Mini-Review

Regulation of Axonal Growth in the Vertebrate Nervous System by Interactions between Glycoproteins Belonging to Two Subgroups of the Immunoglobulin Superfamily

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THE mechanism of formation of connections between neurons by axons and dendrites is one of the crucial and interesting aspects of neural development. To reach their intended targets, extending axons depend on a complex pattern of guidance cues present in their local environment (Dodd and Jessell, 1988). Several distinct mechanisms have been postulated for such guidance functions. (*a*) Neurite growth–promoting proteins exposed either at the surface of neuronal or nonneuronal cells, or localized in the extracellular matrix (ECM)[1] provide pathways along which axons can preferentially elongate (Rathjen and Jessell, 1991; Grumet, 1991; Reichardt and Tomaselli, 1991; Takeichi, 1991). (*b*) Repulsive molecules may block axonal extension into certain territories; e.g., by contact inhibition of the mobility of growth cones. In spatial and temporal concert with molecules favorable to axon growth, such molecules may be involved in guidance (Caroni and Schwab, 1988; Stahl et al., 1990; Raper and Kapfhammer, 1990; Keynes et al., 1991; Chiquet-Ehrismann, 1991). (*c*) Soluble chemoattractants, released by intermediate or final cellular targets, might guide axons by the establishment of concentration gradients (Lumsden and Davies, 1986; Tessier-Lavigne et al., 1988; Heffner et al., 1990; Placzek et al., 1990).

Axon growth on favorable substrata involves specific interactions with cell surface glycoproteins. Three major structural classes of axonal membrane proteins have been described so far to undergo specific interactions with neurite growth–promoting ECM or cell surface molecules, and thus, have been implicated as axonal receptors in the growth-generating machinery: (*a*) integrins of the β_1 and β_3 subfamilies (Reichardt and Tomaselli, 1991), (*b*) cadherins (Takeichi, 1991), and (*c*) axonal membrane proteins of the Ig superfamily (Rathjen and Jessell, 1991; Grumet, 1991; Hortsch and Goodman, 1991). Ig-like proteins concentrated on axons can be further classified by the expression of both Ig- and fibronectin type III (FNIII)-related repeats (referred to as Ig/FNIII-like proteins in the following), or Ig-like domains alone. According to the overall domain organization and primary sequence, the group of Ig/FNIII-like proteins in vertebrates can be further divided into subgroups (Fig. 1).

1. *Abbreviations used in this paper*: ECM, extracellular matrix; FNIII, fibronectin type III; GPI, glycosyl-phosphatidylinositol; NCAM, neural cell adhesion molecule; PSA, polysialic acid.

In this article, we focus on two of the subgroups of Ig/FNIII-like proteins that are predominantly expressed on axons during development (Rathjen, 1991). Other members of the Ig/FNIII family, like neural cell adhesion molecule (NCAM), expressed more widely in the nervous system of vertebrates, are discussed only briefly in the context of the function of the two subgroups of axonal Ig/FNIII-like molecules. For a survey of the field of axonal proteins implicated in growth, the reader is referred to recent reviews (Hortsch and Goodman, 1991; Bixby and Harris, 1991; Reichardt and Tomaselli, 1991; Edelman and Crossin, 1991; Rathjen and Jessell, 1991; Walsh and Doherty, 1991).

Structural Features of Ig/FNIII-like Proteins Concentrated on Axons in Vertebrates

According to the overall domain organization and amino acid sequence homology, the first subgroup of axonal Ig/FNIII-like proteins includes the glycosyl-phosphatidylinositol (GPI)-anchored molecules F11/F3 (Brümmendorf et al., 1989; Gennarini et al., 1989) and axonin-1/TAG-1 (Furley et al., 1990; Zuellig et al., 1992), while the second subgroup is formed by the transmembrane glycoproteins L1/Ng-CAM,[2] Nr-CAM, and neurofascin (Moos et al., 1988; Bur-

