CHAPTER SIX

Does Planar Cell Polarity Signaling Steer Growth Cones?

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Contents

Abstract

Recent studies established the role of planar cell polarity signaling in axon guidance. Signaling mechanisms controlling the direction of axon growth are poorly understood. The possibility that conserved and robust cell polarity signaling pathways may be reused as a key mechanism to convey asymmetric signaling in growth cones will provide insights to solving this long-standing mystery. Insights gained from growth cones can also shed light on general principles of cell polarity signaling. This review also discusses the possibility that this cell polarity signaling-based mechanism may be a general mechanism for mediating directional control by many, if not all, axon guidance molecules.

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1. OUTLINE OF REVIEW

This review summarizes the role of planar cell polarity (PCP) signaling components in axon guidance and whether and how growth cones may use cell polarity pathways (planar and apical–basal) or a subset of cell polarity pathways to detect guidance cues and to turn up or down along gradients of guidance molecules. This review also explores, for the first time, the exciting possibility that this cell polarity signaling-based mechanism (or module) may be a general principle in growth cone guidance.

In stark contrast to our rich knowledge of the identity of axon guidance molecules, how signaling conveys directionality is poorly understood. The possibility that cell polarity signaling may be a key mediator of turning will provide answers to fundamental questions in signaling and cell biological mechanisms of growth cone steering. The systemic and global feature of planar polarity (also referred to as tissue polarity) and apical–basal polarity (A-BP) suggests that this cell polarity-based signaling mechanism may be responsible for establishing the highly organized axonal and dendritic wiring patterns throughout the nervous system, a striking feature of neural circuitry.

Even though the growth cone is a motile structure, apparently distinct from nonmotile epithelial sheets, there are potentially common links at the molecular and cellular levels because the molecular and cellular components are not "stationary" in nonmotile epithelial cells. In fact, recent work showed that the key component in the adherence junction, E-cadherin, is actively turned over while setting up polarity. Ongoing polarized endocytosis and exocytosis in stationary epithelial cells are also widely recognized. Insights gained from cell polarity signaling pathways in growth cones can also shed light on general principles of cell polarity signaling. This review focuses on PCP components, although A-BP will be included because of the intimate interactions of these two cell polarity pathways.

2. PCP SIGNALING COMPONENTS MEDIATE AXON GUIDANCE

Two independent studies published in 2003 converged on the surprising discovery that the Wnt family morphogens are conserved axon guidance molecules (Lyuksyutova et al., 2003; Yoshikawa, McKinnon, Kokel, & Thomas, 2003). In the vertebrate spinal cord, commissural axons that ascend after midline crossing are attracted by Wnts, which are expressed in an anterior-high–posterior-low (rostral high–caudal low) graded fashion

