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Review

GPI-anchored proteins at the node of Ranvier

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ABSTRACT

Contactin and TAG-1 are glycan phosphatidyl inositol (GPI)-anchored cell adhesion molecules that play a crucial role in the organization of axonal subdomains at the node of Ranvier of myelinating fibers. Contactin and TAG-1 mediate axo-glial selective interactions in association with Caspr-family molecules at paranodes and juxtaparanodes, respectively. How membrane proteins can be confined in these neighbouring domains along the axon has been the subject of intense investigations. This review will specifically examine the properties conferred by the lipid microenvironment to regulate trafficking and selective association of these axo-glial complexes. Increasing evidences from genetic and neuropathological models point to a role of lipid rafts in the formation or stabilization of the paranodal junctions.

1. Introduction

Contactin and TAG-1 are glycan phosphatidyl inositol (GPI)-anchored cell adhesion molecules belonging to the immunoglobulin superfamily (Ig-CAMs) and have been studied extensively for their function during neuronal development. Contactin and TAG-1 interact with multiple ligands including Ig-CAMs and extracellular matrix components and participate to neuroblast migration, axonal growth, fasciculation and guidance, and synaptic function [1–4]. Another important role of Contactin and TAG-1 was discovered in the organization of axonal subdomains at the node of Ranvier of myelinating fibers where they mediate axo-glial selective interactions in association with Caspr-family molecules. This review will examine this last role with a special focus on the properties conferred by their lipid anchor and raft-partitioning.

2. Molecular organization of the axonal subdomains at the node of Ranvier

The node of Ranvier is an attracting model for studying the mechanisms of membrane segregation along the axolemma (Fig. 1A). The formation of nodes is induced by contacts with myelinating glial cells that are ensheathing the axon, the oligodendrocytes in central nervous system (CNS) and Schwann cells in peripheral nervous system (PNS). The nodal gap is highly enriched in voltage-gated sodium and KCNQ channels and the Ig-CAM Neurofascin-186. On both sides of the node, the paranodal junctions anchor the terminal cytoplasmic loops of the myelin onto the axolemma. The paranodes are characterized by septate-like junctions that consist in regularly-spaced intermembrane transverse bands. Paranodal junctions depend on interactions among three CAMs, Contactin and Caspr/paranodin on the axon and Neurofascin-155 on the glial cell. Next, the juxtaparanodal regions are enriched in Shaker-type Kv1 channels co-clustered with TAG-1 and Caspr2. How membrane proteins can be confined in these neighbouring domains along the axon has been the subject of intense investigations over the last years [5–7]. Multiple complementary mechanisms may be implicated such as clustering of adhesive complex mediated by glial ligands, anchoring of CAMs and ion channels by axonal cytoskeletal scaffolds and/or selective trafficking and targeting of transport vesicles towards the axonal subdomains [8].

2.1. Role of Contactin at the paranodal region

Deficiency in either Contactin or Caspr/paranodin, induces severe neurological defects, aberrant organization of the paranodal region and reduction of nerve conduction velocity [9,10]. In both these knock-out mice, the septate-like junctions are disrupted and some terminal loops of the myelin are everted not facing the axolemma (Fig. 1B). The distribution of Contactin and Caspr are

Abbreviations: GPI, glycan phosphatidyl inositol; Ig-CAM, cell adhesion molecules of the immunoglobulin superfamily; CNS, central nervous system; PNS, peripheral nervous system; FNIII, fibronectin type III; ER, endoplasmic reticulum; MAL, myelin and lymphocyte protein; CGT, ceramide galactosyl transferase; MS, multiple sclerosis; EAE, experimental allergic encephalomyelitis

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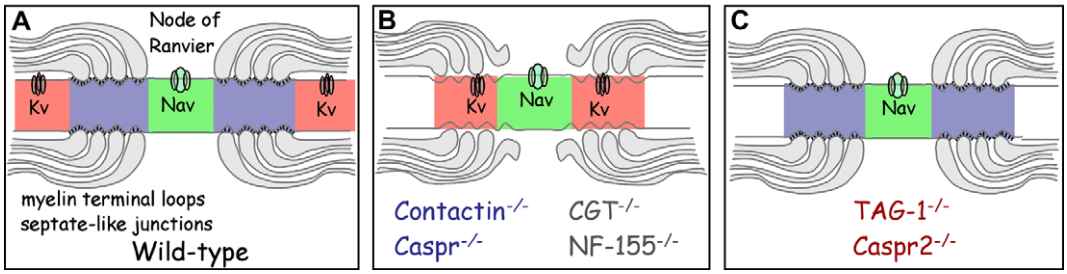