2. The classification of F11/F3 and axonin-1/TAG-1 as species homologues has not been challenged so far. However, it is controversial at present whether the glycoprotein termed predominantly L1 (but also NILE) in mammals and the glycoprotein termed Ng-CAM (but also G4, 8D9, and L1) in the chick are species homologues. Many features in common to both proteins speak in favor of a status as species homologues, but a number of dissimilarities may be interpreted as counterarguments (for a detailed account cf. Burgoon et al., 1991; Grumet, 1992). In brief, features in common to both proteins include (*a*) their gross domain structural organization (Moos et al., 1988; Burgoon et al., 1991); (*b*) their appearance as a pattern of polypeptides probably originated by partial proteolytic cleavage of a single precursor molecule (Grumet et al., 1984; Wolff et al., 1987; Sadoul et al., 1988); (*c*) the occurrence of a phosphorylation site on the cytoplasmic domain (Grumet et al., 1984; Faissner et al., 1985; Sadoul et al., 1989); (*d*) the tissue expression patterns in most areas of the nervous system on which data are available for corresponding developmental stages (Rathjen and Schachner, 1984; Stallcup et al., 1985; Daniloff et al., 1986; but cf. Shiga et al., 1990, for an exception); and (*e*) involvement in biological functions, such as neuronal migration (Lindner et al., 1983; Hoffman et al., 1986), promotion of neurite outgrowth (Lagenaur and Lemmon, 1987; Lemmon et al., 1989; Chang et al., 1990; Kadmon et al., 1990), and fasciculation (Hoffman et al., 1986; Fischer et al., 1986; Rathjen et al., 1987*a*). The argumentation that L1 and Ng-CAM may not be species homologues but relatives derived

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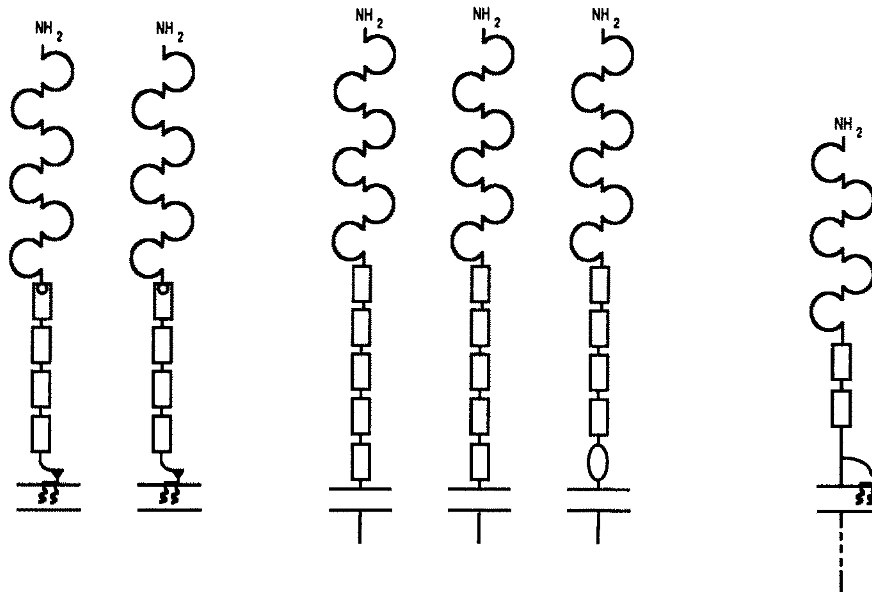

Figure 1. Basic structure and subgroup assignment of axonal proteins containing both Ig- and FNIII-related domains. Based on the overall domain organization and amino acid sequence homology, F11/F3 and axonin-1/TAG-1 form one subgroup, whereas L1/Ng-CAM, Nr-CAM, and neurofascin belong to a second subgroup. L1 of the rat has initially been termed NILE (nerve growth factor–inducible large external) glycoprotein. Other names for chicken Ng-CAM found in the literature are G4, 8D9, and L1. NCAM which contains five Ig- and two FNIII-like repeats can not be assigned to these subgroups. Ig-related domains of the C2 subcategory are drawn as loops; it is thought that each Ig-like domain has a three-dimensional structure similar to that of the immunoglobulin fold of the C2 subcategory. FNIII-like repeats are shown as rectangles; a three-dimensional structure similar to that of the repeated type III domains of fibronectin is assumed. The segment in neurofascin which is rich in proline, alanine, and threonine is indicated by an ellipse. The cytoplasmic segment is shown as a short line, whereas the GPI anchor is indicated by an arrow and two wavy lines attached to the plasma membrane. The circle in the first FNIII-like repeat of F11/F3 and axonin-1/TAG-1 indicates a putative hinge region, as suggested by the amino acid sequence. For variant forms of the members of the L1 subgroup which might be generated by alternative pre-mRNA splicing, see text. NCAM forms generated by differential splicing differ in the plasma membrane attachment and in the size of the cytoplasmic region (indicated by a broken line). For short amino acid segments expressed in specific NCAM forms see Walsh and Doherty (1991). At present, there are no published reports on the involvement of Nr-CAM in axonal growth. However, its localization and its close relationship to neurofascin makes it likely. A chick axonal glycoprotein termed Bravo shares biochemical properties with, and might be identical to, Nr-CAM (De la Rosa et al., 1990). So far, Nr-CAM and neurofascin have only been found in the chick.