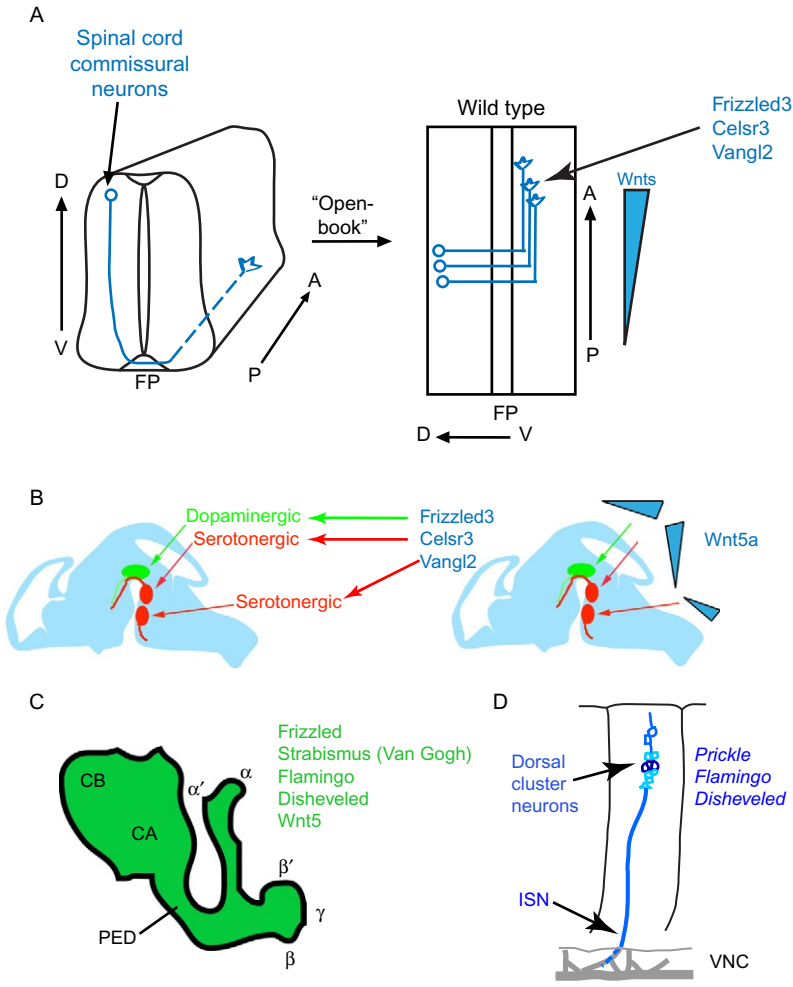

Figure 6.1 Evidence of PCP signaling in axon guidance. (A) Spinal cord commissural axons reply on PCP signaling components for faithful anterior turning after crossing the midline (Lyuksyutova et al., 2003; Shafer, Onishi, Lo, Colakoglu, & Zou, 2011). (B) Dopaminergic and serotonergic axons in the midbrain and hindbrain are guided to project along the anterior–posterior axis by PCP components (Fenstermaker et al., 2010). (C) *Drosophila* mushroom body axon projection patterns are organized by PCP components (Shimizu, Sato, & Tabata, 2011). (D) *Drosophila* sensory axons rely on core PCP components for outgrowth (Mrkusich, Flanagan, & Whitington, 2011).

along the length of the spinal cord and detected by the receptor, Frizzled3, in the commissural neurons (Lyuksyutova et al., 2003) (Fig. 6.1A). Studies in *Drosophila* showed that Derailed-expressing commissural axons only cross the midline along the anterior commissure due to the repulsive function of

Wnt5 expressed at higher levels in posterior commissure (Yoshikawa et al., 2003) (Fig. 6.1B). Derailed is a Wnt binding receptor, which mediates axon repulsion by Wnt5a concentrated in the posterior commissure. Since then, a number of publications reported the role of Wnt signaling in axon guidance in a variety of neuronal types (corticospinal motor neurons, retinal ganglion cells, olfactory sensory neurons, dorsal root ganglion cells, dopaminergic, and serotonergic neurons) (Blakely et al., 2011; Domanitskaya et al., 2010; Fenstermaker et al., 2010; Hilliard & Bargmann, 2006; Hutchins, Li, & Kalil, 2011; Keeble et al., 2006; Kennerdell, Fetter, & Bargmann, 2009; Li, Hutchins, & Kalil, 2009; Liu et al., 2005; Lu, Yamamoto, Ortega, & Baltimore, 2004; Pan et al., 2006; Prasad & Clark, 2006; Rodriguez-Gil & Greer, 2008; Sato, Umetsu, Murakami, Yasugi, & Tabata, 2006; Schmitt et al., 2006; Shimizu et al., 2011; Song et al., 2010). Because morphogens are known for their role in specifying cell types by activating different transcription programs at different concentrations, their function in axon guidance had not been anticipated. This has also led to the investigation on how Wnt family morphogens signal in growth cones to guide axons (Zou, 2004).

In an earlier review in 2004, it was cautiously postulated that "PCP signaling pathway," a "variation of PCP signaling pathway," or a "subset of PCP machinery" might be involved in Wnt-mediated axon guidance. The caution was motivated by the fact that only Frizzled3 had been shown to mediate axon guidance at that time (Zou, 2004). In 2005, another PCP component, Ceslr3, showed similar function to Frizzled3 in axon guidance (Tissir, Bar, Jossin, De Backer, & Goffinet, 2005). However, Flamingo, the *Drosophila* homologue of the Celsrs, has additional function independent of PCP signaling (Berger-Muller & Suzuki, 2011). Therefore, the evidence of PCP signaling in axon guidance was still preliminary.

In more recent years, new work has begun to reveal that cell polarity signaling components may play a central role in Wnt-mediated axon guidance. In 2008, components of A-BP signaling, atypical PKC/Par3/Par6 complex, were first shown to directly mediate Wnt attraction and anterior turning of spinal cord commissural axons (Wolf et al., 2008). In 2010 and 2011, multiple PCP components, Frizzled3, Ceslr3, and Vangl2, were directly tested and found to be required in anterior–posterior guidance of brainstem serotonergic and dopaminergic axons and the spinal cord commissural axons (Fenstermaker et al., 2010; Shafer et al., 2011) (Fig. 6.1A and B). In addition, Vangl2 was found localized on tips of extending filopodia and promoted commissural axon growth in response to Wnt5a

(Shafer et al., 2011). The involvement of multiple PCP signaling components in axon guidance appears to be evolutionarily conserved because in *Drosophila*, frizzled (fz), strabismus (stbm/Van Gogh), flamingo (fmi), and disheveled (dsh) are cooperatively required for axonal targeting and branching of the *Drosophila* mushroom body neurons and Wnt5 was implicated as the ligand (Shimizu et al., 2011) (Fig. 6.1C). In the dorsal cluster neurons, *prickle*, *flamingo*, and *disheveled* promote sensory axon advance in *Drosophila* (Mrkusich et al., 2011) (Fig. 6.1D). These recent studies strongly favor the view that the PCP signaling pathway (and together with A-BP signaling) may provide a major axon steering mechanism in response to Wnts.

