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Molecular mechanisms of growth cone guidance: stop and go?

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Abstract. Evidence from pathfinding studies in both vertebrates and invertebrates indicates that growth cones are not guided by simple stop or go signals. Rather, the navigation of growth cones through the preexisting tissue is controlled by a continuous integration of both positive and negative cues. The path taken by an axon is determined by the continuously changing situation encountered by the growth cone at any given site along the trajectory of the axon to the target. The signals derived from interactions of surface molecules with these cues provided by the environment of the growth cone are constantly changing both temporally and spatially. Therefore, each growth cone encounters a unique set of guidance cues directing it to its specific target, thus allowing for the tremendous complexity required for the guidance of millions of axons in the developing nervous system.

Key words: Growth cones – Axonal pathfinding – Environmental factors – Spinal cord – Molecular mechanisms

Introduction

The establishment of a functional nervous system crucially depends on the correct wiring of its individual parts. Thus, during development, axons have to find their way to and make connections with their appropriate target cells. So far the mechanisms that determine the manner in which growth cones navigate through preexisting tissue to find their correct targets are not very well understood. Intensive efforts to learn more about the establishment of neuron-target connections have concentrated on various problems. For example, what factors provided by the environment promote axon growth and influence the orientation of the growth cone? How does a growth cone discriminate between target and non-target cells? What are the receptors on the growth cone membrane responsible for the recognition of environmental signals, and how are these signals translated into a response from the neuron? Obviously, these questions have to be studied at the molecular level. Therefore, molecules involved in neurite growth promotion, specific cell-cell adhesion, and signal transduction are of great interest to developmental neurobiologists. Because of the enormous complexity of the nervous system, most of our knowledge about protein function has been gained from in vitro experiments in which the role of individual molecules can be studied more easily. However, in order to understand a composite phenomenon, such as pathfinding, we ultimately have to test molecular mechanisms in vivo. Whereas a genetic approach has been very fruitful in invertebrates (for a review, see Seeger 1994), such studies are far more difficult in higher vertebrates, although progress in molecular biology has made possible the creation of transgenic and knockout mice. Investigations of lower vertebrates, such as zebrafish, which can be studied both genetically and with more conventional methods, have become more and more popular (Kuwada 1995). Comparisons between invertebrate and various classes of vertebrate systems have shown that a surprising number of principles are shared during development. For example, the establishment of the general body plan is ruled by homeobox genes, which are related in *Drosophila* and mammals (McGinnis and Krumlauf 1992; Keynes and Krumlauf 1994). More subtle aspects of development are also common between insects and higher vertebrates. Members of the same families of proteins, such as the semaphorins (Kolodkin et al. 1993; Kolodkin 1996; Pueschel et al. 1995) or the immunoglobulin-like cell adhesion molecules (Burden-Gulley and Lemmon 1995; Bieber 1991), are involved in guiding elongating axons toward their target. Taking advantage of these similarities, neurobiologists have gained further insights into the development of the nervous system.

Pathfinding – then and now

Although the idea that growing axons are guided toward their target by gradients of attractive molecules can be

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found in Ramón y Cajal's reports (Ramón y Cajal 1892), neuroscientists at the beginning of this century believed that mechanical forces rather than chemical cues guided axons through the preexisting tissue (Weiss and Taylor 1944). Sperry (1963) rejuvenated the ideas of Cajal concerning the involvement of chemotropism and chemotaxis in pathfinding. He first postulated that axons homed in on their target because the target cells express a matching label. Obviously, the number of labels required for individually tagging every axon would exceed the number of proteins found in the nervous system. However, Sperry countered attacks by his critics in a revised version of the chemoaffinity hypothesis by postulating at least two perpendicular gradients that would specify individual locations in the target area (Sperry 1963). Based on this hypothesis, great efforts were made to discover such gradients in the retina and tectum (for reviews, see Holt and Harris 1993; Kaprielian and Patterson 1994). Although some of the proteins were identified based solely on their graded expression in retina and/or tectum (for example, the TOP antigens; Trisler et al. 1981; Savitt et al. 1995), others were identified by their functional characteristics. Bonhoeffer and colleagues have described a 33-kDa glycoprotein that is enriched in posterior tectal membranes and that causes the collapse of temporal retinal axons (Walter et al. 1987; Stahl et al. 1990). More recently, the cloning of a 25-kDa protein has been reported by the same lab (Drescher et al. 1995). This protein, called RAGS (for repulsive axon guidance signal), is predominantly expressed in the posterior tectum, and induces the collapse of both nasal and temporal axons. Although RAGS does not have the expected functional specificity of inducing the collapse of only temporal retinal growth cones, it is a ligand for the large subfamily of Eph receptor tyrosine kinases and links them to neural development (for recent reviews, see Brambilla and Klein 1995; Friedman and O'Leary 1996; Mueller et al. 1996). The expression of Eph receptor tyrosine kinases and their ligands indicates their involvement in axon guidance and segmentation of the nervous system. Thus, the distribution of Mek4 and ELF-1 in countergradients in retina and tectum strongly suggests that these molecules play a role in the establishment of retino-tectal topography (Cheng et al. 1995). The expected topographic specificity of ELF-1 on retinal axons guidance has recently been shown in an elegant study both in vitro and in vivo (Nakamoto et al. 1996). Its ectopic expression in the tectum demonstrates the repulsive effect of ELF-1 on temporal retinal axons.

