# Comment

# 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 Sonderegger, 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. 2 A) 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 complex and diverse set of results, including their relationship to earlier work from several groups. It takes the liberty of stressing certain aspects of the results and their interpretation to highlight a mechanistic principle, namely a conformation-dependent molecular switch that contributes to a particular pathway choice. While these comments focus on the IgCAMs, the reader should be aware that this choice is influenced by other guidance mechanisms as well, most notably the interaction of the axonal receptor Robo, with the soluble factor Slit secreted by midline cells (see Brose et al., 1999; Kidd et al., 1999).

The pathway choice under consideration involves axons that initially have been guided toward a specialized region of the ventral-most margin of the CNS (called the floorplate) by a floorplate-secreted chemoattractant. The axons then choose whether to pass through the floorplate to the contralateral CNS, or to project ipsilaterally by turning away from the floorplate (Fig. 2 A; for review, see Stoeckli and Landmesser, 1998). The three IgCAMs that appear to be associated with this choice are axonin-1/TAG-1 (Dodd

et al., 1988; Stoeckli et al., 1989), NgCAM/L1 (Grumet et al., 1984; Rathjen and Schachner, 1984), and NrCAM/Bravo (de la Rosa et al., 1990; Grumet et al., 1991). Axonin-1/TAG-1 is expressed by commissural axons (Dodd et al., 1988; Shiga and Oppenheim, 1991), whereas NrCAM/Bravo is found in the floorplate region (Krushel et al., 1993; Moscoso and Sanes, 1995) and NgCAM/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. 2 B), 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 for axons growing through the floorplate region (Bovolenta and Dodd, 1990), 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. 2 B). 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

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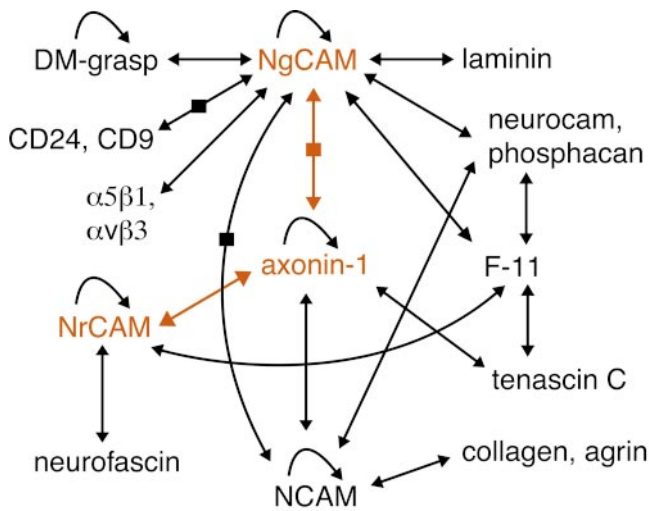
*Figure 1.* Summary of molecular interactions involving Ig super-family CAMs found on vertebrate axons. The IgCAMs featured here are indicated in red. Looped single arrows represent ho-mophilic binding. Double-headed arrows represent heterophilic binding; when a square is also present on the arrow, the binding occurs within the plane of the same membrane (cis). Adapted from Brummendorf and Rathjen (1998).



*Figure 2.* Axon guidance at the floorplate: in vivo, cell culture and molecular aspects. (A) Routes taken in vivo by commissural axons, as defined by a particular pathway choice (*). In control animals, the axonin-1 and NgCAM-expressing axons cross the midline of the ventral spinal cord through the NrCAM-express-ing floorplate (FP) to form a contralateral (Con) projection. In animals treated with antibodies against either axonin-1 or NrCAM, the axons do not enter the floorplate but instead turn to follow an NgCAM-expressing ipsilateral (Ips) pathway. (B) In culture, commissural axons can grow equally well on NgCAM substrates with or without NrCAM, but upon encounter with NrCAM the growth cones increase in size and prefer to remain associated with that substrate. This preference is blocked by anti-bodies against either axonin-1 or NrCAM. (C) Schematic summarizing the proposed molecular states of axonin-1 and their possible consequences for signal transduction. Axonin-1 (red) can exist in an extended or horseshoe conformation. The ex-tended form can interact cis with NgCAM and in this configura-tion, enhanced casein kinase II activity (NgCAM-associated) and reduced fyn activity (axonin-1–associated) is observed. The horseshoe form can interact trans with NrCAM, and in this situa-tion fyn activity is enhanced and casein kinase II is reduced. As these states are believed to reflect the conditions found in the choices shown in A and B, it is proposed that the associated growth cone decisions may involve the observed changes in sig-nal transduction pathways.

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 be-tween axonin-1 and NgCAM (Buchstaller et al., 1996) as well as the aforementioned trans interaction between axo-nin-1 and NrCAM. Homophilic trans binding of both axo-nin-1 and NrCAM are also shown but are more likely to affect interactions among the axons and within the floor-plate, respectively.

The key to relating these activities to generation of growth cone decisions may lie in the fact that the cis bind-ing of axonin-1 to NgCAM and the trans binding of axo-nin-1 to NrCAM can result in distinct signaling properties. Domain-deletion mutants and domain-specific monoclo-nal 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 hetero-dimer with NgCAM, and a horseshoe-like conformation which can form the trans link to NrCAM (Rader et al., 1996). Finally, these mutually exclusive combinations ap-pear to generate distinct patterns of kinase activity, stem-ming 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 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 be-havior 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 stabili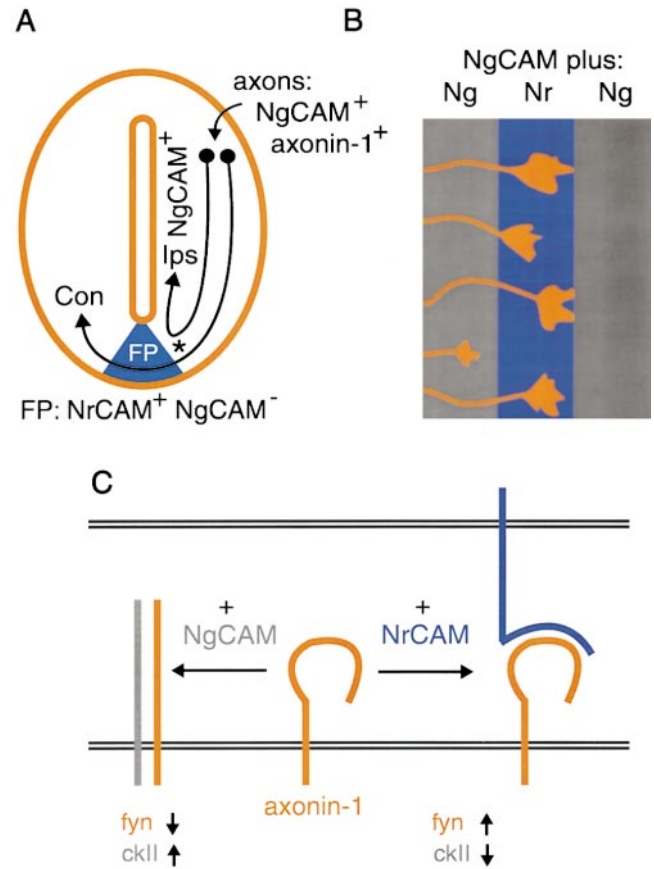ze microtubules via MAP1B, and presum-ably restrict axon choices. However, upon encounter with the NrCAM-expressing floorplate, this process would be reversed, allowing flexibility to make appropriate pathway decisions.

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. 2 C), 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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