In each set of published study, a subset of PCP components, three to four of six, were analyzed. However, if one pools all studies together, five of six PCP components have been shown required for axon guidance, with the exception of Diego. Given the conservation of the PCP pathways in other systems, it is likely that the same set of PCP proteins is involved in axons guidance. Nonetheless, analyses of complete set of "core" PCP genes in each of the systems will be necessary to conclude whether the entire complement of PCP pathway is engaged in the growth cones or only a subset of PCP signaling pathway is sufficient. In addition, analyzing the role of Fat/Dachsous set of PCP genes in axon guidance will further test the idea how similar the PCP signaling in growth cone is to other examples of PCP signaling.

3. CAN PCP SIGNALING BE USED IN MOTILE GROWTH CONES?

PCP is a common structural feature of tissues throughout the animal kingdom, although most of our knowledge of PCP has been derived from studies in *Drosophila* (hairs of wing and abdomen, bristles on the surface of the body, and the ommatidia in the eye) (Goodrich & Strutt, 2011). Elegant fly genetic studies led to the discovery of the key regulatory system, especially the "core" PCP components that control planar polarity. In more recent years, orientation of hair follicles in mammalian skins, polarized stereocilia of inner ear hair cells, and asymmetric position of primary cilia in the ependymal lining of mammalian brain have emerged as examples of planar polarity in vertebrates. Strikingly, the same set of "core" PCP components controls planar polarity in a highly conserved manner, suggesting that these "core" components and their robust interactions are part of the "universal" code for planar polarity. For more complete description of

PCP signaling, please consult other articles in the same issue. So far, only the "core" PCP components have been analyzed in axon guidance. Therefore, this review focuses on the "core" components.

Planar polarity has mostly been studied in stationary cells that form two-dimensional tissue sheets. Therefore, it is surprising that PCP signaling components are involved in directional control of the motile axonal growth cones. In addition to axon guidance, the same conserved genes that control the "classic" PCP events are also important in many types of moving cells or cellular structures, such as in convergent extension and neuronal migration (Fenstermaker et al., 2010; Mrkusich et al., 2011; Shafer et al., 2011; Shimizu et al., 2011; Wada & Okamoto, 2009). The function of PCP components is required for the precise anterior turning of commissural axon growth cones after midline crossing in the spinal cord and proper anterior–posterior guidance of dopaminergic and serotonergic axons in the midbrain and hindbrain. The anterior–posterior guidance of the spinal cord and brainstem neurons, as well as the anterior–posterior migration of *zebrafish* facial motor neurons and mouse branchinomotor neurons, coincides with the anterior–posterior polarity observed in the intercalating cells in *zebrafish* convergent extension (Lyuksyutova et al., 2003; Qu et al., 2010; Shafer et al., 2011; Wada & Okamoto, 2009). In addition to axon guidance, there has been an explosion of papers demonstrating the function of PCP genes in various developmental and disease processes in different tissues (Barrow, 2011; Carroll & Das, 2011; Happe, de Heer, & Peters, 2011; Heinonen, Vanegas, Lew, Krosl, & Perreault, 2011; Ng, 2012; Sugiyama, Lovicu, & McAvoy, 2011; Sundberg et al., 2011; Wu, Ge, Huang, Hua, & Mu, 2011; Yates & Dean, 2011; Zou, 2011).

The rapid expansion of the roles of these PCP genes automatically invites the following questions: Is the term "planar cell polarity" being used too loosely? Are some of the functions separate from the real PCP signaling? Alternatively, this may suggest that a common signaling mechanism which can impart polarity drives many morphogenesis processes, some of which are obviously analogous to the classic PCP described in *Drosophila* and others are not immediately similar at least on the surface. Given the broad nature of planar polarity in tissues from all germ layers (Zou, 2011), it is a formal possibility that the core PCP signaling system, being first characterized in fly wing and eye, may be a widely used signaling module conveying directionality in many cell types during various events of morphogenesis. "PCP signaling" or "PCP-like signaling" or "PCP signaling module" refers to this

common molecular and cellular signaling mechanism in general, including the classic PCP events in the fly. Needless to say, the incredible versatility of the PCP signaling module will need to be achieved by many different upstream input and downstream output in different morphogenesis events, which will be interesting topics to study.

