Developmental Biology (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Review article

The spinal cord shows the way – How axons navigate intermediate targets

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ARTICLE INFO

Keywords:
Axon guidance
Commissural neurons
Midline crossing
Neural development

ABSTRACT

Functional neural circuits depend on the establishment of specific connections between neurons and their target cells. To this end, many axons have to travel long distances to reach their target cells during development. Studies addressing the molecular mechanisms of axon guidance have to overcome the complexity of subpopulation-specific requirements with respect to pathways, guidance cues, and target recognition. Compared to the brain, the relatively simple structure of the spinal cord provides an advantage for experimental studies of axon guidance mechanisms. Therefore, the so far best understood model for axon guidance is the dI1 population of dorsal interneurons of the spinal cord. They extend their axons ventrally towards the floor plate. After midline crossing, they turn rostrally along the contralateral floor-plate border. Despite the fact that the trajectory of dI1 axons seems to be rather simple, the number of axon guidance molecules involved in the decisions taken by these axons is bewildering. Because guidance molecules and mechanisms are conserved throughout the developing nervous system, we can generalize what we have learned about the navigation of the floor plate as an intermediate target for commissural axons to the brain.

1. Introduction

The summary of what we have learned as a field using the dI1 axons as a model has been assembled into the current understanding of axon guidance mechanisms. Both the general mechanisms as well as the function of individual guidance molecules have been confirmed in other areas of the nervous system, both the central as well as the peripheral nervous system.

Axons navigate by integrating the signals derived from interactions between surface receptors on the axon tip, the growth cone, and guidance molecules in their environment (Kolodkin and Tessier-Lavigne, 2011). These guidance cues can be perceived by the growth cone as attractive or repulsive depending on the type of receptor expressed on the surface. To make navigation over long distances possible, or more reliable, axons use different strategies. One strategy is to divide the entire trajectory into shorter segments, each ending with an intermediate target. Obviously, intermediate targets are initially attractive for growth cones but upon contact the growth cones change their behavior. Due to a change in the perception of the intermediate target, the axons exit the intermediate target instead of forming synaptic contacts. The change in behavior is triggered by a change in the repertoire of guidance receptors expressed on the growth cone surface. Another strategy used by growth cones to make long-distance navigation less error-prone is fasciculation. Growing axons team up by bundling with each other along common parts of the trajectory. This way, individual axons are less likely to take erroneous decisions on their way to the target.

In the past, a major challenge was the identification of guidance molecules and their receptors on growth cones (Tessier-Lavigne and Goodman, 1996; Dickson, 2002; Kolodkin and Tessier-Lavigne, 2011). Today the focus has shifted to studies analyzing the temporal and spatial regulation of guidance cues and their receptors, as well as the crosstalk between different signaling pathways. Obviously, the details of temporal and spatial regulation of guidance receptors have to be studied in a context-specific manner, as individual axonal populations need to respond differently to a give intermediate target. In this review, we will provide an overview of the current understanding of axon guidance mechanisms by describing the molecular cues used by dI1 commissural axons.

2. The floor plate is the intermediate target of dI1 axons

The dI1 population of commissural neurons represents a convenient model for axonal navigation, as the initial trajectory is easy to follow experimentally. Axons grow from the dorsal spinal cord towards the floor plate, their intermediate target. Axons cross the ventral midline without delay and turn rostrally along the contralateral floor-plate border. Along this trajectory they are guided by both repulsive

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http://dx.doi.org/10.1016/j.ydbio.2016.12.002
Received 20 July 2016; Received in revised form 26 November 2016; Accepted 1 December 2016

and attractive guidance cues. These cues can be subdivided into long- and short-range guidance cues. By definition long-range guidance cues are secreted and act upon the navigating growth cone to either attract it towards the intermediate target or to repel it from a non-target. Long-range guidance cues provide directional information without specifying the actual pathway of the axons. Axons select a specific pathway based on the interaction between guidance receptors on their tip and short-range guidance cues expressed on cells along their trajectory.

The floor plate as the intermediate target of dI1 axons is a major source of both long- and short-range guidance cues. In contrast to the final target of dI1 axons, which is still ill-defined (Sakai et al., 2012), the guidance mechanisms and guidance cues involved in the navigation of the intermediate target have been characterized in great detail.

3. Long-range axon guidance cues direct pre-crossing axons towards their intermediate target, the floor plate

For dI1 axons both long- and short-range guidance cues have been characterized. Netrin1 was the first long-range axon guidance cue that was identified. Netrin1 is secreted by the floor plate and acts as a long-range attractant for dI1 axons (Kennedy et al., 1994; Serafini et al., 1996; Bin et al., 2015; Yung et al., 2015). The attractive effect of Netrin1 is mediated by Deleted in Colorectal Cancer (DCC; Keino-Masu et al., 1996) and Neogenin (Srinivasan et al., 2003), two closely related transmembrane protein of the immunoglobulin superfamily of cell adhesion molecules (IgSF-CAMs). Neogenin not only acts as a receptor for the repulsive axon guidance cue RGM but also mediates axonal attraction to Netrin in zebrafish (Wilson and Key, 2007, 2006). In chick, Neogenin appears to be the major Netrin receptor as no DCC gene has been found (Phan et al., 2011).

In addition to DCC, DSCAM has been proposed as another receptor for Netrin1 in flies (Andrews et al., 2008) and in vertebrates (Ly et al., 2008). Although DSCAM can bind to Netrin1, its role as a Netrin receptor mediating the attractive response of dI1 axons has not been substantiated. While some in vitro experiments indicated growth impairment and aberrant turning responses of commissural axons in the absence of DSCAM (Ly et al., 2008), no effect on commissural axon outgrowth and guidance was found in *Dscam*-null mice (Palmesino et al., 2012).

Netrin1 appears to be the major chemoattractant for dI1 commissural axons but it may not be the only one. The detailed analysis of the phenotype in the initial mouse model for Netrin loss-of-function revealed residual axon extension towards the floor plate. However, this was attributed to the fact that this mouse was a hypomorph rather than a complete knockout of the *Netrin* gene. Recently, the role of Netrin in dI1 guidance has been reanalyzed in complete knockout mice (Bin et al., 2015; Yung et al., 2015). In these mice, no axons crossed the midline, clearly indicating that Netrin is the major long-range attractant for pre-crossing axons.

Still, there is evidence for additional factors contributing to the attractive effect of the floor plate on pre-crossing commissural axons. Studies in the hypomorphic *Netrin* background indicated a role of Shh signaling in long-range attraction to the floor plate (Fig. 1; Charron et al., 2003). In contrast to Shh's role as a morphogen, its activity as a long-range guidance cue for pre-crossing commissural axons was shown to be transcription independent and to be mediated by the activation of Src family kinases (SFKs; Yam et al., 2009). It was proposed that in pre-crossing axons Boc and Patched would mediate the attractive role of Shh (Okada et al., 2006).

More recently, Vascular Endothelial Growth Factor (VEGF), well known as a strong angiogenic factor, was identified as a long-range chemoattractant in the spinal cord (Ruiz de Almodovar et al., 2011) and in the visual system (Erskine et al., 2011). For dI1 axons Flk1 was identified as the receptor mediating the attractive effect of VEGF by activating Src family kinases similar to Netrin and Shh (Fig. 1; Ruiz de Almodovar et al., 2011).

Commissural axons are not only attracted towards the intermediate target they are also repelled by the roof plate. BMPs (Bone Morphogenetic Proteins) induce dorsal interneurons (Liem et al., 1997), and later also affect the initial trajectory of their axons (Augsburger et al., 1999). Under the influence of roof-plate derived BMP7, dI1 axons extend ventrally towards their intermediate target, the floor plate. Both gain- and loss-of-function approaches showed that the type-I BMP receptors, apart from their function in the specification of cell fate, also mediate commissural axon outgrowth and guidance mainly via BMPRIB. BMP homodimers induce transcription, whereas the heterodimer between BMP7 and GDF7 mediates a guidance signal in the growth cone by a non-transcriptional mechanism (Yamauchi et al., 2008; Augsburger et al., 1999; Fig. 1).

After years without discovering new molecules, Draxin (dorsal repulsive axon guidance protein) was identified as a chemorepulsive axon guidance molecule for dI1 commissural neurons (Islam et al., 2009; Fig. 1). Draxin is not only expressed in the dorsal spinal cord, it is also required for the formation of commissures in the brain. The mechanism of action is not completely understood, but Draxin can bind to multiple Netrin receptors: Deleted in Colorectal Cancer (DCC), Neogenin, UNC5 family members, and Down Syndrome Cell Adhesion Molecule (DSCAM). Of all those, Draxin binds with the highest affinity to DCC but at a different binding site than Netrin. Hence, DCC is a convergent receptor for Netrin and Draxin in long-range axon guidance (Ahmed et al., 2011). In the *Draxin* knockout mouse commissural axons projected toward the floor plate in a defasciculated manner, likely due to a decrease in the repulsive activity in their environment (Islam et al., 2009). In addition, in vitro, Draxin was found to interact directly with Netrin and, therefore modulate Netrin function by competition with Netrin receptors (Gao et al., 2015).

4. Short-range guidance cues are required for midline crossing

Once dI1 axons have reached the floor plate, they enter the floor plate area to cross the midline due to positive interactions derived from cell adhesion molecules, expressed on the growth cone and the floor-plate cell surface. Cell adhesion molecules of the immunoglobulin superfamily (IgSF-CAMs) described to play crucial roles in axon-axon and axon-floor plate contacts were L1/NgCAM, NrCAM, and Contactin2 (also known as Axonin1 or TAG1; Stoeckli and Landmesser, 1995; Stoeckli et al., 1997). Contactin2/Axonin1, expressed on the growth cone of commissural axons, interacts with NrCAM, expressed on floor-plate cells, to promote midline crossing. In addition, Contactin2/Axonin1 also binds NgCAM in cis (in the plane of the same membrane) and trans (between two membranes) (Kunz et al., 1998). The different interactions were shown to modulate downstream signaling (Kunz et al., 1996). In line with its molecular interaction pattern Contactin2/Axonin1 was shown to be required for pathway choice but not growth of commissural axons at the floor plate (Fitzli et al., 2000).

