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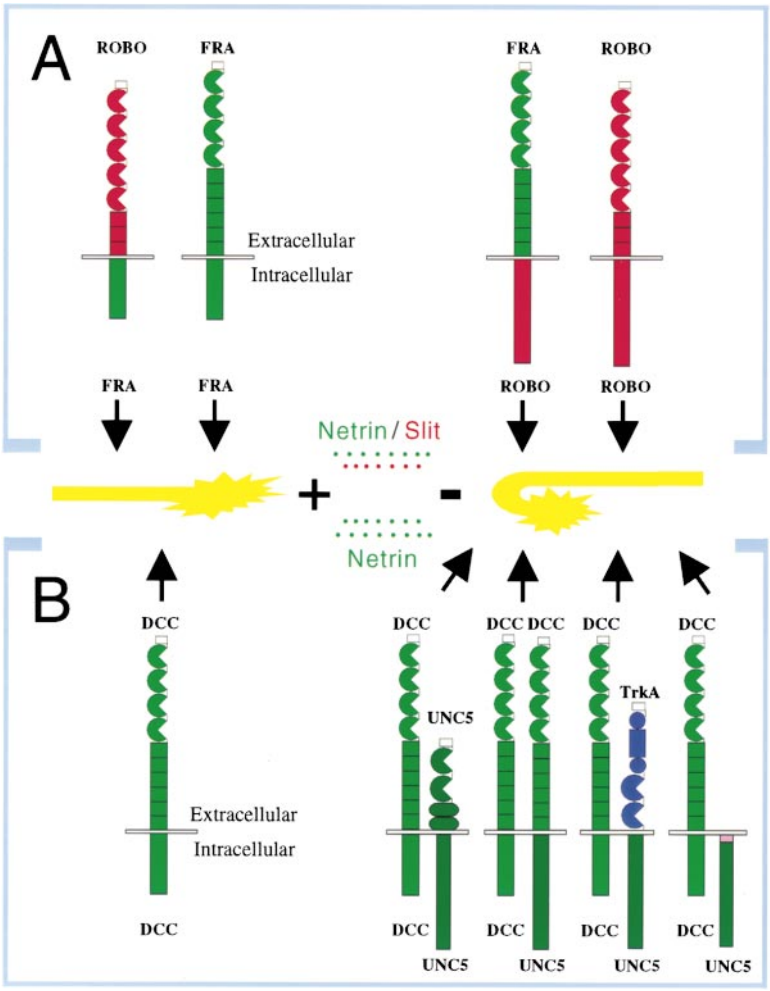
Attracted or Repelled? Look Within

The past several years have seen a revolution in our understanding of how axons find their targets during neural development. These new insights are due in part to a melding of detailed cellular and genetic characterizations of axon guidance events with the molecular description of several families of phylogenetically conserved cues capable of mediating these steering decisions (reviewed by Mueller, 1999). Neurons use these cues, which include both attractants and repellents acting over short and long distances, to direct their axons to appropriate intermediate or final targets. Refinement of these projections is imparted by the ability of individual axons to respond to multiple guidance cues presented at different points along their trajectories. Further complexity in these early guidance events is provided by the bifunctionality of many of these guidance cues: attracting certain populations of axons and repelling others, or even attracting or repelling the same axon depending on the state of certain intracellular signaling molecules. Though it is likely that many families of guidance cues and their receptors remain to be discovered, the tools are now in hand to begin dissecting the molecular basis of attractive and repulsive guidance mechanisms. Two studies published in the June 25 issue of *Cell*, one from the Goodman laboratory (Bashaw and Goodman, 1999) and the other a collaborative effort between the Tessier-Lavigne and Poo laboratories (Hong et al., 1999), provide insight into the logic of how growth cones interpret guidance cues as being attractive or repulsive. Using complementary in vivo and cell culture approaches, and focusing on distinct but overlapping guidance receptor families, both groups demonstrate a key role for the cytoplasmic domains of guidance receptors in mediating attraction and repulsion. In addition, Hong et al. (1999) provide evidence for a novel molecular mechanism whereby a heteromultimeric receptor complex can dictate whether the steering response to a single cue, netrin-1 (Net-1), is attractive or repulsive.

Perhaps there is no better place to investigate attractive and repulsive guidance mechanisms than at the CNS midline (Flanagan and Van Vactor, 1998). In both vertebrates and invertebrates, axons from specific populations of neurons are attracted toward the midline by long-range chemoattractants belonging to the Netrin family. Netrin attractive functions are mediated by receptors belonging to the DCC family, a branch of the immunoglobulin (Ig) superfamily that includes DCC in vertebrates and Frazzled (Fra) in *Drosophila*. Upon arriving at the midline, contralaterally projecting axons undergo a conversion; they cross the midline, lose responsiveness to midline-derived Netrin cues, and do not recross the midline. Recent analyses reveal that Slit proteins, also expressed on the midline, can act as repellents and are likely responsible for moving these contralaterally projecting axons across the midline and keeping them from recrossing (Zinn and Sun, 1999). Slit receptors are members of the Roundabout (Robo) family, also a branch of the Ig superfamily, but they differ from DCC proteins in the number of extracellular Ig and fibronectin domains and share no similarity over their large cytoplasmic domains. Using the well-characterized *Drosophila* midline, Bashaw and Goodman (1999) recognized a great opportunity to test in vivo two important and related questions: are Netrin and Slit receptors—Fra and Robo, respectively—modular such that their cytoplasmic domains are responsible for the nature of the growth cone response to a guidance cue; and are attractive and repulsive intracellular signaling components generally present in diverse cell types? The answer to both questions is yes.

