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Are the Glypiated Adhesion Molecules Preferentially Targeted to the Axonal Compartment?

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Abstract

The question of how the cell surface molecules may be specifically delivered to subdomains of neurons is of particular interest considering that polarized sorting to the axon could enable adhesion glycoproteins to induce fasciculation of axonal tracts, guidance to the target cell, and the establishment of synaptic contacts. It was recently proposed that GPI-anchored molecules undergo preferential delivery to the axonal surface, implicating a similar polarized sorting of glycoproteins in neurons and epithelial cells (Dotti and Simons, 1990; Dotti et al.,

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1991). This review focuses on the cellular and subcellular localization of several glypiated adhesion molecules (Thy-1, TAG-1, F3/F11, P-31) in the developing and adult cerebellar cortex of the mouse. We conclude that the cellular distribution of GPI-anchored adhesion molecules within neurons is very complex and depends on:

1. The neuronal cell types, for example, F3/F11 is localized in axons in granule cells but is present in all compartments of Golgi cells.
2. The molecule itself: Thy-1, TAG-1, and P-31 are present on the granule cell body, whereas at the same developmental stage, F3/F11 is restricted to the axon.
3. The differentiation state: Thy-1 delivery to the axon correlates with postsynaptic target maturation.

Index Entries: Neuron; cerebellum; development; adhesion molecules; GPI-anchor; F3/F11; Thy-1; targeting.

Introduction

The creation of two separate compartments, axons and dendrites, is essential for generating the functional polarity of neurons. Membranous proteins directly implicated in synaptic transmission may be specifically delivered to their site of function. The dendritic membrane that typically receives inputs differs in composition from axonal membrane, particularly with regard to receptors and ion channels. On the other hand, the synaptic vesicle components are abundant in axons, especially in their terminals, but are not detectable in dendrites. During neuroontogeny, the establishment of complex synaptic networks depends on interactions between pre- and postsynaptic structures that are mediated by compatible cell surface ligands. Most of the cell–cell adhesion molecules identified to date in the nervous system belong to the Ig superfamily (Williams and Barclay, 1988). Their relative diversity and their selective expression with respect to time and place could enable them to determine the appropriate neuron–neuron connections. Some of these molecules such as the 180 kDa N-CAM isoform, L1 (Moos et al., 1988; Pershon and Schachner, 1987), TAG-1 (Dodd et al., 1988; Furley et al., 1990), F11 (Brümmendorf et al., 1989), F3 (Gennarini et al., 1989b; Faivre-Sarrailh et al., 1992), and Thy 1 (Morris et al., 1985; Xue et al., 1991) display a restricted expression to subsets of neurons, are eventually specific to the axonal or dendritic compartments, and could possibly only be present in a portion of a defined axonal process. They may be coupled to the membrane by a transmembrane region (L1, N-CAM 180) or anchored to the outer leaflet of the lipid bilayer by covalent linkage to a glycosyl phosphatidylinositol (GPI) (TAG-1, F11, F3, Thy-1). The GPI-anchor may allow the protein to perform specific functions that would be impossible with a conventional transmembrane linkage (review in Low and Saltiel, 1988).

The question of how the cell surface molecules may be specifically delivered to subdomains of neurons is of particular interest considering that polarized sorting to the axon could enable adhesion glycoproteins to induce fasciculation of axonal tracts, guidance to the target cell, and the establishment of synaptic contacts. It was recently proposed that GPI-anchored molecules undergo preferential delivery to the axonal surface, implicating a similar polarized sorting of glycoproteins in neurons and epithelial cells (Dotti and Simons, 1990; Dotti et al., 1991). The situation is quite clear in epithelial cells. Such cells contain two distinct plasma membrane domains segregated by tight junctions, the apical and basolateral surfaces. Both endogenous or transfected GPI-anchored molecules appear to be restricted to the apical surface (Lisanti et al., 1988,1989). The transfer of the signal for GPI attachment to the ectodomain of a basolateral glycoprotein by recombinant DNA methods results in the delivery of the fusion protein to the apical surface of the epithelial cell, indicating that the GPI anchoring mechanism may convey apical targeting information (Lisanti et al., 1989). A physical barrier, such as the tight junction that blocks in the epithelial cells lateral diffusion of lipids and proteins between the two

