Sonic Hedgehog gets another role

Catherine E Krull

A study in this issue finds that the chemoattractant cue, Sonic Hedgehog, can activate a repulsive response of commissural axonal pathfinding to Semaphorins, thereby acting as a 'switch' in axon guidance.

Proper wiring of the brain requires a careful orchestration of various attractive and repulsive guidance cues to which pathfinding axons must wade through with precision. For example, commissural axons in the developing spinal cord must move toward the ventral midline along the ventral-dorsal axis and then cross over to the contralateral side for longitudinal growth along the anterior-posterior axis. One of the molecules that entices growing axons toward its gradient is Sonic Hedgehog (Shh)[1], which is secreted by the cells in the midline floor plate[2]. Parra and Zou[3] found that Shh has another role after midline crossing. Surprisingly, it induces repulsion by switching on the repulsive or inhibitory activities of two Semaphorins, 3B and 3F. This finding links a morphogen/chemoattractive molecule (Shh) with chemorepulsive molecules (Semaphorins), whereby Shh acts as a switch in regulating the actions of other guidance molecules.

Shh and its signaling pathway have been well studied. It acts as an inducer of ventral cell types, including motoneurons in the developing spinal cord[4], and is important for determining the correct number of digits in the developing hand[5]. For commissural axons, floor plate chemoattractants such as Netrin and Shh attract the initial axonal projection toward the ventral midline[1]. Shh then binds to its receptor Patched-1, which in turn regulates the G protein–coupled receptor Smoothened to mediate chemoattraction[1]. After midline crossing, the commissural axons normally make a sharp turn toward the anterior and either project into a structure called the ventral funiculus alongside the floor plate or spread out in a highly organized way to grow laterally into the lateral funiculus. At this time, the axons of commissural neurons acquire responsiveness to the class 3 Semaphorins and Slits, which are known inhibitors of axonal pathfinding, thus regulating axonal exit from the floor plate and restricting now-crossed axons along the anterior-posterior axis of the spinal cord[6].

In addition to its chemoattractant role in commissural axon guidance, Parra and Zou[3] found that Shh signaling can orchestrate the subsequent chemorepulsion response mediated by the Semaphorin signaling pathway. They used an 'open-book' explant system, a type of *in vivo*/*in vitro* hybrid system that allowed for the examination of mechanisms of commissural axonal guidance[3]. This experimental system uses a rat embryonic day 13 (E13) spinal cord whose roof plate has been cut open along the anterior-posterior axis so that the tubal structure of the spinal cord is 'opened' in a two-dimensional plane. This then allows for the visualization of three-dimensional growth of commissural axons (that is, first along the dorsal-ventral axis, then along the anterior-posterior axis) in two-dimensional space that can be imaged and quantified readily. Using this preparation, they disrupted Shh signaling pathway by expressing a dominant-active form of Patched-1 that lacks the Shh binding domain and constitutively inhibits Smoothened. They also knocked down Smoothened itself by shRNA electroporation *in utero* using the open-book preparation. In all cases, the axonal projections of commissural neurons were severely defective. One can even say commissural axons grew out willy-nilly under these conditions; they were stalled at the midline, tied in tortuous knots and sometimes were found backtracking toward their origin or overshooting/defasciculating after midline crossing.

Using a Shh function-blocking antibody, 5E1, the authors found that decreasing Shh signaling itself facilitated the repulsion induced by the midline chemorepellent Semaphorins. Commissural neurons lie in the dorsal margin of the developing spinal cord. At E13 in rats, many anterior axons from commissural neurons have reached and crossed the midline, whereas more posterior populations of axons are still growing toward or just starting to cross the floor plate. The authors used the dorsal-most portion of the developing spinal cords at hindlimb levels to get young, pre-crossing commissural axons. They first used a pre-crossing collagen explant assay to test whether any of the diffusible signaling molecules in the floor plate could activate Semaphorin repulsion in pre-crossing commissural axons. Parra and Zou[3] found that, in the presence of a known chemoattractant (Netrin-1), axons did not turn away from the Semaphorin 3F source unless the neurons were

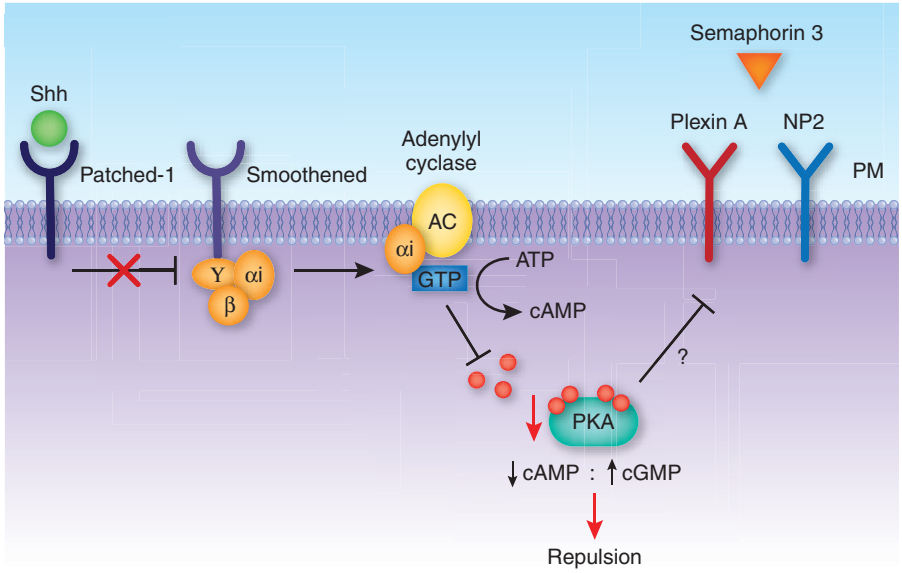

Figure 1 Schematic diagram showing the Shh signaling pathway (adapted from ref. 3). Shh binds it receptor, Patched-1, which then allows Smoothened to activate adenyl cyclase (AC) and inhibit or reduce PKA. PKA either directly or indirectly inhibits the receptors for Semaphorins (PlexinA and Neuropilin2 (NP2)), leading to repulsion. PM, plasma membrane.

