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Morphogens as conserved axon guidance cues

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Morphogen family proteins are now widely appreciated as axon guidance cues. Because their roles as morphogens are highly conserved across phylogeny, their functional conservation in axon guidance is now being rigorously examined. Recent studies suggest that morphogens are important in shaping topographic projections in chick and *Drosophila* visual systems, a process that occurs even later in development.

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Introduction

Axon wiring involves two phases: pathfinding and target selection. During pathfinding, growth cones navigate in developing embryos towards their targets, which can be far away from their cell bodies and often pass through intermediate targets, laying down a scaffolding of axon networks. As they approach their target area, axons select their postsynaptic targets within similar groups of cells. Synaptic connections are organized in topographic, point-to-point or converging patterns and often in a lamina-specific manner. These exquisite patterns of connectivity, essential for all behavioral functions, are established by a collaboration of molecular guidance cues and neural activity.

Studies over the past decade or so identified several classes of classical axon guidance molecules, which have pivotal roles in nervous system wiring [1,2]. A recent development in axon guidance studies has been the finding that morphogens, which are known to specify cell fate by concentration gradients, are axon guidance cues, adding to the repertoire of wiring instructions for navigating growth cones [3–9]. These morphogens, which tend to set up gradients along major body axes, remain or become re-expressed in the region, to provide directional information to axons along major body axes. Because morphogen functions start at an earlier stage, it is reasonable to speculate that they might help to establish the expression gradient of other axon guidance molecules. Therefore, understanding the full function of morphogens in nervous system wiring might provide new insights into the molecular and cellular logic in the organization of neuronal connectivity.