Chimeric Fra or Robo receptors, containing either the extracellular domain of Fra and the intracellular domain of Robo (Fra–Robo) or the extracellular domain of Robo and the intracellular domain of Fra (Robo–Fra), were expressed on all neurons in *Drosophila* embryos. This resulted in repulsive guidance responses to Netrins (Fra–Robo) and attractive responses to Slit (Robo–Fra) (see figure, panel A). For example, ectopic Fra–Robo directs axons that would normally cross the midline away from it, leading to a commissureless phenotype. In addition, Fra–Robo also directs motor axons away from the Netrin-expressing muscles that they would normally innervate. These effects are not likely to be due to dominant-negative effects of Fra–Robo. The chimeric Robo–Fra receptor produces complementary phenotypes; Slit can now function as a midline attractant. Remarkably, muscle precursors that normally migrate away from the midline in response to the Slit repellent can interpret Slit as an attractant when they ectopically express Robo–Fra. These results indicate that the attractive or repulsive nature of a particular guidance cue resides in the cytoplasmic domain of the receptor and that this response can be independent of binding a specific class of ligand. Further, a variety of neuronal and nonneuronal cells are shown to be capable of novel attractive and repulsive responses, demonstrating that the downstream signaling components necessary for Netrin and Slit guidance responses are present in different cell types.

But what about guidance cues that are bifunctional—how can the same cue be both an attractant and a repellent? Hong et al. (1999) directly address this issue. Genetic evidence in *C. elegans* and direct evidence in vertebrates have motivated the search for understanding the molecular basis of Netrin bifunctionality (reviewed by Mueller, 1999). Altering intracellular cyclic nucleotide levels in vertebrate neurons in vitro can convert an attractive Netrin response to a repulsive one in a DCC-dependent fashion. Though this shows that a single receptor can mediate both Netrin attraction and Netrin repulsion, work in *C. elegans* and in vertebrates demonstrates that UNC5 receptors, yet another branch of the Ig superfamily containing members distinct from both DCC and Robo proteins, are involved in Netrin-mediated repulsive guidance events and are also Netrin binding proteins. However, a simple model whereby DCC and UNC5 receptors independently signal Netrin



The Cytoplasmic Domains of Netrin and Slit Receptors Dictate the Choice between Attraction and Repulsion

attraction and repulsion is challenged by observations that UNC5 and UNC40 (a DCC family member) are both required for many Netrin-mediated repulsive guidance events in *C. elegans*.

First, Hong et al. (1999) directly show, by introducing the vertebrate UNC5H2 receptor into *Xenopus* spinal neurons grown in culture, that an UNC5 receptor is required cell-autonomously to elicit a repulsive response to Net-1. These spinal neurons express endogenous DCC, and previous work showed that antibody neutralization of DCC abolishes an attractive response to Net-1 (Ming et al., 1997). UNC5H2-dependent repulsion is also abolished by DCC neutralization, directly demonstrating a requirement for both UNC5 and DCC for Net-1 repulsion and strongly suggesting that the role of UNC5 is to convert an attractive response to a repulsive one.

To understand how UNC5 effects this conversion, a series of chimeric and altered receptors were constructed and introduced into spinal neurons. Interestingly, Net-1 functions as a repulsive cue in the presence of DCC as long as a membrane-associated cytoplasmic domain of UNC5H2 is also present. A chimeric UNC5H2 consisting of a DCC ectodomain and an UNC5H2 cytoplasmic domain, a similar construct with a TrkA ectodomain, or simply the UNC5H2 cytoplasmic domain targeted to the inner plasma membrane by a myristoylation sequence are all capable of converting Net-1 attraction to repulsion in the presence of endogenous DCC (see figure, panel B). These data show that Net-1 need not bind the ectodomain of UNC5 to produce repulsion, and they suggest that the cytoplasmic domains of UNC5 and DCC form a receptor complex. This point is shown directly by an extensive series of coimmunoprecipitation experiments which demonstrate that this association is ligand dependent. Therefore, Net-1 binding to its receptor serves to overcome an inhibition of the association between DCC and UNC5 cytoplasmic domains to activate a molecular switch that signals repulsion. Since an UNC5 ectodomain is not required for this switch to occur, Net-1 binding is likely to induce an intramolecular conformational change in DCC that allows the UNC5 and DCC cytoplasmic domains to associate. Additional experiments show that Net-1 can mediate repulsion by binding to the ectodomains of either UNC5 *or* DCC, so long as both DCC and UNC5 cytoplasmic domains are present. Finally, extensive yeast two-hybrid analysis and in vitro competition experiments define conserved regions of both the DCC and the UNC5 cytoplasmic domains that appear to mediate this association in the absence of additional factors.