The general acceptance of repulsive cues as active factors in growth cone guidance has been rather slow. The discovery of collapsin-1, the first semaphorin described in chicken embryos, was based on in vitro observations of neurites from various neural tissues (Kapfhammer and Raper 1987a,b). Sympathetic growth cones were found to collapse upon contact with retinal neurites. After developing an in vitro assay (Raper and Kapfhammer 1990), Raper and collaborators identified and cloned collapsin-1 as the collapse-inducing active factor from chicken embryonic brain membranes (Luo et al. 1993). Its mouse homolog semaphorin III was shown to contribute to the repulsive effect of ventral spinal cord on the central projections of dorsal root ganglia (DRG) neurons (Fitzgerald et al. 1993; Messersmith et al. 1995). Distinct classes of sensory neurons project axons to specific laminae in the dorso-ventral axis. In vitro, neurotrophin 3 (NT-3) but not nerve growth factor (NGF) can promote the outgrowth of muscle afferents that terminate in the ventral spinal cord (Hory-Lee et al. 1993). The repulsive effect of semaphorin III/collapsin is only found in presence of NGF, and not in presence of NT-3, suggesting that semaphorin III/collapsin could be a selective chemorepellent for subpopulations of sensory neurons in vivo and therefore be involved in their guidance to specific layers in the spinal cord (Messersmith et al. 1995). The final step in the acceptance of repulsive cues as active factors in growth cone guidance was the observation that collapsin-1 could steer growth cones without inducing their collapse (Fan and Raper 1995). After contacting a collapsin-coated bead with a lateral filopodium, growth cones tend to turn away from the bead rather than collapse.

Our current view of the mechanisms involved in the establishment of neuron-target connections therefore implicates both attractive and repulsive guidance cues presented by the environment (Goodman and Shatz 1993; Goodman 1996; Tessier-Lavigne and Goodman 1996). They can either be soluble secreted molecules acting over some distance or more locally active cell-surface or extracellular-matrix-bound molecules. Evidence for long-range chemoattractants secreted by the target tissue has been found in the trigeminal system (Lumsden and Davies 1986), in the pons (Heffner et al. 1990; Sato et al. 1994), and in the spinal cord, where commissural axons have been shown to be attracted toward floor-plate explants (Tessier-Lavigne et al. 1988). Netrin-1, the protein secreted by the floor plate, was the first chemoattractant that could be identified and purified (Kennedy et al. 1994; Serafini et al. 1994). Further functional characterization of netrin-1 has revealed a repulsive effect on trochlear neurons (Colamarino and Tessier-Lavigne 1995). The finding that one guidance cue can be perceived as either attractive or repulsive by different populations of growth cones is intriguing. It remains to be seen whether netrin-1 binds to different receptors to implement attraction versus repulsion as postulated for unc-6, the netrin homolog in *Caenorhabditis elegans* (for a recent review, see Culotti and Kolodkin 1996). Unc-6 partial loss-of-function mutants were impaired either in ventral or dorsal axon pathfinding depending on the loss of interaction with unc-40 or unc-5, respectively (Hedgecock et al. 1990; Wadsworth et al. 1996). Furthermore, it remains to be shown whether the semaphorins, which have so far been classified as chemorepellents, can also have a dual function, like netrin. Recently, a novel class of murine semaphorins containing 7 thrombospondin repeats was cloned by an approach based on the polymerase chain reaction (Adams et al. 1996). Since thrombospondin has been implicated in neurite growth promotion (O'Shea et al. 1991; Arber and Caroni 1995), the two new semaphorins, SemaF and SemaG, might act as positive signals for growth cone guidance.