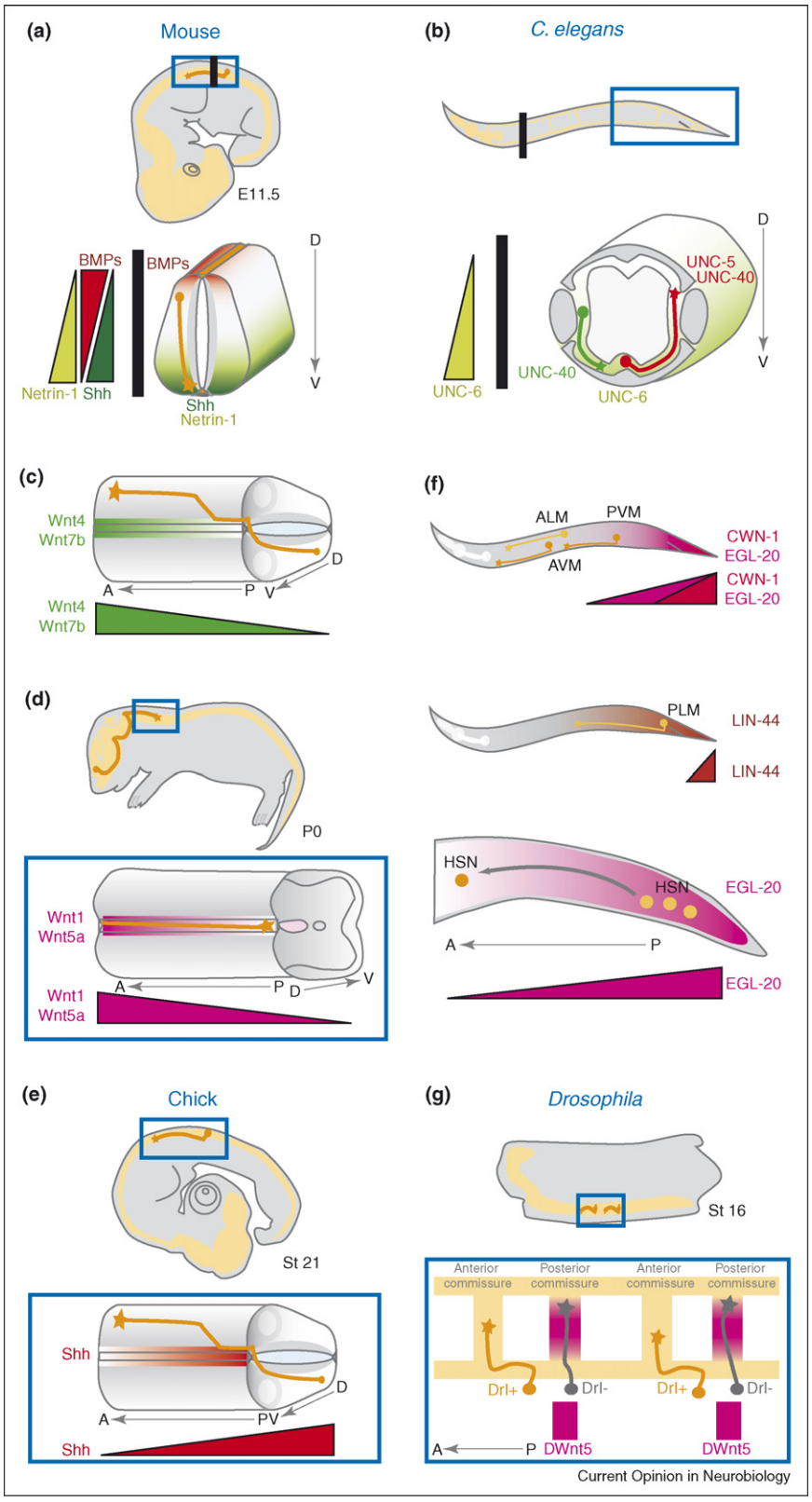
Pathfinding

Several families of morphogen proteins, which are known to specify cell fates along major body axes, are now well-accepted guidance molecules for axonal pathfinding. The nervous system is organized along major body axes, and, interestingly, morphogens are found to be important for guiding axons along these axes in several animal systems.

Along the dorsal–ventral axis in the vertebrate spinal cord, bone morphogenetic proteins (BMPs) and Sonic hedgehog (Shh), together with a conserved classical axon guidance molecule, Netrin-1, control the axonal pathfinding of commissural neurons from the dorsal spinal cord to the ventral midline [10,11] (Figure 1a,b). Along the anterior–posterior axis of the spinal cord, multiple Wnt family proteins guide ascending sensory axons (commissural axons) and descending cortical motor axons (corticospinal tract) along the longitudinal axis (Figure 1c,d). At least two Wnts, Wnt4 and Wnt7b, were found to be expressed in an anterior–posterior gradient within the mouse floor plate, the ventral midline of the spinal cord. Through an attractive response via Frizzled3, this Wnt expression gradient was proposed to provide an anterior turning instruction to the commissural axons after midline crossing, through an anterior 'pull' mechanism [12,13] (Figure 1c). For descending axons, two Wnts, Wnt1 and Wnt5a, are expressed in an anterior–posterior gradient in the dorsal midline of the neonatal spinal cord and repel corticospinal tract axons down the spinal cord via a conserved repulsive Wnt receptor, Ryk [14•,15] through a 'push' mechanism (Figure 1d). Therefore, Wnts might control pathfinding along the anterior–posterior axis of multiple classes of axons in the spinal cord, 'pulling' some axons and 'pushing' others. In chick spinal cord, commissural axons are repelled by Shh after midline crossing, and Shh was found to be expressed in an increasing anterior–posterior gradient and is required for anterior turning [16•] (Figure 1e).

The role of Wnts as key guidance cues along the anterior–posterior axis has been shown to be highly conserved (Figure 1f). In nematodes, three Wnts are expressed at the posterior end of the worm body, and mutations of the

Figure 1



Roles of morphogen family proteins in axon pathfinding. **(a)** BMPs and Shh control dorsal–ventral pathfinding of spinal cord commissural axons. BMPs repel spinal cord commissural axons from the roof plate. Shh attracts spinal cord commissural axons towards the floor plate in collaboration with Netrin-1. **(b)** Conserved function of a classical axon guidance molecule, Netrin, in dorsal–ventral guidance of axons in

Wnt and Frizzled genes were found to cause anterior–posterior guidance and polarity defects of several processes, as well as neuronal migration [17•–19•,20]. These Wnts presumably form an increasing anterior–posterior gradient and guide axons using a repulsive mechanism through the Frizzled receptors.

This posterior–anterior 'push' mechanism appears to function in the chick spinal cord, where Shh was found to be expressed in an increasing anterior–posterior gradient (Figure 1e). Through repulsion, Shh repels postcrossing commissural axons anteriorly via Hedgehog-interacting protein [16•]. It remain to be tested whether Shh has a similar function in guiding postcrossing commissural axons in the mouse, whether Wnts regulate postcrossing commissural axons in the chick spinal cord and whether these two pathways work in both systems – and, if so, whether they operate in the same neurons or in different neuronal populations.