Fig. 1. The axonal subdomains of the node of Ranvier. (A) In wild-type animals, the node of Ranvier (green), which contains high density of voltage-gated sodium channels (Nav) is flanked by the paranodal junctions (blue). Next, the juxtaparanodes (red) are enriched in Kv1 channels. Phenotype of mutants for paranodal junctions (B) and juxtaparanodes (C).

interdependent at paranodes. Indeed, in Caspr-deficient mice, Contactin is not detected at paranodes and becomes enriched at the nodal gap in the CNS. Reciprocally, in Contactin$^{-/-}$ mutant mice, Caspr is not addressed to the axolemma. In the two types of mutants, the clustering of sodium channels at the nodal region is still observed, which is displaying an enlarged distribution. In addition, the distribution of Caspr2 and Kv1.1/1.2 channels is strongly modified since these components are found at paranodes instead of juxtaparanodes. Therefore, paranodal junctions act as a fence separating the lateral domains enriched in sodium and potassium channels. As a consequence, the velocity of nerve conduction is decreased. Genetic disruption of Neurofascin-155 expression in myelinating glial cells prevents clustering of the axonal Caspr/Contactin complex and results in alteration of paranodal junctions [11–13]. In the different genetic animal models generating disruption of septate-like junctions (mutant mice for Caspr, Contactin or Neurofascin-155), axonal swelling and degeneration is observed in Purkinje cells. Axonal transport seems to be disturbed with mis-orientation of microtubules and neurofilaments together with accumulation of mitochondria and smooth endoplasmic reticulum (ER) at the paranodal region [14]. These observations indicate a correlation between the disruption of septate-like junctions and axonal degeneration.

2.2. Role of Contactin at the nodal gap

Expression of axonal CAMs at paranodes and juxtaparanodes is similar in the PNS and CNS, but differs at the node. Neurofascin-186 is found at the node both in the CNS and PNS, Contactin is present at the node only in the CNS and NrCAM only in the PNS [12,15]. In the PNS, the nodal extracellular matrix protein gliomedin is secreted by Schwann cell microvilli and binds Neurofascin-186 and NrCAM on the axon initiating the clustering of the voltage-gated sodium channels through ankyrin$_G$ scaffolding [16]. In the CNS, the nodal extracellular matrix contains Versican V2 secreted by the perinodal astrocyte, which assembles tenascin-R and phosphacan [17,18] (Fig. 2). This complex of matrix components by virtue of its ability to bind Neurofascin-186 and Contactin may be crucial for the clustering of the voltage-gated sodium channels. Indeed, Contactin displays a broad activity of binding and interacts with the β1-subunit of the voltage-gated sodium channels, Neurofascin-186, tenascin-R, and phosphacan/RPTPßz [19–21].

Neuronal sodium channels are heterotrimers composed of the pore-forming α-subunit and two auxiliary β-subunits. The β-subunits contain an extracellular Ig domain with homology with CAMs that allows binding with CAMs and extracellular matrix components. The β2-subunit displays homology with Contactin [22] and interacts with tenascin-C and tenascin-R [23,24]. The β1-subunit shares similarity with the myelin CAM Po and interacts with Neurofascin-186, RPTPß and Contactin [21,25,26]. Contactin co-transfected in mammalian fibroblasts together with the α- and