goon et al., 1991; Grumet et al., 1991; Volkmer et al., 1992). Axonin-1/TAG-1 and F11/F3 also exist as secreted forms, probably derived from an intracellular pool rather than released from the cell surface (Ruegg et al., 1989). The extracellular portion of the polypeptides of both subgroups is composed of six Ig-like domains of the C2 subcategory as defined by Williams and Barclay (1988) and four to five FNIII-related repeats (Fig. 1). Amino acid sequence comparisons of the individual domains in the proteins indicate that colinear domains show the highest degree of similarity. The cytoplasmic segment of ∼110 amino acid residues represents the most conserved region between L1, Ng-CAM, Nr-CAM, and neurofascin. This region may therefore have an important function through interaction with cytoplasmic proteins. In contrast to axonin-1/TAG-1 and F11/F3, several variant forms which might arise via alternative pre-mRNA splicing are known for neurofascin and Nr-CAM, whereas only two cytoplasmic isoforms of L1 and Ng-CAM have been described (Grumet et al., 1991; Volkmer et al., 1992; Miura et al., 1991).

The members of the two subgroups of axonal Ig/FNIII-like proteins are thought to play a critical role in axonal growth because of their predominant expression during the early phases of development, their transient presence on some axons, and their coexistence on many axon systems at specific stages (Rathjen, 1991). Furthermore, several independent in vitro experiments, including antibody perturbation studies and direct neurite outgrowth assays using these proteins, either immobilized on cell culture dishes or expressed by transfection in cell lines, have revealed compelling evidence that these Ig/FNIII-like proteins participate in axonal growth processes (Stallcup and Beasley, 1985; Hoffman et al., 1986; Fischer et al., 1986; Chang et al., 1987; Rathjen et al., 1987*a*,*b*; Lagenaur and Lemmon, 1987; Ruegg et al., 1989; Chang et al., 1990; Furley et al., 1990; Kadmon et al., 1990; Stoeckli et al., 1991; Gennarini et al., 1991).

As most functional aspects known so far implicate Ig/FNIII-like proteins as regulators of cellular contact phenomena, the investigation of the members of this protein family with respect to their molecular mode of function has become interesting for many aspects of developmental biology and neurobiology. In particular, their binding to other

from a common ancestor is mainly based on the fact that their amino acid sequence identity is only 40% (Burgoon et al., 1991; Grumet, 1992). This is low if compared with other putative homologues among the neural Ig/FNIII-like proteins, such as F11/F3, axonin-1/TAG-1, and NCAM of chick and mouse, but not unusual if compared with the evolutionary conservation of other Ig-like molecules involved in cell adhesion; e.g., ICAM-1, for which an amino acid sequence identity as low as 50% is found already when different mammals such as man and mouse are compared (Horley et al., 1989). Since the issue of the interspecies relationship of mouse L1 and chick Ng-CAM can not be settled based on the presently available data, we use the denomination L1/Ng-CAM in the following, which combines the terms most often found in the literature.

macromolecules has become one major field of interest. The combination and the arrangement of several Ig- and FNIII-like domains in one polypeptide might confer the capability of multiple macromolecular interactions. It is conceivable that each individual Ig- or FNIII-like domain selectively interacts with an Ig- or FNIII-like domain of another protein, or related sequence motifs of proteins of another structural group. This might result in complex binding specificities, as is required for selective axonal growth behavior. A direct or indirect linkage between the Ig/FNIII-like proteins of these two subgroups on axons was suggested by the observation that antibodies to the individual Ig/FNIII-like proteins prevent fasciculation of extending axons to a similar degree in in vitro assays (Rathjen et al., 1987*a,b*; Chang et al., 1987; Ruegg et al., 1989). Evidence for a functional linkage has come from recent studies on NCAM, L1, Ng-CAM, and F11, indicating that these Ig/FNIII-like proteins regulate axon fasciculation by a common phosphorylation-dependent mechanism (Cervello et al., 1991). The possibility of a direct physical interaction between neural Ig/FNIII-like proteins has been suggested by investigations on cells of the immune system which revealed that specific Ig-like proteins bind to other members of the Ig superfamily or to proteins of the integrin family to regulate cell–cell interactions (Springer, 1990).

