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Review

Gap junction communication in myelinating glia ☆

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ABSTRACT

Gap junction communication is crucial for myelination and axonal survival in both the peripheral nervous system (PNS) and central nervous system (CNS). This review examines the different types of gap junctions in myelinating glia of the PNS and CNS (Schwann cells and oligodendrocytes respectively), including their functions and involvement in neurological disorders. Gap junctions mediate intercellular communication among Schwann cells in the PNS, and among oligodendrocytes and between oligodendrocytes and astrocytes in the CNS. Reflexive gap junctions mediating transfer between different regions of the same cell promote communication between cellular compartments of myelinating glia that are separated by layers of compact myelin. Gap junctions in myelinating glia regulate physiological processes such as cell growth, proliferation, calcium signaling, and participate in extracellular signaling via release of neurotransmitters from hemijunctions. In the CNS, gap junctions form a glial network between oligodendrocytes and astrocytes. This transcellular communication is hypothesized to maintain homeostasis by facilitating restoration of membrane potential after axonal activity via electrical coupling and the re-distribution of potassium ions released from axons. The generation of transgenic mice for different subsets of connexins has revealed the contribution of different connexins in gap junction formation and illuminated new subcellular mechanisms underlying demyelination and cognitive defects. Alterations in metabolic coupling have been reported in animal models of X-linked Charcot–Marie–Tooth disease (CMTX) and Pelizaeus–Merzbarcher-like disease (PMLD), which are caused by mutations in the genes encoding for connexin 32 and connexin 47 respectively. Future research identifying the expression and regulation of gap junctions in myelinating glia is likely to provide a better understanding of myelinating glia in nervous system function, plasticity, and disease. This article is part of a Special Issue entitled: The Communicating junctions, roles and dysfunctions.

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Contents

Abbreviations: A, astrocyte; O, oligodendrocyte; KO, knockout; dKO, double knockout

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1. Introduction

1.1. Myelinating glia: Schwann cells and oligodendrocytes

Myelin consists of a membrane sheath that wraps around an axon, speeding the conduction of action potentials to provide efficient impulse propagation in large size animals [1]. Since it was first described by Ehrenberg in 1833, the concept of myelin has evolved from being viewed as a static component surrounding the axons to a current understanding of a complex and dynamic process of cell–cell interaction [2,3] that supports axonal integrity and survival [4], and can be modified by functional experience [5].

The structure of myelinated fibers is similar both in the PNS and CNS. Myelinated segments of nerve fibers known as internodes are delimited by areas of naked axons, the nodes of Ranvier [6], where action potentials are generated. The portion of myelin adjacent to the nodes is the paranodal region, which is where the terminal lamellae of non-compact myelin contact the axon (see Fig. 1).

Two different types of glial cells, Schwann cells and oligodendrocytes, are responsible for myelinating the PNS and the CNS respectively. Schwann cells originate from the neural crest and develop into Schwann cell precursors and immature Schwann cells before reaching their mature state [7]. Oligodendrocytes originate from oligodendrocyte precursor cells (OPC) which are generated at the ventral neuroepithelium of the neural tube during embryogenesis or dorsal spinal cord and hindbrain in early post-natal life [8]. Both types of myelinating glial cells contact the axons they are going to myelinate early in development; however, each myelinating Schwann cell associates with a single short axonal segment, whereas a single multipolar oligodendrocyte can interact with up to 40 segments on multiple axons [2]. The initial events in myelination by oligodendrocytes are stimulated by electrical activity in axons. This suggests that electrically active axons will be preferentially myelinated, leading to the possibility that environmental experience may modulate neural development and the functional properties of neural circuits as a result of the increased conduction velocity in myelinated axons [9]. In the CNS, oligodendrocytes are coupled through gap junctions to astrocytes, which are bushy shaped glial cells that participate in brain homeostasis by removing excess neurotransmitter from the synaptic cleft. Astrocytes are also involved in synapse formation and modulation [10]. In the PNS, Schwann cells must exert all the functions of both kinds of glia in the CNS, which indicates a remarkable plasticity of these cells. This heterogeneity of functions is accompanied by changes in gap junction expression and intercellular contacts. Schwann cells express a basal lamina of extracellular matrix that surrounds the node of Ranvier; whereas in the CNS, node structure does not include basal lamina and it is instead contacted by astrocytic processes [11] (see Fig. 1).

Gap junctions in myelinating glia are involved in many physiological processes beyond cell-to-cell communication, including growth control, regulation of cell permeability and calcium signaling. Moreover, in the CNS, gap junctions are hypothesized to play an important role in brain homeostasis by facilitating restoration of membrane potential after axonal activity via electrical coupling and re-distribution of potassium ions [12]. Coupling between myelinating glia and astrocytes constitutes a glial network [13] that promotes the intracellular diffusion of potassium released from axons firing action potentials [14,15].