4. IS THE GROWTH CONE POLARIZED?

Axonal growth cones have been studied for many years. They are known to be very sensitive to concentration differences and are able to turn to areas with higher or lower concentration of molecular guidance cues. However, whether growth cones are polarized or asymmetric has never been established or at least there has not been a widely accepted consensus. The highly motile membrane and cytoskeletal structures tend to lead one to think that growth cones are highly "fluid" and "dynamic" and not polarized. However, when looking inside the growth cone, there is ample evidence of polarity (Fig. 6.2).

First, microtubules are polarized with plus ends pointing toward the distal end of axons and represent the "forward" direction of the growth cone. This polarized organization of microtubule structure is established as early as the axon is formed. This "proximal–distal" growth cone axis controls not only the direction of microtubule polymerization (and depolymerization) but also the direction of vesicular trafficking.

Second, actin filaments also show polarity. The plus ends of actin filaments (barbed ends) point to the tips of filopodia and the minus ends point to the inside of the growth cone. Actin filaments undergo retrograde flow with monomers moving inside the growth cone due to treadmilling (Yang, Zhang, Pollard, & Forscher, 2012).

Third, endo- and exocytosis are found polarized in growth cones and involved in navigation (Itofusa & Kamiguchi, 2011). Whether endo- and exocytosis can provide sufficient membrane translocation to turn growth cones still needs experimental evidence. However, the existence of polarized membrane trafficking suggests that this could at least be a mechanism of setting up an asymmetric signaling gradient within the growth cone.

Based on the aforementioned polarized microtubule and actin organization and membrane trafficking (Etienne-Manneville, 2011; Itofusa & Kamiguchi, 2011), the growth cone is a highly polarized structure.

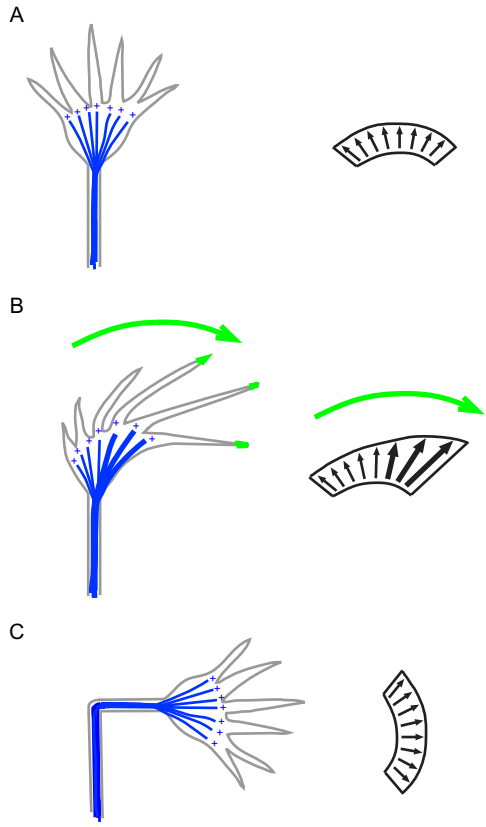

Figure 6.2 A model for growth cone polarity and growth cone turning. Blue lines represent microtubules, which have plus ends pointing toward the distal end of the growth cone. The thickness of microtubules represents their stability. Green indicates the localization of some signaling molecules, which may be asymmetrically localized, such as Vangl2 (Shafer et al., 2011).

Perhaps a unique feature about growth cone polarity is that polarity is much more dynamic than stationary cells and the direction of polarity can change, for example, during turning. Recent studies show that both apical–basal and planar polarity signaling components in epithelial polarity are involved in Wnt-mediated turning (Fenstermaker et al., 2010; Shafer et al., 2011; Wolf et al., 2008). This suggests that the growth cone may engage these potent cell polarity regulators to change its polarity in response to guidance cues to achieve turning.

5. BIOCHEMISTRY AND CELL BIOLOGY OF PCP SIGNALING

PCP components have mostly been identified by genetic analyses. Much less is understood in terms of biochemical and cell biological mechanisms. In addition, neither the upstream regulators of PCP nor the downstream effectors that put out the asymmetry in most cases are well understood. Consistent with that, there has not been reliable or relevant biochemical readout specific for PCP signaling. JNK and Rac1 activations are often used in many studies, and they are definitely involved in PCP signaling. However, their exact roles in PCP signaling are unknown because JNK and Rac1 also respond to many other signaling pathways. Asymmetric localization of PCP components has been shown to be essential to PCP signaling. However, how such asymmetric localization is established and what this asymmetric localization encodes are not clear. The ubiquitin proteosome system is a key mechanism of asymmetric localization of some PCP components, suggesting that selective degradation could be a way to introduce asymmetry (Narimatsu et al., 2009). Recent studies established that endocytosis is required for PCP signaling (Sato, Yamamoto, Sakane, Koyama, & Kikuchi, 2010; Yu et al., 2007). Based on all these findings, it is possible that multiple signaling events may take place in different parts of the cell during PCP signaling at the same time or in sequence. Therefore, it is necessary to understand all the biochemical interactions among PCP components before the complete picture of PCP mechanisms can emerge.