The classical guidance cues for growing axons were thought to be attractive cell surface molecules. Evidence from in vitro studies suggested that growth cone guidance could be explained, at least in part, by differential adhesion (see, for example, Letourneau 1975). However, after identification of a large number of cell adhesion molecules (CAMs) and their functional characterization, simple adhesion seems not to be their major contribution to pathfinding (Lemmon et al. 1992). Rather, the growth-promoting and signaling capabilities of these molecules are important for pathfinding. A great deal of attention has been given to the immunoglobulin (Ig) superfamily of CAMs (Burden-Gulley and Lemmon 1995). These molecules have been studied intensively in connection with neuronal development in vertebrates (Rathjen and Jessell 1991; Burden-Gulley and Lemmon 1995). In vitro assays have been used to demonstrate their capacity to promote neurite growth and their role in fasciculation. Especially intriguing is the complex interaction pattern of these molecules (Sonderegger and Rathjen 1992; Bruemmendorf and Rathjen 1993, 1995, 1996). In vitro assays with purified proteins bound to polystyrene beads, for example, have revealed their various homo- and heterophilic binding capacities, suggesting that the regulation of specific CAM/CAM interactions can directly influence pathfinding decisions of elongating axons. Therefore, much effort has been put into their molecular analysis and the elucidation of their signal transduction pathways (for recent reviews, see Doherty and Walsh 1994; Burden-Gulley and Lemmon 1995). So far, only a small number of in vivo studies confirming the suggested role of CAMs in pathfinding in vertebrates have been published. In some of them, Lynn Landmesser and colleagues describe the role of Ig superfamily CAMs during the innervation of the chicken hindlimb (Landmesser et al. 1988, 1990; Tang et al. 1992, 1994). These studies have elucidated the importance of the polysialic acid component of NCAM and its impact on NgCAM-mediated fasciculation for correctly sorting motoneuron fibers in the plexus region. Older in vivo studies were aimed at investigating the role of NCAM in the development of the retino-tectal system (Thanos et al. 1984; Silver and Rutishauser 1984). They showed that the injection of anti-NCAM Fab fragments into the eye resulted in pathfinding errors of retinal ganglion cell axons. The observed effects were suggested to be attributable to either an interference with fiber/fiber interactions or the absence of proper contacts between the retinal axons and the neuroepithelial endfeet. In both studies, the authors concluded that the effect of NCAM was a general interference with proper cell/cell contacts rather than the perturbation of a specific guidance cue for retinal axon pathfinding.

A specific cue for the interaction of newly formed axons has been identified in the goldfish retina (Vielmetter et al. 1991). Since goldfish grow throughout most of their life, their visual system has to adapt. Therefore, new retinal ganglion cells are constantly added in the periphery of the retina. Axons of each new generation of retinal ganglion cells fasciculate with each other on their way to the optic tectum. E587, a protein homologous to NgCAM and NrCAM, was identified based on its restricted staining of these newly formed axon fascicles of retinal ganglion cells. The injection of Fabs against the E587 antigen into the eye of rapidly growing goldfish disrupted these fascicles, resulting in a tendency of individual axons to change fascicles on their way from the periphery to the center of the eye (Bastmeyer et al. 1995). It remains to be determined whether the disruption of fascicles results in pathfinding errors of these retinal ganglion axons in the tectum or whether they are still capable of contacting their appropriate target. The prevention of fasciculation with pioneer axons does not necessarily induce pathfinding errors (Pike et al. 1992; Stoeckli and Landmesser 1995), although the studies in *Drosophila* and grasshopper that gave rise to the labeled-pathway hypothesis showed that, after ablation of specific axon tracts, pathfinding was impaired, because selective fasciculation was no longer possible (Goodman et al. 1984; Grenningloh and Goodman 1992).