2. Gap junctions

Gap junctions are formed by members of the connexin family of transmembrane proteins which converge evolutionary from innexins, the protein channels responsible for gap junction communication in invertebrates [16]. In addition, three members of a protein channel subtype homologous to innexins, the pannexins, have been identified in the CNS of vertebrates. It remains unclear whether pannexins can form gap junctions in vivo or instead, if they serve as hemichannels, which act as conduits through the plasma membrane to allow release of ATP [17], a neurotransmitter and important cell–cell signaling molecule [18]. Multiple gap junction channels cluster in the cell membrane to form gap junction plaques. Each gap junction results from the docking of two hemichannels or connexons of adjacent cells, which in turn are composed of six connexins. Each connexin contains four transmembrane domains linked by two extracellular loops and one intracellular loop. Single gap junction channels can be made of similar (homotypic) or different (heterotypic) subtypes of connexins [19] (see Fig. 2). Any two compatible connexins can theoretically be coupled, but functional and biochemical experiments have shown that in general, not all connexin pairs are compatible and only connexins that are closely related to each other can form functional heterotypic channels [20]. Heterotypic channels often exhibit distinct electrophysiological and ion selective properties from those found in homotypic channels [21].

The opening of gap junction channels allows communication between neighboring cells by facilitating the exchange of small molecules and metabolites. Connexin channel opening is highly regulated in several different manners, including gating by transmembrane voltage [22], phosphorylation [23], and extracellular calcium concentration. For example, conduction through hemichannels, which mediates communication between the cytoplasm of myelinating glia and the extracellular space, is suppressed by the millimolar concentrations of calcium in the extracellular fluid [24].

In vertebrates, gap junctions are generally permeable to molecules smaller than 1 kDa, including cyclic nucleotides, vitamins and amino acids, as well as ions [25]. The permeability of channels formed by different connexins can exhibit some chemical selectivity beyond exclusion simply by molecular weight, indicating some chemical or charge-specific effects on permeability of different types of molecules [26,27]. Metabolic coupling between cells and transfer of cell signaling molecules is an important function of gap junctions in general, but this has not been studied extensively in myelinating glia. Gap junctions also enable formation of ensembles of cells coupled together into communication compartments that are jointly regulated by the concentration of a second messenger or metabolite. For example, in the neocortex gap junctions are believed to coordinate the activity of inhibitory neurons [28].

3. Gap junction communication in the myelinating glia

3.1. Glial network

Glial cells coupled to each other constitute a network that was firstly known as a “panglial” syncytium [29]. However, it has been recently suggested that the expression “glial network” may be more