5. Navigating the intermediate target: Midline crossing

Once the growing axons reach the intermediate target, where long-range and short-range attractant concentrations are maximal, the growth cones must lose responsiveness to attractive signals in order to continue their growth towards their final destinations (Fig. 2). A switch in responsiveness is achieved by a change in surface receptor expression. To this end, both loss of pre-existing receptors and expression of novel receptors is possible, but the latter is much more prevalent. In any case, tight temporal regulation of the switch in surface receptors is required to ensure smooth navigation of axons. The molecular mechanisms used to control surface expression of receptors are diverse. Commissural axons lose responsiveness to Netrin upon crossing the midline by up-regulating members of the Roundabout

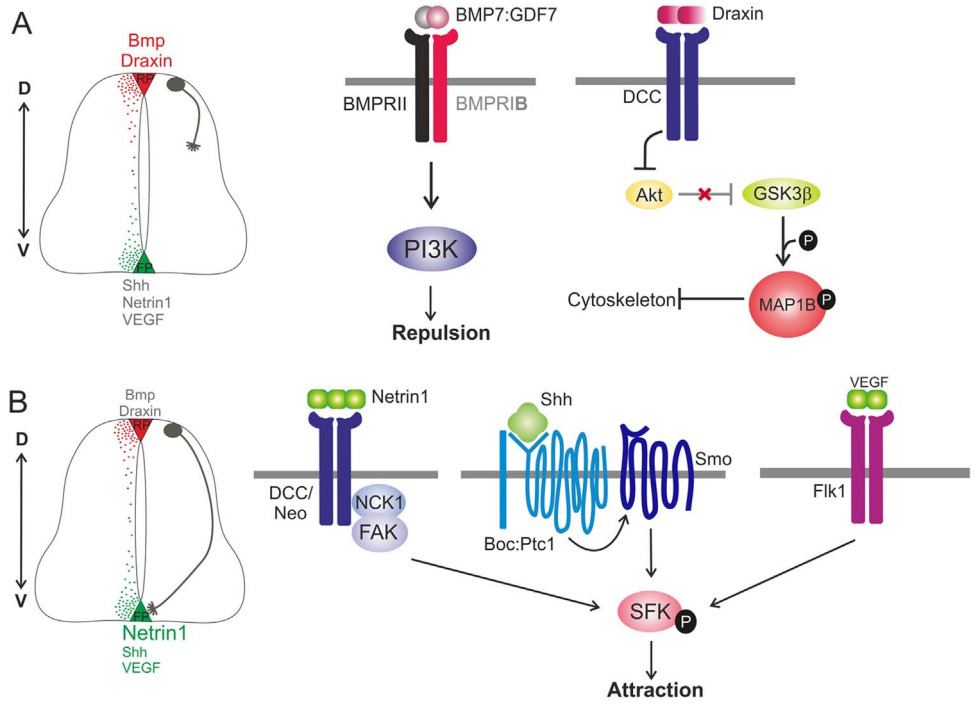

Fig. 1. Long-range guidance cues outline the intermediate target. Axons of dI1 commissural neurons are directed to the floor plate by long-range repellents (A) and long-range attractants (B). Long-range repellents BMP7 and Draxin are derived from the roof plate (red triangle), while the long-range attractants Netrin, Shh, and VEGF are derived from the floor plate (green triangle). (A) The heterodimer BMP7:GDF7 interacts with the receptor complex BMPRII:BMPRIB to mediate the repulsive signal via PI3K. Draxin interacts with DCC on commissural axons resulting in the activation of Akt. Active Akt phosphorylates and thereby inactivates GSK3β preventing it from phosphorylating MAP1B (Meli et al., 2015). The phosphorylation status of Map1B affects cytoskeleton dynamics. (B) The attractive effect of Netrin1, the major chemoattractant secreted by the floor plate, is mediated by DCC or Neogenin. Shh acts as an attractant for pre-crossing commissural axons by a transcription-independent mechanism. VEGF mediates attraction by binding to Flk1 receptors. In all cases, attraction is suggested to include the activation of Src family kinases (SFKs; Ruiz de Almodovar et al., 2011). Note that all the downstream signaling pathways linked to long-range attractants and repellents are not specific to any of the ligand-receptor interactions. Additional studies will have to be done to explain the specificity of the effect.

(Robo) family, which sense the floor plate-associated repellent Slit (see below). A cis-interaction between Robo and DCC silences the attraction by Netrin (Shirasaki et al., 1998; Stein and Tessier-Lavigne, 2001). Similarly, the attractive effect of Shh is overcome by the expression of a new receptor, Hhip (Hedgehog-interacting protein; see below).

Thus, changing guidance receptors is the key to floor-plate navigation. Upon reaching the floor plate, commissural axons use transcriptional and post-translational mechanisms to ensure proper regulation

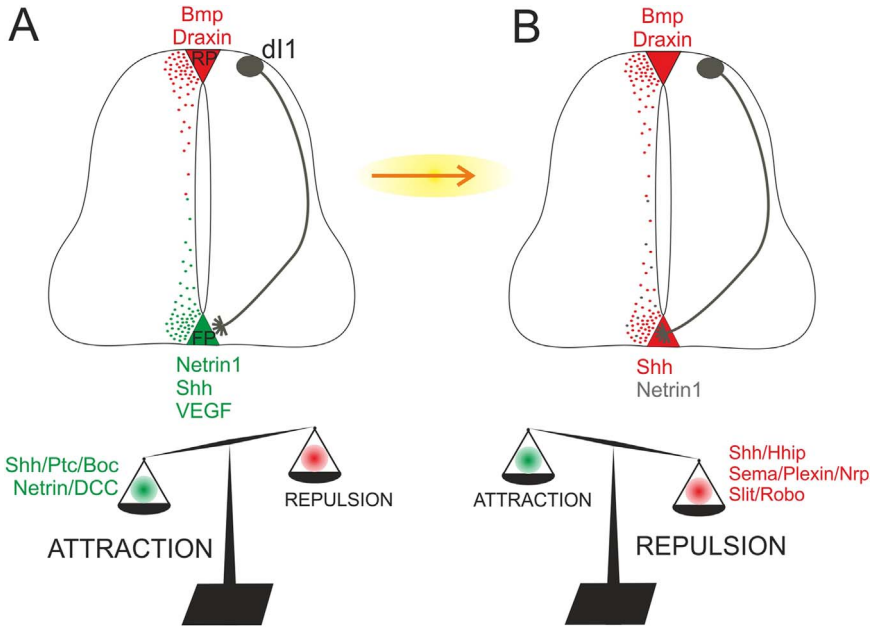

Fig. 2. Navigating the midline requires a switch from attraction to repulsion. Pre-crossing axons are attracted to the intermediate target, the floor plate due to a prevalence of positive signals derived from the interaction between long- and short-range attractants and the respective receptors on the growth cone (A). Repulsive cues associated with the floor plate cannot be detected, as the receptors for these cues are not available on the growth cone surface. Upon arrival of the axon at the intermediate target (B), surface receptors on the growth cone change, repulsive cues are now detectable and result in the expulsion of the growth cone from the floor plate. The regulation of the availability of guidance cue receptors on the growth cone surface is extremely complex and includes transcriptional, translational, as well as post-translational mechanisms (see text for details).

of guidance receptors on the growth cone surface. For midline crossing in the visual system miRNA-based regulation of translation has also been described (Baudet et al., 2011). In general, the precisely controlled timing of surface receptor expression allows for the detection of repulsive cues only after reaching the floor plate. These negative signals expel the axons from the floor plate and, thus, not only complete midline crossing but they also prevent re-crossing (Fig. 2).

One of the major repulsive midline-associated cues both in vertebrates and invertebrates is Slit (Brose et al., 1999; Kidd et al., 1999). There are three Slit proteins in vertebrates, all of them expressed by the floor plate (Long et al., 2004; Sabatier et al., 2004). The receptors for Slits are Roundabouts (Robos), a family of four proteins: Robo1, Robo2, Robo3 (also called Rig-1) and Robo4 (or Magic Roundabout). Robo4 has a different domain structure and is specific for endothelial cells (Huminiecki et al., 2002).

Loss of the only Slit in flies results in axons that fail to leave the midline (Kidd et al., 1999). In vertebrates, midline crossing is significantly affected only in the absence of all three Slits (Long et al., 2004). Loss-of-function phenotypes for vertebrate Robos are more complex than those for Slit (Chen et al., 2008; Long et al., 2004; Sabatier et al., 2004; Zelina et al., 2014).

In both vertebrates and invertebrates, Robo surface expression needs to be tightly regulated to prevent premature sensitivity to Slit, which would interfere with midline crossing. Interestingly, the mechanism used to regulate Robo surface expression differs between flies and vertebrates but regulation is at the post-translational level in both cases. In flies, Robo surface expression is regulated by Comm (Commissureless; Kidd et al., 1998). Comm is transiently expressed in contralaterally, but not ipsilaterally, projecting neurons. According to the sorting model (Keleman et al., 2002, 2005), Comm controls surface expression of Robo before midline contact by preventing its insertion into the growth cone membrane. Instead, Robo is transported directly to the endosomal-lysosomal compartment. Thus, in the presence of Comm, the growth cone cannot sense the midline repellent Slit, and axons are able to cross the midline. After crossing, Comm is down-regulated by an unknown mechanism, resulting in continued Robo surface expression.

In vertebrates, regulation of Robo surface expression could not be explained by Comm, as no ortholog was found in vertebrate genomes. Instead, different mechanisms have been demonstrated to regulate Robo1-mediated repulsion to Slits at the post-translational level.

In contrast to Robo1, which was found only at low levels on pre-crossing axons, Robo3 was highly expressed on pre- and post-crossing commissural axons (Sabatier et al., 2004). In *Robo3* knockout mice all Contactin2-positive commissural neurons failed to cross the midline. This phenotype was explained by a cis-interaction between Robo3 and Robo1 in the growth cone membrane. Robo3 occurs in different isoforms: Robo3.1 was suggested to prevent premature sensitivity to repulsive Slits associated with the floor plate by cis-binding to the low levels of Robo1 found on the growth cone of pre-crossing axons (Chen et al., 2008). Robo1 on post-crossing axons was no longer prevented from binding to Slits because Robo3.1 was replaced by Robo3.2 by an unknown mechanism.

