

Mechanisms of growth cone repulsion

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Abstract

Research conducted in the last century suggested that chemoattractants guide cells or their processes to appropriate locations during development. Today, we know that many of the molecules involved in cellular guidance can act as chemorepellents that prevent migration into inappropriate territories. Here, we review some of the early seminal experiments and our current understanding of the underlying molecular mechanisms.

Introduction and context

During development of multicellular animals, many cells or processes that emanate from them need to migrate from their sites of origin to other positions within the animal that are appropriate for their eventual functions. For example, the neural crest cells that later generate the peripheral nervous system and other derivatives migrate from the dorsal neural tube along precise pathways to specific locations within the embryo. Similarly, the axons and dendrites of both central and peripheral neurons often migrate long distances to reach appropriate synaptic partners. Understanding the forces that drive normal development requires learning the cellular and molecular mechanisms by which migrating cells or their processes are guided to appropriate target locations.

Research from the last century by luminaries such as Santiago Ramon y Cajal and Roger Sperry led to the idea that the growing tips of nerve cell axons, called growth cones, are guided to their targets by a process of chemoaffinity [1]. For example, Sperry and his colleagues disrupted the retina in a variety of animals in which retinal axons can regenerate and found that regenerating retinal axons grew back to their appropriate targets in the optic tectum. From these experiments, Sperry proposed that there are attractive signals that guide axons toward appropriate synaptic partners. He further suggested that chemoaffinity was not unique to the retino-tectal system but constituted a general mechanism of axon guidance.

During the ensuing search for molecular mechanisms underlying retino-tectal chemoaffinity, culture experiments from the mid-1980s revealed, surprisingly, that guidance of retinal axons involves repulsive interactions. For example, Friedrich Bonhoeffer and colleagues established a 'stripe assay' in which retinal axons chose between growing on adjacent stripes of membranes derived from either anterior or posterior tectal cells [2,3]. The results showed clearly that temporal retinal axons were repelled by posterior tectal cell membranes. Isolation and further study of the responsible molecule, called ephrin, revealed that it is a member of a family that can promote attraction or repulsion and is involved in many normal and pathological processes [4,5].

Are repulsive interactions specific to retinal axons, or are they common during development? Experiments done by Roger Keynes, Kathryn Tosney, and their collaborators suggested that repulsive interactions are a common mechanism for guiding migration. These experiments revealed that migration of motor axons and neural crest cells through the somite is essentially nature's 'stripe assay'. The segmental arrangement of motor axons and migrating neural crest cells arises because these cells are repelled by posterior somites and thus migrate only through anterior somites [6] (reviewed in [7]).

Other hints about cellular mechanisms underlying axon repulsion came from experiments in the lab of Jonathan

Raper. These studies revealed that when retinal and sympathetic explants were cultured together, growth cones that encountered axons of the other cell type typically lost motility, collapsed, and sometimes began growing in a different direction [8]. The lab isolated a signal capable of causing growth cone collapse from brain membrane extracts in the early 1990s and named it collapsin [9]. Interestingly, collapsin caused the collapse of dorsal root ganglion neuron growth cones; dorsal root ganglion neurons, like sympathetic neurons, are derived from the neural crest. But collapsin did not cause the collapse of retinal growth cones. Collapsin was later found to be a member of a family of signaling molecules called semaphorins, which are now known to regulate a myriad of normal and pathological processes (for recent reviews, see [10-12]).

The many functions of both semaphorins and ephrins have been extensively reviewed [4,5,10-12]. Here, we focus on several recent discoveries about semaphorin 3A (the original collapsin-1; *Sema3A*) [13] and ephrins [14] which provide new insights into the molecular mechanisms underlying growth cone repulsion and how these result in proper nervous system wiring.

Major recent advances

It has been known for some time that the ability of *Sema3A* to repel growth cones of cultured *Xenopus laevis* spinal neurons can be converted to attraction by activation of cyclic nucleotides [15]. Growth cone collapse, retraction, and turning all require cytoskeletal reorganization that depends on regulation of intracellular calcium levels that are mediated through Rho family GTPases [16]. What is the relationship between *Sema3A* signaling, cyclic nucleotides, intracellular calcium levels, and growth cone repulsion or attraction? Recent studies from the lab of Kyonsoo Hong using cultured *X. laevis* spinal neurons provide important new insights into this question. In an elegant series of experiments, Nishiyama and colleagues [17] discovered that *Sema3A* not only repels growth cones, but also causes their membranes to become hyperpolarized, whereas attractants such as Netrin and brain-derived neurotrophic factor cause membrane depolarization. Growth cone membrane hyperpolarization requires neuropilin, one of the major classes of semaphorin receptors which often acts together in a complex with the plexin class of receptors [10,12,18-20]. This study [17] showed that growth cone membrane hyperpolarization is mediated by chloride entry through channels that are activated by a small increase in intracellular cyclic guanosine monophosphate (cGMP) that follows *Sema3A*-mediated receptor activation. Membrane hyperpolarization leads to a small elevation of intracellular

calcium on the side of the growth cone exposed to *Sema3A* and to repulsion of the growth cone from the *Sema3A* source. Importantly, larger increases in intracellular cGMP lead to activation of protein kinase G, which depolarizes the growth cone membrane by activating sodium channels, leading to higher levels of intracellular calcium and converting repulsion to attraction. Thus, changes in levels of cGMP regulate shifts in membrane potential that act as a switch between repulsion and attraction by gating calcium entry into the growth cone.

