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Should I Stay or Should I Go: Wnt Signals at the Synapse

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Several extracellular factors, including Wnt proteins, have been reported to induce synapse formation. In this issue, Klassen and Shen (2007) report that Wnt proteins can also act as antisynaptogenic signals to prevent synapse formation in certain parts of the worm *Caenorhabditis elegans*. The differential response of axon populations to local Wnt proteins may contribute to the patterning of synaptic connections.

The establishment of functional circuits in the nervous system requires great specificity in the formation of synaptic connections. Recently, considerable progress has been made in understanding how neurons use positive synaptogenic signals to make synapses on appropriate target cells. Examples of such positive mediators of synaptic specificity are SYG-1 and SYG-2, discovered in the worm *C. elegans*. These two molecules serve as a guide for the HSNL motor neuron, one of four neurons responsible for egg laying. Without SYG-1 (located in the surrounding epithelial cells where the HSNL neuron should form a synapse) or SYG-2 (the receptor for SYG-1 located on the HSNL axon), synaptic specificity is compromised and ectopic synapses are formed onto incorrect target cells (Shen and Bargmann, 2003 and Shen et al., 2004). Whether such positive signals are balanced by negative signals that prevent synapse formation in incorrect target zones has not been extensively explored. In this issue of *Cell*, Klassen and Shen (2007) show that Wnt proteins can exert an antisynaptogenic effect to prevent innervation of inappropriate target cells. Interestingly, Wnt proteins are known to promote synapse formation suggesting that Wnts might exert pro- and antisynaptogenic effects on distinct cell populations to influence innervation patterns.

Klassen and Shen (2007) used the DA9 neuron in *C. elegans* to demonstrate how a neuron might send an axon through an asynaptic zone to reach its target area without making inappropriate synapses. The DA9 neuron has a well-preserved position within *C. elegans*. The axon extends from the cell body on the ventral side of the worm and runs toward the tail. Just short of the tail, the axon turns, crosses to the dorsal side, and runs anterior in order to form synapses with the dorsal body wall muscles and the VD class of inhibitory neurons (Figure 1). Although this neuron targets the body wall muscles and inhibitory neurons on the dorsal side of the worm, it does not form presynaptic terminals at the posteriormost end of the axon. Klassen and Shen (2007) show that this lack of synapse formation is a result of Wnt signaling, thus indicating that Wnts may be responsible for the recognition of synaptic zones.

Wnt Proteins in *C. elegans*

Wnts are a large class of signaling molecules implicated in a variety of processes throughout development such as body axis specification, stem cell self renewal, cell-fate determination, axon guidance, and synaptogenesis. There are over 16 Wnt proteins in vertebrates, which all have a conserved cysteine residue that is palmitoylated before the proteins are secreted.

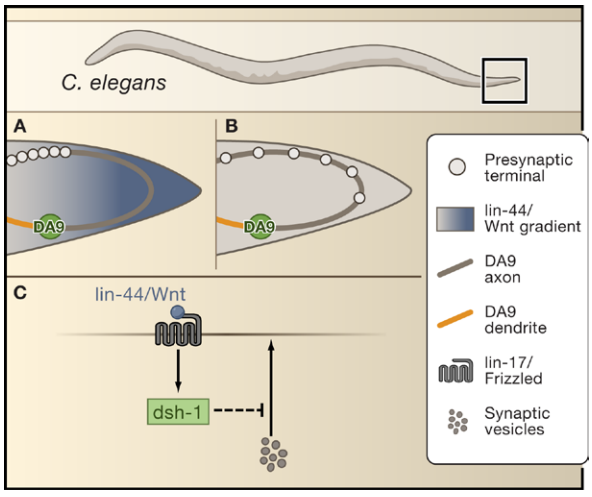

Figure 1. Wnt Activity and Synapse Formation in the Worm
(A) As the axon of the DA9 neuron passes through the tail and through a zone of Wnt expression, presynaptic termini are noticeably absent. However, when the axon passes out of the Wnt expression zone, presynaptic termini are formed.
(B) Mutations in *lin-44* (*wnt*) or *lin-17* (*frizzled*) lead to synapse formation within the asynaptic region of the tail.
(C) A model of how Wnt might prevent synapse formation within the asynaptic zone. Lin-44/Wnt binds to lin-17/frizzled, which signals through dsh-1 to prevent recruitment of synaptic vesicles to the putative active zone.