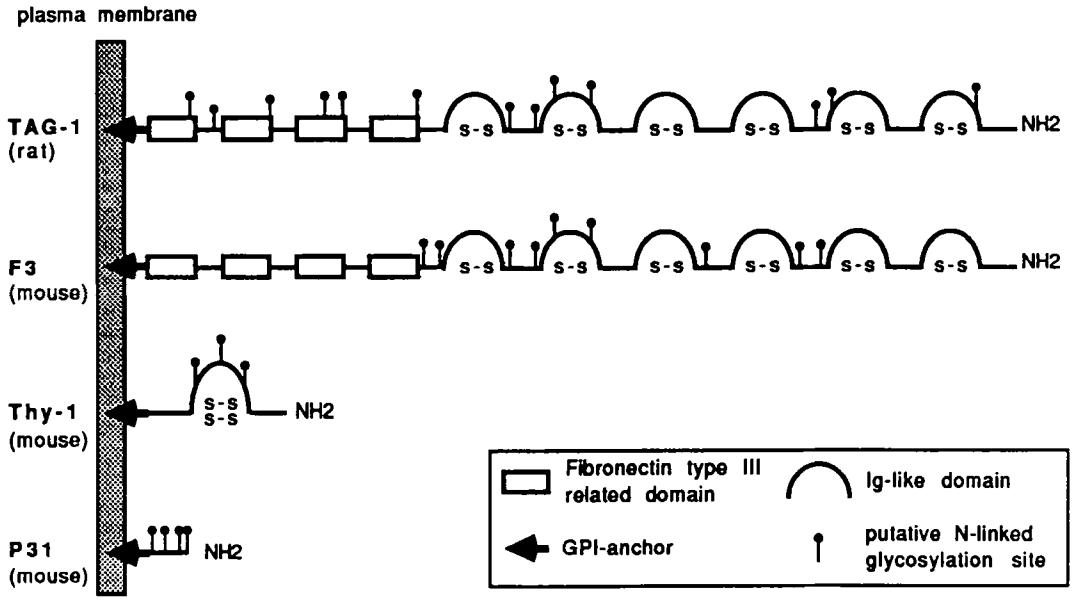

Fig. 1. Schematic representation of the molecular structure of several glypiated neuronal adhesion molecules. TAG-1, F3, and Thy-1 belong to the immunoglobulin superfamily and P-31 is a glycopeptide. Double S symbols indicate the possible position of disulfide bonds.

membrane compartments, has not been described between the axonal and somatodendritic domains of neurons, but the compartmentalization of the Golgi elements (Peters et al., 1976) and cytoskeleton (Matus et al., 1983; Black and Baas, 1989) in nerve cells raises the possibility of common molecular mechanisms of surface protein sorting in the two cell types.

Dotti et al. (1991) have used the model of rat hippocampal neurons, which undergo a well-defined morphological and functional polarization after 10–14 d of culture to investigate the sorting of GPI-anchored molecules in nerve cells. In this system, Thy-1, a glypiated molecule of the Ig superfamily, has an exclusively axonal localization. As Thy-1 itself has been found to be present on the dendrites of hippocampal pyramidal neurons and cerebellar Purkinje cells in tissue sections (Morris et al., 1985; Xue et al., 1990,1991), the preferential delivery of glypiated proteins to axons may apply to only certain types of neurons or may depend on the in vivo establishment of neuronal networks. Recently, the cellular and subcellular localization of several glypiated adhesion molecules (Thy-1, TAG-1, F3/F11, P-31) during development of the nervous system was described in great detail, and so it is now possible to analyze the functional role of the GPI-anchor in neurons more thoroughly (Fig. 1). P-31 antigen, which does not belong to the Ig superfamily, is a highly glycosylated peptide and is thought to be involved in cell–cell interactions. The cellular distribution of N-CAM 120, the glypiated isoform of N-CAM, has not been specifically analyzed since anti-N-CAM antibodies recognize the extracellular domain that is common to both the transmembrane isoform N-CAM 140 and the glypiated isoform N-CAM 120 (Pershon and Schachner, 1987). This article will focus on the developing and adult cerebellar cortex of the mouse, mainly because this structure is formed from identifiable neuronal types that establish relatively simple circuitry (Palay and Chan-Palay, 1974) and has been extensively studied with respect to the immunocytochemical distribution of glypiated adhesion molecules.