Neuronal activity and cyclic nucleotide levels have also been shown to be important regulators of ephrin signaling in an *in vitro* model of retino-tectal map formation developed by Patricia Gaspar and her colleagues [21]. Like semaphorin signaling, signaling by ephrins and their Eph receptors can be either attractive or repulsive and is often mediated through Rho GTPase family effects on the cytoskeleton [4,5]. This study [21] found that oscillations in spontaneous activity are needed for the ephrin-mediated repulsive interactions that cause the elimination of exuberant retinal axons. Retinal growth cones normally collapse and then retract in response to ephrin-A5; however, these responses are prevented by blocking sodium channels with tetrodotoxin. Cyclic adenosine monophosphate (cAMP) can rescue this blockade, but only if it is administered in a periodic fashion, mimicking normal cAMP oscillations.

These and other studies from culture systems suggest that extension to correct targets requires exquisite regulation of growth cone sensitivity to molecules that can act either as repellents or as attractants. What regulates growth cone sensitivity and how does this translate into appropriate pathfinding? Several recent studies provide important new perspectives on this question. Chick motoneurons express the *Sema3A* receptor, neuropilin-1, and respond to *Sema3A* from surrounding tissues. Work from the lab of Valerie Castellani showed, surprisingly, that specific populations of chick motoneurons also express *Sema3A* [22]. Using gain-of-function and loss-of-function experiments, Moret and colleagues [22] found that intrinsic *Sema3A* decreases the availability of neuropilin-1 at the growth cone membrane, thereby decreasing the sensitivity of motor growth cones to extrinsic *Sema3A*. This decreased sensitivity enables the growth cones to extend into a region bordered by tissues that express high levels of *Sema3A*, rather than being entirely repelled from this region, and thus to extend to specific muscle targets. Similarly, interactions between ephrins and Ephs expressed by the same cell have been shown to modulate the response to ephrin-mediated repulsion in cultured retinal axons [4,23].

Axons of olfactory neurons also have to extend to specific targets and to form a map in the olfactory bulb, although it is different from the topographic map formed by retinal axons in the optic tectum in which axons from a particular part of the retina extend to a specific region of the tectum. In the olfactory map, olfactory neurons each express a single odorant receptor; cells expressing the same receptor can be located anywhere within the olfactory epithelium but their axons all terminate in the same position of the olfactory bulb. New research from the lab of Hitoshi Sakano showed that, in mice, odorant receptors expressed by olfactory neurons regulate production of cAMP and that this in turn regulates expression levels of neuropilin-1 and Sema3A in olfactory growth cones [24]. Thus, axons that express the same odorant receptor also have the same neuropilin or Sema3A expression pattern. Repulsive interactions among neuropilin-expressing and Sema3A-expressing growth cones during pathfinding result in presorting of the axons before they reach their targets, so that axons of cells expressing the same odorant receptor travel together. Sema3A is also expressed by some of the cells surrounding the axons during pathfinding, and this may contribute to proper establishment of the topographic map. A similar mechanism is used during sorting of olfactory axons in fruit flies [25,26], suggesting that this could be a general mechanism for map formation.

Earlier work from the lab of Richard Axel showed that ephrin signaling is involved in formation of the olfactory map [27]. Subpopulations of olfactory neurons that express different olfactory receptors also differentially express ephrin-A5 or ephrin-A3 on their axons. Altering ephrin levels alters formation of the olfactory map; thus, like semaphorins, ephrins are critical for map formation. More recently, elegant work from the lab of Hitoshi Sakano has shown that, like semaphorins, ephrins form part of a neuronal identity code for targeting olfactory axons to the appropriate position in the olfactory bulb [28]. In this case, ephrin-A5 and its receptor, EphA5, are expressed in complementary patterns in axon terminals. These expression patterns are correlated with expression of the cyclic nucleotide-gated channel gene A2 (CNGA2), suggesting that the ephrin and Eph levels are regulated by neuronal activity.

Future directions

Like any groundbreaking research, these studies raise a host of important questions. For example, what is the relationship between semaphorin signaling and ephrin signaling during formation of the olfactory map? How common is it for a cell to regulate sensitivity to a ligand by co-expressing the ligand and the receptor? In addition to semaphorins and ephrins, there are other repellents,

such as slits, that have also been demonstrated to be either repulsive or attractive. Similarly, there are attractants, such as netrins, that have been demonstrated to be either attractive or repulsive. How common is it for this switch between repulsion and attraction to be regulated by activity-mediated changes in membrane polarization? We look forward to learning the answers to these questions from future studies.

Abbreviations

cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; Sema3A, semaphorin 3A.

Competing interests

The authors declare that they have no competing interests.

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