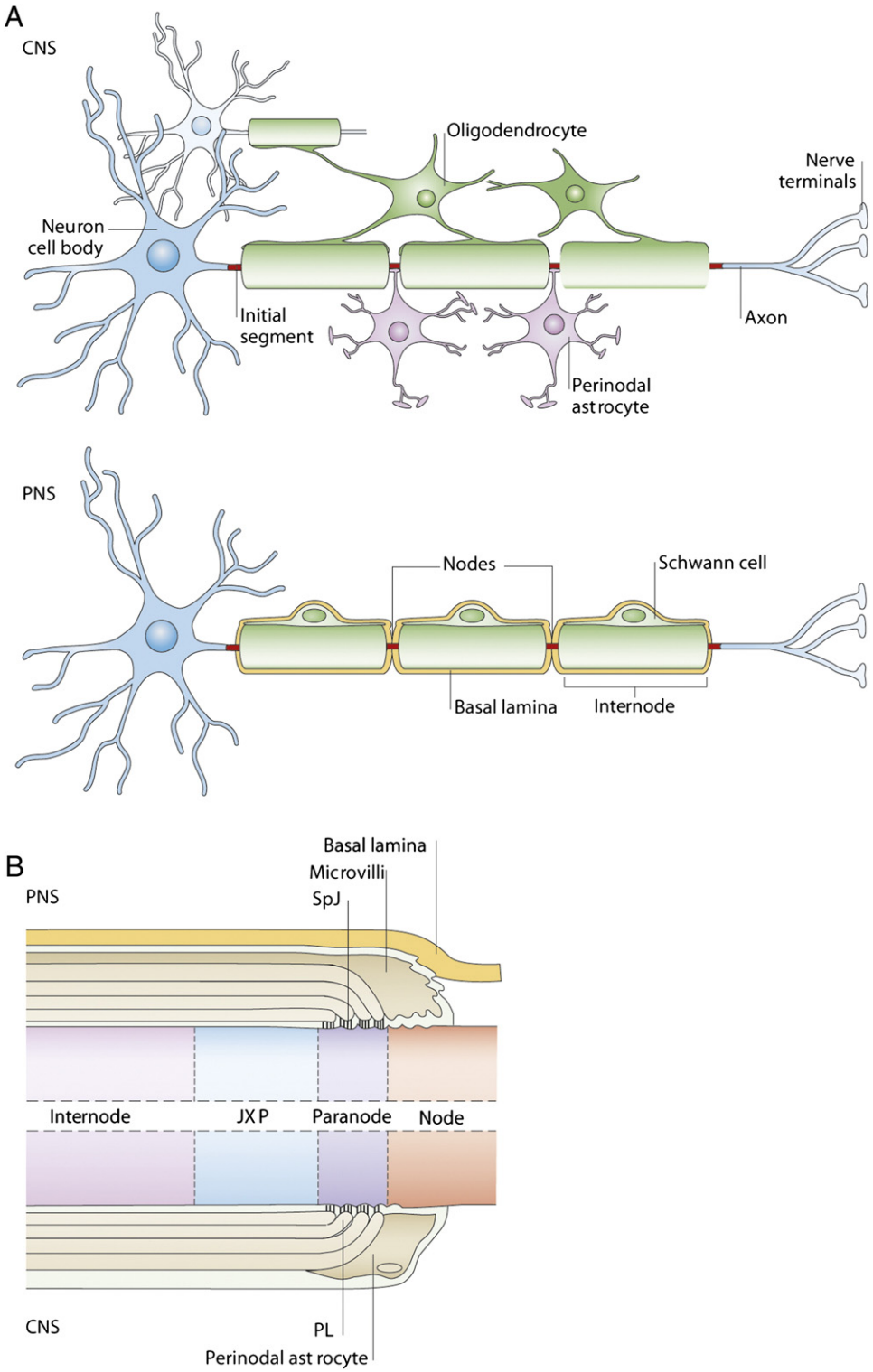

Fig. 1. Illustration modified from Poliak and Peles et al. [11] showing the differences between myelinated fibers in the Peripheral Nervous System (PNS) and the Central Nervous System (CNS). A. Oligodendrocytes myelinate the CNS wrapping their processes around multiple axonal segments. Schwann cells are the myelinating cell type in the PNS and contact single axonal segments. Discontinuities of the myelin sheath along the axon known as nodes of Ranvier are contacted by perinodal astrocytes in the case of the CNS, whereas in the PNS Schwann cells extend microvilli to the node that is surrounded by basal lamina, highlighting the multifunctional capacity of these cells. B. Representation of a longitudinal cut of the myelinated fiber surrounding a node of Ranvier in the PNS (top) and CNS (bottom). The paranode, adjacent to the nodes, is formed by non-compact myelin and contains reflexive gap junctions establishing a communication compartment across the membranous myelin layers. The juxtaparanode (JXP) is composed of compact myelin and divides the paranodal region of the internodal region.

adequate to describe the functional and plastic properties exhibited by heterocellular glial coupling [30].

The work of Rouach et al. [31] in hippocampal slices demonstrated the existence of a metabolic astroglial network in which the degree of connectivity among astrocytes is activity dependent being enhanced by glutamatergic synaptic activity. These findings also encourage consideration of neuroglial and gliovascular interactions at a network level [32].

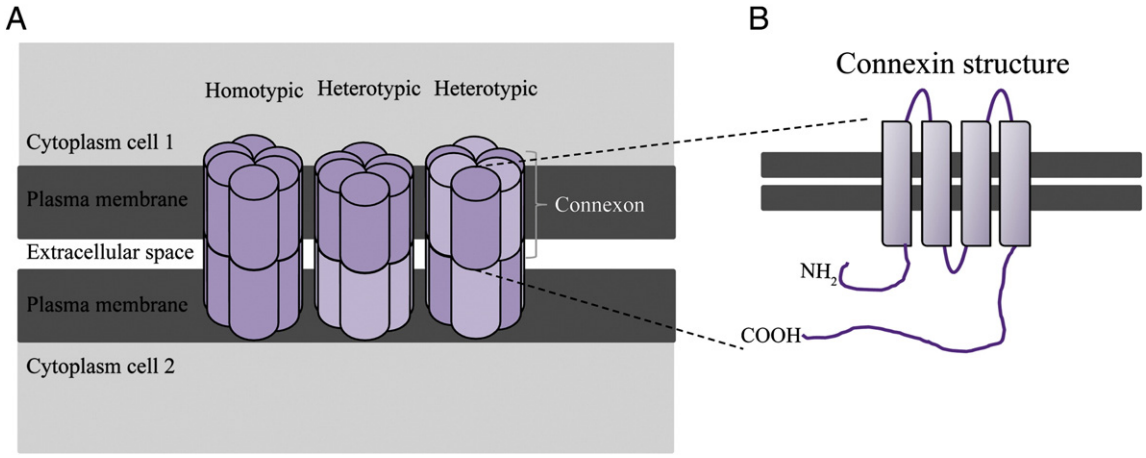

Fig. 2. A. Schematic representation of hemichannels or connexons from neighboring cells docking to form functional gap junctions that enable communication between the cytoplasm of cell 1 and the cytoplasm of cell 2. Connexons can be assembled from the same subset of connexins (homomeric) or different subsets of connexins (heteromeric). Moreover, gap junctions can present either identical connexon composition of connexin subtypes (homotypic) or different connexon composition of connexin subtypes (heterotypic). B. Connexins are transmembrane proteins composed of four transmembrane domains with alpha helix, two extracellular loops and an intracellular loop. Both N- and C-terminals are intracellular. The two extracellular loops contain three highly conserved cysteine residues responsible for the selectivity of hemichannel interactions.

Apart from metabolic support, glial networks mediate clearance of potassium and other ions after axon excitation, which is crucial to ensure the normal resting potential in axons and essential for electrical excitation and neuronal viability. Accumulation of potassium would lead to an osmotically driven water gradient resulting in pathological axonal swelling [33]. This becomes more critical when axons are tightly wrapped by myelin; highlighting the importance of gap junction communication across myelinating glia to provide a diffusion pathway from the axon out to the extracellular space. This may be the main function of the gap junctions formed between neighboring oligodendrocytes, astrocytes, between astrocytes and oligodendrocytes, and between the layers of myelin membrane in compact myelin formed by oligodendrocytes [34] and Schwann cells [35]. Gap junctions between axons and myelinating glia are not known to occur in adult mice but they have been described in brain slices of neonatal rats [36] and in cultures of human fetal brain cells [37] and embryonic rat brain cultures [38,39].