Table 1
Presence of Immunoreactivity for Several Glypiated Adhesion Molecules in the Soma, Dendrite, and Axon of Different Neuronal Cell Types of Developing and Adult Mouse Cerebella*

	Thy-1		F3/F11		TAG-1		P31	
	10 Days	/adult	10 Days	/adult	10 Days	/adult	10 Days	/adult
Purkinje cell								
Soma	+	+	–	–	–	–	–	–
Dendrite	+	+	–	–	–	–	Faint	–
Axon	+	+	ni	ni	–	–	–	–
Granule cell								
Soma	–	Faint	–	–	+	–	+	–
Dendrite	–	ni	–	–	–	–	–	–
Axon	–	Faint	+	+	+	–	+	–
Golgi cell								
Soma	+	+	+	+	–	–	ni	–
Dendrite	ni	ni	+	+	–	–	ni	–
Axon	ni	ni	+	+	–	–	ni	–
Deep cerebellar neuron								
Soma and dendrite	+	+	ni	ni	ni	–	ni	–
Axon (mossy fiber)	–	+	+	+	–	–	ni	–
White matter axons	+	+	+	+	–	–	+	–

*ni: not investigated, -: no immunoreactivity, +: good immunoreactivity, faint: faint immunoreactivity. Results were taken from Morris et al., 1985, Xue et al., 1990, 1991 for Thy-1, from Faivre-Sarrailh et al., 1992 for F3/F11, from Yamamoto et al., 1990 for TAG-1, and from Kuchler et al., 1989 for P-31.

The first striking observation is that glypiated adhesion molecules are differently expressed at precise developmental stages in restricted neuronal cell types, indicating that these molecules may exert multiple functions during neuronal morphogenesis. This developmentally regulated expression complicates the comparative analysis of their selective distribution in the cellular compartments of a defined neuron (Table 1). Nevertheless, several of these glycoproteins have been localized within a single cell, the granule cell, allowing us to determine whether axonal sorting depends on neuronal cell type or is related to the extracellular part of the glypiated molecule.

Thy-1 Glycoprotein Is Expressed in Both Somato-Dendritic and Axonal Compartments in Most Neurons

Thy-1 is an adhesion molecule, as demonstrated using thymocytes (He et al., 1991), that contains a single Ig domain of the V type and is linked to the membrane via a GPI-anchor (Homans et al., 1988). Thy-1 glycoprotein is present in both the somato-dendritic and axonal compartments of most mature neurons (Fig. 2) (Morris et al., 1985; Xue et al., 1990,1991; Xue

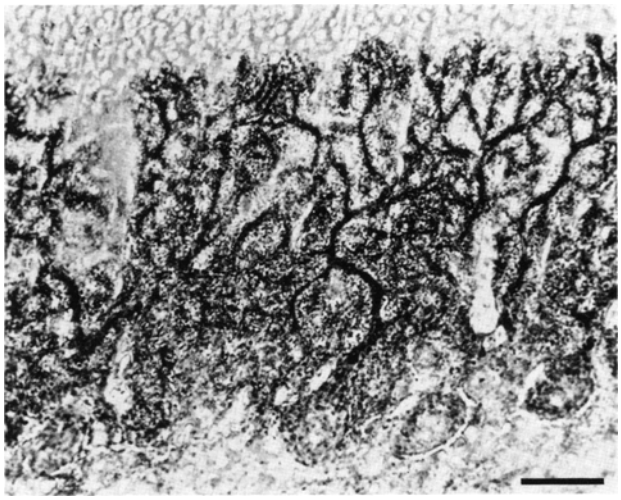

Fig. 2. Immunoreactivity for Thy-1 in the cerebellar cortex of 12-d-old rat. Purkinje cell dendrites are intensely labeled. Bar: 50 μm. (Courtesy of J. Morris).

