Short review

Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS

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Abstract

This review article summarizes early and recent literature on the structure, distribution and composition of gap junctions between astrocytes and oligodendrocytes, and the differential expression of glial connexins in adult and developing mammalian CNS. In addition to an overview of the topic, discussion is focused on the organization of homologous gap junctional interactions between astrocytes and between oligodendrocytes as well as on heterologous junctional coupling between astrocytes and oligodendrocytes. The homotypic and heterotypic nature of these gap junctions is related to the connexins known to be produced by glial cells in the intact brain and spinal cord. Emphasis is placed on the ultrastructural level of analysis required to attribute gap junction and connexin deployment to particular cell types and subcellular locations. Our aim is to provide a firm basis for consideration of anticipated rapid advances in understanding of structural relationships of gap junctions and connexins within the glial gap junctional syncytium. Conclusions to date suggest that the glial syncytium is more complex than previously appreciated and that glial pathways of junctional communication may not only be determined by the presence of gap junctions, but also by the connexin composition and conductance regulation of junctional channels. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Gap junctions provide pathways for direct intercellular communication via channels that link the cytoplasmic compartment of cells and allow exchange of ions, metabolites and water. These channels are composed of six subunits of transmembrane gap junction proteins. Because these proteins connect head to head across the extracellular space, they were termed connexins, and the hemichannels they formed are referred to as connexons. Connexons embedded in the plasma membranes of adjacent cells form appositions with each other and cluster together to create gap junctional plaques consisting of six to 10,000 or more connexons. Gap junctions occur between the same or different cell types (homologous vs. heterologous junctions) and functional channels can consist of apposing connexons that contain the same or different connexins (homotypic vs. heterotypic channels).

Gap junctions were originally conceived to be monolithic structures composed of a single class of identical protein subunits. However, about 15 connexins (‘’Cx’’ followed by molecular weight designation) have been identified to date, ranging from 26 kDa to about 60 kDa, and sorted into three gene families (α, β and γ). These connexins have considerable amino acid sequence homology, exhibit diverse tissue distributions, and show substantial selectivity in their ability to form functional heterotypic channels with each other. There is also evidence that some connexins form heteromeric channels, where individual connexons contain a mixture of different connexins [39,103]. Several reviews on gap junctions and connexins have appeared in recent years [14,15,35,91,101], including those devoted to the subject of gap junctional intercellular communication (GJIC) in the CNS [13,34,111]. The present review covers selected topics on glial gap junctions and connexins and is expanded upon elsewhere [67].

About nine members of the connexin family have so far been reported to be expressed in adult and/or developing CNS; members of the α and β subgroups are expressed in glia, the γ subgroup, of which Cx36 is the first example, appears to be expressed in neurons [17]. Given the cellular heterogeneity and morphological complexity of neural tissue, major goals are to establish the cells that form gap junctions, to identify the connexins expressed by particular cell types, and to determine the subcellular sites to which they deploy their connexins. While considerable advances have been made along these lines with respect to glial cells, progress has been slow due to the technically challenging nature of the work and the level of analysis required to resolve important, functionally relevant issues.

For example, visualization and site designation of gap junctions requires detailed ultrastructural examination by electron microscopy (EM) with clear identification of the cell types linked by gap junctions. However, ultrastructural examination is often laborious and allows analysis of relatively small areas of tissue compared with studies by light microscopy (LM) (Fig. 1). Cellular and subcellular localization of connexins also requires immunolabelling techniques at the EM level to confirm the localization of specific connexins in individual gap junctions and to identify the cell types that form the immunolabelled junctions (Figs. 2 and 3). EM immunolocalization is often necessary since LM immunolabelling for connexins by fluorescence or immunoperoxidase usually reveals only small dots or spots.
puncta (Fig. 1). Moreover, connexins are only occasionally detected within somata by LM immunohistochemical methods, thereby usually precluding cell type identification of protein expression by LM. Given observations that, for example, astrocytic processes bearing gap junctions are frequently found within less than a micrometer of neuronal elements (Fig. 3A,B), it is impossible to assign these tiny puncta to particular cells types in the absence of ultrastructural data [88,114,115].

2. Gap junctional communication between astrocytes

2.1. Astrocytic gap junctions

Astrocytes form structural barriers at vascular surfaces, ensheathe neuronal elements and separate regions of dissimilar or fluctuating ionic composition, thereby physically and/or metabolically compartmentalizing various neuronal components of the CNS [see Ref. [67]]. Metabolically, astrocytes in brain and spinal cord are thought to contribute to extracellular K⁺ homeostasis through, in part, a process termed spatial buffering [81,82], for which there is evidence in the retina [78]. This involves the cell-to-cell redistribution of excess extracellular K⁺ through the cytoplasm of vast networks of contiguous astrocytes coupled by gap junctions [see Ref. [68]]. Ever since astrocytes were assigned the property of buffering K⁺ around neurons, it has been assumed that gap junctions in this system provide a direct pathway into perivascular compartments for disposal of K⁺ [77,82] and accompanying water [90]. Equally plausible, however, was the related notion that coupling would increase the volume of the glial compartment around active neurons [45].

Ultrastructural, dye-transfer and electrophysiological studies have provided strong support for the generally accepted view that astrocytes are extensively coupled to form what are defined as “functional syncytia” [63,67,88]. In addition, gap junctions are particularly numerous between astrocytic processes that ensheathe chemical synapses, glomeruli and nodes of Ranvier, as well as between those at brain and vascular surfaces [for refer-
ences, see Ref. [67]). While these reports suggest that astrocytic gap junctions are ubiquitous in the CNS, the functional significance of the observed local heterogeneities has remained largely unclear. The global organi-
zation of astrocytic gap junctions has been difficult to deduce using traditional methods of junction visualization by EM. This early work has now been considerably extended by the discovery of connexins and identification of the particular connexin proteins expressed by astrocytes and other support cells in the CNS.