In a broader sense, the midline pathfinding of the *Drosophila* commissural axons is reminiscent of anterior–posterior guidance choice (Figure 1g). In each segment in the ventral nerve cord, Derailed-positive axons avoid the posterior commissure and only cross the midline along the anterior commissure. DWnt5 is expressed at a higher level in the posterior commissure, and Derailed (also known as Ryk in vertebrates) mediates repulsion and directs the subset of commissural axons expressing Derailed to traverse the midline along the anterior commissure [21]. Similar to this, in the vertebrate spinal cord, Ryk also mediates repulsion by Wnt1 and Wnt5a, although from anterior to posterior [14•] (Figure 1d). In this sense, the segmented fly ventral nerve cord can be considered as a number of miniature spinal cords compressed together in a reverse anterior–posterior direction or many shorter *Caenorhabditis elegans* bodies in the same orientation, although repulsion in *C. elegans* is mediated by Frizzled receptors.

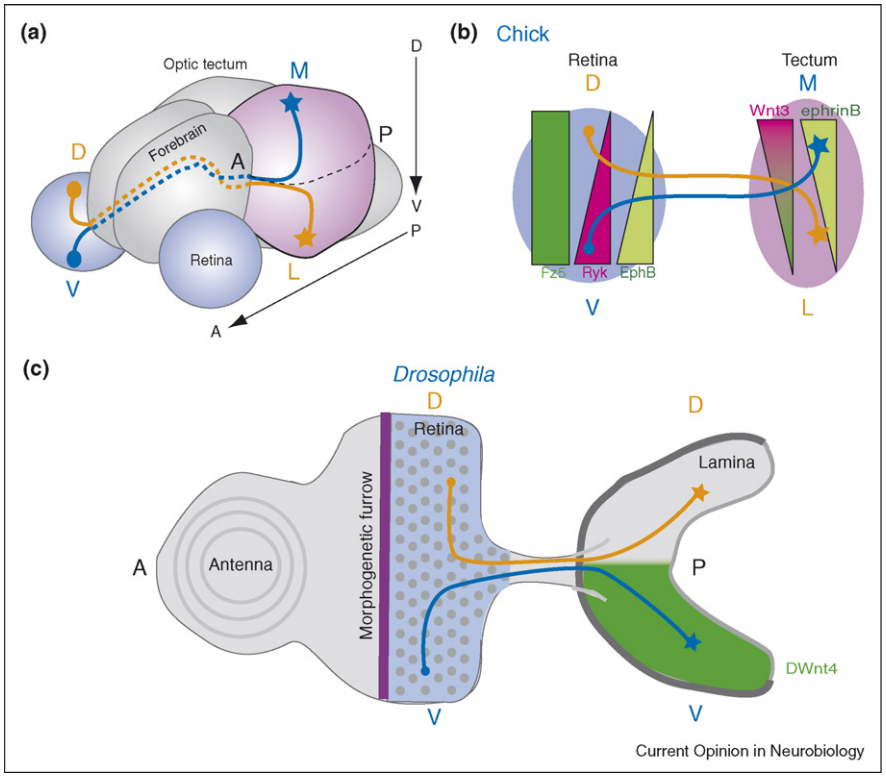
Topographic mapping

Intriguingly, Wnt family proteins might also have a role in organizing synaptic connections by conveying topographic position information during axon target selection (Figure 2). Topographic mapping connects two areas of the nervous system in such a way that the positional information of one area becomes represented continuously to the other. This is a highly recurring wiring paradigm in multiple nervous systems. Sperry [22] postulated that molecular gradients might be ideal candidates for positional cues. Two coordinate systems perpendicular to each other, dorsal–ventral and rostral–caudal, specify the two-dimensional positional information on the retina. The two-dimensional positional information, medial–lateral and anterior–posterior, is then recapitulated in the midbrain and forebrain targets via topographic projections of retinal ganglion cell (RGC) axons (the dorsal–ventral map is shown in Figure 2a). Ephrin family guidance cues are required for both the anterior–posterior- (ephrinAs) and medial–lateral dimension (ephrinB1) [23–28]. Computational modeling proposes that to create a smooth and complete map, two counterbalancing forces are necessary in each dimension, so that axons originating from different topographic positions can find their corresponding addresses. If only one mapping force is present, the entire map will collapse to one end [29–32]. Wnt3 protein is expressed in a decreasing medial–lateral (dorsal–ventral) gradient in the chick optic tectum, and RGC axons from different dorsal–ventral positions have position-dependent responses to various concentrations of Wnt3 (Figure 2b). Dorsal RGCs are attracted by Wnt3 at lower concentrations but repelled by Wnt3 at higher contractions. Ventral RGCs are repelled by Wnt3 at both higher and lower concentrations. Therefore, all RGC axons are directed laterally by Wnt3, with ventral axons being repelled more strongly, and the dorsal-most axons are encouraged to growth towards the lateral end of the map, where lower levels of Wnt3 protein are present. This lateral mapping activity has been shown to be counterbalanced by ephrinB1–EphB function in a positionally dependent way because higher attraction by ephrinB1, which peaks at the medial tectum, was detected in the ventral RGCs. When Wnt3 repulsion is blocked, RGC axon termination zones shift medially [33•]. It had previously been shown that when ephrin1–EphB signaling is ablated, the termination zones shift laterally [28]. In this context, Wnt3 acts as one of the essential mapping cues along the dorsal–ventral axis, together with a classical axon guidance molecule, ephrinB1, for RGC axons during topographic mapping, consistent with computational modeling (Figure 2b).