L1/Ng-CAM, a Neuronal Receptor for Substratum-induced Neurite Outgrowth, Is Activated by Both Homophilic Interaction with L1/Ng-CAM and Heterophilic Interaction with Axonin-1

Studies on interactions between individual axonal Ig/FNIII-like glycoproteins have recently revealed that L1/Ng-CAM, expressed at the surface of cultured neurons, promotes neurite outgrowth not only by homophilic (like-like) binding to an L1/Ng-CAM substratum, but also by heterophilic (like-unlike) interactions with axonin-1 (Kuhn et al., 1991). Both the homophilic interaction of L1/Ng-CAM molecules and the heterophilic interaction of L1/Ng-CAM with axonin-1 have been demonstrated directly at the molecular and functional level in neurite growth assays. A physical association between isolated L1/Ng-CAM molecules has been demonstrated using artificial membrane vesicles, into which these molecules had been incorporated, and fluorescent microspheres, onto which the proteins had been covalently coupled (Grumet and Edelman, 1988). Such procedures have become popular in the past years because they represent the most readily applicable assays for the identification of weak macromolecular interactions. To determine whether L1/Ng-CAM of the neuronal membrane is part of the neurite growth–promoting machinery as a receptor binding to L1/Ng-CAM substratum in a homophilic interaction, Lemmon et al. (1989) used a cross-species approach based on the finding that both L1/Ng-CAM from mouse and from chick each promote neurite extension from neurons of both species. When they cultured chick neurons on mouse L1/Ng-CAM they found that antibodies specific for chick L1/Ng-CAM inhibited neurite outgrowth. Since the antibodies specific for chick L1/Ng-CAM did not recognize mouse L1/Ng-CAM, the observed blockage of neurite outgrowth was attributed to selective binding of the antibodies to L1/Ng-CAM receptors on the growing neurites (containing the chick form of L1/Ng-CAM). Under the assumption that L1 of the mouse and Ng-CAM of the chick are species homologues (for a discussion on this issue see above), these results indicate that the substratum-receptor interaction relevant for neurite outgrowth in this experimental situation is homophilic; i.e., both substratum and receptor are L1/Ng-CAM molecules. Thus, the homophilic binding between L1/Ng-CAM molecules observed with membrane vesicles or the microspheres assay is functionally involved in the recognition and transduction of neurite growth signals emitted by L1/Ng-CAM molecules.

The function of axonin-1 as a second substratum to induce neurite outgrowth by interaction with neuronal L1/Ng-CAM has been demonstrated by Kuhn et al. (1991). Physical binding of isolated axonin-1 and L1/Ng-CAM was demonstrated using fluorescent microspheres with covalently coupled axonin-1 and L1/Ng-CAM, respectively. If microspheres conjugated with axonin-1 or L1/Ng-CAM were incubated together, they formed mixed aggregates, indicating heterophilic binding of the two glycoproteins. Within the sensitivity of the microsphere assay, no interaction of axonin-1 with axonin-1 could be detected. Axonin-1–coated microspheres also bound to the neurites of cultured dorsal root ganglia neurons. This interaction was exclusively mediated by neuritic L1/Ng-CAM, as indicated by complete binding suppression by monovalent antibodies to L1/Ng-CAM. The interaction between neuritic L1/Ng-CAM and immobilized axonin-1 was found to mediate the promotion of neurite growth on axonin-1, as evidenced by the virtually complete arrest of neurite outgrowth in the presence of antibodies to L1/Ng-CAM. Furthermore, if soluble axonin-1 was present in the culture medium of dorsal root ganglia neurons, axonal fasciculation was completely suppressed, probably by inactivation of both the homophilic binding of L1/Ng-CAM molecules and the heterophilic binding of L1/Ng-CAM and axonin-1 across axonal membranes (Stoeckli et al., 1991).

Thus, L1/Ng-CAM of the growth cone membrane can bind to and receive neurite growth–promoting signals from two distinct glycoproteins of the Ig/FNIII subgroups: L1/Ng-CAM and axonin-1 (Fig. 2).