and Morris, 1992). This result would contradict the hypothesis of Dotti et al. (1991) concerning the specific axonal sorting of glypiated glycoproteins were it not for the possibility that Thy-1 may exist under a transmembrane isoform specifically delivered to dendrites. At the present time, there is no evidence for alternative splicing of the Thy-1 pre-mRNA as has been found for the N-CAM gene, which is able to produce two transmembrane isoforms (N-CAM 180 and N-CAM 140) and one GPI-anchored isoform N-CAM 120 (Cunningham et al., 1987; He et al., 1987). However, as mentioned by Xue et al. (1991), biosynthesis of such an isoform might be possible. GPI-anchored molecules are transcribed with an amino acid transmembrane tail that is cleaved and replaced by the lipid anchor in the endoplasmic reticulum (Doering et al., 1990; Ferguson and Williams, 1988). Association of the precursor with a specific polypeptide chain may protect it from the transmembrane tail cleavage resulting in a surface expressed transmembrane molecule (Kurosaki and Ravetch, 1989).

Thy-1 Synthesis Is Correlated with Cessation of Axogenesis: Thy-1 Possibly Stabilizes Mature Neuronal Processes

Thy-1 synthesis occurs in virtually all neurons of the central nervous system at the time when the soma has migrated to its definitive position and started to elaborate dendrites, and when the axon has elongated to its target site. Correlation between Thy-1 expression and cessation of axonal growth is certainly of functional importance in stabilizing the mature neuronal network, since Thy-1 expression in a neural cell line inhibits neurite outgrowth on mature astrocytes (Tiveron et al., 1992). Its level of expression varies strongly from one neuronal type to another. In the cerebellum, Purkinje cells display intense immunostaining, especially over the dendritic arborization as opposed to granule cells that express very low levels of Thy-1 (Fig. 2) (Morris et al., 1985). Purkinje cells already express Thy-1 strongly at birth. This unusual early staining with respect to cerebellar cortex neurons could be explained by the fact that the Purkinje cell axon connects with its target in the deep cerebellar nuclei as soon as the perikaryon starts to migrate toward the cerebellar cortex. Contrastingly, granule cells migrate postnatally from the germinative external layer to the internal granular layer and at the same time, their axons, the parallel fibers, are elongating and making synaptic contacts in the molecular layer mainly during the second and third postnatal weeks. Thy-1 immunoreaction appears on the deeper-lying granule cells (most mature) and their axons at postnatal day 8 and on all cells by postnatal day 28 (Xue and Morris, 1992).

Axonal Delivery of Thy-1 Is Correlated with Target Differentiation

Thy-1 is expressed in the cell body and dendrite when the axon has elongated to its target

site, but axonal delivery of the molecule is correlated with the formation of synaptic terminals and depends on the differentiation of the postsynaptic target. The appearance of Thy-1 immunoreactivity in the mossy fibers is very informative. Thy-1 is present in the cell body and dendrites of pontine neurons from the first postnatal week, but it appears in its axon, the mossy fiber, later a full week after it has elongated. That is when it forms presynaptic terminals called rosettes that are in contact with late-developing granule cells (Xue et al., 1991). The sequence of Thy-1 appearance during the axogenesis of vestibular ganglion neurons is even more complex. Their axon bifurcates, the proximal branch connecting with vestibular nuclei in the brain stem before birth, the distal one elongating to the cerebellar cortex where it has to wait for 2 wk before forming its synaptic terminals that are in contact with granule cells (Xue et al., 1991). The proximal region of this axon connected with vestibular nuclei becomes Thy-1 positive about a week before its synaptic terminals in the cerebellar cortex. Thus, a transient compartmentalization seems to occur between the proximal and distal portion of the axon tract. A signal might be transduced from the postsynaptic target to the axonal terminals, inducing axonal transport of Thy-1 from the cell body, where it has been accumulated during the axonal differentiation period. The axonal carrier vesicles are likely to be delivered via kinesin-mediated fast axonal transport along microtubules (Vale et al., 1985,1986). The induction of axonal transport of Thy-1 may result from maturation changes in the microtubule components of the axon, such as the phosphorylation or detyrosylation of tubulin, a decrease in microtubule-associated protein MAP5 and the modification of tau isoform expression (Cambray-Deakin, 1991; Nunez, 1988; Riederer et al., 1986), that may affect the interaction between microtubules and carried organelles.