Though this work defines certain minimal requirements for converting Netrin attraction to repulsion, it raises

several important questions. What roles do UNC5 ectodomains play in Netrin responses, and do all Netrins within a species interact similarly with DCC and UNC5? Regulation of Netrin-mediated guidance may also result from modulatory events that serve to spatially segregate UNC5 from DCC proteins in those portions of an axon's trajectory where attraction occurs. Future structure–function analyses of DCC and UNC5 extracellular domain associations with DCC, UNC5, Netrin, and possibly other families of proteins will begin to shed light on these issues. In addition, both the Bashaw and Goodman (1999) and Hong et al. (1999) studies raise crucial questions about the nature of repulsive and attractive guidance mechanisms. Might the conversion of the sign of the response to other guidance cues also employ heteromultimeric receptor switches, such that making Semaphorins or Slits attractive is dependent upon the addition or loss of unidentified receptor components? And, finally, though alteration of cyclic nucleotide levels in the growth cone can convert attraction to repulsion and vice versa, does this mean that the signaling outputs for diverse guidance cues and their equally diverse receptors converge on only one or two common signaling pathways? Continued inwardly directed experimental reflection will undoubtedly address these questions.

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Selected Reading

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The Narp Hypothesis?

Efficient synaptic transmission requires the enrichment and specific localization of receptors on the postsynaptic membrane apposed to the transmitter release sites. To date, the wealth of information on the vertebrate neuromuscular junction (NMJ) has provided us with the most thorough paradigms of synaptogenesis and synaptic organization (Sanes and Lichtman, 1999). A central player in NMJ formation is agrin, an extracellular heparan sulfate proteoglycan. Agrin is deposited into the synaptic basal lamina by the motor nerve terminal, where it signals transsynaptically through the receptor tyrosine kinase MuSK. Agrin signaling leads to AChR clustering and many other aspects of postsynaptic differentiation. Thus, the agrin hypothesis as proposed by McMahon (1990) has been well substantiated. Although the exact agrin-induced signaling pathway has yet to be delineated, a key effector protein is rapsyn, a peripheral membrane protein of muscle. Rapsyn can induce clusters of the AChR upon coexpression in heterologous cells and is thought to bind directly to the AChR. Genetic studies in mice have demonstrated the necessary roles of agrin, MuSK, and rapsyn in synaptic differentiation at the NMJ.

Our understanding of mechanisms of receptor clustering at postsynaptic sites on central neurons has been greatly advanced in recent years by identification of CNS receptor binding proteins that may function in an analogous manner to rapsyn. Gephyrin binds to the inhibitory glycine receptor β subunit and is required for postsynaptic clustering of glycine receptors in spinal cord. At glutamatergic synapses, PDZ domain proteins are thought to function in receptor localization and scaffolding to downstream signal transducing proteins (Kim and Huganir, 1999). PDZ domains of the PSD-95 family bind to the C termini (-ESDV) of NMDA receptor NR2 subunits, while PDZ domains of the GRIP family and PICK1 bind to the C termini (-SVKI) of AMPA receptor GluR2/3 subunits. Although direct evidence is lacking for a function of these PDZ domain proteins in localization of NMDA or AMPA receptors at vertebrate glutamatergic synapses, a function in localization of membrane protein ligands and formation of signal transduction complexes has been demonstrated for other PDZ domain proteins in *Drosophila* and *C. elegans*.

In spite of this progress on receptor anchoring/scaffolding proteins of CNS synapses, there has been little progress to date in identifying CNS molecules analogous to agrin, extracellular transsynaptic signaling proteins involved in synaptic differentiation. Agrin itself, though widely expressed in the CNS, is dispensable for the formation of glutamatergic and GABAergic synapses (Serpinskaya et al., 1999). Considering the smaller dimensions of the synaptic cleft at CNS synapses versus at the NMJ, key transsynaptic signaling proteins may be either extracellular or transmembrane. A few transmembrane proteins, notably cadherins, neuroligin, and densin-180, have been localized specifically to CNS synapses, but their function in synaptogenesis has yet to be determined. Enter O'Brien et al. (1999 [this issue of *Neuron*]) with a report of an extracellular protein, Narp (neuronal activity–regulated pentraxin), that can induce clustering of AMPA-type glutamate receptors. Narp was originally cloned by Tsui et al. (1996) as a novel immediate-early gene (IEG) induced by seizure in rat hippocampus. Narp is a member of the pentraxin family of secreted lectins. Classic pentraxins, many of which are acute phase proteins of the immune system, assemble into single symmetric pentameric rings or two such rings interacting face to face.

O'Brien et al. (1999) present several lines of evidence to support a synaptogenic signaling function for Narp at a subset of glutamate synapses. First, Narp is enriched at excitatory synapses on most aspiny but not spiny hippocampal and spinal cord neurons. Second, by analyzing endogenous distribution patterns of surface versus total Narp in hippocampal and spinal cultures, by expressing and localizing myc-tagged Narp in individual