Defining a Role and Mechanism for IgCAM Function in Vertebrate Axon Guidance

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The past two decades of research on vertebrate neural adhesion molecules of the immunoglobulin superfamily class (IgCAMs) have produced an bewildering maze of molecules and potential binding activities (for review, see Sonneregger, 1998). If one takes at equivalent face value the full range of these interactions that might influence even a particular aspect of neural development, such as axonal pathfinding, the complexity is daunting (Fig. 1). This issue of JCB contains an article describing a set of studies that helps to break through this dilemma by relating the molecular and cellular properties of three interacting IgCAMs (indicated in red in Fig. 1) to a specific pathway choice (Fig. 2A) made by vertebrate CNS commissural axons in vivo (Fitzli et al., 2000). Not only does the new information focus attention on a manageable subset of interactions, but it also suggests a novel functional relationship between multiple IgCAMs on neural cells.

This synthesis has been achieved through a combination of studies at the animal, cell culture, and biochemical level. This commentary provides an abbreviated account of a set of studies that, through the floorplate region (Krushel et al., 1988; Shiga and Oppenheim, 1991), whereas NrCAM/Mr/Bravo is found in the floorplate region (K rushel et al., 1993; Moscoso and Sanes, 1995) and NgCAM/M/L1 is abundant on the axons and along the ipsilateral pathway (Shiga and Oppenheim, 1991). Previously, axonin-1 and NrCAM have been functionally implicated in this decision through the demonstration that antibodies against either of these CAMs can shift the choice from the contralateral to the ipsilateral pathway (Stoeckli and Landmesser, 1995), suggesting that the known affinity of axonin-1 for NrCAM (Suter et al., 1995) might be involved.

Fitzli et al. (2000) continue from this base of knowledge, first by examining axonal behavior in an in vitro assay featuring alternating stripes of substrate containing different purified CAMs. When the axonin-1–expressing axons grow over alternating stripes containing a mixture of NrCAM and NgCAM or of NgCAM alone, their growth cones prefer to associate with the NrCAM-containing substrate (Fig. 2B), and this choice is blocked by antibodies against axonin-1. (See the full text of Fitzli et al. [2000] for discussion of the use of an NgCAM/NrCAM mixture in this assay.) Moreover, as described in vivo (Fitzli et al., 2000), the growth cones become enlarged when in contact with the NrCAM. It is important to note that the ability of axons to elongate is not affected by these substrate alternatives. That is, NgCAM and NrCAM are equivalent and redundant in their ability to support axon outgrowth, and outgrowth is not affected by anti-axonin-1. Therefore, the choice being made between the two environments appears to reflect an instructive, axonin-1–dependent signal to the growth cone and not simply a change in the ability to promote axon growth.

The next step was to confirm in vivo that axonin-1 is involved in providing distinct growth cone guidance signals, rather than affecting the outgrowth properties of the axons (Fig. 2B). In fact, as in the stripe assay, antibodies against axonin-1 prevent axon choice without affecting elongation, and elongation is affected only when antibodies against both NrCAM and NgCAM are used.

The final link in the analysis was made between the axon behavior studies and previous work on the binding
properties between CAMs on the same cell membrane (cis) and between two cells (trans). Included among the many activities illustrated in Fig. 1 are a cis binding between axonin-1 and NgCAM (Buchstaller et al., 1996) as well as the aforementioned trans interaction between axonin-1 and NrCAM. Homophilic trans binding of both axonin-1 and NrCAM are also shown but are more likely to affect interactions among the axons and within the floorplate, respectively.

The key to relating these activities to generation of growth cone decisions may lie in the fact that the cis binding of axonin-1 to NgCAM and the trans binding of axonin-1 to NrCAM can result in distinct signaling properties. Domain-deletion mutants and domain-specific monoclonal antibodies were used in this study to demonstrate that the binding sites on axonin-1 for both NgCAM and NrCAM are overlapping regions of the molecule (within Ig domains 1–4). Moreover, axonin-1 has previously been shown to exist in two distinct conformations: an extended conformation that is capable of forming the cis heterodimer with NgCAM, and a horseshoe-like conformation which can form the trans link to NrCAM (Rader et al., 1996). Finally, these mutually exclusive combinations appear to generate distinct patterns of kinase activity, stemming from the association of fyn with axonin-1 and casein kinase II with NgCAM. With the cis binding, fyn activity is reduced and casein kinase II is enhanced; with the trans binding, fyn activity is enhanced and that of casein-kinase II is reduced (Kunz et al., 1996).

The authors speculate that these signaling alternatives are directly or indirectly involved in the differential behavior of growth cones at the floor plate. They point out that in axon bundles, in which NgCAM is abundant but NrCAM is absent, the resulting activation of casein kinase II could stabilize microtubules via MAP1B, and presumably restrict axon choices. However, upon encounter with the NrCAM-expressing floorplate, this process would be reversed, allowing flexibility to make appropriate pathway decisions.

Regardless of the precise consequences of the cis versus trans interactions of axonin-1, a principle appears to be
emerging from this body of work that helps to make sense of at least some of the interactions shown in Fig. 1. That is, axonin-1 appears to exist in several distinct states (Fig. 2C), defined partly by intrinsic properties (the intramolecular associations involved in the horseshoe conformation), and partly by extrinsic influences (association with cis NrCAM or trans NgCAM). As a part of this process, the intrinsic and extrinsic parameters can interact via their effects on the stability of the different protein conformations and on the activity of associated kinases. If in fact the consequence of those states is to alter fundamental signals to the cell, then axonin-1 is in effect the core of a switching mechanism controlled by the composition of the environment. The proof of this hypothesis would be a major step forward in understanding the mechanism of neural IgCAM function. It would also provide a base from which to investigate the relationship of these IgCAMs to other relevant guidance mechanisms (such as Slit/Robo-mediated chemorepulsion), as well as to help define potential roles for the remaining interactions shown in Fig. 1.

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