F3/F11 Neuronal Cell Surface Molecule Promotes Neurite Outgrowth

The mouse F3 glycoprotein that displays high sequence homology with chicken F11 is considered as a species homolog of F11 and is termed F3/F11. This molecule contains six Ig domains of the C2 type (Williams and Barclay, 1988), a premembrane region with sequence similarity to fibronectin type III repeats and a GPI membrane anchor (Gennarini et al., 1989b). It is coded for by a single gene and no indication for alternative splicing of the pre-mRNA has been found (Gennarini et al., 1989a; G. Gennarini and C. Goridis, unpublished observations). It is therefore unlikely that transmembrane isoform of F3/F11 is expressed. F3/F11 is strongly expressed in the brain at the time of differentiation (Gennarini et al., 1990) and has been shown to promote in vitro neurite outgrowth (Chang et al., 1987; Gennarini et al., 1991; Durbec et al., 1992). However, F3/F11 remains present in adult brain whereas the TAG-1 molecule, another GPI-anchored molecule that displays strong structural similarities with F3/F11 and promotes in vitro neurite elongation, is transiently expressed (Furley et al., 1990). This indicates that F3/F11 may have an additional function, possibly in the establishment and stabilization of synaptic connections being that it has been localized in synaptic terminals (Fig. 3) (Faivre-Sarrailh et al., 1992).

F3/F11 Expression Is Restricted to the Axon Within Granule but not Within Golgi Cells

F3/F11 shows a complex distribution pattern in the cerebellar cortex, being restricted to neuronal subpopulations and to particular subcellular compartments depending on the neuronal type expressing it. F3/F11 is present in granule

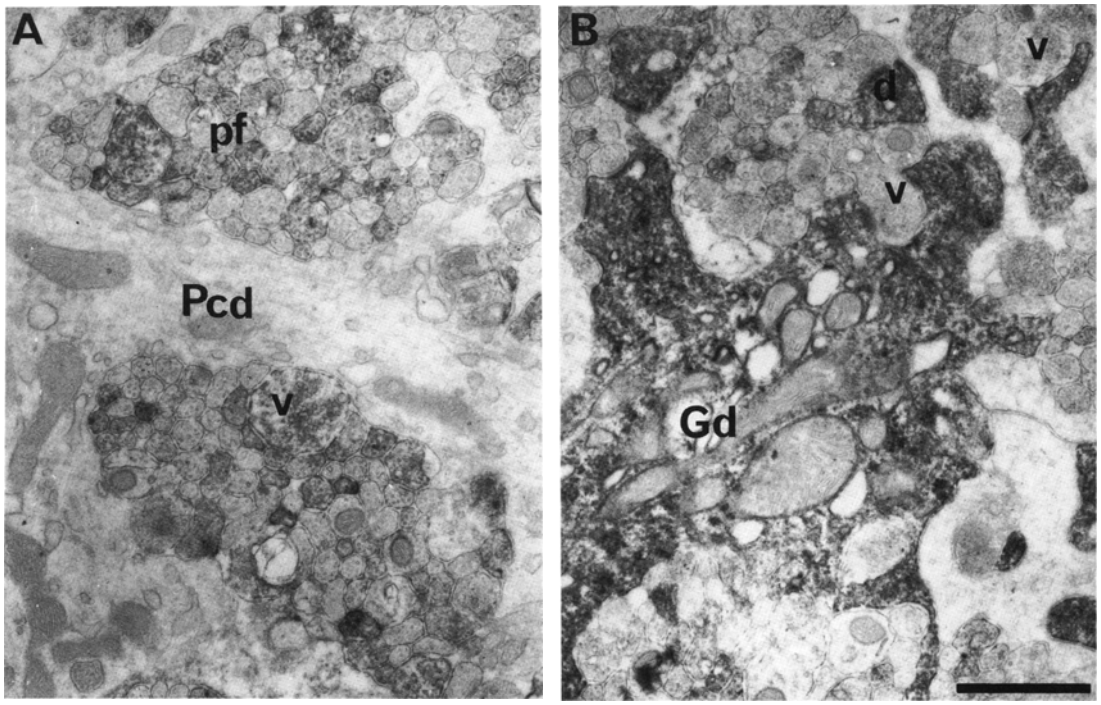

Fig. 3. Immunoelectron microscopic localization of F3/F11 in the molecular layer of 10-d-old mouse cerebellum. A. Region of a Purkinje cell dendrite (Pcd), which is unstained. The parallel fibers (pf) and their presynaptic varicosities (v) are labeled. B-region of a Golgi cell dendrite (Gd) that is labeled, including in dendritic spines (d). The presynaptic varicosities (v) contacting them are unstained. Bar: 1 μm.