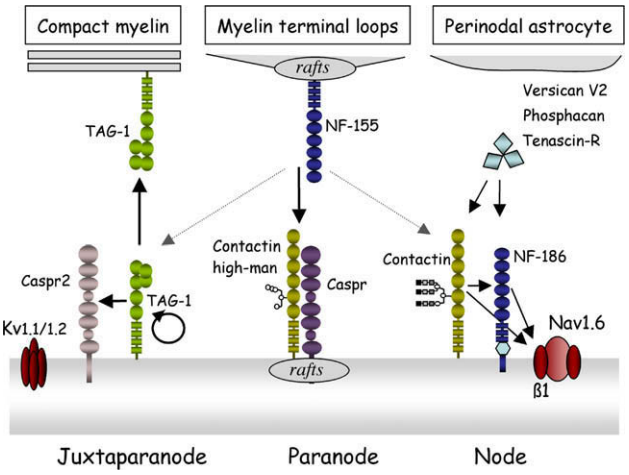

Fig. 2. Segregation of ion channels and CAMs in distinct axonal subdomains of the node of Ranvier in the CNS. Members of the Ig-CAM family show broad binding activity (arrows). Their selective association requires fine-tuning by N-glycosylation (Contactin), alternative splicing (Neurofascin) or raft-partitioning.

β1-subunits of the sodium channel Nav1.2, increases the sodium currents due to a higher density of sodium channels at the surface membrane as assessed by ^{3}H-saxitoxin binding [27]. These results point out the role of Contactin for enhancing sodium channel expression at the cell surface through interactions with the β1-subunit, by increasing channel insertion or stabilization in the membrane.

2.3. Role of TAG-1 at the juxtaparanodes

TAG-1/Axonin-1/Contactin-2, originally described as a protein transiently expressed in axons during development [3], is the juxtaparanodal counterpart of Contactin (48% amino acid identity), which has the particularity to be expressed by both neurons and myelinating glial cells [28]. The phenotype of TAG-1-deficient mice indicates that this protein is crucial for juxtaparanodal organization and required for Caspr2 and Shaker-type Kv1 channels enrichment in this region in the CNS and PNS (Fig. 1C) [29]. Similarly, Caspr2-deficient mice display alteration of TAG-1 and Kv1 clustering at juxtaparanodes [30]. From these studies, a tripartite complex has emerged at juxtaparanodes formed by cis-interaction (heterophilic) between TAG-1 and Caspr2 within the axolemma and by trans-interaction (homophilic) of neuronal TAG-1 with TAG-1 present on the glial membrane. The nodal gap and paranode organization do not appear to be affected in both the sciatic and optic nerves of TAG-1-deficient mice [29], but recently some more quantitative studies indicated shorter internodes in particular in the optic nerve [31,32]. Interestingly, the myelin sheath thickness and

compaction are not modified in TAG-1$^{-/-}$ ventral spinal cord [29] whereas the TAG-1$^{-/-}$ retinal ganglion cell axons appear hypomyelinated, revealing region-specific cues for TAG-1-induced myelination [32]. The behavioural phenotype of TAG-1-deficient mice is characterized by an increased sensitivity to convulsive stimuli attributed to upregulation of adenosine A1 receptors in the hippocampus [33] and also by an impairment in learning and memory as assessed by the Morris water maze and novel object recognition tests [31]. This last neuro-behavioural abnormality might be linked to juxtaparanodal disorganization in the hippocampus and entorhinal cortex known to be involved in learning and memory [31]. This link between an abnormal axonal subdomain organization and neurological behaviours is quite surprising. Nevertheless, another study recently described a link between abnormal paranodal organization, decreased conduction velocity and schizophrenia-related behaviours in proteolipid protein 1 transgenic (plp1$^{tg/-}$) mice [34].

3. Mechanisms for the segregation of the GPI-anchored CAMs along myelinated axons

3.1. Cooperation between cis- and trans-interactions to generate adhesive complexes

The homophilic binding activity of TAG-1 is mediated through two distinct binding sites in the Ig and Fibronectin type III-like (FNIII) domains and a model of multimodal interactions has been proposed [35]. The formation of a cis-dimer via the fourth FNIII repeats may strengthen trans-binding via the Ig1-4 domains simultaneously [35–37]. In transfected cells TAG-1 associates in cis with Caspr2, but it is unknown whether this association is mediated via the FNIII or Ig domains of TAG-1 and whether the cis-heteromeric association with Caspr2 may favor the trans-homophilic interaction of TAG-1. The sequential assembly of the juxtaparanodal complex is unknown. In contrast to Caspr, which requires association with Contactin before ER exit, Caspr2 can reach the plasma membrane without TAG-1. Thus, it is unknown whether Caspr2 and TAG-1 associate in cis at the axonal membrane as a prerequisite for their clustering by the trans-interaction with glial TAG-1 at juxtaparanodes. In addition, as TAG-1 also interacts with several CAMs, N-CAM, NrCAM, L1, β-integrin, and the proteoglycans neurocan and phosphacan/RPTPβ/z [38–41], these various molecular interactions may regulate its function during axogenesis and myelination [32].