A recent study on the biochemical interactions of the core PCP components led to a possibly general mechanism for setting up and/or maintaining the asymmetric localization of PCP components (Shafer et al., 2011) (Fig. 6.3). The distribution of Frizzled3 protein appears to depend on its state of phosphorylation. Frizzled3 protein is mostly localized in intracellular vesicles, and hyperphosphorylation of Frizzled3, induced by Disheveled1, causes Frizzled3 to be targeted to the plasma membrane (Fig. 6.3B). Vangl2, which antagonizes Disheveled1, reduces Frizzled3 phosphorylation and membrane localization on the cell surface (Shafer et al., 2011) (Fig. 6.3C). These findings are consistent with the observations that Van Gogh and Prickle tend to have opposite functions from Frizzled and Disheveled in PCP signaling and suggest that the antagonism may be achieved by opposite effects on Frizzled phosphorylation/membrane localization

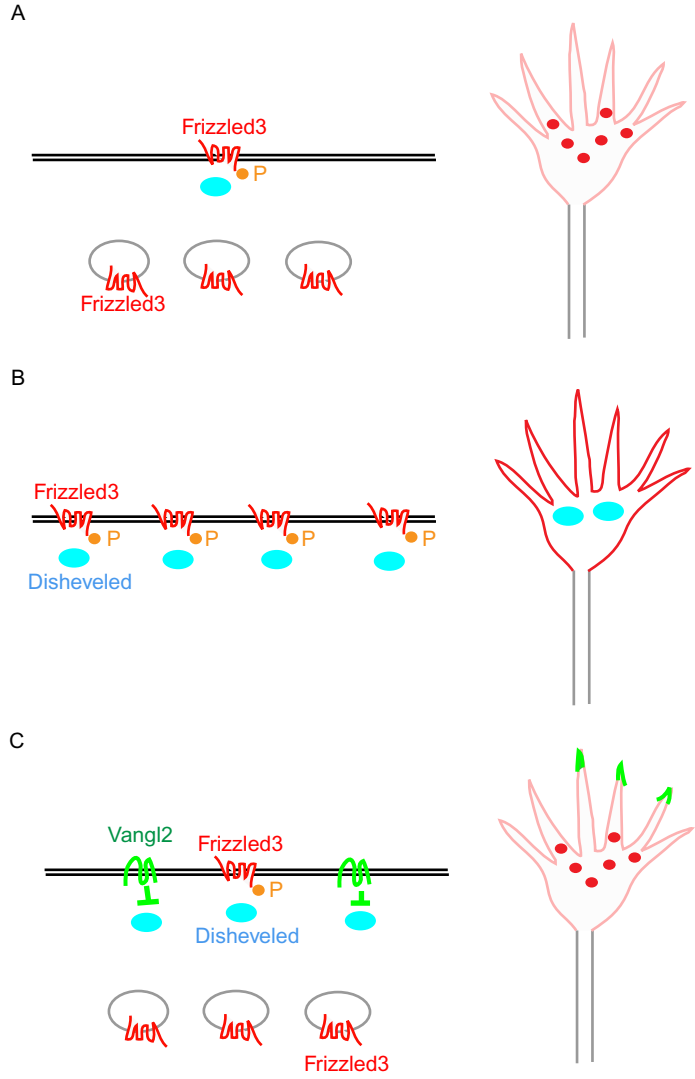

Figure 6.3 Regulation of Frizzled3 membrane localization by phosphorylation. (A) Frizzled3 exists mostly in intracellular vesicles. (B) Disheveled1 can cause Frizzled3 hyperphosphorylation and accumulation on plasma membrane, which stops PCP signaling. (C) Vangl2 antagonizes Disheveled1-mediated Frizzled3 inactivation by promoting Frizzled3 endocytosis. Vangl2 is enriched on tips of extending filopodia not in retracting filopodia, suggesting Vangl2 activates PCP signaling in a subset of filopodia (Shafer et al., 2011).

mediated by Disheveled and Van Gogh. In addition, Frizzled and Disheveled are localized on the distal membrane of fly wing epithelial cells and Van Gogh and Prickle are localized on the proximal membrane shortly before the appearance of morphological asymmetry along the proximal–distal axis. It is possible that Van Gogh may be activated on the proximal membrane only and therefore Frizzled is removed from the cell surface on the proximal membrane. It is also possible that Disheveled is highly activated on the distal membrane such that it is able to keep Frizzled on the plasma membrane. Vangl2 itself undergoes complex phosphorylation as well (Gao et al., 2011), which may represent an input of another regulatory signal. However, it is currently unknown whether Frizzled3 hyperphosphrylation induced by Disheveled inhibits endocytosis or promotes exocytosis. Both cases are consistent with the current findings, and determining which case is true will certainly shed more light on PCP signaling.