Pathfinding in the developing spinal cord

Although the retinotectal system traditionally has been the center of a great deal of attention, the commissural axons in the spinal cord are currently one of the best understood systems for pathfinding at the molecular level. Currently, they represent the only model system for which both long-range and short-range guidance cues have been identified. Using a three-dimensional collagen gel matrix system (originally used by Ebendal and Jacobson 1977), Tessier-Lavigne and colleagues demonstrated a chemoattractive effect of floor-plate explants on commissural axons. The purification of netrin-1, a protein produced and secreted by the floor plate was based on its trophic effect. Its tropic effect was shown in vitro when expressed in COS cells (Kennedy et al. 1994). Furthermore, the function of netrin as a guidance molecule was also supported by the analysis of netrin-deficient mice (Serafini et al. 1996; Skarnes et al. 1995). In these mice, only very few commissural axons reached the floor plate, as seen by immunohistochemical staining with a TAG-1 antibody (Serafini et al. 1996) and by visualizing commissural axons with a lipophilic dye (E.T. Stoeckli and M. Tessier-Lavigne, unpublished). Furthermore, these mice lacked several commissures in the brain, such as the corpus callosum and the hippocampal commissure, whereas others, such as the anterior commissure, were severely defective. However, some commissures were not affected at all: both the posterior and the habenular commissures were present in netrin-deficient mice (Serafini et al. 1996). Interestingly, the pathfinding of trochlear neurons was largely normal in netrin-deficient mice, although a chemorepellent effect of netrin-1 on trochlear motor axons was shown in vitro (Colamarino and Tessier-Lavigne 1995), suggesting that additional long-range cues are involved in the guidance of these axons.

CAMs of the Ig superfamily have been implicated as short-range guidance cues in pathfinding based on their complex interaction pattern found in vitro. It has been

suggested that preferences for some CAM/CAM interactions over others would allow a growth cone to make pathfinding choices. These preferences could be modulated by the interaction of CAMs in the plane of the growth cone membrane (cis-interaction). Evidence for the importance of cis-interactions for axon growth was found in cultures of sensory neurons (Stoeckli et al. 1996; Buchstaller et al. 1996). In the absence of axonin-1/NgCAM interactions in the plane of growth cone membranes, axon outgrowth on both NgCAM and axonin-1 substrata was blocked. The cis-interaction of axonin-1 and NgCAM is not required on a laminin substratum, where neurite growth is mediated by integrin receptors. Connected with the cis-interaction of axonin-1 and NgCAM was their substratum-dependent redistribution from the apical to the substratum-facing membrane of the growth cone. This redistribution was found whenever axonin-1 and NgCAM were involved in the promotion of axon growth, i.e., on axonin-1, NgCAM, or mixed axonin-1/NgCAM substrata, but not on laminin. The redistribution of axonin-1 to the substratum-facing membrane of growth cones growing on NgCAM was independent of a trans-interaction with the substratum and therefore is probably induced by a cis-interaction with NgCAM in the plane of the growth cone membrane (see also a review by P. Sonderegger, this issue).

Evidence for a modulatory role on CAM/CAM interactions was also found for the soluble form of axonin-1 (Stoeckli et al. 1991). Axonin-1 is a unique member of the Ig superfamily of CAMs, since it occurs predominantly as a soluble molecule. It was originally identified as an axonally secreted protein in cultures of embryonic chicken DRG neurons (Stoeckli et al. 1989, 1991). If purified soluble axonin-1 was added to the culture medium of intact DRG explants, they no longer formed fascicles on a collagen substratum, but rather extended single neurites (Stoeckli et al. 1991). Thus, soluble axonin-1 caused a defasciculation of neurites by saturating binding sites for membrane-bound axonin-1. In addition, soluble axonin-1 could also interfere with interactions of other CAMs on the neuritic surface. Therefore, soluble axonin-1 might modulate the adhesive strength between growth cones and a preexisting fascicle, such that growth cones could leave, turn away, and join a new fascicle on their way to their target.

Taken together, these pieces of evidence prompted a study to test the involvement of Ig superfamily CAMs in growth cone guidance in vivo. By using the commissural neurons of the embryonic chicken spinal cord as a model system, different roles for axonin-1, NrCAM, and NgCAM in axonal pathfinding were identified (Stoeckli and Landmesser 1995). The injection of function-blocking antibodies or purified soluble axonin-1 into the central canal of the embryonic chicken spinal cord in ovo was used to perturb CAM/CAM interactions. The resulting effects on commissural axons were analyzed by injection of lipophilic dyes into transverse Vibratome sections or whole-mount preparations of the spinal cord. Whereas axonin-1 and NgCAM interactions were shown to be important for the fasciculation of commissural axons, the perturbation of NgCAM interactions did not induce pathfinding errors. However, the interaction of growth cone axonin-1 with NrCAM expressed by the floor plate was shown to be important for the proper guidance of commissural axons across the midline. The perturbation of both axonin-1 and NrCAM interactions with function-blocking antibodies or the injection of soluble axonin-1 induced pathfinding errors in vivo (Fig. 1). The injection of soluble axonin-1 had the most dramatic effect on commissural pathfinding (Fig. 1b). This result is consistent with the idea that soluble axonin-1 saturates binding sites for membrane-bound CAMs. Since soluble axonin-1 can interfere with binding partners of all CAMs that are capable of binding axonin-1, the effect of soluble axonin-1 is more pronounced than the effect of anti-axonin-1 antibodies (Fig. 1d). For instance, it is possible for soluble axonin-1 to interfere with the interaction between floor-plate NrCAM and a binding partner on commissural growth cones other than axonin-1 by saturating all NrCAM-binding sites. These results were consistent with observations made earlier in cultures of DRG explants where soluble axonin-1 was found to induce a defasciculated growth pattern (see above).