Gap junctions are relatively common between oligodendrocytes and astrocytes (O/A) [40,41], but the coupling between these cell types is weak. Some evidence suggests directional coupling, with dye flowing more freely from astrocytes into oligodendrocytes than in the reverse direction [41] [42]. This is consistent with the heterotypic coupling between different gap junction hemichannels in each cell, as astrocytes and oligodendrocytes do not share any of the same connexins. Dye coupling between adjacent oligodendrocytes varies widely in different parts of the brain and under different conditions, and when dye-coupling is observed, only a relatively small number of cells are coupled. Oligodendrocytes can exchange metabolites, ions and other gap-junction permeable molecules among themselves much easier than with astrocytes [55].

Coupling between oligodendrocytes (O/O) occurs through adjacent cell bodies [43]. Little if any gap junction coupling between oligodendrocytes had been observed in white matter of the corpus callosum [44] and spinal cord previously [45], but more recent evidence supports O/O communication through gap junctions (see below). 20% of oligodendrocytes in spinal cord gray matter are dye coupled [45]. Electrical coupling is seen in 3/4 of oligodendrocyte cell pairs tested in cell culture [46].

3.2. Reflexive gap junction across the myelin sheath

Reflexive gap junctions are gap junctions formed between different processes of the same cell. Such coupling is particularly important in myelin because of the unique topology presented by the multilaminar spiral wrapping of compacted membrane around axons, which presents a long and restricted pathway for diffusion of cytoplasmic components. In the PNS, connexin 32 (Cx32) is expressed at the paranodes together with myelin-associated glycoprotein (MAG) and E-cadherin. The functionality of this particular location of Cx32 was explored by injecting intracellular dyes into living myelinating cells. This experiment revealed that only low molecular mass dyes, such as 5, 6-carboxyfluorescein (not high molecular mass dyes) diffuse across the myelin sheath through the paranodes and the periodic expansions in compact myelin, also known as Schmidt–Lanterman incisures (see Fig. 3). This was the first functional evidence that gap junctions mediate a radial pathway of diffusion across the myelin layers, which provides a shortcut to diffusion that is one million times faster than the circumferential pathway [25]. However, this gap junction pathway is still present in Cx32 KO mice, suggesting that other connexins must be present at these locations as well. Nevertheless, these other connexins are not sufficient to preserve the functional or structural integrity of myelin, as Cx32 KO mice develop peripheral demyelinating neuropathy.

In the CNS, Cx32 is localized to the paranodes where it most likely forms reflexive channels (Cx32/Cx32). However, it is not known if they form reflexive channels in any other regions as is the case in peripheral myelin [47].

4. Types of connexins expressed in myelinating glia

Schwann cell protein expression of connexin subtypes Cx32, Cx43, Cx29 and Cx46 is regulated during development. Nonmyelinating Schwann cells are dye-coupled, but this abates when the cells begin to myelinate [48]. Schwann cells express Cx46 early in development while they are still proliferating and re-express it again after nerve injury [49]. Cx46 expression seen during the proliferating phase ceases when the cells undergo myelination [50]. Moreover, Cx29 is not expressed in neural crest cells, but later it is expressed in Schwann cell precursors both in vivo and in vitro, and as such, is used as a marker in Schwann cell lineage progression in mice [51].

Immunofluorescence studies show positive staining for Cx32 in Schwann cells at postnatal stages coinciding with the onset of myelination [51]. Cx32 enhances the proliferative response of Schwann cells to neuregulin-1 (NRG1), highlighting its role in primary myelinating and remyelinating events [52].

Oligodendrocytes and astrocytes express distinct sets of connexin proteins. Oligodendrocytes express Cx47 [53–55], Cx32 [56,57], and Cx29 [58,59]. Astrocytes express Cx43 and Cx30 [60,61], and possibly