3.2. The differential binding affinities of Ig-CAMs may underlie their segregation in neighbouring adhesive complexes

In contrast to TAG-1, Contactin does not exhibit any homophilic binding activity. Contactin displays a broad binding specificity for Ig-CAMs (L1, NrCAM, Neurofascin, β1-subunit of the Na+ channels) [1,21,42] or extracellular matrix components (tenascin-R, tenascin-C, phosphacan/RPTPβ/z) [19,20,43]. Due to their high homology, TAG-1 and Contactin share a series of common binding partners expressed by glial cells like Neurofascin, phosphacan/RPTPβ/z, and as recently reported mCD24 [44]. This raises the puzzling question of the molecular basis for the efficient segregation of neighbouring axo-glial complexes at the node, paranode or juxtaparanode (Fig. 2). CAMs may cluster together depending on their relative binding affinities and in a determined cis- or trans-configuration. For example, both TAG-1 and Contactin can interact with Neurofascin as analyzed in vitro [45]. However, the homophilic binding affinity of TAG-1 when associated with Caspr2 at the juxtaparanode may be favored. Likewise, the glial Neurofascin-155 may display a much higher affinity for Contactin associated with Caspr at the paranode than for Contactin expressed at the node. In addition, the generation of specific splice variants, such as the glial isoform Neurofascin-155 expressed at paranodes and the neuronal isoform Neurofascin-186 at the nodal gap, provides a way to regulate the binding activities of Neurofascin [46]. The presence of other extracellular ligands such as tenascin-R, phosphacan or versican at the node may also modulate interactions between Ig-CAMs at that site [17,18,45].

3.3. The selective processing and N-glycosylation of Caspr/Contactin underlie their targeting at paranodes

Other types of mechanisms may be crucial for the segregation of membrane domains at the node of Ranvier, such as the selective trafficking and exocytosis of CAMs along the axons. The trafficking of Caspr and Caspr2 strongly differs. The cell surface delivery of Caspr is a tightly controlled process, which requires N-glycosylation and depends on the lectin chaperones calnexin/calreticulin [47]. Chaperone-based ER retention is a widely used mechanism that prevents the cell surface delivery of unassembled subunits of membrane channels or receptors. The cis-association of Contactin with Caspr is strictly required for its ER exit and transport to the cell surface via the lipid rafts [48]. A motif consisting in Pro-Gly-Tyr (PGY) repeats in the Caspr ectodomain is responsible for its ER retention and deletion of this motif results in the cell surface targeting of Caspr in the absence of Contactin. Interestingly, structural prediction studies suggest that the PGY region has the ability to adopt a stable organized β-sheet structure. Since Contactin does not interact directly with PGY, an attractive hypothesis would be that Contactin might act as a chaperone and induce a disorder-to-order transition of the PGY-rich sequence, allowing to by-pass the calnexin/calreticulin checkpoint before ER export [49].

Caspr associated with Contactin may traffic via an unconventional pathway that leads to the expression of the two glycoproteins exhibiting mannose-rich N-glycans at the cell surface [47]. By contrast, the Contactin glycoform expressed at the node may bear complex N-glycans [15]. Mutant CHO lines affected in the processing of N-linked carbohydrates have been used to decipher whether Neurofascin-155 may specifically interact with Contactin bearing either high-mannose residues or complex oligosaccharide chains. The Lec1 and Lec23 lines mutated for the N-acetylglucosamine transferase I and α-glucosidase I, respectively, produce N-glycans with the high-mannose configuration [50]. Neurofascin-155 strongly binds Contactin expressed by these mutated lines by comparison with Contactin expressed by control CHO cells. Thus the relative affinity of Neurofascin-155 for Contactin associated in complex with Caspr at paranodes may be strongly increased due to their specific N-glycan processing [49] (Fig. 2).