6. LOCALIZATION OF VANGL2 PUNCTA ON FILOPODIA TIPS SUGGESTS THAT PCP SIGNALING MAY STEER GROWTH CONES

In live growth cones, Vangl2 protein is highly enriched on the tips of extending filopodia but not the shrinking filopodia. This suggests that at least one aspect of PCP-like signaling is selectively activated on those filopodia tips and not in the rest of the growth cones, which is inactivated by Disheveled1 (Shafer et al., 2011) (Fig. 6.3C). In other words, the asymmetry in growth cones may be manifested by differences among filopodia, the ones with Vangl2 versus those without. This opens up the opportunity to understand how PCP signaling takes place in growth cones. First, this provides a clue that the filopodia tips, not the entire filopodia, are sensors of the growth cone; second, this also suggests that Frizzled3, whose phosphorylation is reduced by Vangl2, may be endocytosed from the tips, carrying signal into the growth cone. It will be very informative to follow Frizzled3 trafficking and decipher the information Frizzled3 may bring into the growth cone. On the other hand, following the question on how Vangl2 is localized to the tips can potentially lead to the answers of how upstream activators of PCP signaling regulate polarity.

In a previous study, aPKC/Par3/Par6 complex, a key component of the A-BP signaling, was found to mediate Wnt attraction during anterior turning of commissural axons (Wolf et al., 2008). There has been evidence that A-BP and PCP signaling pathways interact with each other intimately. First,

aPKC can inhibit Frizzled/PCP signaling by directly phosphorylating the intracellular domain of Frizzled (Djiane, Yogev, & Mlodzik, 2005); second, Disheveled directly binds to Lgl, a substrate of aPKC, and regulates the localization of Lgl (Dollar, Weber, Mlodzik, & Sokol, 2005). Therefore, there may exist a connection that integrates these two polarity signaling pathways in epithelial cells and in axonal growth cones. It is now possible to study the mechanisms of how A-BP signaling components may interact with PCP components in commissural axon growth cones. How, for example, does aPKC affect Frizzled3 phosphorylation and trafficking will provide clues to how signaling events take place in growth cones. Understanding what effect these signaling events have on cytoskeletal structures (the microtubules and the actin system) during growth cone turning will eventually solve this century-old mystery of axon guidance.

Growth cones are also repelled by Wnts via a different receptor Ryk (Keeble et al., 2006; Liu et al., 2005). Ryk signaling is still relatively unclear although it is thought to involve the src family kinases in *Drosophila* (Wouda, Bansraj, de Jong, Noordermeer, & Fradkin, 2008). In vertebrate, the Wnt/Calcium signaling has been implicated in Ryk signaling (Hutchins et al., 2011; Li et al., 2009; Li, Hutchins, & Kalil, 2010). The relationship of Ryk with PCP signaling components has been unknown. However, a recent study suggests that Ryk signaling may converge with Frizzled/PCP via interacting with Vangl2 (Macheda et al., 2012). This raises the interesting question whether a common core mechanism causes both attraction and repulsion or whether attraction and repulsion are mediated by totally different mechanisms. The remarkable observation that growth cone attraction and repulsion can be switched by the ratio of cAMP and cGMP suggests a common core may exist (Song et al., 1998; Song, Ming, & Poo, 1997).

7. ARE THERE GROWTH CONE–GROWTH CONE INTERACTIONS DURING PCP-MEDIATED TURNING?

Cell–cell interaction is an essential component in PCP signaling. Several PCP components have both cell-autonomous and cell-nonautonomous functions. If PCP signaling module functions in the growth cones, are there any similar cell–cell interactions?

Neurons of the same type are often born at the same time and have similar time course in their developmental program. Neurons and axons certainly have the opportunities to interact with each other during pathfinding. In the spinal cord, commissural neurons that are born at the

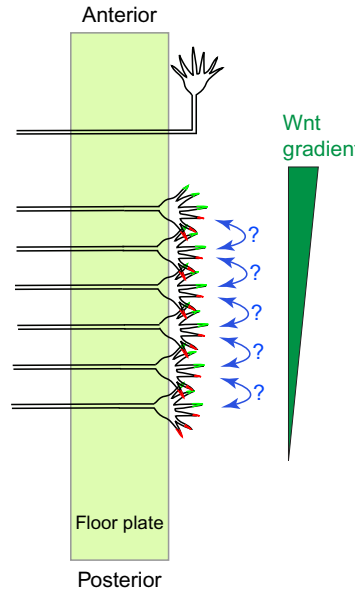

Figure 6.4 Possible growth cone–growth cone interactions during midline crossing and anterior turning of commissural axons.

same time cross the midline at the same time and turn at the same time (Fig. 6.4). It takes many hours (8–9 h) to cross the midline and 1–2 h to turn anteriorly (Y. Zou lab unpublished results). There is plenty of time for commissural neurons and their axons to interact with each other, although there have been no studies addressing this possibility so far. If commissural axon growth cones interact with each other, the global anterior turning of this "sheet" of commissural axon growth cones is highly reminiscent of planar polarity. Furthermore, a sheet of axons may detect greater concentration drop than individual growth cones if they interact with each other. Alternatively, growth cones may not talk to each other. But rather, they work individually because they have sensitive filopodia to detect large enough concentration drops. These intriguing possibilities deserve further investigation.