Two different models for commissural axon pathfinding were proposed that were consistent with the results of the in vivo study and observations made in other species (Bernhardt et al. 1992; Clarke et al. 1991; Bovolenta and Dodd 1991). The two models differ in their explanation of why commissural axons ignore the guidance cues directing them into the longitudinal axis before they have crossed the midline, but follow the instructions given by the same guidance cues after they have reached the contralateral border of the floor plate (Fig. 2). Because of the symmetry of the spinal cord, these cues are probably the same on both sides of the floor plate. Model I postulates that the floor plate is a more attractive substratum for the commissural axons than the adjacent spinal cord tissue. Therefore, axons enter the floor plate when they reach the ventral border of the floor plate. Reaching the contralateral border of the floor plate, the growth cones have to choose between growing straight onto a less favorable substratum or making a turn in order to keep maximal contact with the floor plate surface. Model II is based on observations made in vitro about the substratum-dependent redistribution of CAMs on the growth cone surface (see above and Stoeckli et al. 1996). According to this model, the growth cones would have to change the distribution of their surface molecules in order to be able to read and interpret the guidance cues directing them into the longitudinal axis. The distribution of surface molecules appropriate for reading the guidance cues would only be acquired by contact with the floor-plate surface. Therefore, a growth cone would ignore the guidance cue on the ipsilateral side of the floor plate but readily follow the instructions on the contralateral side. Both these models have in common that contact with the floor plate is crucial for the correct guidance of the commissural growth cone. Consequently, the mechanism of growth cone guidance at the floor plate was addressed in an in vitro study (Stoeckli et al. 1997). A two-dimensional coculture system of commissural neurons and floor-plate