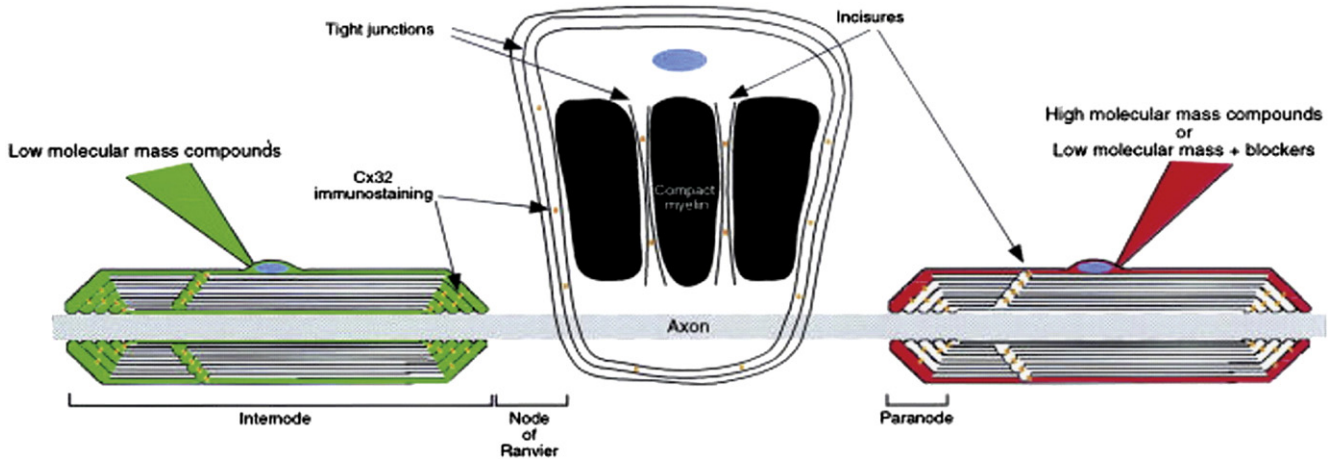

Fig. 3. Schematic representation from Balice-Gordon et al. [25] showing the diffusion of low (green) and high (red) molecular mass compounds (or low molecular mass compounds in the presence of gap junction blockers) across the PNS myelin sheath following perinuclear dye injection. Low molecular mass dyes as 5, 6-carboxyfluorescein diffuse across the myelin sheath through the paranodes and the periodic expansions in compact myelin, also known as Schmidt–Lanterman incisures. High molecular mass dyes are not able not cross the myelin layers and accumulate outside the myelin sheath. This study provided the first evidence of gap junctions mediating a radial pathway across the myelin sheath in the PNS which is one million times faster than the circumferential pathway. A Schwann cell has been unwrapped in the middle of the figure showing the location of Cx32 in the regions of non-compact myelin (Schmidt–Lanterman incisures and paranodes).

Cx26 [62,60]. Oligodendrocytes couple not only to astrocytes and other oligodendrocytes, but also to OPCs [63,64].

Cx47 is expressed in oligodendrocytes in early embryonic periods and shows local and temporal restrictions in the corpus callosum, the striatum, the cerebellum, and the spinal cord in adult animals [55]. Cx29 and Cx32 expression levels are detectable at the beginning of myelination and expression increases in adult brain [55]. However, Cx29 has not been shown to form hemichannels in the adaxonal membrane [14,65].

It was believed that oligodendrocytes were only coupled to astrocytes [12] using Cx47/Cx43 and Cx32/Cx30 heterotypic channels [66,58]. However, Wasseff and Scherer [67] report that oligodendrocytes can also couple to each other (O/O) in the corpus callosum, corroborating the finding by Maglione et al. [64]. These recent findings suggest that the earlier failure to find O/A or O/O coupling in the corpus callosum [44,68,45,40] may be explained in part by the inability of Lucifer Yellow to cross Cx32/Cx30 channels [69]. This is consistent with other research showing that biocytin did not label oligodendrocytes [70] when injected into astrocytes. Whether O/O coupling is mediated primarily by Cx32/Cx32 or by Cx47/Cx47 homotypic channels is not yet established, but evidence suggests that Cx47, but not Cx32, is required for O/A coupling [67].

Maglione et al. [64] report that the number of oligodendrocytes coupled to other oligodendrocytes in white matter is significantly reduced in Cx47 KO mice. Moreover, no O/A coupling remains after Cx47 ablation. After Cx32 ablation O/A remains but Cx30 is vastly reduced [71]. Intercellular coupling was absent in Cx32/Cx47 dKO mice and the loss of oligodendrocyte gap junctions results in an increase in the oligodendrocytic input resistance [64]. O/A coupling was almost absent in Cx43/Cx30 dKO mice [72,73], but some O/A coupling remained in Cx43 deficient animals even though no coupling from oligodendrocytes to OPCs was observed (see Table 1) [64]. In hippocampus, lack of astroglial Cx43 or Cx30 caused a reduction of 50% [74] or 20% [75] in A/A coupling respectively. Apart from the nervous system, Cx43 is located in other tissues like heart and epithelial cells. Mutations in the gene GJA1 encoding for Cx43 cause a rare autosomal dominant inherited disorder known as occulodentodigital dysplasia syndrome (ODDD). Dye transfer assessment in acute brain slices and dual patch clamp measurements in cells cotransfected with WT Cx43 and G60S, a dominant Cx43 mutation that cause ODDD, did

Table 1
Coupling characteristics in KO and dKO mice for the major oligodendrocytic and astrocytic gap junctions that participate in glial networks. A. There are some discrepancies about the major connexin subtype mediating O/O coupling. Maglione et al. [64] found a pronounced reduction in O/O coupling in Cx47 deficient mice but it was unaffected in Cx32 deficient mice while Wasseff and Scherer [67] results suggest the opposite. The later authors discuss that discrepancies can be due to methodological aspects as the dye used in the permeability assays was different in both studies as well as the genetic background of the mice used as control. However, both investigations agree in the finding that no O/O coupling remains in either the corpus callosum or neocortex of Cx47/Cx32 dKO mice. Maglione et al. [64] further inspected O/O coupling in Cx30/Cx43 dKO which was found to be reduced. The lack of Cx43 alone diminished the coupling of oligodendrocytes to immature oligodendrocyte subpopulation suggesting a role for astrocytic Cx43 in precursor population progression. B. Studies from Maglione et al. [64] and Wasseff and Scherer [67] failed to find robust O/A coupling in the corpus callosum in contraposition to the traditional view that oligodendrocytes were only coupled to astrocytes. However, the weak O/A coupling observed in control mice was reduced in Cx47 deficient mice and totally abolished in Cx47/Cx32 dKO and Cx30/Cx43 dKO. C. Conditional deletion of Cx43 in astrocytes results in 50% decay [74] of the A/A coupling in the hippocampus while loss of Cx30 causes 20% reduction [71] on the astroglial hippocampal coupling. However, A/A coupling is completely impaired by the deletion of both astrocytic connexins [72].