A role for Robo3 that differed from the role of Robo1 and Robo2 in midline crossing was confirmed more recently (Zelina et al., 2014). The function of Robo3 was found to have changed during evolution. In mammals, Robo3 lost its ability to bind to Slits. Robo3 levels were increased in the presence of Netrin1, an effect that was not seen with non-mammalian Robo3. Instead, mammalian Robo3 was found to bind to Dcc and, thus, contribute to Netrin-mediated attraction to the floor plate. A recent screen for proteins interacting with Robo3 identified Nell2 (neural epidermal growth factor-like-like 2), a secreted glycoprotein with some structural similarity to Netrin (Jaworski et al., 2015). Nell2 was originally identified as a protein involved in differentiation of sensory neurons (Nelson et al., 2004) and later shown to repel retinal ganglion cell axons (Jiang et al., 2009). During the time window of commissural axon pathfinding Nell2 is not only expressed in dI1 neurons themselves but also in motoneurons (Jaworski et al., 2015). Nell2 was shown to repel commissural axons in a Robo3-dependent manner in vitro, whereas Robo1 and Robo2 were not required. Nell2 derived from the motoneurons was suggested to keep pre-crossing commissural axons out of the motor columns. However, no major effect on midline crossing was observed in mice lacking both alleles of NELL2 either in Robo3 wildtype or heterozygous background, despite the fact that many pre-crossing axons extended aberrantly through the motor columns (Jaworski et al., 2015).

6. RabGDI and Calsyntenin1 regulate trafficking of guidance receptors

Despite the findings that Robo3 can limit the sensitivity of Robo1 to Slit, these cis-interactions did not explain why there are only low levels of Robo1 protein on pre-crossing growth cones, as mRNA levels of Robo1 remain unchanged before and after commissural axon contact with the floor plate (Sabatier et al., 2004; Mambetisaeva et al., 2005; Reeber et al., 2008; Jaworski et al., 2010). In vitro and in vivo studies in chicken embryos demonstrated that Robo1 surface levels on commissural axons are regulated by specific trafficking of vesicles containing guidance receptors as cargo (Philipp et al., 2012; Alther et al., 2016; Fig. 3).

Mutations in RabGDI (Rab Guanine Nucleotide Dissociation Inhibitor, GDI1), a component of vesicular transport and recycling machinery, were identified as a cause of human mental retardation and

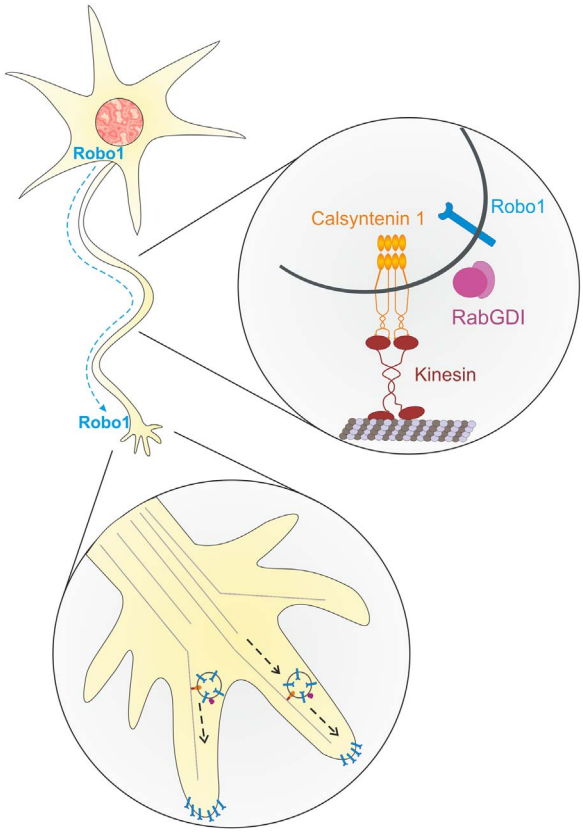

Fig. 3. Specific trafficking of guidance receptors contributes to their precisely timed expression on the growth cone surface. The availability of Robo1, the receptor mediating the repulsive signal of midline-associated Slits, must be tightly regulated. Premature expression on the growth cone surface would prevent axons from crossing the midline. Specific trafficking of vesicles containing Robo1 was shown to depend on Calsyntenin1 and RabGDI (Alther et al., 2016). Calsyntenin1 serves as a linker between the vesicle and the kinesin motor required for anterograde transport along microtubules. RabGDI appears to regulate timing of Robo1 insertion but its own regulation is not yet known (Philipp et al., 2012).

associative memory deficits in mice (D'Adamo et al., 1998, 2002). Loss of RabGDI function during midline crossing prevented trafficking of Robo1 receptors to the growth cone surface and resulted in a failure of axonal exit from the midline (Philipp et al., 2012; Alther et al., 2016).

RabGDI was found to collaborate with Calsyntenin1 in the precise timing of Robo1 expression on dI1 commissural axons. Calsyntenins are a family of three transmembrane proteins (Vogt et al., 2001). Like RabGDI, they are involved in learning and memory in C. elegans (Hoerndli et al., 2009), mice (Lipina et al., 2016), and humans (Preuschhof et al., 2010). Furthermore, a link between Calsyntenin and APP processing was reported (Steuble et al., 2012, 2010; Vagnoni et al., 2011).

Calsyntenin1 plays a role in anterograde vesicular transport due to its capacity to bind to the Kinesin motor (Fig. 3). In the cytoplasmic domain, Calsyntenin1 contains two binding sites for the interaction with the tetratricopeptides of the Kinesin Light Chain 1 (KLC1; Konecna et al., 2006). This trafficking function is not only required during axon guidance (Alther et al., 2016; Ponomareva et al., 2014) but also during synapse formation and plasticity (Pettem et al., 2013; Ster et al., 2014; Um et al., 2014). Robo1 was not the only guidance receptor that was regulated via specific Calsyntenin1-mediated trafficking, as the insertion of the Wnt receptor Fzd3 into post-crossing but not pre-crossing commissural growth cones also depended on Calsyntenin1 function, explaining why dI1 axons respond to the Wnt gradient only after exiting the floor plate (Alther et al., 2016; see below). Interestingly, trafficking of Fzd3-containing vesicles depended only on Calsyntenin1, but not on RabGDI.

7. The role of semaphorin signaling in axon guidance at the floor plate

In addition to Robo/Slit signaling, axons rely on semaphorin signaling to exit the floor plate (Fig. 4). Class-3 Semaphorins derived from the floor plate provide a repulsive signal to post-crossing axons expressing Neuropilin2-containing receptor complexes (Zou et al., 2000). Sema3B signals through Neuropilin2 and its co-receptor PlexinA1 in order to promote the exit of commissural neurons from the floor plate. Pre-crossing axons are insensitive to Sema3B because PlexinA1 is processed by the protease Calpain1 and, therefore, is unavailable for the formation of a receptor complex with Neuropilin2 (Nawabi et al., 2010). Once axons reach the floor plate, NrCAM suppresses Calpain1 function, thus, PlexinA1 is stabilized and enables semaphorin signaling. More recently, additional details on the regulation of Calpain activity were provided, as GDNF, a neurotrophic factor expressed by the floor-plate, was discovered to activate NCAM/GFRa1 receptors on axons and consequently, suppress Calpain1 activity (Charoy et al., 2012).

Interestingly, PlexinA1 provides a link between the Robo/Slit- and the Semaphorin-dependent repulsion, as it can bind Slit directly (Delloye-Bourgeois et al., 2015). Differences between the phenotypes seen in the absence of all Slits compared to those seen in the absence of all Robos suggested that an additional receptor for Slit might play a role in midline exit. Indeed, the C-terminal fragment of Slit generated by an unknown proteolytic process was shown to signal through PlexinA1 (Delloye-Bourgeois et al., 2015). Thus, proteolytic processing not only regulates the function of PlexinA1 receptors but also of Slit ligands.

In contrast to the repulsive class-3 Semaphorins other members of the Semaphorin family have been studied less extensively. Only recently, a member of the class-6 Semaphorins, Sema6B, was shown to act as a receptor in commissural axon guidance (Andermatt et al., 2014). Sema6B is expressed by commissural axons only during the time window when they cross the floor plate. Based on in vivo and in vitro studies, axonal Sema6B was suggested to act as a receptor for PlexinA2 expressed on floor-plate cells. In addition, Sema6B interacting in cis with axonal PlexinA2 was concluded to prevent sensitivity of

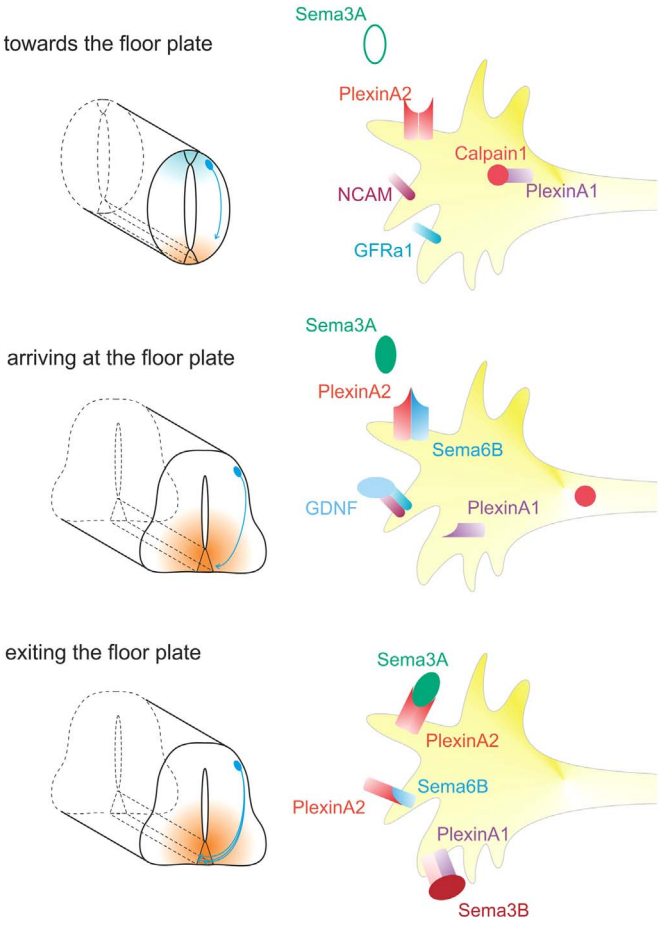

Fig. 4. Semaphorin signaling contributes to midline navigation. Although pre-crossing commissural axons on their way to the floor plate express PlexinA2, they are not repelled by class-3 Semaphorins, as their concentration is low in the dorsal and intermediate spinal cord. Furthermore, PlexinA1 is destabilized by Calpain1 and therefore cannot form a receptor complex with Neuropilin, required for Sema3-mediated repulsion (Nawabi et al., 2010; Charoy et al., 2012). When pre-crossing commissural axons get close to the floor plate and therefore concentrations of class-3 Semaphorins increase, PlexinA2 is prevented from forming a receptor complex with Neuropilin by cis-interacting Semaphorin6B (Andermatt et al., 2014). However, upon floor-plate contact PlexinA1 is no longer cleaved by Calpain1 and starts to be inserted into the growth cone membrane due to the inhibition of Calpain by GDNF binding to GFRa1/NCAM (Charoy et al., 2012) and NrCAM (Nawabi et al., 2010). PlexinA2 from the floor plate outcompetes axonal PlexinA2 for interaction with Sema6B (Andermatt et al., 2014). Thus, axonal PlexinA2 is accessible to Neuropilins for the formation of receptor complexes mediating the repulsive activity of class-3 Semaphorins. Thus, post-crossing axons are no longer attracted by the floor plate and grow along the longitudinal axis. Note Neuropilins have been omitted from the drawing to reduce complexity.