A role for Wnt proteins in topographic mapping now receives support from invertebrate studies showing a conservation of such functions. In the fruitfly, DWnt4–dFz2 signaling controls dorsal–ventral retinotopic connections of the photoreceptor axons in the visual target in the brain (Figure 2c). *Drosophila* compound eyes contain 750 ommatidial units. Each of these units includes eight

(Figure 1 Legend Continued) *C. elegans*. UNC-6 (Netrin) attracts axons ventrally through UNC-40 receptor and repels axons dorsally through UNC-40 and UNC5 receptor complex. **(c)** Wnt4 and Wnt7b, expressed in an anterior–posterior decreasing gradient, attract mouse spinal cord commissural axons to turn anteriorly along the ventral midline after midline crossing. **(d)** Wnt1 and Wnt5a, expressed in an anterior–posterior decreasing gradient, repel cortical spinal tract axons to grow posteriorly in the spinal cord along the dorsal midline. **(e)** Shh, expressed in an anterior–posterior increasing gradient in the chick spinal cord midline, repels spinal cord commissural axons to grow anteriorly. **(f)** Multiple Wnts in *C. elegans*, expressed in an anterior–posterior increasing gradient, repel several classes of neuronal processes anteriorly, control anterior–posterior polarity of processes and neuronal migration. **(g)** In each embryonic segment in *Drosophila*, Derailed-positive axons are repelled by DWnt5, expressed in the posterior commissure, and cross the midline along the anterior commissure. A, anterior; P, posterior; D, dorsal; V, ventral.

Figure 2



Roles of Wnts in dorsal–ventral topographic mapping. **(a)** Dorsal–ventral topographic organization of visual map in vertebrates. Dorsal retinal ganglion cell axons find the targets in the lateral tectum/superior colliculus and ventral retinal ganglion cell axons target to the medial tectum, forming a smooth topographic representation of the visual world from the retinal to the tectum/superior colliculus. **(b)** Two molecular gradients of guidance cues, ephrinB1 and Wnt3, are required for dorsal–ventral topographic mapping. Both ephrinB1 and Wnt3 are expressed in medial–lateral decreasing gradients. Both EphB and Ryk receptors are expressed in dorsal–ventral increasing gradients. Frizzled receptors are expressed at the same levels along the dorsal–ventral axis in retinal ganglion cell axons. Ryk is a higher-affinity Wnt receptor than Frizzleds and mediates repulsion in RGC growth cones. Frizzleds mediate attraction. Dorsal axons expressing fewer Ryk receptors are attracted by lower concentrations of Wnt3 but repelled by higher concentration of Wnt3. Ventral axons have higher Ryk expression and are repelled by Wnt3 at both lower and higher concentrations. Along the dorsal–ventral axis, all RGC cells prefer to grow laterally. However, ephrinB–Eph signaling attracts RGC axons to grow medially in a position-dependent manner, counterbalancing the position-dependent effect of Wnt–Ryk signaling, allowing the formation of a complete and smooth topographic map. **(c)** DWnt4–DFz2 controls dorsal–ventral retinotropic mapping. Photoreceptor axons in the *Drosophila* compound eyes project in a topographic manner to their brain targets. Axons from dorsal ommatidia project to the dorsal aspect of the lamina and those originating from ventral ommatidia project to the ventral lamina, forming a topographic map. DWnt4 is enriched in the ventral lamina and in *Dwnt4*, *DFz2* and *dsh* mutants, ventral axons mistarget to the dorsal lamina. A, anterior; P, posterior; D, dorsal; V, ventral.