4. Role of rafts in the paranodal complex clustering and formation of septate-like junctions

The Caspr2/TAG-1 and Caspr/Contactin complexes, in spite of their high degree of homology, give rise to different ultrastructural axo-glial contacts. It would be instructive to understand the molecular basis for such difference. Their selective partitioning into the lipid rafts may be of importance. The mode of association of GPI-anchored proteins to the lipid rafts is still unclear. It is proposed that the lipid remodelling of GPI-anchored proteins within the Golgi that generates saturated acyl chains is essential for their incorporation into lipid microdomains [51]. Also, oligomerization of GPI-anchored proteins depending on cholesterol may induce their stabilization in the lipid rafts. In addition, TAG-1 has been shown to interact with gangliosides GM3, GM1, GD1B and GD3 [52,53]. As expected for GPI-anchored proteins, Contactin and TAG-1 expressed alone in COS cells are partitioned within the low-density

Triton-X100-insoluble fractions which corresponds to the lipid rafts. Association with Contactin drives Caspr within the low-density lipid raft fractions whereas on the contrary, TAG-1 is constrained by Caspr2 in the high-density fractions [29,48]. The glial partner for Caspr/Contactin, Neurofascin-155, is recruited into the lipid rafts due to its palmitoylation [54]. The partitioning of Neurofascin-155 in the detergent-insoluble fraction increases with brain maturation and may be correlated with the formation of axo-glial junctions [55]. The paranodal glycoproteins on both sides of the axo-glial junctions are partitioning with rafts. The selective recruitment within lipid rafts of the paranodal but not juxtaparanodal components may participate to their lateral segregation. As detailed in the following paragraphs, increasing evidences from genetic and pathological models point to a role of the lipid rafts in the formation or stabilization of paranodal axo-glial junctions.

4.1. The MAL-deficient mouse

MAL (myelin and lymphocyte protein) is a tetraspan proteolipid characterized as a raft component implicated in the apical secretion of membrane proteins in epithelial cells [56]. In MAL-deficient mice, the initial formation of paranodes is normal but the axo-glial junctions become altered in adult animals [57]. A reduction of the clusters positive for Caspr and Neurofascin-155 is observed at paranodes of myelinated tracts and at the ultrastructural level, some paranodal loops are everted and the typical transverse bands disorganized. These data indicate that the glial MAL proteolipid may be critical for the trafficking of Neurofascin-155 to the paranodal loops and that such trafficking may occur through the raft-machinery.

4.2. The CGT-deficient mouse

Alterations of the paranodal junctions have been reported in mutant mice for the ceramide galactosyl transferase (CGT), involved in the biosynthesis of myelin galactocerebroside and sulfatide (the oligodendrocyte antigen markers GalC and O4, respectively), with absence of transverse bands, and diffuse expression of Caspr along the internode [58,59]. The mechanisms underlying the paranodal defects in these mice are not well understood, but the galactolipids might be implicated either in cell–cell interactions or in the targeting of paranodal components. The fraction of Neurofascin-155 partitioning with the lipid rafts is decreased in the CGT mutant brain [55]. Since galactolipids are enriched in rafts, they might be implicated in the raft-dependent clustering of Neurofascin-155.

4.3. The ganglioside-deficient mouse

Autoantibodies to the gangliosides GM1 and GD1 are implicated in a subtype of Guillain-Barré syndrome, an acute neuropathy that disrupts nodes of Ranvier in peripheral motor nerves and causes acute limb weakness [60]. The mutant mice deficient for the β1,4-N-acetylgalactosaminyltransferase lack complex gangliosides and have abnormally lengthened nodes and alteration of paranodal junctions [61]. In these mutant mice, some paranodal loops are facing away from the axon, and transverse bands are missing in the innermost loops correlated with attenuation of Caspr and Neurofascin-155 immunostaining at paranodes. GM1 which is a raft-component enriched at paranodes might be involved in the raft partitioning of both axonal Caspr/Contactin and glial Neurofascin-155.