8. IS THIS CELL POLARITY-BASED SIGNALING SYSTEM A GENERAL GROWTH CONE STEERING MECHANISM?

By studying how Wnts signal in growth cones, we learned that the growth cone uses both A-BP and PCP signaling pathways that are essential for cell and tissue polarity in epithelia to control the direction of turning

(Fig. 6.5A and B). Looking into the molecular and cellular anatomy of the growth cone, the growth cone, particularly the filopodia, has similar compositions as adherens junction. Growth cones have both aPKC and N-cadherin in the filopodia. N-Cadherin is found in adherens junctions in chick cardiac muscle cells and lens epithelium (Volk & Geiger, 1984, 1986). Cadherins also interact with aPKC and regulate its activity and

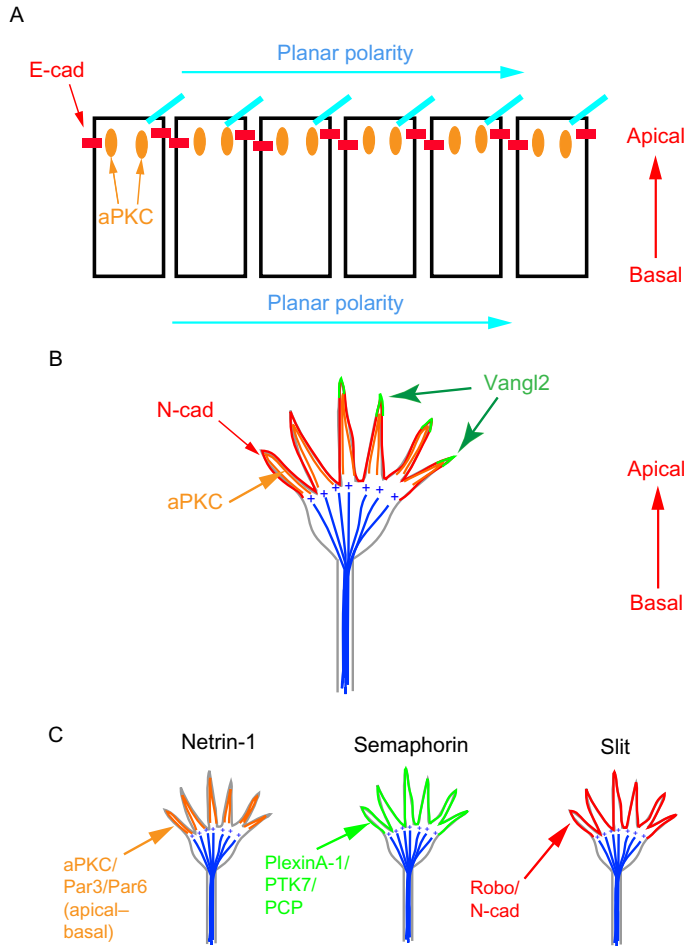

Figure 6.5 A cell polarity-signaling-based machinery for growth cone turning. (A) In epithelia, E-cadherin and aPKC defines the apical basal polarity and planar polarity is perpendicular to apical–basal axis. (B) In neuronal growth cones, N-cadherin and aPKC may define the distal–proximal (apical–basal) axis and PCP signaling may be asymmetrically activated in a subset of filopodia and steer turning in axis perpendicular to the distal–proximal (apical–basal) axis. (C) Other axon guidance signaling may access this

therefore may regulate cell polarity signaling during gastrulation (Seifert, Ibrahim, Stodtmeister, Winklbauer, & Niessen, 2009). I would like to propose that the growth cone filopodia can be viewed as "mobile adherens junction" searching for the missing half and can respond to many cues (Fig. 6.5B).

The cell polarity signaling pathways offer an opportunity to understand signaling mechanisms for growth cone steering. Because of the intimate interactions of A-BP and PCP signaling, it is likely that one may depend on the other. For example, one possibility is that A-BP specifies the proximal–distal axis of the growth cone and PCP components may polarize growth cones perpendicular to the proximal–distal axis (Fig. 6.5B). An alternative to this model is that PCP and A-BP are not organized along these perpendicular axes in growth cones but rather may function in collaboration to amplify each other's signaling level in certain selected filopodia, causing a massive turning signal.

