Expression of the Wnt signaling system in central nervous system axon guidance and regeneration

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GRADED EXPRESSION OF Wnts GUIDE AXONS ALONG THE ANTERIOR–POSTERIOR AXIS IN CNS DEVELOPMENT

During development of the brain and the spinal cord, growing axons are directed by multiple guidance cues to their synaptic targets forming axon networks along the major anatomical axes, anterior–posterior (A–P), dorsal–ventral axes, and interior–superior. Axons along the A–P or rostrocaudal axis of the spinal cord establish the circuitry required for supraspinal control of motor function as well as for relaying sensory information to the brain. For long-distance projections, such as along the A–P axis, global gradients of molecular cues are necessary. Understanding the development of these long-distance connections between brain and spinal cord may provide insights for developing useful therapeutic interventions to repair these axons after injury.

The vertebrate commissural axons within the spinal cord are an excellent specimen to identify global A–P guidance cues. These axons have an initial dorsal–ventral trajectory to reach and cross the midline. Secreted attractants, Netrin-1, and Sonic Hedgehog (Shh) from the ventral midline, the floor plate, provide the dorsal–ventral cues (Kennedy et al., 1994; Serafini et al., 1994, 1996; Charron et al., 2003). Upon reaching the midline, commissural axons decussate and post-crossing commissural axons are repelled by Slits and Semaphorin 3B secreted from the floor plate (Zou et al., 2000). A global A–P guidance mechanism ensures the precise 90° turn of commissural axons immediately after midline crossing. A decreasing anterior-to-posterior mRNA gradient of the diffusible morphogen Wnts (Wnt4, Wnt7b, Wnt5a, and Wnt7a) dictates the appropriate anterior turning of post-crossing commissural axons via the Frizzled3 receptor (Figure 1; Lyuksyutova et al., 2003). Disruption of the Wnt gradient or the loss of the Frizzled3 receptor leads to A–P guidance defects of post-crossing commissural axons (Lyuksyutova et al., 2003). Wnt proteins are also expressed in an A–P gradient (Onishi and Zou, unpublished results). The responsiveness of Frizzled3 expressing commissural axons is dependent upon the activation of phosphatidylinositol-3-kinase (PI3K) and atypical protein kinase C, a key regulator of the apical–basal polarity signaling pathway (Wolf et al., 2008). In addition, planar cell polarity (PCP) signaling is required for anterior turning (Shafer et al., 2011). Therefore, Wnt attraction may be mediated by both apical–basal and PCP signaling cascades present in the growth cones of post-crossing commissural axons.

In contrast to the global gradients of Wnt proteins along the A–P axis in the spinal cord, Wnts 5α and 7b are expressed in more complex gradients along the A–P axis in the developing hindbrain and midbrain (Fenstermaker et al., 2010; Blakely et al., 2011). These expression gradients control the orientation and growth of dopaminergic and serotonergic axons (Fenstermaker et al., 2010; Blakely et al., 2011), subpopulations of which contribute to supraspinal motor control (Hudspeth and Kaypers, 1987; Jordan et al., 2008). Orientation of midbrain monoaminergic axons also depends on the expression of components of the PCP signaling pathway (Fenstermaker et al., 2010).

Wnt5 and Wnt5a mRNAs are expressed in decreasing anterior-to-posterior gradients within the early postnatal spinal cord during corticospinal tract development and repel corticospinal axons (Lin et al., 2005). The receptor protein tyrosine kinase member Derailed (Dvl) mediates Wnt repulsion in Drosophila (Yoshikawa et al., 2003). Wnt5, expressed in the posterior commissure, repels anterior commissural axons that express Dvl, while the posterior...
While work in the vertebrate spinal cord and invertebrate systems has demonstrated a role for Wnt morphogens in axon pathfinding, the vertebrate visual system utilizes Wnt expression gradients as positional cues to establish topographic connections. Wnt3 mRNA outgrowth as well as repulsion of corticospinal axons along the A–P axis of the developing spinal cord (Liu et al., 2005; Li et al., 2008). The Wnt3 gradient was proposed to counterbalance the ephrin B1 mapping force. The specificity of retinal projections in D. melanogaster are also governed by the expression of Wnts, with DWnd4 expression guiding retinal axons to the ventral lamina to maintain a retinotopic map (Sato et al., 2006). Therefore, Wnt signaling may have a conserved role in topographic map formation. How Wnt expression gradients are established in these brain areas are currently unknown.