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grown on a bed of Shh-expressing COS cells. What the authors saw was a true repulsion. Without Shh, axons did not bend away from the Semaphorin sources, consistent with the authors' previous observations[6]. Tracing the trajectory of commissural axons using the lipophilic dye DiI injected into the dorsal part of the developing spinal cord, Parra and Zou[3] then found that most commissural axons stalled at the midline as if they could not leave the midline properly or overshot past the anterior-posterior axis when the Shh function-blocking antibody was added. As with the Patched-1 constitutive-active constructs or Smoothened disruptions, commissural axons exhibited unbundled behavior from their normal trajectory and failed to respond to Semaphorins.

Finally, Parra and Zou[3] examined whether cyclic nucleotides are important for this phenomenon. PKA activity has been shown to be coupled with Semaphorin-plexin repulsion *in vivo*[7]. First, Parra and Zou[3] examined whether cAMP/PKA activity was needed for proper midline axon pathfinding. Explant cultures were treated with the adenyl cyclase activator forskolin, which increases the level of cAMP; this manipulation caused stalling defects on commissural axons, many of which formed knots, whereas others wandered or turned widely in a similar fashion as seen with disrupted Shh signaling. To test their idea that reduced PKA activity is required for inducing responsiveness to class 3 Semaphorins by Shh, the authors returned to their assay of pre-crossing commissural axons in collagen gels. They found that Shh-induced Semaphorin repulsion was indeed reduced in the presence of forskolin. These findings suggest that Shh regulates the sensitivity of growth cones to Semaphorins by regulating cAMP levels. Moreover, these findings are, to the best of our knowledge, the first example of a diffusible morphogen/chemoattractant acting as a switch for other more repulsive or inhibitory axon guidance molecules.

This study brings up a whole host of interesting avenues to explore (**Fig. 1**). Does downregulating cAMP/PKA signaling affect the function of other molecules involved in silencing Semaphorin signaling, such as the A-kinase anchor proteins (AKAPs)? Answering this question would determine whether cAMP/PKA acts directly on Semaphorins or indirectly through AKAPs. Are plexins and neuropilins (known receptors for Semaphorins) involved and how do they work mechanistically? In addition, what is the timing of the switch that gets turned on after commissural axons cross the midline so that ipsilateral axons aren't responsive to Semaphorins before crossing? Stay tuned, as there is more to come about Shh.

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Polar exploration

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The frontal pole cortex is thought to be the most complex of all frontal cortex areas. Overcoming technical obstacles to direct recordings, a study in this issue finds that neurons in this area have unexpectedly simple response properties.

The polar explorer Ernest Shackleton said "Difficulties are just things to overcome, after all." The last decade has seen rapid progress in our understanding of the functional organization of the frontal lobe. Much of our knowledge of this organization has come from studies recording the electrical activity of single neurons in awake, behaving monkeys. One area, however, has resisted exploration. The frontal pole (Brodmann area 10) is the most anterior region of the frontal cortex (**Fig. 1**). Until now, no one has been able to record neuronal activity from this area because it lies underneath a bony air sinus. In this issue, Tsujimoto *et al.*[1] treated this difficulty as just an obstacle to overcome and their intrepid exploring has produced some surprising results. They found that frontal pole neurons encoded a subject's earlier decision, but only at the time when the subject was receiving feedback as to whether they had made the correct response.

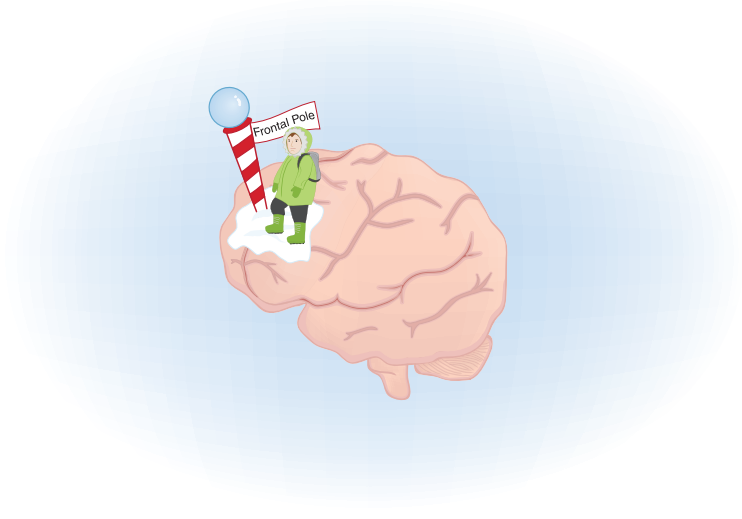

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Figure 1 The frontal pole cortex (Brodmann area 10) is the most anterior region of the frontal lobe.

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Integral to understanding the organization of the frontal cortex has been the idea of hierarchy. As the complexity of cognitive processes and behaviors increases, control is thought to shift toward progressively more anterior regions of the frontal cortex[2].