Modulation of the Function of the Ig/FNIII-like Proteins on Axons by Specific Macromolecular Interactions at the Cell Surface

The dualistic substratum recognition specificity of L1/Ng-CAM and its relatively stable expression on all major long-projecting axon tracts throughout development implicates L1/Ng-CAM as a possible molecular sensor during axon extension. In contrast to exclusively homophilic interactions, as previously assumed for L1/Ng-CAM, a combined heterophilic/homophilic axonal recognition system might allow to translate growth cone interactions with different substrata, composed of L1/Ng-CAM, axonin-1, or mixtures thereof, into distinctive intracellular growth signals. Conceivably, such a mechanism could give the growth cone the ability to explore its local environment with respect to neurite growth–promoting molecules, in order to select the most suitable pathway for elongation. Thus, several questions concerning the mode of action of L1/Ng-CAM arise.

First, does the homophilic binding among L1/Ng-CAM molecules exclude a heterophilic interaction of L1/Ng-CAM with axonin-1 or is it possible that both binding modes can occur simultaneously? And, directly related to that question: Does the binding between L1/Ng-CAM and axonin-1 occur

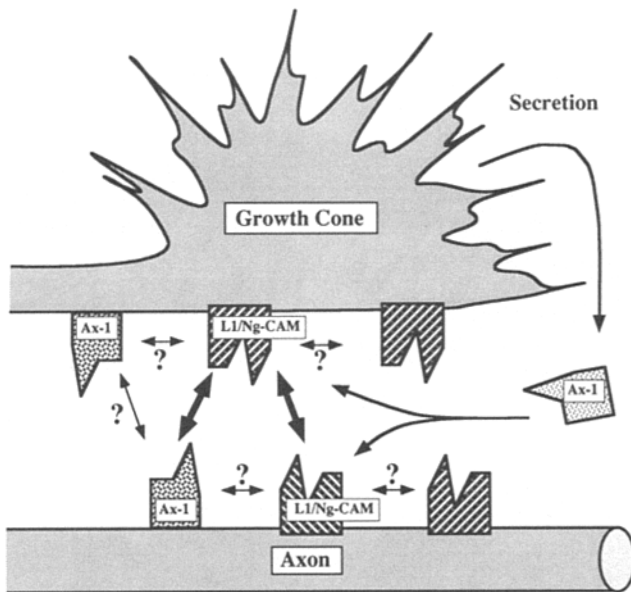

Figure 2. Established and hypothetical interactions of Ll/Ng-CAM with other Ll/Ng-CAM molecules and/or axonin-l on a growth cone moving along an axon. Ll/Ng-CAM on growth cones has been characterized as a neurite growth-promoting receptor with dual substratum specificity; it can mediate neurite outgrowth on Ll/Ng-CAM and on axonin-1. Lateral interactions between adjacent molecules within the plane of the same membrane (*cis* interactions) are hypothetical; they may occur in the membranes of the growth cone and/or that of the axon and may influence the substratum preference of Ll/Ng-CAM receptor proteins. In particular situations, axonin-l may also homophilically interact with axonin-l in the plane of the same membrane or with axonin-l of an apposed membrane, since a homophilic interaction has been reported to occur with TAG-1, the presumptive rat homologue of axonin-1. Secreted axonin-1 has been found to prevent neurite fasciculation in vitro; it might generate substratum preference by differential interference with both the homophilic and the heterophilic interaction of Ll/Ng-CAM. It has been demonstrated that soluble axonin-l is indeed released by axons; whether the growth cone is the site of release remains to be determined.

only across different plasma membranes (*trans*-binding) or also within the same membrane (*cis*-binding)? The expression of both multiple Ig- and FNIII-like domains in both proteins suggests that several physical interactions might be possible simultaneously within and across plasma membranes. Such multiple interactions are known for the T cell receptor/CD3/CD4(CD8) complex where several Ig-like proteins function in concert in antigen-specific responses (Klausner et al., 1990). Thus, it is conceivable that complexes of Ig/FNIII-like proteins will be found on growth cones. If so, this would imply that the binding between axonin-l and Ll/Ng-CAM or between different Ll/Ng-CAM molecules may be localized to a specific Ig- or FNIII-like domain within Ll/Ng-CAM and axonin-l. That a specific binding can be mapped to a single domain has been demonstrated in studies on the Ig-like protein ICAM-1, which is expressed on a wide variety of cells and is implicated in interactions of T cells with their target cells. Mutational analysis of the interaction of ICAM-1 (CD54) with LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), which are members of the integrin protein family, indicates that the first Ig-like domain mediates binding to LFA-1, whereas the third domain involves Mac-1 binding (Staunton et al., 1990; Diamond et al., 1991). Furthermore, the most NH_2-terminal Ig-like domain of CD2, another adhesion receptor of T lymphocytes, binds to LFA-3, another Ig superfamily member, acting as a counter-receptor on target cells (Recny et al., 1990). The three-dimensional structure of CD2 has recently been determined as an immunoglobulin fold, which characteristically is formed of a twisted stack of two β-pleated sheets, composed of antiparallel β strands (Driscoll et al., 1991). In conjunction with previous studies on mutations that disrupt the binding to LFA-3 (Peterson and Seed, 1987), it has been suggested that the two molecules interact through the face of a β sheet (Driscoll et al., 1991; Springer, 1991). This type of binding is reminiscent of the type of interaction found in the dimerization of the Ig domains of immunoglobulins, and distinct from the interaction involved in antigen binding, which is accomplished by loops connecting the β-sheet strands (Amzel and Poljak, 1979).