**RE-INDUCED Wnts FORM DIFFERENT GRADIENTS AFTER INJURY**

In the intact adult spinal cord, Wnt mRNA expression is unde-tectable, however after spinal cord injury, re-induction of Wnts 1, 4, and 5a occurs as evidenced by expression of mRNA in the cells immediately surrounding the lesion (Liu et al., 2008). The re-induced Wnts form gradients that peak at the lesion sites and decrease both anteriorly and posteriorly relative to the lesion sites. In addition to Wnts, other guidance molecules have also been found to be re-induced by spinal cord injury. Class 3 semaphorins and ephrins are both re-expressed in spinal cord lesion, although the role of these guidance cues in the injury response has not been defined (Pasterkamp et al., 1999; Bundesen et al., 2003; Benson et al., 2005; Carmichael et al., 2005; Pasterkamp and Ver- haagen, 2006). Wnt expression at the spinal cord injury site is coupled with re-expression of the repulsive receptor Ryk in corticospinal motor neurons where it is trafficked to the distal tip of the lesioned corticospinal axons (Liu et al., 2008). Inhibition of Wnt–Ryk signaling after spinal cord injury reduces the retraction of lesioned corticospinal axons from the injury site while concur-rently promoting the sprouting of corticospinal axon collaterals within the spared spinal cord tissue (Liu et al., 2008). Another study showed similar results using a contusion model (Miyashita et al., 2009). Therefore, the re-induced Wnt gradients may be responsible for the long-range retraction of corticospinal tract axons following spinal cord injury.

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**Wnt GRADIENTS IN TOPOGRAPHIC MAPPING**

While work in the vertebrate spinal cord and invertebrate systems has demonstrated a role for Wnt morphogens in axon pathfinding, the vertebrate visual system utilizes Wnt expression gradients as positional cues to establish topographic connections. Wnt3 mRNA and protein are both expressed in a decreasing gradient along the medial (high) to lateral (low) axis within the developing chick tectum and mouse superior colliculus (Schmitt et al., 2006). This gradient of Wnt3 is used to establish a retinotopic map through the expression of the repulsive Wnt receptor Ryk in a ventral to dorsal decreasing gradient in retinal ganglia cells (Schmitt et al., 2006). The Wnt3 gradient was proposed to counterbalance the ephrin B1 mapping force. The specificity of retinal projections in D. melanogaster are also governed by the expression of Wnts, with DWnd4 expression guiding retinal axons to the ventral lamina to maintain a retinotopic map (Sato et al., 2006). Therefore, Wnt sig-naling may have a conserved role in topographic map formation. How Wnt expression gradients are established in these brain areas are currently unknown.

**FIGURE 1** | Anterior-posterior Wnt gradients in the developing spinal cord guide growth of ascending sensory and descending motor axons.

(A) Schematic depicting anterior (high) to posterior (low) gradients of Wnts in the developing mouse spinal cord at E11.5 and P0. At E11.5, post-crossing commissural axons (red) are attracted anteriorly up a gradient of Wnt4 (purple) through the receptor Frizzled3. At P0, descending corticospinal motor axons (red) are repelled posteriorly down a gradient of Wnt1 and Wnt5a (purple). In situ hybridization showing Wnt4 anterior–to-posterior gradient in the floor plate at E13.5. Whole mount in situ hybridization showing Wnt4 anterior–to-posterior gradient in the floor plate at E11.5. In situ images are from Lyuksyutova et al. (2005a) and Liu et al. (2008).