pre-crossing axons to midline-associated Sema3B by preventing the formation of a receptor complex between PlexinA2 and Neuropilin2. Upon reaching the floor plate, the cis-complex between PlexinA2 and Sema6B would be outcompeted by floor-plate PlexinA2 binding Sema6B in trans. The interaction between floor-plate PlexinA2 and axonal Sema6B would be possible because floor-plate PlexinA2 was not prevented from trans-interactions by cis-binding to Sema6B. Thus, axonal PlexinA2 would be released from Sema6B and be available for receptor complexes with Neuropilins, allowing axon to sense repulsive class-3 Semaphorins. Together with the studies on PlexinA1 cleavage by Calpain, these studies point to another type of post-translational regulation of guidance receptor expression. The possibility to form a Plexin/Neuropilin receptor complex, which mediates repulsive signals derived from floor plate-associated class-3 Semaphorins, is regulated by modulating protein stability through enzymatic cleavage or protein availability through sequestration by cis-interacting partners.

However, regulation of axonal sensitivity to Semaphorin appears to

be regulated also by Shh signaling (Parra and Zou, 2010). The interaction between floor-plate Shh and axonal Ptc1 was shown to downregulate PKA activity and thus reduce cAMP levels. Lowering cAMP levels was required but not sufficient to turn on growth cone sensitivity to Sema3B. Because cAMP is a second messenger in many processes its specific role in axon guidance is difficult to characterize and more detailed studies will be required.

8. Turning into the longitudinal axis

In addition to their role as promoters of midline crossing members of the IgSF-CAMs were also found to contribute to the turning of post-crossing commissural axons at the floor-plate exit site (Joset et al., 2011; Niederkofler et al., 2010). SynCAMs, also called Nectin-like proteins or Cadms, form an IgSF-CAM subfamily originally identified as synapse-inducing molecules (Biederer et al., 2002). More recently, they were shown to contribute to axon guidance of CNS (Niederkofler et al., 2010) and PNS (Frei et al., 2014) axons. In line with findings for other subfamilies of IgSF-CAMs, the SynCAMs regulate axonal behavior by their complex interaction pattern, where different cis-interactions regulate the choice of trans-binding partners (Frei et al., 2014). So far, it is unclear how SynCAMs direct post-crossing commissural axons rostrally, as gradients along the longitudinal axis of the spinal cord have not been found.

9. Shh – double duty in commissural axon guidance

Shh is a multifaceted molecule for commissural axon guidance. It not only acts as an attractant for pre-crossing commissural axons mediated by Smo downstream of Ptc and Boc (Fig. 1) and triggers growth cones' sensitivity to Sema3B, as described above, it also directs post-crossing commissural axons. Shh expressed in a caudal^{high} to rostral^{low} gradient along the longitudinal axis of the spinal cord was shown to act as a repellent for post-crossing commissural axons (Fig. 5; Bourikas et al., 2005). In contrast to pre-crossing commissural axons, post-crossing axons did not require Smo for their rostral turn along the contralateral floor-plate border suggesting that the repulsive activity of Shh was mediated by a different receptor. Indeed, the switch in axonal responsiveness to Shh was shown to depend on a change in receptor expression, as Hhip (Hedgehog-interacting protein) was shown to be responsible for the repulsive activity of Shh on post-crossing commissural axons (Bourikas et al., 2005).

More detailed studies of the mechanism underlying the switch in Shh receptor expression identified Glypican1 as a Shh-binding molecule responsible for the induction of Hhip expression (Fig. 5; Wilson and Stoeckli, 2013). Downregulation of Glypican1 in dI1 neurons produced pathfinding errors at the midline, resembling the ones seen in the absence of Shh signaling. Indeed, combinatorial knockdown of both molecules exhibited a genetic interaction between the two. Biochemical analysis confirmed a physical interaction between these two molecules, implying that Shh binding to Glypican1 was needed for proper axon guidance. Finally, several knockdown and rescue experiments demonstrated that Glypican1 was required for the transcriptional regulation of Hhip (Wilson and Stoeckli, 2013). Thus, Shh acts as a chemoattractant for pre-crossing axons (Charron et al., 2003) and at the same time prepares neurons for the post-crossing phase of axon guidance by inducing the expression of its own receptor, Hhip, used by post-crossing axons (Bourikas et al., 2005; Wilson and Stoeckli, 2013).

An alternative explanation was provided by in vitro experiments for the adoption of repulsive activity of Shh on post-crossing axons (Yam et al., 2012). Rather than a switch in Shh receptor, commissural axons were suggested to use a timer to change responsiveness to Shh in vitro. Cultured rat commissural neurons exposed to a Shh gradient were shown to respond with attraction towards Shh for the first two days, while after 3–4 days in culture, they changed direction and turned away from high levels of Shh. Additional experiments demonstrated that the modulation of protein 14-3-3 levels were correlated with the change in axonal behavior (Yam et al., 2012). In particular, an increase in 14-3-3 levels was related to a decrease in active PKA levels (Fig. 5; Yam et al., 2012). So far, this report has been the only one to address a purely cell-intrinsic mechanism for the change in axon behavior at the intermediate target. It is unclear how reaching a threshold concentration of an intracellular molecule is tightly regulated to ensure the switch in axonal behavior precisely when required at floor-plate exiting. It is very likely that 14-3-3 levels are controlled by the interaction of surface molecules expressed on the growth cone and the floor plate, respectively.

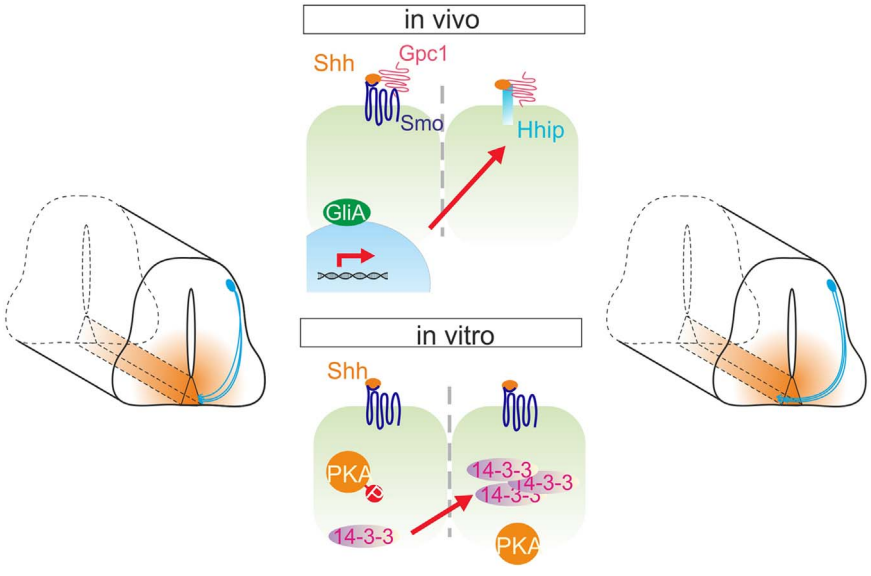

Fig. 5. Shh plays a dual role in commissural axon guidance. Shh not only attracts pre-crossing commissural axons by transcription-independent (non-canonical) signaling (see Fig. 1), it also acts as a repellent for post-crossing commissural axons. This switch in axonal responsiveness to Shh is possible due to a change in surface receptors. While pre-crossing axons express Ptc1 and Boc as Shh receptors (see Fig. 1), Shh induces the expression of its own receptor on post-crossing axons in a Glypican1-dependent manner (Wilson and Stoeckli, 2013). Using an in vitro system, Yam and colleagues (Yam et al., 2012) demonstrated an age-related upregulation of the 14-3-3 protein in commissural neurons and a concomitant decrease of PKA activity resulting in repulsion. A decrease in PKA activity was found to trigger sensitivity of growth cones to repulsive class-3 Semaphorins (Parra and Zou. 2010).

10. Wnt signaling and post-crossing commissural axon guidance

Based on explant cultures of developing mouse spinal cords, a rostral^{high} to caudal^{low} gradient of Wnts was shown to direct post-crossing commissural axons rostrally along the contralateral floor-plate border (Lyuksyutova et al., 2003). The Wnt receptor Frizzled3 was found in the commissural neurons and its functional contribution to post-crossing commissural axon guidance was confirmed by the analysis of *Fzd3* knockout embryos.

The Wnt network involves a complex set of signaling pathways involved in development and impaired in several types of cancer. Despite many years of research, many questions concerning the nature of this ligand and the specific signaling output in each context remain open (Niehrs, 2012; van Amerongen, 2012). The complexity in this field comes firstly from the large number of Wnt ligands (19 genes encoded in humans), Frizzled receptors (10 family members) and co-receptors; and secondly from the complex molecular interactions that each member in the different pathways establishes. Traditionally, Wnt signaling was divided up into three distinct pathways, the canonical or β-Catenin pathway, which is the best studied, the planar cell polarity (PCP) pathway (Tissir and Goffinet, 2013), and the WNT-Ca^{2+} pathway (Kohn and Moon, 2005). More recently, the separation between the pathways has been questioned, especially for Wnt functions beyond morphogenesis (Avilés et al., 2013; van Amerongen and Nusse, 2009).