Vertebrate Wnts bind to the Frizzled family of 7-transmembrane domain proteins (reviewed in Miller, 2001). Wnts signal through several different pathways: the Wnt canonical pathway, the planar cell polarity pathway, and the calcium pathway. In the canonical pathway, Wnt binds to the LRP/Frizzled coreceptor, which signals downstream to Disheveled (Dvl). Dvl then inhibits a trio of proteins, GSK3β, Axin, and APC. Inhibition leads to dephosphorylation of β-catenin, which triggers its activation and allows its translocation to the nucleus, where it binds to TCF/LEF transcription factors. Noncanonical pathways in vertebrates also involve the signaling protein Dvl and act through either JNK or CaMKII and PKC (reviewed in Ciani and Salinas, 2005).

As in vertebrates, Wnts in *C. elegans* have been implicated in a variety of processes such as cell-fate specification and migration of neuroblasts. There are five Wnts (lin-44, egl-20, mom-2, cwn-1, and cwn-2) and four Frizzled receptors (lin-17, mom-5, mig-1, and cfz-2) in *C. elegans*. As in vertebrates, there are both canonical and noncanonical pathways. The canonical pathway is conserved between invertebrates and vertebrates with nematode homologs of Dvl (dsh-1, dsh-2, and mig-5), GSK3β (gsk-3), Axin (pry-1), and APC (apr-1). There are three homologs of β-catenin in *C. elegans* (bar-1, wrm-1, and hmp-2) and only one homolog of TCF/LEF (pop-1). Bar-1 is involved in the *C. elegans* canonical pathway, where it binds to pop-1 to activate transcription. Wrm-1, however, is involved in the *C. elegans* noncanonical pathway and inhibits pop-1. Although the canonical pathway in nematodes is similar to the pathway in vertebrates, noncanonical pathways vary considerably between vertebrates and invertebrates (reviewed in Eisenmann, 2005; Herman and Wu, 2004).

Wnts Negatively Regulate Synaptogenesis

In their new study, Klassen and Shen (2007) show that Wnt acts as a negative regulator of synaptogenesis in DA9 neurons and can contribute to target specificity. They used a synaptobrevin-1 (snb-1) reporter gene expressed in the DA9 neuron to examine the distribution of presynaptic terminals in the DA9 neuron. In a candidate-based approach to identify signals that regulate the distribution of synapses made by DA9 neurons, the authors focused on Wnts because *lin-44* (a C. *elegans wnt* gene) is expressed by four hypodermal cells in the tail, which creates a putative Wnt gradient in the asynaptic zone. The authors found that mutations in *lin-44* led to an alteration in presynaptic puncta distribution, indicated by a redistribution of snb-1 positive puncta in the previously asynaptic zone (Figure 1). This phenotype was exacerbated by mutation of another Wnt, egl-20, which is expressed on the anterior and ventral side of the tail. Mutations in *lin-17* (which encodes a Wnt receptor) mimicked the phenotype. Lin-17 was found to be localized to the asynaptic region of the DA9 axon, strengthening the interpretation that local Wnt signaling negatively regulates synaptogenesis (Figure 1). Furthermore, Klassen and Shen (2007) found that they could manipulate the location of this asynaptic region by misexpressing lin-44 under the *egl-20* promoter. They found that the asynaptic region expanded to include regions overlapping with the new pattern of lin-44 expression. Less striking, however, was their finding that when they inverted the Wnt gradient, only 20% of worm mutants showed ectopic presynaptic puncta.