Connexins	(A) O/O coupling	(B) O/A coupling	(C) A/A coupling
Cx47 KO	Pronounced reduction [64] but other studies did not find differences [67]	Reduced [64]	Not determined
Cx32 KO	Present [64] but other studies found partial disruption [67]	Present [64]	Loss of Cx30 in gray matter astrocytes [71]
Cx29 KO	Present [64]	Present [64]	Not determined
Cx43 (fl/fl): Hgfap-Cre	Coupling of oligodendrocytes to immature oligodendrocyte subpopulation impaired [64]	Present [64]	50% reduced in hippocampus Cx30 upregulated partially compensate the loss of Cx43[74]
Cx30 KO	Not determined	Not determined	20% reduced in hippocampus hippocampal slices [75]
Cx32/Cx47 dKO	No coupling remains in the neocortex [67] and the corpus callosum [64]	No coupling remains in the neocortex [67] and the corpus callosum [64]	
Cx30/Cx43 dKO	Reduced [64]	Almost abolished [64]	Absent [72]

not reveal any reduction in A/A coupling [76]. These results open a possibility that connexins may play other roles beyond intercytoplasmatic cell communication.

Potassium buffering among astrocytes, oligodendrocytes, and between oligodendrocytes and astrocytes through gap junction coupling are important for myelin maintenance [73,72]. This glial network is mediated predominantly by oligodendrocytes in white matter coupled among each other through Cx47 and Cx32 [64]. The formation of a glial network in the corpus callosum and also in the neocortex [28], reflects the importance of distributing metabolites intercellularly through gap junctions during myelin formation and development.

5. Hemichannel permeability to ATP in physiology and disease

During the biogenesis of gap junctions, connexons reach the plasma membrane and find their appropriate locations by an unknown mechanism. However it is a matter of discussion if these individual connexons or hemichannels are present in the plasma membrane as transient structures or if they play a physiological role [77].

The work done by Geoffrey Burnstock unveiled the role of ATP as a neurotransmitter when released or co-released after synaptic vesicle exocytosis [78]. The action of ATP and other adenine derivatives not only plays a role in neuronal communication, but also in glial activity. In this regard, calcium waves recorded in cultured astrocytes are triggered by ATP-induced ATP release, generating an extracellular propagation wave of ATP that, in turn, activates the intracellular calcium wave [79]. There is growing evidence that the activation of astrocytes is related to synaptic plasticity, and ATP-dependent activation of astrocytes modulates distant synaptic activity [80]. Much astrocytic release of ATP does not fit with an exocytotic source, and it was suggested that ATP would reach the extracellular medium by crossing the hemichannels built up by Cx43, a subtype of connexin present in different cells and organs throughout the body and expressed at very high levels in the central nervous system, specifically in astrocytes. Single channel recording in combination with luciferin–luciferase assay provided direct evidence that the large single channel conductance of Cx43 was accompanied with an increase of luminescence due to ATP crossing the hemichannel [81]. HeLa cells transfected with Cx43 mimicked the astrocytic intracellular calcium waves mediated by extracellular ATP, indicating that ATP crossed the plasma membrane using the intramolecular tunnel of Cx43 [82]. HeLa cells also release ATP under low extracellular calcium concentrations that induce an increase of permeability of Cx43 [83]. In C6 glioma cell line, the release of ATP is strongly decreased when Cx43 is knocked down with siRNA [84].

Interestingly, astrocytes isolated from Cx43 null mice do not release ATP when stimulated by Benzoyl-ATP (BzATP), a $P2X_7R$ agonist [85]. This is attributed to a lower cytoplasmic ATP concentration in Cx43-null astrocytes, but because knockdown of Pannexin 1 (Panx1) prevented ATP release, the authors conclude that Panx1 and not Cx43 hemichannels provide sites of ATP release. However, other authors have not obtained similar results when transfecting with Panx1 [84]. Pannexins are a group of membrane proteins that differ from connexins in amino acid sequence, but they have similar organization within the lipidic membrane. They have four transmembrane regions, two extracellular loops, one intracellular loop and intracellular N and C termini [86]. Panx1 is ubiquitous in tissues and organs, and has been implicated in the controlled ATP release in many cell and tissues including erythrocytes, which lack secretory granules, and astrocytes [87–91]. It has been suggested that Panx1 is always and exclusively forming hemichannels "in vivo", but the possibility that they might also form gap junction channels remains. The work of Bruzzone et al. [92] pointed out that when expressed in paired *Xenopus laevis* oocytes, they form a large conductance connection. Because of the high conductance of Panx1 and the purinergic receptor P2X7, it was suggested that some kind of direct interactions could explain how P2X7 receptors may support low and high conductance open states; but as a matter of fact, experimental results favored the view that they correspond to two independent structures [93–95]. However, activation of Panx1 delivers ATP to different kinds of purinergic receptors [96] and it seems that among other physiological roles, Panx1 may be involved in cell apoptosis by controlling ATP release [97]. Panx1 can also trigger neuronal death being activated through excessive ATP and glutamate release from astrocytes in proinflammatory conditions [98].