Frizzled proteins were implicated as receptors in Wnt signaling for all three pathways (Bovolenta et al., 2006). The specificity was thought to be given by the co-receptors. Lrp5 and Lrp6 co-receptors can only signal via the canonical pathway (Fig. 6). In this β-Catenin-dependent pathway, the Wnt ligand signals through Dishevelled and promotes the stabilization of the cytoplasmic β-Catenin, which in Wnt OFF conditions is degraded by a 'destruction complex'. In the Wnt ON condition, β-Catenin can enter the nucleus and bind to the Tcf/Lef transcription factors, promoting gene expression usually thought to regulate proliferation and differentiation.

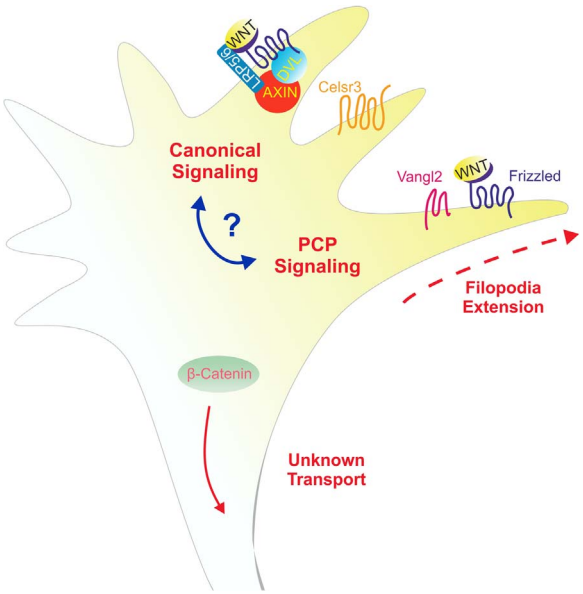

Fig. 6. Wnt signaling in post-crossing commissural axons is mediated by canonical and non-canonical signaling components. Wnt signaling in post-crossing commissural axons was shown to depend on PCP (planar cell polarity) components, as loss of Vangl2 or Celsr3, as well as the intracellular mediators, Prickle and Daam1, resulted in pathfinding errors (Shafer et al., 2011; Avilés and Stoeckli, 2016). In addition, both in vitro and in vivo studies confirmed a role of canonical Wnt signaling in axon guidance (Avilés and Stoeckli, 2016). Currently, it is unknown whether these signaling pathways influence each other and how Wnt components in the growth cone communicate with canonical Wnt signaling events in the cell body. The Wnt/PCP pathway is activated in the filopodia tips close to the Wnt source in a Vangl2-dependent manner (Shafer et al., 2011). This activation leads to the inhibition of Dvl2 and Fzd3 endocytosis in an Arf6-dependent manner, which is correlated with filopodia elongation and turning of the growth cone. In addition, LRP5 and LRP6, obligatory receptors for canonical Wnt signaling, are also required for post-crossing commissural axon pathfinding.

The other Wnt signaling pathways are β-Catenin independent. In the planar cell polarity (PCP) pathway a set of core proteins participate (Fig. 6). The Wnt ligand binds to the transmembrane receptors Frizzled, Celsr (vertebrate orthologue of Flamingo/Starry night) and Van Gogh-like proteins. The signal is then mediated by the cytosolic components Dishevelled, Prickle and Ankrd6 (vertebrate orthologue of Diego) which lead to the activation of the small GTPases Rac1 and RhoA, as well as the JNK pathway. In vertebrates, activation of PCP signaling regulates cell movement during gastrulation, neural tube closure and the orientation of the stereocilia in the inner ear (Tissir and Goffinet, 2013).

In the Wnt/Ca^{2+} pathway, the Wnts trigger calcium release via PI3K and activate CaMKII and PKC which leads to complex and dynamic responses (Kohn and Moon, 2005). This Wnt signaling pathway is perhaps the least understood. In most of the cases the specific activation of one of the Wnt pathways leads to the inhibition of the others, however, recent evidence suggests that in some cases several pathways can be activated and coordinated at the same time (van Amerongen and Nusse, 2009; van Amerongen, 2012).

The finding that Wnts were involved in commissural axon guidance raised the question about the Wnt signaling pathways activated in this novel Wnt task, as well as the regulation of Wnt activity between pre- and post-crossing commissural axons. In vitro studies in combination with the analysis of knockout mice suggested that the PCP pathway was involved in post-crossing commissural axon guidance (Lyuksyutova et al., 2003). Core PCP components were expressed in dI1 commissural neurons and found to contribute to axon guidance in the spinal cord and in the brain (Fenstermaker et al., 2010; Shafer et al., 2011). Moreover, increased levels of phosphorylated JNK, a direct downstream effector of the PCP pathway, were found in post-crossing axons (Onishi et al., 2013; Shafer et al., 2011). These results were confirmed by the analysis of different knockout mice. The Looptail mouse is a well known PCP mutant, with a point mutation in the *Vangl2* gene which makes the mutant protein unstable. Commissural axons in these embryos lost the anteroposterior direction after crossing the midline. The same phenotype was observed for *Celsr3* knockout mice, with several axon guidance defects (Shafer et al., 2011; Tissir et al., 2005). Taken together, these analyses confirmed that PCP components were necessary for proper post-crossing commissural axon guidance.

The molecular details of PCP signaling are difficult to study due to the complexity of the interactions. There are three different Dishevelled proteins with different interaction partners. Dvl1 was demonstrated to play an inhibitory role in the activation of PCP signaling by hyper-phosphorylating Fzd3. Vangl2 inhibits Dvl1 phosphorylation of Fzd3. The unphosphorylated form of Fzd3 is internalized and promotes PCP signaling in the growth cone (Shafer et al., 2011). In a follow-up study rapid endocytosis of Fzd3 upon Wnt5a binding mediated by Arf6 was shown (Onishi et al., 2013). Arf6 binds preferentially to unphosphorylated Fzd3. Interestingly, the small GTPase Arf6 was also suggested to promote the function of β-Catenin in the regulation of transcription (Grossmann et al., 2014).

The advantage of precise temporal control of gene silencing in the developing chicken embryo allowed for the identification of a contribution of canonical/β-Catenin-dependent Wnt signaling in post-crossing commissural axon guidance (Avilés and Stoeckli, 2016). As described in mice, the PCP components Celsr3, Vangl2, Prickle and Daam1 were also expressed in dI1 commissural neurons in the chicken embryo by the time axons turn longitudinally. Interfering with these genes' functions resulted in axon guidance defects, indicating that the PCP signaling was required for post-crossing commissural axon guidance also in chicken embryos. In contrast to the Lrp6 knockout mouse which was mentioned to have normal axon guidance along the antero-

posterior axis (Lyuksyutova et al., 2003), the silencing of Lrp6 in chicken resulted in severe pathfinding errors of post-crossing commissural axons (Avilés and Stoeckli, 2016). This discrepancy is most likely explained by the presence of the co-receptor Lrp5 that may compensate for Lrp6 in the mouse. Lrp5/6 double knockout mice do not develop past gastrulation and therefore preclude the analysis of axon guidance. The acute loss of canonical Wnt signaling, that is, the silencing of canonical Wnt signaling only during axon guidance in the chicken embryo could not be compensated and therefore resulted in axon guidance defects. The requirement for canonical Wnt signaling was confirmed by in vitro and in vivo experiments.

When taken together the studies on Wnt signaling in axon guidance result in a complex picture. Clearly the separation of Wnt signaling into three distinct pathways is not supported. Rather a complex network of receptors and downstream components was found to mediate the axonal response to Wnt gradients (Avilés and Stoeckli, 2016; van Amerongen and Nusse, 2009).

The Wnt signaling role in axon guidance is compelling, although only the most common elements in these pathways have been tested. The complexity of the Wnt world leaves many molecules unexplored, such as other non-Wnt activators and several types of receptors which can act independently of Frizzled. This is the case of Ryk, a receptor tyrosine kinase known to bind Wnt5a and to mediate repulsive axon guidance in corticospinal tracts (Bovolenta et al., 2006). Ryk is not expressed in commissural neurons discarding a role in commissural axon guidance. However, there is plenty of Wnt co-receptors unexplored, Ror, Ptk7 and Syndecans, molecules that could also be involved in the cooperation between PCP and canonical signaling.

11. Wnts and Shh – from antagonists to collaborators

The Wnts and Shh gradients exhibit an antagonistic role during morphogenesis by patterning ventral versus dorsal fate in the spinal cord (Ulloa and Martí, 2010). However, this antagonistic effect turns into cooperation during post-crossing commissural axon guidance (Domanitskaya et al., 2010; Avilés et al., 2013).

In contrast to the mouse spinal cord, Wnt5a and Wnt7a were not found to be expressed in a marked gradient along the floor plate in the chicken embryo (Domanitskaya et al., 2010). Still, after downregulation of Wnts most axons exhibited abnormalities and failed to turn rostrally after midline crossing. These results confirmed that Wnts were required for post-crossing commissural axons also in the developing chicken spinal cord, despite the absence of a detectable expression gradient. The apparently contradictory result was explained by the finding that the endogenous Wnt antagonists Sfrp1 and Sfrp2 were expressed in and adjacent to the floor plate at high levels and with a caudal^{high} to rostral^{low} gradient. In fact, downregulation and localized overexpression of Sfrp1 reproduced the phenotypes observed after downregulation of Wnts in agreement with its role in shaping a Wnt activity gradient (Domanitskaya et al., 2010).

Interestingly, and in agreement with findings in mesoderm (Lee et al., 2000), Shh was found to induce sFRP expression in the developing neural tube (Domanitskaya et al., 2010). Thus, the caudal^{high} to rostral^{low} gradient of Shh not only repels post-crossing commissural axons directly (Bourikas et al., 2005) but it also acts indirectly by shaping a rostral^{high} to caudal^{low} activity gradient of Wnt via Sfrp1 (Domanitskaya et al., 2010).