To understand how Wnt signaling regulates synapse formation, Klassen and Shen (2007) mutated several components downstream of lin-17. They first investigated the role of Dvl, a key downstream effector of Wnt signaling. Consistent with its role in Wnt signaling, they found that dsh-1 mutants had increased numbers of presynaptic puncta within the previously asynaptic region. They also examined mutations in a number of Wnt signaling components: two β-catenin homologs (bar-1 and wrm-1); a TCF/LEF-1 homolog (pop-1); a nematode-like kinase, lit-1 (which regulates pop-1); and an axin homolog (pry-1). None of these mutants affected the distribution of synapses in asynaptic regions within the DA9 axon, suggesting that a new *C. elegans* Wnt pathway is involved in regulating synapse formation.

To determine if Wnt signaling is also involved in regulating synapse formation in other neurons, Klassen and Shen (2007) examined synapse formation in the DB7 motor neuron. Although DB7 neurons have a different position in the worm and send an axon posterior in the dorsal nerve cord, they also fail to make synapses in the tail region of the worm. The authors mutated either lin-44 or lin-17 and found a redistribution of presynaptic puncta within the posterior-most region. This shows that other motor neurons in *C. elegans* might use the same mechanism to recognize correct synaptic target zones, but it remains to be determined if a similar mechanism holds true in vertebrates. It should also be noted that the analysis of redistribution of synapses after Wnt manipulation is based on immunofluorescent localization of synaptic protein. Eventually, ultrastructural analysis will be required to determine if the ectopic presynaptic puncta indeed represent bona fide synapses.

The work by Klassen and Shen (2007) is consistent with two distinct interpretations for the way in which Wnts regulate synaptogenesis. In one case Wnts could simply act as antisynaptogenic signals. This would explain the complete absence of synapses in the region where Wnts are expressed. An alternate possibility is that since loss of Wnt leads to a redistribution, rather than an absolute increase, in the number of presynaptic puncta in the previously asynaptic region of the animal, the effects of Wnts could be dose-dependent. High levels of Wnts might inhibit presynaptic differentiation, and low levels might promote presynaptic differentiation. In

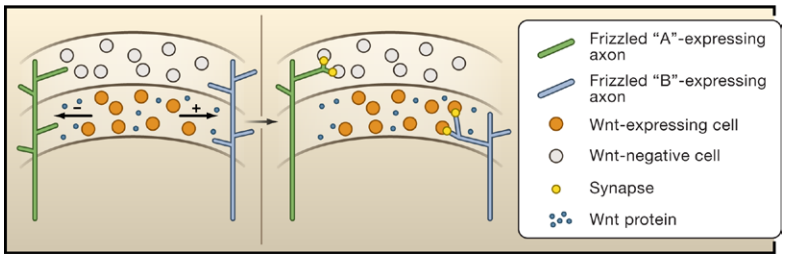

Figure 2. Wnt Signaling and Segregation of Synaptic Inputs

The axons expressing different Wnt receptors (Frizzled "A" and Frizzled "B") are initially intermixed in Wnt-positive and Wnt-negative zones. Frizzled "A" mediates an antisynaptogenic Wnt response, whereas Frizzled "B" mediates a prosynaptogenic response. This leads to segregation of the synapses from two axon populations to the Wnt-negative and Wnt-positive zones.

such a case Wnts might act as morphogens (synaptomorphogens) to induce different outcomes based on local concentration.

Pro- and antisynaptogenic Wnt Signaling

Previous studies in both invertebrates and vertebrates have implicated Wnts as positive regulators of synaptogenesis, which is why the findings of Klassen and Shen (2007) are so exciting. Studies in cultures of mouse cerebellum show that Wnt7a conditioned medium increases the number and size of mossy fiber terminals (Hall et al., 2000). This signal is thought to act through Dvl, which colocalizes with presynaptic markers. Cerebellum cultures derived from mice lacking Dvl also show a decrease in presynaptic puncta (Ahmad-Annuar et al., 2006). Studies in vivo show a decrease in the size of presynaptic terminals at postnatal day 10, but this difference is not seen at postnatal day 15 (Hall et al., 2000), perhaps due to the presence of other Wnts in the cerebellum (Salinas et al., 1994). This decrease is exacerbated slightly in mice lacking both Wnt7a and Dvl (Ahmad-Annuar et al., 2006). Although these experiments support a positive role for Wnts in synaptogenesis, there is still limited data on whether Wnts affect the number of functional synapses.