The relationship between different types of connexins and ATP release has been assessed using not only glial or neural cell lines but also other cell types. In *X. laevis* oocytes, activation of the endogenous Cx38 with low extracellular divalent concentration triggers the release of ATP [99]. Cx26, a CO_2 dependent connexin found in astrocytes from the respiratory centers of the medulla oblonga, is permeable to ATP under CO_2 acidifying conditions [100,101]. In colonic epithelial cells, Cx26 becomes permeable to ATP when interacting with Shigella [102]. In organotypic cultures of mouse cochlea, ATP release is linked to the activation of Cx26 and also with Cx30 [103]. The adhesion of macrophages to endothelial cells is mediated by a release of ATP by means of Cx37 [104].

There is evidence of the presence of functional connexin hemichannels in the plasma membrane of oligodendrocytes [105] and Schwann cells [107] communicating the cytoplasm of the cell with the extracellular space. This evidence is supported by the selective permeability and reduction of the permeability after treatment with gap junction blockers exhibited by several cell types [106]. It is hypothesized that under physiological conditions, connexin hemichannels remain closed to prevent leakage of cytoplasmatic components, metabolites and ions. Nevertheless, mutations S85C [106] and F235C [107] affecting different parts of the Cx32 protein induce a greater conductance of the mutant hemichannels present in the plasma membrane with a reduction in the threshold of opening when expressed in *Xenopus* oocytes. Both mutations were cloned from patients suffering from X-linked Charcot–Marie–Tooth disease (CMTX), a peripheral neuropathy caused by mutations in the gene encoding for Cx32. The data obtained from these mutations suggest that in pathological conditions hemichannels may be opened at resting membrane potentials inducing metabolic stress due to hemichannel leakage and therefore leading to Schwann cell death.

There is an open question about the presence of Cx32 hemichannels at the node or Ranvier and their hypothetical permeability to ATP. It would be of interest to know if Cx32 mutations causing CMTX show differences in conducting ATP. Results obtained in Cx32 transfected C6 glioma cell line are in accordance with the view that ATP reaches the extracellular space crossing connexin hemichannels, which in turn are activated by an increase of cytoplasmatic calcium concentration [108]. Moreover, repetitive electrical field stimulation of isolated sciatic nerve provokes the release of ATP; glutamate also triggers the release of ATP [109]. Cultured Schwann cells also release important amounts of ATP under UTP stimulation [110], which mimic the release of ATP from astrocytes, suggesting that glial cells from CNS and PNS may share some mechanisms for releasing ATP and activating a pathway of purinergic signaling.

6. Mutations in gap junctions lead to demyelinating neuropathy in both PNS and CNS

Further understanding of the functional importance of gap junctional coupling in myelination and axonal survival comes from diseases resulting from mutations in genes encoding for Cx47 (GJC2) and Cx32 (GJB1), which are the causes of Pelizaeus–Merzbarcher-like disease (PMLD) and CMTX respectively.

6.1. Pelizaeus–Merzbarcher-like disease (PMLD)

PMLD is a recessive inherited severe leukoencephalopathy in humans caused by mutations in the gene GJC2 encoding for Cx47. Patients affected share many clinical features with Pelizaeus–Merzbarcher disease

(PMD) patients, including nystagmus, progressive spasticity, ataxia and hypomyelination on MRI imaging. PMD is an X-linked disease caused by mutations in the major membrane protein of the CNS myelin, Proteolipid Protein 1 (PLP1). Therefore, PMD and PMLD are caused by mutations in myelin proteins (Cx47 and PLP1 respectively) and patients show similar symptomatology [111].

Twenty-four mutations compromising different parts of Cx47 protein have been described, but despite this genetic heterogeneity, the degree of impairment shown by the patients is the same. Mutations can cause the protein to be retained in intracellular compartments such as the endoplasmic reticulum, or can impair the docking of hemichannels thus impeding the passage of molecules between cells and leading to loss of function [112].

A mutation that disrupts the SOX10 transcriptional activation site in the GJC2 promoter region has been described in a family with a mild PMLD phenotype. The fact that another mutation in the binding site of SOX10 in GJB1 is linked to CMTX suggests that transcriptional regulation of GJC2 and GJB1 genes may be critical in myelination of both the CNS and the PNS, respectively [113].

The generation of Cx47 KO mice showed no significant alterations in the CNS apart from minor ultrastructural changes, such as vacuolation of the myelinated fibers in the optic nerve [54]. The generation of a mouse expressing the Cx47 M282T mutation showed impaired motor function, reduced myelin basic protein (MBP) expression, and astrogliosis in the cerebellum of juvenile mice, a phenotype that was completely restored in three-month-old mice [114]. However, Cx32/Cx47 dKO or Cx32 KO mice expressing M282T mutation exhibit a severe phenotype with tremors and tonic seizures as a result of devastating broad demyelination of the CNS that causes death by the sixth postnatal week [53,114]. These observations lead to the conclusion that Cx47 and Cx32 play a key role in myelination of the CNS and display redundant functionality in the mice CNS, which would not happen in humans considering the affection of PMLD patients. The phenotype exhibited by dKO animals also suggests that the main role of these connexins is to ensure homeostasis of CNS tissue by coupling oligodendrocytes and astrocytes into a network for potassium clearance after nervous activity.