photoreceptor neurons, which project axons through the optic stalk into the lamina (R1–6) and then medulla (R7,8) in the brain. Dorsal photoreceptor axons project to the dorsal lamina, and ventral photoreceptors project to the ventral lamina, forming a topographic map. DWnt4 is expressed in the ventral lamina and is required for ventral photoreceptors to project to the correct topographic position. The latter process appears to occur via an attractive mechanism, involving DFz2, because both DWnt4 and DFz2 mutations caused a shift of axons towards the dorsal lamina, where Wnt4 is not expressed [34•]. This suggests that Wnts can act as attractive molecules in topographic mapping, and that there might exist another mapping activity along the dorsal–ventral axis of the fly visual system.

Comparing notes and open questions

Multiple lines of studies suggest that morphogens have important and conserved roles in nervous system wiring. Although some common findings corroborate each other, there remains an abundance of interesting differences and gaps in knowledge.

Directions of morphogen action

An intriguing observation is that morphogens tend to provide directional instructions for axon guidance along the same axes where they exert their initial functions. BMPs and Shh specify cell fate along the dorsal–ventral axis of the spinal cord and also direct commissural axons along the dorsal–ventral axis. The Wnt family proteins are known for their patterning function along

the anterior–posterior axis as well as dorsal–ventral axis. Along the anterior–posterior axis, Wnt8a posteriorizes the neural tube in *Xenopus*, and *Drosophila* Wingless controls cell fate in each segment. The functions of the Wnt family proteins are diverse. Some Wnts appear to act as morphogens and signal through the canonical pathway, whereas others are thought to be noncanonical Wnts, such as those controlling planar cell polarity. However, in the case of axon guidance, both classes of Wnts can act as directional cues. At midgestation, Wnt4 and Wnt7b were found to be expressed in a decreasing anterior–posterior gradient in the ventral midline of the spinal cord, guiding the anterior turn of commissural axons. At a later stage (neonatal), Wnt1 and Wnt5a were found to be expressed in an anterior–posterior gradient in the dorsal midline of the spinal cord and to direct the posterior growth of corticospinal tract axons. Among these Wnts, Wnt1 has been shown to be a morphogen but it can also act as guidance molecule here. It is possible that the classical morphogenetic types of Wnts set up anterior–posterior patterns early on, and that the nonmorphogen function of Wnts, which acts at a later stage, takes over to guide axon pathfinding along the anterior–posterior axis in *Drosophila*. Wingless and Hedgehog also control cell fate along the anterior–posterior axis in each segment. At a later stage, DWnt5 regulates pathway selection of derailed-positive commissural axons [21]. Interestingly, in the case of chick spinal cord, an increasing anterior–posterior gradient of Shh was reported and proposed to provide directional instruction to chick commissural axons along the anterior–posterior axis [16•]. It will be interesting to test whether Hedgehog regulates anterior–posterior guidance of other axons in *Drosophila*. Along the dorsal–ventral axis, Wnt3, which acts as a morphogen, is a dorsalizing factor in the neural tube. At a later stage (postnatal day 0), its dorsal–ventral gradient in the midbrain serves as a positional instruction for topographic mapping of visual axons with a nonmorphogenetic function. Because the direction of the morphogen gradients determines the direction of axon growth and targeting, the question of how these gradients are initially established and then maintained to a much later stage, when the animal sizes increase, is of great importance.