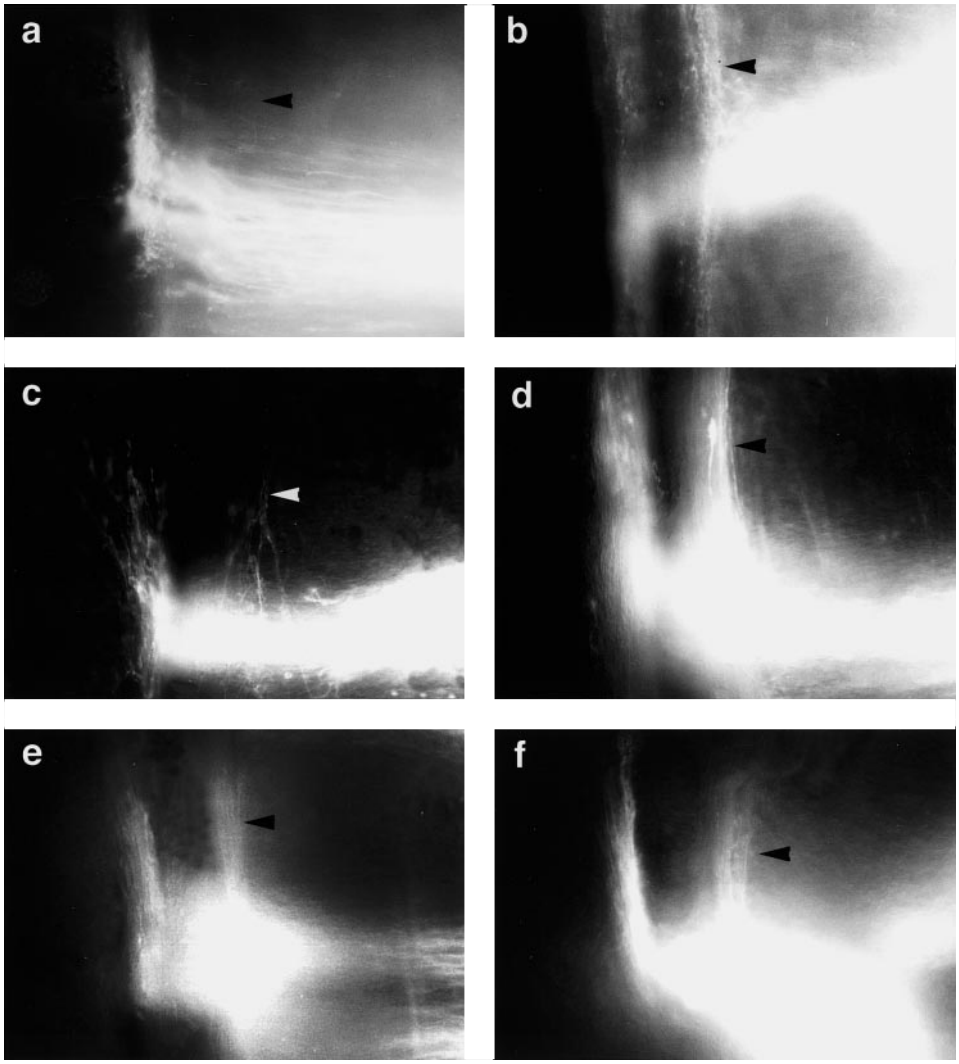

Fig. 1a–f. The perturbation of axonin-1 and NrCAM interactions induces pathfinding errors of commissural axons in vivo. Soluble axonin-1 (**b**) or function-blocking antibodies against NrCAM (**c**) or axonin-1 (**d**) were repeatedly injected into the central canal of embryonic chicken spinal cords in ovo throughout the time of commissural axon growth. The embryos were sacrificed at stage 25 to 25 1/2, and commissural axons were visualized in whole-mount preparations by injection of DiI into the area of the commissural cell bodies (for details, see Stoeckli and Landmesser 1995). In control embryos (**a**), all the commissural axons crossed the midline and turned into the longitudinal axis along the contralateral border of the floor plate. The injection of soluble axonin-1 (**b**), anti-NrCAM antibodies (**c**), anti-axonin-1 antibodies (**d**), a mixture of both anti-axonin-1 and anti-NrCAM antibodies (**f**), or soluble axonin-1 together with anti-NrCAM (**e**) induced pathfinding errors of the commissural axons. Instead of crossing the midline, some axons turned into the longitudinal axis prematurely along the ipsilateral floor-plate border. The effect of soluble axonin-1 (**b**) was more pronounced than the effect of anti-axonin-1 (**d**), or anti-NrCAM (**c**) alone, or in combination (**f**). The effects seen after injection of soluble axonin-1 together with anti-NrCAM antibodies (**e**) were comparable to the effect caused by soluble axonin-1 alone (see also Stoeckli and Landmesser 1995). *Arrowheads*, Ipsilateral floor-plate border. Magnification 210×

explants was developed to study the behavior of growth cones upon floor-plate contact. Of particular interest was the behavior of growth cones in the presence and absence of antibodies interfering with axonin-1 and NrCAM interactions. The observation that commissural axons committed pathfinding errors when axonin-1 and NrCAM interactions were perturbed could be interpreted as a failure of the growth cone to make appropriate contact with the floor plate. As a consequence, they would turn into the longitudinal axis prematurely along the ipsilateral border of the floor plate. This idea was indeed supported by the observations made in co-culture experiments. Whereas the presence of anti-axonin-1 and anti-NrCAM antibodies did not decrease the chemoattractive effect of floor-plate explants, it prevented commissural axons from entering the floor-plate explants. Surprisingly, the mechanisms by which anti-axonin-1 and anti-NrCAM antibodies hindered commissural axons from entering the floor-plate explants differed. In a time lapse study, the presence of anti-axonin-1 resulted in a collapse of commissural growth cones upon floor-plate contact, whereas the presence of anti-NrCAM prevented commissural growth