The fact that FNIII-like repeats expressed in Ll/Ng-CAM and axonin-l have been found in a variety of vertebrate and invertebrate proteins implicated in axonal extension suggests that these domains might be as common and as versatile for interaction and recognition functions as Ig-like domains. Although the three-dimensional structure of the FNIII-like domains has not been studied in detail, recent analysis of the 10th type III module in fibronectin by nuclear magnetic resonance spectroscopy reveals interesting similarities to the immunoglobulin fold. The investigated type III domain is formed from seven β strands in two antiparallel β sheets with a topology reminiscent of immunoglobulin C domains (Baron et al., 1992). Based on these structural similarities, one may speculate that FNIII-like repeats interact with Ig-like domains or other FNIII-like domains within the axonal proteins. This would increase the combinatorial binding possibilities among Ig/FNIII-like proteins. Furthermore, it would enable the Ig/FNIII-like molecules to interact with other axonal proteins that contain FNIII-like domains in their extracellular region like the recently discovered tyrosine phosphatases (Tian et al., 1991; Yang et al., 1991). In the best studied case, however, binding of the 10th FNIII repeat in fibronectin occurs through the tripeptide Arg-Gly-Asp sequence to a protein belonging to the integrins (Ruoslahti, 1988). It is not yet clear whether FNIII-like repeats in the axonal proteins bind to adhesion receptors of the integrin type, although several Ig/FNIII-like proteins including TAG-1, Ng-CAM, and neurofascin contain the Arg-Gly-Asp sequence in an amino acid environment that resembles the 10th type III repeat of fibronectin; and, moreover, an integrin α subunit, termed α_8, with preferential axonal localization has been described (Bossy et al., 1991).

Second, if the homophilic and the heterophilic binding activities of Ll/Ng-CAM to Ll/Ng-CAM or axonin-1, respectively, are exclusive and therefore competitive, Ll/Ng-CAM receptor bears the potential to select between the Ll/Ng-CAM and axonin-1 substrata in situations where both are accessible. Assuming a constant ratio of the affinities to Ll/Ng-CAM and axonin-1, one would expect that Ll/Ng-CAM expressing growth cones at decision points would give preference to the axonal membrane evoking a more intense neurite growth-promoting signal, as determined by the absolute and relative density of the two ligands. If, however, the homophilic and

heterophilic binding activities of Ll/Ng-CAM are not competitive, it is conceivable that both bindings act additively, and combinations of Ll/Ng-CAM and axonin-1 support a more effective neurite outgrowth.

