; Temporal retina—anterior tectum (13.33)

Before the molecular nature of this guidance was understood, experiments showed that there were repulsive molecules present in the tectum that nasal axons could grow on, but that temporal axons could not (13.34). When a growth cone contacts a "repulsive surface", it collapses. Eventually it grows in a different direction, turning to avoid repulsive molecules. The molecules now known to be responsible in the tectum are the Ephrins and Eph receptors. There are many ligands and receptors, expressed in gradients across both the retina and tectum. Receptor molecules are on the RGC axons, and the level of receptors corresponds to the region in the retina the axons originate from. Ligands are expressed in gradients in the tectum.

The model (13.35):

Ephrins and their receptors are found at high concentrations in temporal retina and posterior tectum; Ephrins and their receptors are found at low concentration in nasal retina and anterior tectum.

--The high concentration of receptors on T axons stops them as quickly as they encounter a few ligands at the A tectum (inhibitory interaction)

--Nasal axons (lower concentration of receptors) don't stop until they reach the higher Ephrin concentration in the posterior tectum.

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--Eph mutants: nasal axons go through tectum without making synapses. Overexpression of Eph receptors in the anterior tectum leads temporal axons to avoid these patches.

--The levels of Eph and EphR expression are positively regulated by transcription factors: Engrailed (en) in the posterior tectum; BF-1 in nasal retina, BF-2 in temporal retina.

Summary

Axon guidance encompasses many kinds of guidance molecules. Each axon contains a specific collection of various receptors for guidance molecules; thus different axons respond differentially to sets of guidance cues. In this way, axons are able to target very specific areas in a repeatable fashion. The retina extends axons to specific regions of the tectum via Ephrin signals, and a precise map is developed based on the axon's "net repulsion" in a particular region of the tectum. The Ephrin ligands (in the tectum) and the Ephrin receptors (on the retinal ganglion axons) guide each axon to a specific destination. This is just one example of the complex system that allows individual axons to target specific destinations.

Review Questions

1. What extracellular matrix components are important in axon extension?
2. How might cadherins and N-CAM be relevant to pathfinding by non-pioneer axons?
3. What are the distinctions between chemotaxis and haptotaxis?
4. What sort of assay was used to obtain evidence for netrin activity?
5. Describe the classic experiment in axon pathfinding (the frog eye rotation expt); why did the results suggest that the axons inherently knew where to go?
7. How was it determined that retinal axons had specificity for particular regions of the tectum, and what are the specificities?
8. Describe the assay used to identify the molecules involved in the retinotectal projection.
9. What are the molecules involved in directing retinal ganglion axons to the tectum, and how do they work? How is the expression of ephrins and their receptors regulated?