4.4. The ether lipid-deficient mouse

Defects in myelination and paranode disorganization have been reported in knock-out mice for the peroxisomal dihydroxyacetone-phosphate acyltransferase [62]. This enzyme is required for the biosynthesis of ether-linked glycerolipids, including essentially plasmalogens. In addition, selected GPI-anchored proteins contain at the sn-1 position, a long alkyl ether chain in their GPI-anchor and their synthesis or raft distribution may be altered in the ether lipid-deficient mouse [63].

The ether lipid-deficient mouse displays alterations of the paranodal junctions with an increased paranodal length and the lack of transverse bands in some paranodes. In addition, axonal swellings and ER accumulation in Purkinje cell axons were observed like in mice deficient for Caspr or Contactin. Other alterations have been reported in the cerebellar cortex including defects in the foliation patterning, delay in the granule cell migration, and alteration of the Purkinje cell innervation by both climbing and parallel fibers. These last defects point to a neuronal contribution for the mutant phenotype. Strikingly, since Contactin is expressed at the synaptic level and implicated in the cerebellar microorganization [64–68], it may be a good candidate to account for part of the alterations generated by the ether lipid-deficiency.

In the ether lipid-deficient mouse, it seems that Caspr and Contactin are clustered at paranodes even when the typical transverse bands are partially or completely missing in some paranodal junctions. This raises the intriguing question of whether Contactin might be specifically ether-linked with glycolipids to allow organization of the junctional complex into septate-like arrays.

4.5. Paranodal junctions as a target for multiple sclerosis

Neurofascin autoantibodies have been identified in patients with multiple sclerosis (MS) [69] and early alteration in Neurofascin-155-positive paranodal structures occurs within and adjacent to actively demyelinating white matter lesions that are associated with damaged axons [70]. In chronic experimental allergic encephalomyelitis (EAE), an animal model for MS, the concentration of Neurofascin-155 is not changed but its raft-association is reduced. In addition, the immunoreactivity of Neurofascin-155 is dramatically increased in EAE lesion sites indicating an enhanced epitope accessibility that may be due to the breakdown of blood–brain barrier and fibronectin infiltration [71,72]. In cultured oligodendrocytes, fibronectin perturbs the membrane localization and raft-association of Neurofascin-155. Therefore, it is likely that alteration of raft assembly may induce abnormality of paranodal junctions in EAE lesions.

5. MS and TAG-1

Recently, Edgar Meinl and his colleagues identified TAG-1 as an autoantigen recognized by both autoantibodies and T helper Th1/Th17 T cells in MS patients [73]. The adoptive transfer of TAG-1-specific T cells is sufficient to induce EAE in the rat with a preferential inflammation in the grey matter of both spinal cord and cortex. However, in this TAG-1-induced EAE, demyelination and axonal injury were only observed when MOG (myelin oligodendrocyte glycoprotein)-specific antibodies were added. TAG-1-induced EAE is the first animal model for cortical grey matter involvement in MS patients revealing a new role for TAG-1 in anti-neuronal immunity [74].

6. Evolutionarily conserved function of GPI-anchored molecules

Contactin and TAG-1 belong to the Contactin subfamily that includes six genes in mammals [4] and a single representative in *Drosophila*. *Drosophila* Contactin plays a conserved function in the formation of septate junctions, the fly counterparts of vertebrate paranodal junctions [75,76]. Septate junctions have been first char-

acterized in invertebrates and act as a paracellular barrier between epithelial cells and between glial cells insulating axons. The CAMs implicated in the formation of *Drosophila* septate junctions includes Contactin, Neurexin IV, the homolog of Caspr, and Neuroglian, the homolog of Neurofascin-155 [77]. Whether the establishment or stabilization of fly septate junctions depends on lipid rafts is still unknown. Despite the chemical differences between *Drosophila* and mammalian lipids, detergent-insoluble fractions have been isolated in *Drosophila*, which contain ergosterol, sphingolipids and GPI-linked proteins [78,79]. This raises the possibility that rafts may play preserved functions across widely separated phyla in providing a special microenvironment that regulates protein trafficking or anchoring and allows future genetic analyses in *Drosophila*.

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