(B) Schematic depicting anterior (high) to posterior (low) gradients of Wnts along the anterior–posterior axis probed for Wnts 1, 4, and 5a. Wnt4 is expressed in a decreasing anterior-to-posterior gradient within the floor plate (arrow). Wnts 1 and 5a are expressed in decreasing anterior-to-posterior gradients in the dorsal spinal cord. Scale bar = 100 μm.

(C) Whole mount in situ hybridization showing Wnt4 anterior–to-posterior gradient in the floor plate at E13.5. In situ images are from Lyuksyutova et al. (2005a) and Liu et al. (2008).

In addition to Wnts, other guidance molecules have also been found to be re-induced by spinal cord injury. Class 3 semaphorins and ephrins are both re-expressed in spinal cord lesion, although the role of these guidance cues in the injury response has not been defined (Pasterkamp et al., 1999; Bundesen et al., 2003; Benson et al., 2005; Carmichael et al., 2005; Pasterkamp and Verhaagen, 2006). Wnt expression at the spinal cord injury site is coupled with re-expression of the repulsive receptor Ryk in corticospinal motor neurons where it is trafficked to the distal tip of the lesioned corticospinal axons (Liu et al., 2008). Inhibition of Wnt–Ryk signaling after spinal cord injury reduces the retraction of lesioned corticospinal axons from the injury site while concurrently promoting the sprouting of corticospinal axon collaterals within the spared spinal cord tissue (Liu et al., 2008). Another study showed similar results using a contusion model (Miyashita et al., 2009). Therefore, the re-induced Wnt gradients may be responsible for the long-range retraction of corticospinal tract axons following spinal cord injury.

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In addition to the up-regulation of Ryk in injured corticospinal motor neurons, peripheral injury of the sciatic nerve results in the expression of Ryk in primary sensory neurons within the dor-sal root ganglia (DRG; Li et al., 2008). Peripheral injury results in an increased intrinsic growth capacity of large-diameter sensory neurons in the DRG with a corresponding alteration of expression levels of thousands of genes (Richardson and Iossa, 1984; Stam et al., 2007). This increased intrinsic growth capacity, due to the conditioning effect of the peripheral injury, allows for the regen-eration of the central branch of primary sensory neurons along a permissive substrate (Richardson and Iossa, 1984). Additionally, Ryk siRNA expression in developing DRG neurons reduces neurite outgrowth *ex vivo* and attenuates the Wnt3a-mediated outgrowth response of cultured DRG explants (Li et al., 2004). In contra-
of the regenerating axonal marker growth-associated protein 43 (GAP-43) near the lesion site (Suh et al., 2011). In corticospinal motor neurons: Wnt–Ryk signaling is able to promote both axon outgrowth as well as repulsive guidance through distinct signaling cascades (Li et al., 2009). This bifunctionality of Ryk may be active in DRG neurons as well, though it is currently unknown which neurons express Ryk after injury and what role the increased expression of the repulsive Wnt receptor Ryk may play in the peripheral conditioning lesion, if any.

OTHER MORPHOGENS AFTER INJURY

Wnts are not the only morphogens that are re-induced after spinal cord injury. Motor nuclei have been demonstrated to increase bone morphogenetic protein (BMP) production following peripheral axotomy and potentially re-activate BMP-2 protein expression (Jin et al., 2003; Wang et al., 2007). Downstream of BMP-2 and 4 activation, BMP type I receptor mediates signaling through Smads and is responsible for central regeneration of large-diameter proprioceptive axons already demonstrated an enhancement of axonal plasticity of corticospinal motor axons after injury (Liu et al., 2008). This induced axonal plasticity may provide a substrate for the formation of novel supraspinal motor circuits and improved functional recovery after injury. Understanding how Wnt expression is regulated will provide additional therapeutic tools.

REFERENCES


Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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