6.2. X-linked Charcot–Marie–Tooth disease (CMTX)

CMTX is a dominant inherited sensory and motor peripheral neuropathy caused by mutations in the gene GJB1 encoding for Cx32 linked to the X chromosome. This is the second most common form of demyelinating Charcot–Marie–Tooth disease type 1 (CMT1), representing 10–15% of all cases. CMTX is characterized by progressive weakness and atrophy of the distal limb muscles that can result in severe deformities like feet drop [115]. Males are uniformly affected but female carriers show variable clinical features due to random X-chromosome inactivation [116]. More than 300 mutations for the gene GJB1 have been described (http://www.molgen.ua.ac.be/CMTMutations/default.cfm; Inherited Peripheral Neuropathies Mutation Database) leading to impaired Cx32 trafficking [117], voltage gating defects [106,118] and inability to form functional gap junctions across the myelin sheath once inserted into the plasma membrane [119].

Patients affected by CMTX do not show severe CNS symptoms suggesting that Cx47 can compensate for the loss of Cx32 function. However, some studies show subtle central alterations and few mutations have been suggested to involve CNS dysfunction [120,121]. There are a few mutations related to CMTX that do not directly affect the GJB1 gene but instead affect the binding of the transcription factors SOX10 [122] or EGR2/Knox20 to the P2 promoter that regulates Cx32 expression in Schwann cells [123].

A useful tool for the study of CMTX came from the generation of Cx32 KO which shows a late-onset demyelinating neuropathy that resembles human CMTX [116]. During the first months of life these mice show only dysfunctions in the liver where Cx32 is abundantly expressed [124,125]. The progressive peripheral demyelination starts at 3 months of age and it is characterized by unusually thin myelin sheaths, cellular onion-bulb formations, increased Schwann cell proliferation and enlarged periaxonal collars. Motor fibers are more severely affected than sensory fibers [116], but conduction velocity is only slightly decreased [126].

Strong evidence that CMTX is caused by mutations of Cx32 in Schwann cells and not in other cell types was provided by Scherer et al. [127] by expressing human Cx32 in Cx32 KO under the myelin protein zero (MPZ) promoter specific for Schwann cells, and rescuing the pathologic phenotype observed in peripheral nerves, but not in liver or spinal cord of the Cx32 KO mice [127]. Further characterization of Cx32 KO revealed new features, including alterations in the distribution of proteins such as potassium channels Kv1.1 [128], increased expression of GFAP [129], and increased number of OPCs [130].

How the lack of Cx32 leads to disease is not fully understood. The main function attributed to Cx32 is to form reflexive gap junctions across the peripheral myelin sheath mediating a faster pathway of diffusion to the adaxonal cytoplasm than would be possible without a pathway for radial diffusion across the myelin lamellae. However, analysis of the diffusion rate of fluorescent dyes in Cx32 KO was not slower than in the wild-type, suggesting that other gap junctions may mediate this pathway [25]. Moreover, the fact that reflexive channels exhibit different permeabilities to molecules such as cAMP suggests that gap junctions may regulate or sustain signaling cascades that favor the survival and myelination of the axons. For example, gap junctions together with ATP, mediate calcium signaling between the networks of branched Schwann cells covering the lanceolate ending of the rat hair follicles [131].

It might be interesting to further explore the consequences of changes in permeability or block of signaling cascades induced by the lack or deficiencies in the channels formed by mutated proteins. Previous studies have shown that ionophoresis or changes in transjunctional voltage in Cx43 and Cx45 can change the permeability of these gap junctions to intracellular injected dyes [132]. Therefore it would be important to determine if defects on the voltage gating of Cx32 mutants induce new selective properties in the channels that can lead to disease.

7. Conclusions

Gap junction communication in myelinating glia is crucial for myelination and axonal survival in both the PNS and the CNS. There are many open questions about the signaling pathways and functions sustained by gap junctions in myelinating glia. Connexin expression changes during differentiation of Schwann cells, indicating the diverse roles that these junctions play in glial cell biology. Reflexive coupling via gap junctions solves a unique problem presented by the diffusion barriers in compact myelin, but many other important roles of gap junctions in myelinating glia are not well understood. This is evidenced by the failure to understand the pathophysiological basis for many myelin disorders associated with genetic mutations in connexins in myelinating glia.

The fundamental facts about gap junction coupling among myelinating glia in the CNS are only now emerging. Coupling among oligodendrocytes and between oligodendrocytes and astrocytes has not been studied extensively, considering the critical importance of communication among these cells and with axons in maintaining normal physiological function. Beyond potassium buffering, gap junction coupling among oligodendrocytes could be important in maintaining axonal excitability, providing nutritional support, transfer of intercellular signaling molecules necessary for maintenance of myelin, and remodeling myelin under appropriate circumstances, as could coupling between astrocytes and oligodendrocytes. Why OPCs are coupled through gap

junctions to oligodendrocytes is unclear, but these progenitor cells are highly responsive to neural injury, suggesting a possible role in intercellular communication during nervous system repair or remyelination. In addition to the functional significance of gap junction coupling among myelinating glia, the physiological regulation of these channels is not well explored. Future research to elucidate the function and regulation of gap junctions in myelinating glia could lead to development of new therapeutic treatments for CMTX and PMLD and other myelin disorders, while deepening understanding of the means by which myelinating glia contribute to nervous system function and plasticity.

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