Bifunctionality and signaling pathways

Most classical axon guidance cues are bifunctional, attractive to some axons and repulsive to others. Although some morphogens appear to behave similarly to the classical axon guidance molecules, acting as both attractants and repellents, others have so far been found to be only repulsive. Shh is an attractant for precrossing commissural axons but repels postcrossing commissural axons in chick spinal cord and inhibits the growth of chick retinal ganglion cell axons. BMPs are repellents, with no reports of any attractive functions. Within the Wnt family, there are attractants and repellents. However, most individual Wnts have so far only been found to be attractive or repulsive. Vertebrate Wnt4 attracts postcrossing commissural axons and DWnt4 attracts photoreceptor axons. Wnt5a repels descending corticospinal tract axons and *Drosophila* Wnt5 repels commissural axons. However, Wnt3 displays biphasic function in RGC axons, attracting dorsal RGC axons at lower concentrations and repelling ventral RGC axons at all concentrations but repelling dorsal axons at higher concentrations. In *C. elegans*, all Wnts appear to be found repulsive so far. It remains to be seen whether any *C. elegans* Wnts are attractive. Therefore, more examples need to be analyzed for a clear picture to evolve, particularly for BMPs and Wnts. This information will be of importance when we consider the signal transduction mechanisms mediating morphogens in axon guidance. Bifunctionality increases the numbers and classes of axons being patterned. The same concentration gradients of guidance cues can guide multiple classes of axons.

Integration of morphogen signaling?

Morphogen signaling pathways operate simultaneously during morphogenesis, and interact among each other. These interactions are likely to be important in establishing spatial patterns of structures during patterning. For example, BMP signaling is known to antagonize Shh signaling in pattern formation [35] but cooperate with each other in tissue differentiation [36]. BMP-2 can modulate Wnt and Frizzled and enhance canonical Wnt signaling [37]. An interesting question is whether and how the signaling pathways of these morphogens interact with each other during growth cone guidance. Although the signaling environment is different during growth cone guidance, certain signaling components might persist, enabling potential interactions. Signal integration can increase the functional diversity by regulating the outcome or the strength of signaling. This might also provide a possible avenue to understand the dynamic nature of growth cone guidance. These morphogens are frequently enriched at the border of a morphogenetic field, where axons often change responsiveness to guidance cues and take a new direction in migration.

Shaping maps with morphogens?

Topographic connections are a common way of organizing synapses. Gradients of guidance cues are essential for the formation of spatially continuous connection patterns. It makes sense that morphogens, which are known to have graded distributions, are capable of conveying positional information to axons within their target areas. Wnts have been shown to be topographic mapping cues along the dorsal–ventral axis in both vertebrate and invertebrate visual systems. A natural question is whether other topographic maps are also established by Wnts and whether other morphogens participate in map formation in a similar fashion.

Morphogens and extracellular matrix?

Cell surface molecules, including morphogen receptors and heparan sulfate proteoglycans (HSPGs), are important

for shaping morphogen gradients and affecting morphogen signaling itself. Particularly interesting are the HSPGs, which are abundant, contribute to the extracellular matrix and are shown to be important for Wg, Dpp and Hh signaling in *Drosophila* [38]. The graded distribution of morphogens is clearly important for their functions in axon guidance, and evidence already exists in fly, mouse, nematode and zebrafish that HSPGs also have a key role in modulating the signaling of axon guidance systems [39]. Some of the *in vivo* functions of HSPGs are likely to be associated with classical axon guidance molecules because many of the latter bind to HSPGs. In addition, all major morphogens bind to HSPGs. Some of the defects seen in mutations affecting HSPG function could be due to morphogen function in axon guidance. Proteoglycans are mostly inhibitory to axons in the adult central nervous system. Perhaps some of the ingredients consist of such morphogens, which could either have been retained in the system or even reinduced after injury.