cells and Golgi cells, whereas Purkinje cell bodies and their dendritic arborizations, as well as stellate cells are not immunoreactive (Fig. 3). In 10-d-old and adult cerebella, the granule cell bodies and dendrites are not immunoreactive whereas their axons, the parallel fibers, and notably their presynaptic terminals are labeled. Contrastingly, F3/F11 is present in all compartments of another cerebellar neuron, the Golgi cell, in the juvenile and adult cerebellum (Faivre-Sarrailh et al., 1992). If we exclude the possibility of a transmembrane isoform, this indicates that a glycolipid-anchored protein can show a polarized localization in one type of neuron, and a uniform distribution in another. This might be because of differences in the organization and composition of the dendritic microtubule apparatus of the two cell types or alternatively because of different environmental cues imposed by the synaptic afferences, as was observed in the axonal delivery of the Thy-1 molecule. We can raise another hypothesis that suggests that the structure of the GPI-anchor of a defined molecule varies according to the cell type expressing it. One of the features of the GPI-anchored molecule is that it can be released in soluble form upon treatment with bacterial PI-PLC enzyme. However, structural variations of the anchor, such as a modification of the inositol rings by fatty acids, might result in the insensitivity of the molecule to PI-PLC treatment (Low et al., 1988). Several observations on N-CAM 120 (Théveniau et al., 1991,1992) and acetylcholinesterase suggest that the sensitivity of GPI-anchor to PI-PLC depends on the cell type expressing the anchor rather than on the anchored molecule itself. It is therefore possible

that the sorting mechanism that depends on the structure of the GPI-anchor may vary from one cell type to another.

Early Polarized Expression of F3/F11 Within the Granule Cell: Comparison with the Distribution of Other Glypiated Adhesion Molecules

The polarized distribution of F3/F11 within the granule cell occurs very early during ontogenesis. Granule cell axons strongly express F3/F11 as soon as they begin to grow, i.e., just when the granule cell body begins to migrate across the molecular layer. Even at this early stage of differentiation, the premigratory or migratory perikaryon is devoid of staining (Faivre-Sarrailh et al., 1992). It is interesting to note that at this stage, the granule cell body and its growing axon are immunoreactive for two other glypiated neuronal cell surface molecules, TAG-1 (Yamamoto et al., 1990) and P31 (Kuchler et al., 1989). Moreover, Thy-1 is present in both somato-dendritic and axonal compartments of postmigratory granule cells at the adult stage. It seems that in this cell system the mechanism of sorting into a subcellular compartment is more dependent on the extracellular domain of the molecule than on its GPI-anchor. However, as mentioned, we have to bear in mind that the structure of the GPI-anchor may vary and exhibit different physical, biochemical, and immunological properties (Ferguson and Williams, 1988) that could explain the differences observed in the intracellular distribution of these glycoproteins.

The TAG-1 Glycoprotein Is Transiently Expressed and Promotes Neurite Outgrowth

TAG-1 (or SNAP) is a glypiated molecule of the Ig superfamily that is closely related to F3/F11. It contains six immunoglobulin C2 domains and four domains homologous to the type III repeats of fibronectin. It is anchored to neuron surface via a GPI linkage. TAG-1 immobilized as a substrate promotes the extension of neurites in vitro (Furley et al., 1990). Its expression during a brief developmental period in restricted sets of axon systems in the central and peripheral nervous systems suggests that TAG-1 plays a role in the initial extension of developing axons and in selective axon fasciculation (Furley et al., 1990; Yamamoto et al., 1986). It would be interesting to know whether the decrease of TAG-1 expression is regulated by an axon's interactions with components of its target, or by its interactions with components present along the path that leads it to its target. In the commissural neurons of developing spinal cord, TAG-1 expression ceases as the axon trajectory is modified, crosses the floor plate, and turns orthogonally. At the same time L1, a transmembrane adhesion molecule of the Ig family, is expressed at all points of the axon except those in the initial portion that was previously immunoreactive for TAG-1. In the cerebellar cortex, TAG-1 is expressed on granule cell bodies of the external layer. Only postmitotic and premigratory granule cells are labeled. Their axons, the parallel fibers, are immunoreactive during their very initial outgrowth (Yamamoto et al., 1990). At the same stage, the axons of granule cells are also L1 positive, indicating that the correlation between loss of TAG-1 and expression of L1 depends on cell type.