12. Conclusion

Despite the fact that this review summarized the molecular mechanisms used by dI1 commissural axons to navigate their intermediate target, the floor plate, these mechanisms can be generalized to other neuronal populations and their specific intermediate targets. Although some other or additional molecules may be involved in their pathfinding the general theme is conserved: axons are attracted to the intermediate target and then need to overcome this attraction in order to move on. To this end, the growth cones change their response to the intermediate target by changing the guidance receptors on their surface. This can be achieved by changes in transcription or translation but also by post-translational mechanisms, such as specific trafficking of guidance receptors, regulation of protein stability, or regulation of protein-protein interaction by specific sequestration of receptor components. After identification of all these possibilities in dI1 neurons in the spinal cord, it will be easier to verify these mechanisms in neural circuit formation in the brain, where circuits are more difficult to study at the molecular level due to their increased complexity.

References

Ahmed, G., Shinmyo, Y., Ohta, K., Islam, S.M., Hossain, M., Naser, I.B., Riyadh, M.A., Su, Y., Zhang, S., Tessier-Lavigne, M., Tanaka, H., 2011. Draxin inhibits axonal outgrowth through the netrin receptor DCC. J. Neurosci. 31, 14018–14023. http://dx.doi.org/10.1523/JNEUROSCI.0943-11.2011.

Alther, T.A., Domanitskaya, E., Stoeckli, E.T., 2016. Calsyntenin1-mediated trafficking of axon guidance receptors regulates the switch in axonal responsiveness at a choice point. Development 143, 994–1004. http://dx.doi.org/10.1242/dev.127449.

Andermatt, I., Wilson, N.H., Bergmann, T., Mauti, O., Gesemann, M., Sockanathan, S., Stoeckli, E.T., 2014. Semaphorin 6B acts as a receptor in post-crossing commissural axon guidance. Development 141, 3709–3720. http://dx.doi.org/10.1242/dev.112185.

Andrews, G.L., Tanglao, S., Farmer, W.T., Morin, S., Brotman, S., Berberoglu, M.A., Price, H., Fernandez, G.C., Mastick, G.S., Kidd, T., 2008. Dscam guides embryonic axons by Netrin-dependent and independent functions. Development 135, 3839–3848. http://dx.doi.org/10.1242/dev.023739.

Augsburger, A., Schuchardt, A., Hoskins, S., Dodd, J., Butler, S., 1999. Bmps as mediators of roof plate repulsion of commissural neurons. Neuron 24, 127–141. http://dx.doi.org/10.1016/S0896-6273(00)80827-2.

Avilés, E.C., Stoeckli, E.T., 2016. Canonical wnt signaling is required for commissural axon guidance. Dev. Neurobiol. 76, 190–208. http://dx.doi.org/10.1002/dneu.22307.

Avilés, E.C., Wilson, N.H., Stoeckli, E.T., 2013. Sonic hedgehog and Wnt: antagonists in morphogenesis but collaborators in axon guidance. Front. Cell. Neurosci. 7, 86. http://dx.doi.org/10.3389/fncel.2013.00086.

Baudet, M.-L., Zivraj, K.H., Abreu-Goodger, C., Muldal, A., Armisen, J., Blenkiron, C., Goldstein, L.D., Miska, E.A., Holt, C.E., 2011. miR-124 acts through CoREST to control onset of Sema3A sensitivity in navigating retinal growth cones. Nat. Neurosci. 15, 29–38. http://dx.doi.org/10.1038/nn.2979.

Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalali, E.T., Südhof, T.C., 2002. SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science 297, 1525–1531. http://dx.doi.org/10.1126/science.1072356.

Bin, J.M., Han, D., Lai Wing Sun, K., Croteau, L.P., Dumontier, E., Cloutier, J.F., Kania, A., Kennedy, T.E., 2015. Complete loss of Netrin-1 results in embryonic lethality and severe axon guidance defects without increased neural cell death. Cell Rep. 12, 1099–1106. http://dx.doi.org/10.1016/j.celrep.2015.07.028.

Bourikas, D., Pekarik, V., Baeriswyl, T., Grunditz, A., Sadhu, R., Nardó, M., Stoeckli, E.T., 2005. Sonic hedgehog guides commissural axons along the longitudinal axis of the spinal cord. Nat. Neurosci. 8, 297–304. http://dx.doi.org/10.1038/nn1396.

Bovolenta, P., Rodriguez, J., Esteve, P., 2006. Frizzled/RYK mediated signalling in axon guidance. Development 133, 4399–4408.

Brose, K., Bland, K.S., Wang, K.H., Arnott, D., Henzel, W., Goodman, C.S., Tessier-Lavigne, M., Kidd, T., 1999. Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell 96, 795–806. http://dx.doi.org/10.1016/S0092-8674(00)80590-5.

Charoy, C., Nawabi, H., Reynaud, F., Derrington, E., Bozon, M., Wright, K., Falk, J., Helmbacher, F., Kindbeiter, K., Castellani, V., 2012. Gdnf activates midline repulsion by Semaphorin3B via NCAM during commissural axon guidance. Neuron 75, 1051–1066. http://dx.doi.org/10.1016/j.neuron.2012.08.021.

Charron, F., Stein, E., Jeong, J., McMahon, A.P., Tessier-Lavigne, M., 2003. The morphogen sonic hedgehog is an axonal chemoattractant that collaborates with Netrin-1 in midline axon guidance. Cell 113, 11–23. http://dx.doi.org/10.1016/S0092-8674(03)00199-5.

Chen, Z., Gore, B.B., Long, H., Ma, L., Tessier-Lavigne, M., 2008. Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. Neuron 58, 325–332. http://dx.doi.org/10.1016/j.neuron.2008.02.016.

D'Adamo, P., Menegon, A., Lo Nigro, C., Grasso, M., Gulisano, M., Tamanini, F., Bienvenu, T., Gedeon, a.K., Oostra, B., Wu, S.K., Tandon, A., Valtorta, F., Balch, W.E., Chelly, J., Toniolo, D., 1998. Mutations in GDI1 are responsible for X-linked non-specific mental retardation. Nat. Genet. 19, 134–139.

D'Adamo, P., Welzl, H., Papadimitriou, S., Raffaele di Barletta, M., Tiveron, C., Tatangelo, L., Pozzi, L., Chapman, P.F., Knevett, S.G., Ramsay, M.F., Valtorta, F., Leoni, C., Menegon, A., Wolfer, D.P., Lipp, H.-P., Toniolo, D., 2002. Deletion of the mental retardation gene Gdi1 impairs associative memory and alters social behavior in mice. Hum. Mol. Genet. 11, 2567–2580.

Delloye-Bourgeois, C., Jacquier, A., Charoy, C., Reynaud, F., Nawabi, H., Thoinet, K., Kindbeiter, K., Yoshida, Y., Zagar, Y., Kong, Y., Jones, Y.E., Falk, J., Chédotal, A., Castellani, V., 2015. PlexinA1 is a new Slit receptor and mediates axon guidance