Wnts have also been reported to positively regulate synapses at the fly neuromuscular junction. In this case, Wnts are thought to be secreted by the presynaptic terminal and act on both active zone assembly and postsynaptic organization (Packard et al., 2002). Wingless (Wnt) mutants show a decrease in the number of transmitter release sites (boutons) in the presynaptic terminals and a change in the cytoskeleton of these terminals. A subset of Wingless mutants exhibit only an assembly of synaptic vesicles, but no active zone complex—a collection of proteins at the presynaptic terminal that are responsible for vesicle priming and fusion. The mutants also show irregular glutamate receptor expression. Both Wnts and their receptor (Dfz2) are expressed in presynaptic neurons and postsynaptic muscle cells, although rescue experiments suggest that presynaptic Wnt expression may be sufficient to mediate the synaptogenic effects of Wnts at the neuromuscular junction (Packard et al., 2002).

Whereas the effects of Wnts in *Drosophila* suggest a role for Wnt signaling in organizing the presynaptic cytoskeleton, the observations of Klassen and Shen (2007) suggest a different role for Wnts in synaptic development. In *lin-44* and *lin-17* worm mutants, defects in axon guidance and morphology of the DA9 neuron are noticeably absent. Klassen and Shen's work instead suggests that Wnts might act locally to prevent recruitment of synaptic vesicles to the presynaptic terminal. This could be due to a defect in the assembly or organization of the active zone. Proteins that could be targeted by the Wnt signal include Liprin-α or RIM-1, which have been implicated in active zone assembly (reviewed in Zhen and Jin, 2004) (Figure 1C).

Patterning of Synaptic Inputs

How might Wnts exert both positive and negative effects on synapse formation? Given the large diversity of Wnt proteins, one possibility is that different Wnt proteins act as pro- or antisynaptogenic signals. Another interesting possibility is that the same Wnt protein could exert pro- and antisynaptogenic effects on different populations of neurons that express different Frizzled receptors or downstream target proteins. Thus, two neuronal populations expressing different Frizzled receptors that transduce pro- and antisynaptogenic Wnt responses would form synapses in different Wnt-expressing domains. Such a mechanism might allow initially intermixed axon populations to segregate into distinct zones (Figure 2). Finally, analogous to the action of morphogens, the effects of Wnts on synaptogenesis could be dose dependent.

An important contribution of the Klassen and Shen study is the identification of Wnts as antisynaptogenic signals. Such antisynaptogenic signals might be broadly used in the specification of synaptic contacts, and it is likely that signals other than Wnts also provide antisynaptogenic signals for target zone recognition. In fact, there is evidence that Toll may act as a negative synaptogenic signal at the *Drosophila* neuromuscular junction. Toll is a cell-surface molecule that is expressed in the *Drosophila* embryo in muscles 15 and 16 around the time of synaptogenesis. During normal development, the RP3 neuron extends an axon past muscles 15 and 16 to innervate muscles 6 and 7. When Toll is knocked out, RP3 axons stop and innervate muscles 15 and 16, suggesting that Toll normally inhibits RP3 synapse formation on those muscles (Rose and Chiba, 1999; Rose and Chiba, 2000). However, Toll is not a general antisynaptogenic signal

because other neurons in a different class than RP3 can make synapses on muscles 15 and 16, even in the presence of Toll (Kraut et al., 2001). Thus, differential responsiveness to antisynaptogenic signals can allow for the development of specific innervation patterns. It will be interesting to determine how Wnts, Toll, and other antisynaptogenic signals cooperate with pro-synaptogenic signals to influence the patterning of connections in the developing brain.

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