The strong immunoreactivity of granule cell bodies compared to that of embryonic cortex neurons is quite surprising. It may be related to the fact that granule cells migrate and extend their axons simultaneously.

Developmental Pattern of Expression of the P-31 Antigen in the Cerebellar Cortex

The P-31 antigen, which is similar to the heat-stable antigen, is a 30 amino acid highly glycosylated peptide linked to the membrane via

a GPI-anchor (Rougon et al., 1991). Like Thy-1, P-31 is expressed both in the immune and nervous systems, and is believed to be involved in cell–cell interactions. It is transiently expressed in embryonic and postnatal brain from embryonic day 13 to postnatal day 7 (Nedelec et al., 1992), indicating a possible function in neuronal morphogenesis.

In the juvenile cerebellum, external layer postmitotic granule cells are heavily immunoreactive. Premigratory and migratory granule cells as well as the elongating parallel fibers are stained. In contrast, postmigrating granule cells of the internal layer are unlabeled. The myelinated axons of white matter transiently express P-31 until 14 d postnatal. No immunoreactivity is present in the adult cerebellum (Kuchler et al., 1989). This restricted and transient expression of P-31 indicates that it is probably involved in morphogenetic events such as cell migration and axonal outgrowth.

Unfortunately, the brief expression of these two last glypiated molecules, TAG-1 and P-31, during the embryonic and perinatal period is not conducive to their being they are assessed for a specific delivery to the axonal compartment of differentiated neurons.

Summary

In summary, we have seen that the cellular distribution of GPI-anchored adhesion molecules within neurons is quite complex and depends on:

1. The neuronal cell types, for example, F3/F11 is localized in axons in granule cells but is present in all compartments of Golgi cells.
2. The molecule itself: Thy-1, TAG-1, and P-31 are present on the granule cell body, whereas at the same developmental stage, F3/F11 is restricted to the axon.
3. The differentiation state: Thy-1 delivery to the axon correlates with postsynaptic target maturation.

Such a regulated sorting of glypiated molecules in neurons may depend at least on two possible molecular mechanisms. First, the structure of the GPI-anchor may vary, leading to the incorporation of glycoproteins in different types of carrier vesicles. Second, the microtubule-based transport of vesicles may be regulated by the modifications of cytoskeletal components that occur during neuronal maturation.

The available data indicate that F3/F11, TAG-1, and P-31 are strongly expressed on subsets of developing axons, although they are occasionally present on cell bodies and dendrites of some neuronal types. The case of Thy-1 is quite different since it is expressed in the somato-dendritic and axonal compartments of most differentiated neurons and refutes the hypothesis of specific axonal sorting of GPI-anchored molecules. It will therefore be of great interest to determine whether or not a transmembrane isoform of Thy-1 is expressed in dendrites. Either way, the lack of available data on a series of GPI-anchored molecules expressed by a given neuron prevents us from drawing a conclusion about the role of GPI in axonal targeting. However, the presence of the GPI-anchor can dramatically affect protein localization in epithelial cells. This posttranslational modification certainly represents a crucial point of regulation for the sorting of adhesion molecules in neurons.

Another major functional role for the GPI-anchor during neuronal morphogenesis has been recently proposed. It is based on the cleavage and release of glypiated molecules into the extracellular compartment. Spontaneous release of glypiated molecules has been observed both in vivo as indicated by its presence in the cerebrospinal fluid (Durbec et al., 1992), and in vitro in the culture medium of a sensory neuron cell line (Théveniau et al., 1992). Soluble F3/F11 cleaved from a transfected CHO cell line using PI-PLC enzyme strongly stimulates neurite elongation when added to the culture medium of dorsal root ganglia neurons (Durbec et al., 1992). It is thus possible that soluble forms are able to act at some distance from their site of release, much in the same way as local and trophic factors. The physiological importance of this mecha-

nism remains to be established, but it indicates that lipid modification of cell adhesion glycoproteins may be a powerful way of controlling neuronal morphogenesis.

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