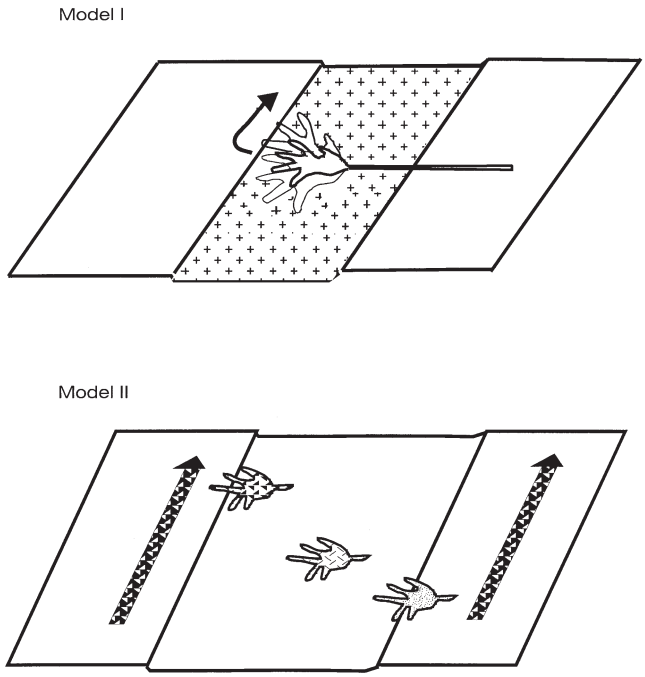

Fig. 2. Two different models for commissural axon pathfinding are consistent with the results from the in vivo studies. *Model I* proposes that the floor plate is a more attractive substratum for commissural axons than the adjacent spinal cord tissue. Therefore, commissural axons at the ventral border of the spinal cord readily enter the floor plate. Reaching the contralateral border, they would have to switch to a less favorable substratum when growing straight, or, as seen in control embryos, they have to turn into the longitudinal axis along the contralateral border of the floor plate to keep maximal contact with the more favorable floor-plate surface. A pause at the floor-plate border may make them susceptible to the guidance cues directing them rostrally into the longitudinal axis. *Model II* is based on observations made in vitro with sensory neurons that showed substratum-specific distribution of axonin-1 and NgCAM on the growth cone surface. Similarly, commissural growth cones could change the distribution of their surface molecules, thereby becoming susceptible to the guidance cue(s) directing them into the longitudinal axis. Since the spinal cord is symmetrical, the guidance cue(s) for the longitudinal axis are probably the same on both sides of the floor plate. However, commissural axons do not respond to these cues on the ipsilateral side of the floor plate but only after they have crossed the midline. This could be explained by a rearrangement of the surface molecules on the growth cone. Growth cones would neglect the guidance cue(s) on the ipsilateral border, because the arrangement of their surface molecules would not allow them to read the cues. On the contralateral side of the floor plate, they would have rearranged their surface molecules as a result of their contact with the floor plate, enabling them to respond to the longitudinal guidance cues

cones from entering the floor plate without inducing their collapse. In order to explain this discrepancy, the existence of at least one additional binding partner for axonin-1 was suggested in a refined model for commissural axon pathfinding (Fig. 3). According to this model, the behavior of growth cones at the floor plate is determined by a balance between positive and negative signals. Each growth cone integrates all the signals derived from interactions of its surface molecules with molecules expressed by the floor plate, the adjacent spinal cord tissue, or other axons. Based on the outcome of this “computation”, the