function of Slit C-terminal fragments. Nat. Neurosci. 18, 36–45. http://dx.doi.org/10.1038/nn.3893.
Dickson, B.J., 2002. Molecular mechanisms of axon guidance. Science 298, 1959–1964. http://dx.doi.org/10.1126/science.1072165.
Domanitskaya, E., Wacker, A., Mauti, O., Baeriswyl, T., Esteve, P., Bovolenta, P., Stoeckli, E.T., 2010. Sonic Hedgehog guides post-crossing commissural axons both directly and indirectly by regulating Wnt activity. J. Neurosci. 30, 11167–11176. http://dx.doi.org/10.1523/JNEUROSCI.1488-10.2010.
Erskine, L., Reijntjes, S., Pratt, T., Denti, L., Schwarz, Q., Vieira, J.M., Alakakone, B., Shewan, D., Ruhrberg, C., 2011. VEGF signaling through Neuropilin 1 guides commissural axon crossing at the optic Chiasm. Neuron 70, 951–965.
Fenstermaker, A.G., Prasad, A.A., Bechara, A., Adolfs, Y., Tissir, F., Goffinet, A., Zou, Y., Pasterkamp, R.J., 2010. Wnt/Planar cell polarity signaling controls the anterior – posterior organization of monoaminergic axons in the brainstem. J. Neurosci. 30, 16053–16064. http://dx.doi.org/10.1523/JNEUROSCI.4508-10.2010.
Fitzli, D., Stoeckli, E.T., Kunz, S., Siribour, K., Rader, C., Kunz, B., Kozlov, S.V., Buchstaller, A., Lane, R.P., Suter, D.M., Dreyer, W.J., Sonderegger, P., 2000. A direct interaction of axonin-1 with NgCAM-related cell adhesion molecule (NrCAM) results in guidance, but not growth of commissural axons. J. Cell Biol. 149, 951–968. http://dx.doi.org/10.1083/jcb.149.4.951.
Frei, J.A., Andermatt, I., Gesemann, M., Stoeckli, E.T., 2014. The SynCAM synaptic cell adhesion molecules are involved in sensory axon pathfinding by regulating axon-axon contacts. J. Cell Sci. 127, 5288–5302. http://dx.doi.org/10.1242/jcs.157032.
Gao, X., Metzger, U., Panza, P., Mahalwar, P., Alsheimer, S., Geiger, H., Maischein, H.M., Levesque, M.P., Templin, M., Söllner, C., 2015. A floor-plate extracellular protein-protein interaction screen identifies Draxin as a secreted Netrin-1 antagonist. Cell Rep. 12, 694–708.
Grossmann, A.H., Yoo, J.H., Clancy, J., Sorensen, L.K., Tong, Z., Ostanin, K., Rogers, A., Grossmann, K.F., Tripp, S.R., Thomas, K.R., Souza-schorey, C.D., Odelberg, S.J., 2014. The small GTPase ARF6 stimulates β-catenin transcriptional activity during WNT5A-mediated melanoma invasion and metastasis. Invasion Metastasis 6, 1–29. http://dx.doi.org/10.1126/scisignal.2003398.
Hoerndli, F.J., Walser, M., Hoier, E.F., De Quervain, D., Papassotiropoulos, A., Hajnal, A., 2009. A conserved function of C. elegans CASY-1 calsyntenin in associative learning. PLoS One 4, e4880. http://dx.doi.org/10.1371/journal.pone.0004880.
Huminiecki, L., Gorn, M., Suchting, S., Poulsom, R., Bicknell, R., 2002. Magic roundabout is a new member of the roundabout receptor family that is endothelial specific and expressed at sites of active angiogenesis. Genomics 79, 547–552. http://dx.doi.org/10.1006/geno.2002.6745.
Islam, S.M., Shinmyo, Y., Okafuji, T., Su, Y., Naser, I., Bin, Ahmed, G., Zhang, S., Chen, S., Ohta, K., Kiyonari, H., Abe, T., Tanaka, S., Nishinakamura, R., Terashima, T., Kitamura, T., Tanaka, H., 2009. Draxin, a repulsive guidance protein for spinal cord and forebrain commissures. Science 323, 388–393. http://dx.doi.org/10.1126/science.1165187.
Jaworski, A., Long, H., Tessier-Lavigne, M., 2010. Collaborative and specialized functions of Robo1 and Robo2 in spinal commissural axon guidance. J. Neurosci. 30, 9445–9453.
Jaworski, A., Tom, I., Tong, R.K., Gildea, H.K., Koch, A.W., Gonzalez, L.C., Tessier-Lavigne, M., 2015. Operational redundancy in axon guidance through the multifunctional receptor Robo3 and its ligand NELL2. Science 350, 961–965. http://dx.doi.org/10.1126/science.aad2615.
Jiang, Y., Obama, H., Kuan, S.L., Nakamura, R., Nakamoto, C., Ouyang, Z., Nakamoto, M., 2009. In vitro guidance of retinal axons by a tectal lamina-specific glycoprotein Nel. Mol. Cell Neurosci. 41, 113–119. http://dx.doi.org/10.1016/j.mcn.2009.02.006.
Joset, P., Wacker, A., Babey, R., Ingold, E.A., Andermatt, I., Stoeckli, E.T., Gesemann, M., 2011. Rostral growth of commissural axons requires the cell adhesion molecule MDGA2. Neural Dev. 6, 22. http://dx.doi.org/10.1186/1749-8104-6-22.
Keino-Masu, K., Masu, M., Hinck, L., Leonardo, E.D., 1996. Deleted in colorectal cancer (DCC) encodes a Netrin receptor. Cell 87, 175–185.
Keleman, K., Rajagopalan, S., Cleppien, D., Teis, D., Paiha, K., Huber, L.A., Technau, G.M., Dickson, B.J., 2002. Comm sorts Robo to control axon guidance at the Drosophila midline. Cell 110, 415–427. http://dx.doi.org/10.1016/S0092-8674(02)00901-7.
Keleman, K., Ribeiro, C., Dickson, B.J., 2005. Comm function in commissural axon guidance: cell-autonomous sorting of Robo in vivo. Nat. Neurosci. 8, 156–163. http://dx.doi.org/10.1038/nn1388.
Kennedy, T.E., Serafini, T., de la Torre, J., Tessier-Lavigne, M., 1994. Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. Cell 78, 425–435. http://dx.doi.org/10.1016/0092-8674(94)90421-9.
Kidd, T., Bland, K.S., Goodman, C.S., 1999. Slit is the midline repellent for the robo receptor in Drosophila. Cell 96, 785–794. http://dx.doi.org/10.1016/S0092-8674(00)80589-9.
Kidd, T., Russell, C., Goodman, C.S., Tear, G., 1998. Dosage-sensitive and complementary functions of roundabout and commissureless control axon crossing of the CNS midline. Neuron 20, 25–33. http://dx.doi.org/10.1016/S0896-6273(00)80431-6.
Kohn, A.D., Moon, R.T., 2005. Wnt and calcium signaling: b-Catenin-independent pathways. Cell Calcium 38, 439–446. http://dx.doi.org/10.1016/j.ceca.2005.06.022.
Kolodkin, A.L., Tessier-Lavigne, M., 2011. Mechanisms and molecules of neuronal wiring: a primer. Cold Spring Harb. Perspect.. http://dx.doi.org/10.1101/cshperspect.a001727, pii:a001727.
Konecna, A., Frischknecht, R., Kinter, J., Ludwig, A., Steuble, M., Meskenaite, V., Indermühle, M., Engel, M., Cen, C., Mateos, J.-M., Streit, P., Sonderegger, P., 2006. Calsyntenin-1 Docks Vesicular Cargo to Kinesin-1. Mol. Biol. Cell 17, 3651–3663. http://dx.doi.org/10.1091/mbc.E06.
Kunz, S., Spirig, M., Ginsburg, C., Buchstaller, A., Berger, P., Lanz, R., Rader, C., Vogt, L., Kunz, B., Sonderegger, P., 1998. Neurite fasciculation mediated by complexes of axonin-1 and Ng cell adhesion molecule. J. Cell Biol. 143, 1673–1690.
Kunz, S., Ziegler, U., Kunz, B., Sonderegger, P., 1996. Intracellular signalling is changed after clustering of the neural cell adhesion molecules axonin-1 and NgCAM during neurite fasciculation. J. Cell Biol. 135, 253–267. http://dx.doi.org/10.1083/jcb.135.1.253.
Lee, C.S., Buttitta, L.A., May, N.R., Kispert, A., Fan, C.M., 2000. SHH-N upregulates Sfrp2 to mediate its competitive interaction with WNT1 and WNT4 in the somitic mesoderm. Development 127, 109–118.
Liem, K.F., Tremml, G., Jessell, T.M., 1997. A role for the roof plate and its resident TGFb-related proteins in neuronal patterning in the dorsal spinal cord. Cell 91, 127–138. http://dx.doi.org/10.1016/S0092-8674(01)80015-5.
Lipina, T.V., Prasad, T., Yokomaku, D., Luo, L., Connor, S.A., Kawabe, H., Wang, Y.T., Brose, N., Roder, J.C., Craig, A.M., 2016. Cognitive deficits in Calsyntenin-2-deficient mice associated with reduced GABAergic transmission. Neuropsychopharmacology 41, 802–810. http://dx.doi.org/10.1038/npp.2015.206.
Long, H., Sabatier, C., Ma, L., Plump, A., Yuan, W., Ornitz, D.M., Tamada, A., Murakami, F., Goodman, C.S., Tessier-Lavigne, M., 2004. Conserved roles for Slit and Robo proteins in midline commissural axon guidance. Neuron 42, 213–223. http://dx.doi.org/10.1016/S0896-6273(04)00179-5.
Ly, A., Nikolaev, A., Suresh, G., Zheng, Y., Tessier-Lavigne, M., Stein, E., 2008. DSCAM Is a Netrin receptor that collaborates with DCC in mediating turning responses to Netrin-1. Cell 133, 1241–1254. http://dx.doi.org/10.1016/j.cell.2008.05.030.
Lyuksyutova, A.I., Lu, C.-C., Milanesio, N., King, L. a., Guo, N., Wang, Y., Nathans, J., Tessier-Lavigne, M., Zou, Y., 2003. Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. Science 302, 1984–1988. http://dx.doi.org/10.1126/science.1089610.
Mambetisaeva, E.T., Andrews, W., Camurri, L., Annan, A., Sundaresan, V., 2005. Robo family of proteins exhibit differential expression in mouse spinal cord and robo-slit interaction is required for midline crossing in vertebrate spinal cord. Dev. Dyn. 233, 41–51.
Meli, R., Weisová, P., Propst, F., 2015. Repulsive axon guidance by Draxin is mediated by protein Kinase B (Akt), glycogen synthase kinase-3β (GSK-3β) and microtubule-associated protein 1B. PLoS One 10, e0119524. http://dx.doi.org/10.1371/journal.pone.0119524.
Nawabi, H., Briançon-Marjollet, A., Clark, C., Sanyas, I., Takamatsu, H., Okuno, T., Kumanogoh, A., Bozon, M., Takeshima, K., Yoshida, Y., Moret, F., Abouzid, K., Castellani, V., 2010. A midline switch of receptor processing regulates commissural axon guidance in vertebrates. Genes Dev. 24, 396–410. http://dx.doi.org/10.1101/gad.542510.
Nelson, B.R., Claes, K., Todd, V., Chaverra, M., Lefcort, F., 2004. NELL2 promotes motor and sensory neuron differentiation and stimulates mitogenesis in DRG in vivo. Dev. Biol. 270, 322–335.
Niederkofler, V., Baeriswyl, T., Ott, R., Stoeckli, E.T., 2010. Nectin-like molecules/SynCAMs are required for post-crossing commissural axon guidance. Development 137, 427–435. http://dx.doi.org/10.1242/dev.042515.
Niehrs, C., 2012. The complex world of WNT receptor signalling. Nat. Rev. Mol. Cell Biol. 13, 767–779. http://dx.doi.org/10.1038/nrm3470.
Okada, A., Charron, F., Morin, S., Shin, D.S., Wong, K., Fabre, P.J., Tessier-Lavigne, M., McConnell, S.K., 2006. Boc is a receptor for sonic hedgehog in the guidance of commissural axons. Nature 444, 369–373. http://dx.doi.org/10.1038/nature05246.
Onishi, K., Shafer, B., Lo, C., Tissir, F., Goffinet, A.M., Zou, Y., 2013. Antagonistic functions of Dishevelleds regulate Frizzled3 endocytosis via Filopodia Tips in Wnt-Mediated Growth Cone Guidance. J. Neurosci. 33, 19071–19085. http://dx.doi.org/10.1523/JNEUROSCI.2800-13.2013.
Palmesino, E., Haddick, P.C.G., Tessier-Lavigne, M., Kania, A., 2012. Genetic analysis of DSCAM's role as a Netrin-1 receptor in vertebrates. J. Neurosci. 32, 411–416. http://dx.doi.org/10.1523/JNEUROSCI.3563-11.2012.
Parra, L.M., Zou, Y., 2010. Sonic hedgehog induces response of commissural axons to Semaphorin repulsion during midline crossing. Nat. Neurosci. 13, 29–35. http://dx.doi.org/10.1038/nn.2457.
Pettem, K., Yokomaku, D., Luo, L., Linhoff, M., Prasad, T., Connor, S., Siddiqui, T., Kawabe, H., Chen, F., Zhang, L., Rudenko, G., Wang, Y., Brose, N., Craig, A., 2013. The specific α-Neurexin Interactor Calsyntenin-3 promotes excitatory and inhibitory synapse development. Neuron 80, 113–128.
Phan, K.D., Croteau, L.P., Kam, J.W.K., Kania, A., Cloutier, J.F., Butler, S.J., 2011. Neogenin may functionally substitute for Dcc in chicken. PLoS One 6, e22072. http://dx.doi.org/10.1371/journal.pone.0022072.
Philipp, M., Niederkofler, V., Debrunner, M., Alther, T., Kunz, B., Stoeckli, E.T., 2012. RabGDI controls axonal midline crossing by regulating Robo1 surface expression. Neural Dev. 7, 36. http://dx.doi.org/10.1186/1749-8104-7-36.
Ponomareva, O.Y., Holmen, I.C., Sperry, A.J., Eliceiri, K.W., Halloran, M.C., 2014. Calsyntenin-1 regulates axon branching and endosomal trafficking during sensory neuron development in vivo. J. Neurosci. 34, 9235–9248. http://dx.doi.org/10.1523/JNEUROSCI.0561-14.2014.
Preuschhof, C., Heekeren, H.R., Li, S.C., Sander, T., Lindenberger, U., Bäckman, L., 2010. KIBRA and CLSTN2 polymorphisms exert interactive effects on human episodic memory. Neuropsychologia 48, 402–408.
Reeber, S.L., Sakai, N., Nakada, Y., Dumas, J., Dobrenis, K., Johnson, J.E., Kaprielian, Z., 2008. Manipulating Robo expression in vivo perturbs commissural axon pathfinding in the chick spinal cord. J. Neurosci. 28, 8698–8708.
Ruiz de Almodovar, C., Fabre, P.J., Knevels, E., Coulon, C., Segura, I., Haddick, P.C.G., Aerts, L., Delattin, N., Strasser, G., Oh, W.J., Lange, C., Vinckier, S., Haigh, J., Fouquet, C., Gu, C., Alitalo, K., Castellani, V., Tessier-Lavigne, M., Chedotal, A., Charron, F., Carmeliet, P., 2011. VEGF mediates commissural axon chemoattraction