Conclusions

The exciting finding that the morphogen family proteins play important roles in axon guidance opens up new avenues to understand nervous system wiring. Questions like how these gradients are established and maintained at much later stages, how specificity of signaling is achieved, how morphogen signaling pathways integrate among each other and with classical axon guidance molecules, how signaling translates extracellular gradients into intracellular signaling asymmetry, the role of these proteins in axon and synaptic patterning, and how neural activity may interface with these guidance systems are all wide open and await further investigation.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Tessier-Lavigne M, Goodman CS: **The molecular biology of axon guidance**. *Science* 1996, **274**:1123-1133.
2. Dickson BJ: **Molecular mechanisms of axon guidance**. *Science* 2002, **298**:1959-1964.
3. Osterfield M, Kirschner MW, Flanagan JG: **Graded positional information: interpretation for both fate and guidance**. *Cell* 2003, **113**:425-428.
4. Schnorrer F, Dickson BJ: **Axon guidance: morphogens show the way**. *Curr Biol* 2004, **14**:R19-R21.
5. Yoshikawa S, Thomas JB: **Secreted cell signaling molecules in axon guidance**. *Curr Opin Neurobiol* 2004, **14**:45-50.
6. Zou Y: **Wnt signaling in axon guidance**. *Trends Neurosci* 2004, **27**:528-532.
7. Ciani L, Salinas PC: **WNTs in the vertebrate nervous system: from patterning to neuronal connectivity**. *Nat Rev Neurosci* 2005, **6**:351-362.
8. Charron F, Tessier-Lavigne M: **Novel brain wiring functions for classical morphogens: a role as graded positional cues in axon guidance**. *Development* 2005, **132**:2251-2262.
9. Fradkin LG, Garriga G, Salinas PC, Thomas JB, Yu X, Zou Y: **Wnt signaling in neural circuit development**. *J Neurosci* 2005, **25**:10376-10378.
10. Augsburger A, Schuchardt A, Hoskins S, Dodd J, Butler S: **BMPs as mediators of roof plate repulsion of commissural neurons**. *Neuron* 1999, **24**:127-141.
11. Charron F, Stein E, Jeong J, McMahon AP, Tessier-Lavigne M: **The morphogen sonic hedgehog is an axonal chemoattractant that collaborates with netrin-1 in midline axon guidance**. *Cell* 2003, **113**:11-23.
12. Lyuksyutova AI, Lu CC, Milanesio N, King LA, Guo N, Wang Y, Nathans J, Tessier-Lavigne M, Zou Y: **Anterior–posterior guidance of commissural axons by Wnt-frizzled signaling**. *Science* 2003, **302**:1984-1988.
13. Imondi R, Thomas JB: **Neuroscience. The ups and downs of Wnt signalling**. *Science* 2003, **302**:1903-1904.
14. • Liu Y, Shi J, Lu CC, Wang ZB, Lyuksyutova AI, Song X, Zou Y: **Ryk-mediated Wnt repulsion regulates posterior-directed growth of corticospinal tract**. *Nat Neurosci* 2005, **8**:1151-1159.
Showing that Wnts are likely guidance cues for multiple axons along the A–P axis of the rodent spinal cord, pushing corticospinal tract axons posteriorly and pulling spinal cord commissural axons anteriorly.
15. Dickson BJ: **Wnts send axons up and down the spinal cord**. *Nat Neurosci* 2005, **8**:1130-1132.
16. • Bourikas D, Pekarik V, Baeriswyl T, Grunditz A, Sadhu R, Nardo M, Stoeckli ET: **Sonic hedgehog guides commissural axons along the longitudinal axis of the spinal cord**. *Nat Neurosci* 2005, **8**:297-304.
Showing that Shh is an A–P guidance cue for a class of chick spinal cord commissural axons.
17. • Pan CL, Howell JE, Clark SG, Hilliard M, Cordes S, Bargmann CI, Garriga G: **Multiple Wnts and frizzled receptors regulate anteriorly directed cell and growth cone migrations in *Caenorhabditis elegans***. *Dev Cell* 2006, **10**:367-377.
Demonstrating that Wnts are conserved directional cues along the A–P axis for cell and growth cone migration in *C. elegans*.
18. • Hilliard MA, Bargmann CI: **Wnt signals and frizzled activity orient anterior-posterior axon outgrowth in *C. elegans***. *Dev Cell* 2006, **10**:379-390.
Demonstrating that Wnt-Frizzled signaling controls cell polarization and axon outgrowth along the A–P axis in *C. elegans*.
19. • Prasad BC, Clark SG: **Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in *C. elegans***. *Development* 2006, **133**:1757-1766.
Demonstrating that Wnt signaling controls A–P polarity and axon outgrowth and involvement of retromer in *C. elegans*.
20. Zou Y: **Navigating the anterior–posterior axis with wnts**. *Neuron* 2006, **49**:787-789.
21. Yoshikawa S, McKinnon RD, Kokel M, Thomas JB: **Wnt-mediated axon guidance via the *Drosophila* Derailed receptor**. *Nature* 2003, **422**:583-588.
22. Sperry RW: **Chemoaffinity in the orderly growth of nerve fiber patterns and connections**. *Proc Natl Acad Sci USA* 1963, **50**:703-710.
23. Flanagan JG, Vanderhaeghen P: **The ephrins and Eph receptors in neural development**. *Annu Rev Neurosci* 1998, **21**:309-345.
24. Frisen J, Yates PA, McLaughlin T, Friedman GC, O'Leary DD, Barbacid M: **Ephrin-A5 (AL-1/RAGS) is essential for proper retinal axon guidance and topographic mapping in the mammalian visual system**. *Neuron* 1998, **20**:235-243.
25. Feldheim DA, Vanderhaeghen P, Hansen MJ, Frisen J, Lu Q, Barbacid M, Flanagan JG: **Topographic guidance labels in a sensory projection to the forebrain**. *Neuron* 1998, **21**:1303-1313.
26. Feldheim DA, Kim YI, Bergemann AD, Frisen J, Barbacid M, Flanagan JG: **Genetic analysis of ephrin-A2 and ephrin-A5 shows their requirement in multiple aspects of retinocollicular mapping**. *Neuron* 2000, **25**:563-574.