Third, it would be interesting to know whether a putative substratum preference of Ll/Ng-CAM is subject to regulatory changes. It is conceivable that the dynamics of an extending growth cone requires rapid transitions between different adherence states without altering the level of adhesive proteins on its surface. There is evidence that the affinity state of integrins can be modulated at the cell surface of neuronal cells. For example, a monoclonal antibody, called TASC, has been described which promotes adhesion of retinal cells to laminin but inhibits attachment to vitronectin. TASC, which binds to the integrin β_1 subunit, probably induces a conformational change in the extracellular domain (Neugebauer and Reichardt, 1991). Several other examples of the modulation of the functions of integrins through conformational changes upon ligand binding have been found for nonneural cells (Hynes, 1992). It is plausible that the axonal adhesion molecules of the Ig/FNIII type assume different affinity states by interreacting. For example, contacts with adjacent axonin-1 or Ll/Ng-CAM molecules of the same growth cone membrane could modulate the substratum affinity of Ll/Ng-CAM receptor molecules. Similarly, axonin-1 and Ll/Ng-CAM proteins in axonal membranes might exert variable substratum functions depending on their lateral ligand. Furthermore, in a situation where both Ll/Ng-CAM and axonin-1 are expressed in apposed membranes, each individual molecule may potentially interact, both laterally with adjacent molecules of the same membrane (*cis* interaction) and across the intermembrane space, with molecules exposed at the surface of the other membrane (*trans* interaction). Thus, networks of molecules interconnected by *cis* and *trans* bindings may be formed. The protein molecules in such networks may assume different conformations, depending on the pattern of ligands and the conformation of each ligand. In such ligand pattern-determined conformations, molecules may assume distinct ligand pattern-determined functional states, for example, of functions such as the affinities and/or the specificities for ligand binding, or transmembrane signaling. In view of the currently known structural and functional characteristics of Ig/FNIII-like molecules, it is likely that interactions occur also between other family members, such as F11/F3, Nr-CAM, and neurofascin. Then, extended networks including multiple Ig/FNIII molecules may be formed. The notion of ligand pattern-determined function of molecules involved in such networks would be especially intriguing in the context of axon guidance as a means of encoding preference for a large number of decision situations by a relatively small number of molecules. The expression of specific mini-exons, which has been described for several Ig/FNIII-like proteins, including neurofascin and Nr-CAM, might also influence the functional state of a network, and thus may increase the number of combinatorial possibilities. Much of the evidence for the functional relevance of mini-exons comes from experiments on NCAM. For example, the inclusion of a 10–amino acid residue insert encoded by an exon in the NCAM gene, called VASE, changes the predicted structure of the fourth domain of NCAM from C2 type characteristics to a domain resembling more a V type (Small and Akeson, 1990). Expression of this exon, which appears to be developmentally regulated, results in a downregulation of the neurite outgrowth–promoting activity of NCAM (Doherty et al., 1992).

Several observations indicate that Ll/Ng-CAM might not be the only binding partner of axonin-1, and that Ll/Ng-CAM may interact with ligands other than Ll/Ng-CAM and axonin-1. Studies on the expression of Ll/Ng-CAM and TAG-1, the presumptive rat homologue of axonin-1, show that these molecules are not colocalized on all axons. A good example is provided by commissural axons in the developing rat spinal cord. As they project ipsilaterally towards the floor plate, the commissural axons express TAG-1, but not, or only weakly, Ll/Ng-CAM. Further distally, when they project contralaterally, L1 appears on their surfaces, whereas TAG-1 is downregulated (Dodd et al., 1988). This suggests that on the ipsilateral side TAG-1 might undergo interactions with a binding partner other than L1. Cell aggregation studies have raised evidence that TAG-1 may bind homophilically (Rathjen and Jessell, 1991). Although homophilic binding was not found with axonin-1 conjugated to fluorescent microspheres (Kuhn et al., 1991), it remains to be determined whether axonin-1 is also capable of binding homophilically when embedded in the more physiological environment of a cell membrane. A dual or multiple ligand binding of axonin-1 would add additional cross-linking capability and advance the formation of networks of Ig/FNIII molecules at the cell surface of axons and growth cones. Evidence for multiple ligand binding capability of Ll/Ng-CAM comes from studies on NCAM, which as an isolated molecule can support axonal growth through homophilic activities (Doherty et al., 1990). When Ll/Ng-CAM and NCAM are preincubated and the mixture is then offered as a substratum, a much more effective promotion of axon outgrowth is seen than when either molecule alone is used. Binding studies using microspheres suggest that interactions of NCAM and Ll/Ng-CAM potentiate the homophilic binding of Ll/Ng-CAM. Such interactions result in enhanced neurite outgrowth (Kadmon et al., 1990). A potentially less specific interference with Ll/Ng-CAM function has recently been demonstrated to arise from the polysialic acid (PSA) moiety of NCAM (Landmesser et al., 1990). In a study aimed at elucidating the function of PSA on NCAM, Landmesser et al. (1990) found that axon fasciculation mediated by Ll/Ng-CAM of chick motor neurons is enhanced and nerve branching reduced, when PSA is cleaved off the NCAM by endoneuraminidase. Whether or not the observed modulation of Ll/Ng-CAM function occurs by direct physical interaction of NCAM containing PSA with Ll/Ng-CAM is unknown at present. It has recently been demonstrated that the high negative charge carried by the PSA moiety of NCAM affects the width of the intercellular space and so might influence the efficiency of adhesive interactions in general (Yang et al., 1992). The F11 protein, which is the closest relative of axonin-1, would be especially interesting as a potential ligand to Ll/Ng-CAM, because it undergoes direct heterophilic interactions with restrictin, a neural ECM protein containing multiple FNIII-like repeats (Rathjen et al., 1991; Nörenberg et al., 1992). Simultaneous binding of Ig/FNIII proteins to other Ig/FNIII members and to ECM molecules would enlarge the number of molecules involved in adhesion molecule networking at the cell surface and provide the basis for further functional refinement.