through its receptor Flk1. Neuron 70, 966–978.
Sabatier, C., Plump, A.S., Ma, L., Brose, K., Tamada, A., Murakami, F., Lee, E.Y., Tessier-Lavigne, M., 2004. The divergent robo family protein Rig-1/Robo3 is a negative regulator of slit responsiveness required for midline crossing by commissural axons. Cell 117, 157–169. http://dx.doi.org/10.1016/S0092-8674(04)00303-4.
Sakai, N., Insolera, R., Sillitoe, R.V., Shi, S.H., Kaprielian, Z., 2012. Axon sorting within the spinal cord marginal zone via Robo-mediated inhibition of N-cadherin controls spinocerebellar tract formation. J. Neurosci. 32, 15377–15387. http://dx.doi.org/10.1523/JNEUROSCI.2225-12.2012.
Serafini, T., Colamarino, S.A., Leonardo, E.D., Wang, H., Beddington, R., Skarnes, W.C., Tessier-Lavigne, M., 1996. Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. Cell 87, 1001–1014. http://dx.doi.org/10.1016/S0092-8674(00)81795-X.
Shafer, B., Onishi, K., Lo, C., Colakoglu, G., Zou, Y., 2011. Vangl2 Promotes Wnt/Planar Cell polarity-like signaling by antagonizing Dvl1-mediated feedback inhibition in growth cone guidance. Dev. Cell 20, 177–191. http://dx.doi.org/10.1016/j.devcel.2011.01.002.
Shirasaki, R., Katsumata, R., Murakami, F., 1998. Change in chemoattractant responsiveness of developing axons at an intermediate target. Science 279, 105–107. http://dx.doi.org/10.1126/science.279.5347.105.
Srinivasan, K., Strickland, P., Valdes, A., Shin, G.C., Hinck, L., 2003. Netrin-1/neogenin interaction stabilizes multipotent progenitor cap cells during mammary gland morphogenesis. Dev. Cell 4, 371–382. http://dx.doi.org/10.1016/S1534-5807(03)00054-6.
Stein, E., Tessier-Lavigne, M., 2001. Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. Science 291, 1928–1938. http://dx.doi.org/10.1126/science.1058445.
Ster, J., Steuble, M., Orlando, C., Diep, T.-M., Akhmedov, A., Raineteau, O., Pernet, V., Sonderegger, P., Gerber, U., 2014. Calsyntenin-1 regulates targeting of dendritic NMDA receptors and dendritic spine maturation in CA1 hippocampal pyramidal cells during postnatal development. J. Neurosci. 34, 8716–8727. http://dx.doi.org/10.1523/JNEUROSCI.0144-14.2014.
Steuble, M., Diep, T.-M., Schätzle, P., Ludwig, A., Tagaya, M., Kunz, B., Sonderegger, P., 2012. Calsyntenin-1 shelters APP from proteolytic processing during anterograde axonal transport. Biol. Open 1, 761–774.
Steuble, M., Gerrits, B., Ludwig, A., Mateos, J.M., Diep, T.M., Tagaya, M., Stephan, A., Schätzle, P., Kunz, B., Streit, P., Sonderegger, P., 2010. Molecular characterization of a trafficking organelle: dissecting the axonal paths of calsyntenin-1 transport vesicles. Proteomics 10, 3775–3788. http://dx.doi.org/10.1002/pmic.201000384.
Stoeckli, E.T., Landmesser, L.T., 1995. Axonin-1, Nr-CAM, and Ng-CAM play different roles in the in vivo guidance of chick commissural neurons. Neuron 14, 1165–1179. http://dx.doi.org/10.1016/0896-6273(95)90264-3.
Stoeckli, E.T., Sonderegger, P., Pollerberg, G.E., Landmesser, L.T., 1997. Interference with axonin-1 and NrCAM interactions unmasks a floor-plate activity inhibitory for commissural axons. Neuron 18, 209–221. http://dx.doi.org/10.1016/S0896-6273(00)80262-7.
Tessier-Lavigne, M., Goodman, C.S., 1996. The molecular biology of axon guidance. Science 274, 1123–1133.
Tissir, F., Bar, I., Jossin, Y., De Backer, O., Goffinet, A.M., 2005. Protocadherin Celsr3 is crucial in axonal tract development. Nat. Neurosci. 8, 451–457. http://dx.doi.org/10.1038/nn0106-147a.
Tissir, F., Goffinet, A.M., 2013. Shaping the nervous system: role of the core planar cell polarity genes. Nat. Rev. Neurosci. 14, 525–535. http://dx.doi.org/10.1038/nrn3525.
Ulloa, F., Martí, E., 2010. Wnt won the war: antagonistic role of Wnt over Shh controls dorso-ventral patterning of the vertebrate neural tube. Dev. Dyn. 239, 69–76. http://dx.doi.org/10.1002/dvdy.22058.
Um, J.W., Pramanik, G., Ko, J.S., Song, M.Y., Lee, D., Kim, H., Park, K.S., Südhof, T.C., Tabuchi, K., Ko, J., 2014. Calsyntenins function as synaptogenic adhesion molecules in concert with neurexins. Cell Rep. 6, 1096–1109.
Vagnoni, A., Rodriguez, L., Manser, C., De Vos, K.J., Miller, C.C., 2011. Phosphorylation of Kinesin Light Chain 1 at Serine 460 modulates binding and trafficking of Calsyntenin-1. J. Cell Sci. 124, 1032–1042.
van Amerongen, R., 2012. Alternative Wnt pathways and receptors. Cold Spring Harb. Perspect. Biol., 4. http://dx.doi.org/10.1101/cshperspect.a007914.
van Amerongen, R., Nusse, R., 2009. Towards an integrated view of Wnt signaling in development. Development 136, 3205–3214. http://dx.doi.org/10.1242/dev.033910.
Vogt, L., Schrimpf, S.P., Meskenaite, V., Frischknecht, R., Kinter, J., Leone, D.P., Ziegler, U., Sonderegger, P., 2001. Calsyntenin-1, a proteolytically processed postsynaptic membrane protein with a cytoplasmic calcium-binding domain. Mol. Cell. Neurosci. 17, 151–166. http://dx.doi.org/10.1006/mcne.2000.0937.
Wilson, N.H., Key, B., 2007. Neogenin: one receptor, many functions. Int. J. Biochem. Cell Biol. 39, 874–878.
Wilson, N.H., Key, B., 2006. Neogenin interacts with RGMa and Netrin-1 to guide axons within the embryonic vertebrate forebrain. Dev. Biol. 296, 485–498. http://dx.doi.org/10.1016/j.ydbio.2006.06.018.
Wilson, N.H., Stoeckli, E.T., 2013. Sonic Hedgehog regulates its own receptor on postcrossing commissural axons in a glypican1-dependent manner. Neuron 79, 478–491. http://dx.doi.org/10.1016/j.neuron.2013.05.025.
Yam, P.T., Kent, C.B., Morin, S., Farmer, W.T., Alchini, R., Lepelletier, L., Colman, D.R., Tessier-Lavigne, M., Fournier, A.E., Charron, F., 2012. 14-3-3 Proteins regulate a cell-intrinsic switch from Sonic Hedgehog-mediated commissural axon attraction to repulsion after midline crossing. Neuron 76, 735–749. http://dx.doi.org/10.1016/j.neuron.2012.09.017.
Yam, P.T., Langlois, S.D., Morin, S., Charron, F., 2009. Sonic Hedgehog guides axons through a noncanonical, Src-Family-Kinase-Dependent signaling pathway. Neuron 62, 349–362. http://dx.doi.org/10.1016/j.neuron.2009.03.022.
Yamauchi, K., Phan, K.D., Butler, S.J., 2008. BMP type I receptor complexes have distinct activities mediating cell fate and axon guidance decisions. Development 135, 1119–1128. http://dx.doi.org/10.1242/dev.012989.
Yung, A.R., Nishitani, A.M., Goodrich, L.V., 2015. Phenotypic analysis of mice completely lacking netrin. Development 142, 3686–3691. http://dx.doi.org/10.1242/dev.128942.
Zelina, P., Blockus, H., Zagar, Y., Péres, A., Friocourt, F., Wu, Z., Rama, N., Fouquet, C., Hohenester, E., Tessier-Lavigne, M., Schweitzer, J., Crollius, H., Chédotal, A., 2014. Signaling switch of the axon guidance receptor Robo3 during vertebrate evolution. Neuron 84, 1258–1272. http://dx.doi.org/10.1016/j.neuron.2014.11.004.
Zou, Y., Stoeckli, E., Chen, H., Tessier-Lavigne, M., 2000. Squeezing axons out of the gray matter: a role for slit and semaphorin proteins from midline and ventral spinal cord. Cell 102, 363–375.