Because A-BP and PCP components are expressed in all neurons and these signaling components can regulate actin, microtubule and membrane trafficking, a spontaneous question is whether this mechanism, or "growth cone compass," is universal. Is there any evidence that other axon guidance signaling system than the Wnt system can also access this "compass?" The following findings are beginning to provide encouraging clues:

1. Par3, Par6, and aPKC are required for axon outgrowth-promoting effects of Netrin-1 and NGF, and Par3 and Par6 are required for ventrally directed growth cone commissural axons to the rat spinal cord midline (Hengst et al., 2009). This study suggests that Netrin-1 can access this potentially universal machinery via A-BP components (Fig. 6.5C).
2. PTK7 is a newly identified Wnt coreceptor in PCP signaling (Peradziryi et al., 2012). Its *Drosophila* orthologue, OTK, forms a complex with

machinery. aPKC/Par3/Par6 is required for Netrin-1-stimulated axon outgrowth (Hengst, Deglincerti, Kim, Jeon, & Jaffrey, 2009). PTK7, a Wnt coreceptor in PCP signaling is a coreceptor for PlexinA1, which mediates semaphorin signaling (Peradziryi, Tolwinski, & Borchers, 2012; Toyofuku et al., 2004; Wagner, Peradziryi, Wehner, & Borchers, 2010). N-Cadherin, a potent stimulator for axon out growth, is found in the adherens junction of chick cardiac muscle cells and lens epithelial cells. Slit–Robo can inhibit adhesion by both E-cadherins and N-cadherins and regulate retinal neurite outgrowth, adhesion, or retinal ganglion cell apical process retraction (Rhee, Buchan, Zukerberg, Lilien, & Balsamo, 2007; Rhee et al., 2002; Santiago-Martinez, Soplop, Patel, & Kramer, 2008; Wong, Baudet, Norden, Leung, & Harris, 2012).

PlexinA1 to mediate repulsion by Sema 1a. PTK7 also interacts with PlexinA1 to regulate cranial neural crest migration in *Xenopus* (Wagner et al., 2010). In chick, KLG/Otk is a coreceptor with PlexinA1 and VEGFR2 to respond to Sema6D in cell migration (Toyofuku et al., 2004). These studies suggest that Semaphorin signaling may access this machinery via PTK7 through the PCP side (Fig. 6.5C).

3. N-Cadherin is long known to be a potent stimulator for axon outgrowth. N-Cadherin was found in the adherens junction of chick cardiac muscle cells and lens epithelial cells and therefore can exert similar adhesion and cell polarization functions as E-cadherin (Volk & Geiger, 1984, 1986). Recent studies show that a classic guidance system, Slit–Robo system, can inhibit adhesion by both E-cadherins and N-cadherins and regulate retinal neurite outgrowth, adhesion, or retinal ganglion cell apical process retraction (Rhee et al., 2007, 2002; Santiago-Martinez et al., 2008; Wong et al., 2012). Cadherin can interact with aPKC and regulate its function (Seifert et al., 2009). Therefore, Slit may access the cell polarity-based steering machinery via the cadherin complex, which may affect both A-BP and PCP signaling (Etienne-Manneville, 2011) (Fig. 6.5C).
4. In addition to adherens junction, focal adhesion can also affect aPKC (Itoh et al., 2010). Therefore, axon guidance signaling which affects focal adhesion kinase may also access this cell polarity-signaling-based turning machinery.

Therefore, it is possible that growth cone signaling can be unified under a common cell polarity-based machinery. If this is true, this common cell polarity-based compass in growth cone is analogous to the core cell cycle mechanism (CDKs and cyclins), which controls cell cycle of all cell types but can be regulated by many different factors and at different checkpoints in different cell types.

9. SUMMARY

It is somewhat surprising that dynamic growth cones can utilize a signaling system that establishes and maintains polarity in nonmoving cell sheets such as in epithelia. However, at the molecular and cellular level, cell polarity signaling may be similarly dynamic in both motile and nonmotile cells. Recent studies suggest that components of adherens junctions, even E-cadherin, are actively turned over even in these nonmoving cells (Baum & Georgiou, 2011). The robustness of cell polarity signaling

pathways imparting asymmetry throughout the entire cells makes them ideally suitable for building the stunningly organized neuronal morphology that is essential for neural circuit function. PCP and A-BP signaling, which intimately interact with each other, are used for axon wiring, and they can, in principle, also be used to regulate designs such as dendrites, axonal and dendritic branches, and even spines (Moreau et al., 2010). These discoveries will not only provide insights of brain development but also identify the components that may be affected in developmental disorders causing nervous system disorders. When there is a need to steer growth cones to repair brain circuits, the knowledge of how PCP signaling may guide growth cones will be instrumental.

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