27. Mann F, Ray S, Harris W, Holt C: **Topographic mapping in dorsoventral axis of the *Xenopus* retinotectal system depends on signaling through ephrin-B ligands**. *Neuron* 2002, **35**:461-473.

28. Hindges R, McLaughlin T, Genoud N, Henkemeyer M, O'Leary DD: **EphB forward signaling controls directional branch extension and arborization required for dorsal-ventral retinotopic mapping**. *Neuron* 2002, **35**:475-487.

29. Prestige MC, Willshaw DJ: **On a role for competition in the formation of patterned neural connexions**. *Proc R Soc Lond B Biol Sci* 1975, **190**:77-98.

30. Gierer A: **Model for the retino-tectal projection**. *Proc R Soc Lond B Biol Sci* 1983, **218**:77-93.

31. Fraser SE, Hunt RK: **Retinotectal specificity: models and experiments in search of a mapping function**. *Annu Rev Neurosci* 1980, **3**:319-352.

32. Fraser SE, Perkel DH: **Competitive and positional cues in the patterning of nerve connections**. *J Neurobiol* 1990, **21**:51-72.

33. Schmitt AM, Shi J, Wolf AM, Lu CC, King LA, Zou Y: **Wnt-Ryk**
• **signalling mediates medial-lateral retinotectal topographic mapping**. *Nature* 2006, **439**:31-37.
Showing that Wnt signaling is involved in topographic mapping in neural circuit development.

34. Sato M, Umetsu D, Murakami S, Yasugi T, Tabata T: **DWnt4**
• **regulates the dorsoventral specificity of retinal projections in the *Drosophila melanogaster* visual system**. *Nat Neurosci* 2006, **9**:67-75.
Showing that Wnts are conserved cues for topographic mapping in *Drosophila* [33•].

35. Sterneckert JL, Hill CM, Palmer R, Gearhart JD: **Bone morphogenetic proteins produced by cells within embryoid bodies inhibit ventral directed differentiation by Sonic Hedgehog**. *Cloning Stem Cells* 2005, **7**:27-34.

36. Yuasa T, Kataoka H, Kinto N, Iwamoto M, Enomoto-Iwamoto M, Iemura S, Ueno N, Shibata Y, Kurosawa H, Yamaguchi A: **Sonic hedgehog is involved in osteoblast differentiation by cooperating with BMP-2**. *J Cell Physiol* 2002, **193**:225-232.

37. Yang L, Yamasaki K, Shirakata Y, Dai X, Tokumaru S, Yahata Y, Tohyama M, Hanakawa Y, Sayama K, Hashimoto K: **Bone morphogenetic protein-2 modulates Wnt and frizzled expression and enhances the canonical pathway of Wnt signaling in normal keratinocytes**. *J Dermatol Sci* 2006, **42**:111-119.

38. Tabata T, Takei Y: **Morphogens, their identification and regulation**. *Development* 2004, **131**:703-712.

39. Lee JS, Chien CB: **When sugars guide axons: insights from heparan sulphate proteoglycan mutants**. *Nat Rev Genet* 2004, **5**:923-935.