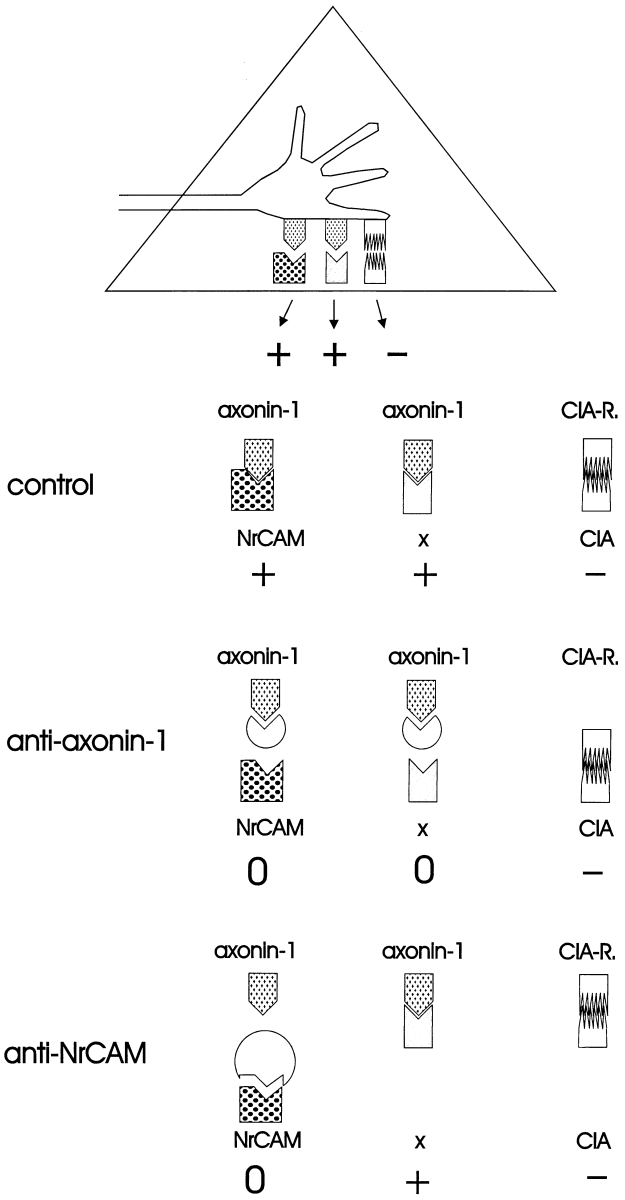

Fig. 3. Pathfinding of commissural axons across the midline is determined by a balance between positive and negative signals. The behavior of commissural growth cones is the result of the integration of all the signals derived from interactions of growth cone molecules with molecules expressed by the floor plate. The model predicts positive influences (+) contributed by axonin-1/NrCAM interactions and by interactions of axonin-1 with a not yet identified molecule of the floor plate (*x*). These interactions mask a negative collapse-inducing activity (*CIA*) of the floor plate. In *control* embryos, commissural axons readily enter the floor plate, since the resulting signal is *positive*. In the presence of *anti-axonin-1* antibodies, both interactions contributing positive signals are eliminated; therefore, the growth cone expressing a receptor for the collapse-inducing activity of the floor plate (*CIA-R.*) collapses upon floor-plate contact. In the presence of *anti-NrCAM*, only interactions between *axonin-1* and *NrCAM* are eliminated, whereas the interaction between *axonin-1* and molecule *x* is still possible. Since this interaction contributes a *positive* signal, the *CIA* of the floor plate is still masked. Thus, observation with time lapse video microscopy showing a collapse of commissural growth cones in the presence of *anti-axonin-1* but not *anti-NrCAM* antibodies can be explained (for details, see Stoeckli et al. 1997). Although the presence of *anti-NrCAM* did not induce the collapse of commissural growth cones, they were still prevented from entering the floor plate, consistent with a neutral balance predicted by this model

growth cone either enters the floor plate and crosses the midline or it avoids the floor plate and turns along the ipsilateral floor-plate border. On the assumption that growth cones interpret the cues provided by the floor plate differently or respond to a different subset of molecules expressed by the floor plate, this model system can also explain pathfinding of neuronal populations other than the commissural neurons. For instance, axons would project ipsilaterally, because their integration of the signals would prevent them from entering the floor plate. Likewise, pathfinding errors committed by normally contralaterally projecting commissural axons in the presence of anti-axonin-1 or anti-NrCAM antibodies can be explained by a shift in the balance from positive to more negative signals. In the presence of anti-axonin-1 antibodies, both interactions contributing positive signals are eliminated. The behavior of the growth cones is determined by the negative signal emanating from the collapse-inducing activity of the floor plate, resulting in a collapse of the growth cone upon floor-plate contact. In contrast, in the presence of anti-NrCAM, only the axonin-1/NrCAM interaction is eliminated, whereas the interaction of axonin-1 with a yet unidentified molecule(s) of the floor plate still contributes a positive signal masking the negative influence of the collapse-inducing activity of the floor plate. In this case, a neutral balance prevents growth cones from entering the floor-plate explants without inducing their collapse.

A balance between positive and negative signals directing the behavior of growth cones at the midline has also been observed in *Drosophila* (Seeger et al. 1993). Two mutants, *roundabout (robo)* and *commissureless (comm)*, were found in which the balance was shifted such that either no axons crossed the midline *(comm)* or additional, normally ipsilaterally projecting axons crossed the midline *(robo*; Tear et al. 1993, 1996). Similarly, the behavior of the Q1 growth cone in grasshopper suggested that a negative repelling activity was associated with the midline (Myers and Bastiani 1993). Q1 growth cones overcame this repulsive cue by contacting the contralateral Q1 growth cone. Therefore, although the exact details and the molecules involved differ, the behavior of the commissural axons at the ventral midline of the CNS seems to be determined by a balance between positive and negative signals in both vertebrates and invertebrates.

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