Fourth, axonin-1, which is anchored to the plasma mem-

brane by GPI, could use L1/Ng-CAM to transmit signals into the interior of the growth cone (for a report on direct association of GPI-anchored membrane proteins with protein tyrosine kinases cf. Štefanová et al., 1991). Although the primary sequences of the cytoplasmic domains of the L1/Ng-CAM subgroup of the Ig/FNIII-like proteins and NCAM exhibit no recognizable kinase or other enzyme domains, nor are they known to associate with enzymes implicated in second messenger systems, there is evidence that L1/Ng-CAM and NCAM might function as a signal-transducing transmembrane protein. Antibodies to L1/Ng-CAM were found to affect the intracellular levels of inositol phosphates and Ca^{2+} ions in PC12 cells in a pertussis toxin–sensitive manner, by binding to cell surface–expressed L1/Ng-CAM. These results suggest that a G protein is involved in the transduction process (Schuch et al., 1989). Recent investigations indicate that promotion of neurite outgrowth on L1/Ng-CAM substratum indeed involves G protein–dependent activation of L- and N-type calcium channels (Williams et al., 1992). A similar or identical mechanism has previously been found involved in neurite outgrowth mediated by NCAM (Doherty et al., 1991). The binding of GPI-linked molecules to transmembrane proteins as a means to transmit signals has been proposed for T lymphocytes in mitogen- and antigen-specific responses (Robinson, 1991). The GPI linkage of axonin-1 makes it a possible candidate for a coreceptor function: After an activation of a program mediated by L1/Ng-CAM into the interior of a growth cone, cleavage of the GPI anchor of axonin-1 might result in an interruption of the signaling mechanism permitting the growth cone to undergo new interactions with other cells or ECM proteins surrounding the growth cone.

Conclusion

An essential requirement for growth cones is the expression of cell surface receptors capable of distinguishing between different pathways in order to reach their cellular targets by responding to environmental cues. These recognition events must then be translated into signals that control growth cone responses. Research in the past decade has led to the identification of several proteins combining repeated Ig-like and FNIII-like domains in one polypeptide that are predominantly located on axons and have been implicated in axonal growth. They can now be categorized (Fig. 1). Previously, it has been discussed that molecules which are restricted to a subset of axons might be implicated in selective axon behavior, whereas proteins with a more general axonal distribution, like L1/Ng-CAM, play only a generally permissive role in neurite extension. However, the recent finding that L1/Ng-CAM can mediate neurite outgrowth by binding to two distinct substrata, L1/Ng-CAM and axonin-1, has opened novel perspectives on the function of L1/Ng-CAM and other Ig/FNIII-like proteins (Fig. 2). If the substratum affinity of L1/Ng-CAM for the two substrata would be subject to modulatory influences determined by the interaction pattern of L1/Ng-CAM with axonin-1 or other L1/Ng-CAM molecules at the cell surface, L1/Ng-CAM could be involved in decision functions at pathway branch points. In situations where both L1/Ng-CAM and axonin-1 are expressed in each of two apposed membranes, e.g., of a growth cone and underlying axon, networks of molecules interconnected in the plane of each membrane and across the intermembrane space may be formed. Molecules in such networks might assume distinct conformations and functions, depending on the nature of their ligand pattern. In view of the tendency of Ig/FNIII-like molecules to interact, we extend this hypothesis and postulate interconnection to extended networks including molecules of both growth cones and underlying axons as a general feature by which Ig/FNIII-like molecules exert their function. The specific combination of Ig/FNIII-like proteins on different axons at pathway bifurcations, in conjunction with the combination of interacting molecules expressed in the membrane of a growth cone, could lead to network configurations that are more or less favorable to axon elongation. This would generate preference among the possible contacts of an extending growth cone with axons leading in different directions and so exert a guiding function. Specification of selective axon behavior by interaction of Ig/FNIII-like proteins at the cell surface acting in concert with regulatory influences on the synthesis or activity of such molecules by cell-intrinsic or environmental mechanisms (Karagogeos et al., 1991; De la Rosa, 1990; Jones et al., 1992; Mayford et al., 1992) might be a powerful and dynamic means of regulating the growth and the guidance of axons in the developing vertebrate nervous systems.

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