2.2. Connexin43 in astrocytes in vivo

It is now well established that astrocytes in vivo express Cx43 [67]. It appears to be present at virtually all astrocytic gap junctions and has not been detected ultrastructurally in neurons or at neuronal gap junctions [114,115]. Cx43 is widely but heterogeneously distributed in rat CNS, suggesting regional variation in astrocytic gap junction density, which is supported by correlations between Cx43 protein expression and counts of Cx43-positive puncta among brain regions [74,114,115]. Studies of Cx43 mRNA by in situ hybridization histochemistry (ISHH) indicated it to be regionally heterogeneous [62] corresponding partly to differences in Cx43 protein levels among brain regions [74,114,115]. Ultrastructural studies with well-characterized antibodies have indicated Cx43 in brain to be relatively more concentrated at gap junctions between astrocytic processes surrounding neuronal somata, dendrites and synaptic glomeruli [114,115]. Within cells, Cx43 was detected by EM largely at or in the vicinity of astrocytic gap junctions (Fig. 2C), and virtually all labelling by LM was punctate. Thus, Cx43-positive puncta seen by EM correspond largely to labelled gap junctions. This was best illustrated in the cerebellar cortex where linear arrays of puncta in the molecular layer were associated with gap junctions between Bergmann glial cells [115]. Such findings suggest that it will be possible to draw conclusions about the global organization of gap junctions in the CNS, particularly after detailed localization to cell type and to junctions between morphologically identified cellular elements is firmly established at the EM level.

In spinal cord, Cx43 was present throughout gray and white matter, but most concentrated in the substantia gelatinosa [79]. Unlike results in brain, astrocyte processes in gray matter were often labelled at both their homologous gap junctions and non-junctional membranes surrounding synaptic glomerular. In white matter, Cx43 was localized to gap junctions between rostrocaudally as well as between radially oriented processes of fibrous astrocytes, suggesting the presence of an extensive gap junctionally coupled syncytium extending into and along the cord. Branches of astrogial radial processes were seen forming gap junctions with oligodendrocytic processes at the outer surface of myelinated fibers (Fig. 3D) and at oligodendrocyte somata (Fig. 4A,B). Such junctions were labelled for Cx43 only on the astrocytic side (Fig. 4F) and were thus heterotypic. Curiously, some radial processes formed junctions with Cx43 labelling only on one side of the junction [79], suggesting that an astrocytic subtype in spinal cord may not express Cx43, although this requires further investigation.

2.3. Connexin30 in astrocytes in vivo

Connexin30 (Cx30) has only recently been identified and found to be highly expressed in brain [20]. It is closely related to Cx26 (77% sequence identity) and some anti-Cx26 antibodies were found to cross-react with Cx30 [72][743]. Based on use of these antibodies, it was suggested that astrocytes produce a second connexin that was unlikely Cx26, but almost certainly Cx30 [72]. This was subsequently confirmed with Cx30-specific antibody which showed immunolabelling for Cx30 in adult rat brain to be restricted to astrocytes and, as in the case of Cx43, largely localized at gap junctions formed by these cells [73]. Inter-astrocytic gap junctional membranes were symmetrically labelled (Fig. 2A) and oligodendrocytic–astrocytic junctional membranes were asymmetrically labelled only on the astrocyte side. In addition, Cx30 was colocalized with Cx43 at gap junctions by EM (Fig. 2D,E) and by double immunofluorescence (Fig. 1). Levels of Cx30 expression are far greater in diencephalic and more caudal CNS areas than in forebrain, which may be related to the different developmental origin of astrocytes in these areas or, alternatively, to regionally specific regulation and functional contributions of Cx30 and Cx43 to glial GJIC. The latter possibility is suggested by the greater prevalence of Cx30, compared with Cx43, at gap junctions between perivasculary astrocytic endfeet on subcortical blood vessels. Functional differences are further supported by the absence of Cx30 in white matter tracts [73], which generally contain an abundance of Cx43 [114,115]. This implies that protoplasmic gray matter astrocytes express Cx30, while fibrous white matter astrocytes do not, thus distin-
Fig. 4. Heterologous astrocyte-to-oligodendrocyte gap junctions. (A,B) Oligodendrocyte soma (A) with a process extending to the lower right and another cross-fractured at upper left. This cell forms numerous gap junctions (black arrowheads) one of which (boxed area, magnified in B) is fractured to display an oligodendrocyte P-face (OP) with an astrocyte E-face (As E). E- and P-face images of aquaporin4 square arrays (white arrows) identify astrocyte plasma membranes. (C) Astrocyte-to-oligodendrocyte gap junctions immunogold-labelled for Cx32. (D) Immunogold labelling for Cx32 at a small gap junction in an oligodendrocyte plasma membrane at the outer tongue of myelin. OP, oligodendrocyte P-face; My, cross-fractured myelin. (E) Oligodendrocyte-to-astrocyte gap junctions. OE, oligodendrocyte E-face; As P, astrocyte P-face. (F) Oligodendrocyte-to-astrocyte gap junctions (E-face) labelled for Cx43. The margins of a large and a small gap junction are indicated by arrowheads. Labels are attached to cytoplasmic Cx43 determinants in the subjacent astrocyte indicating that this oligodendrocyte E-face (OE) forms a heterologous gap junction with an astrocyte. (A), (B) and (E) were modified from Ref. [89]; (C) was modified from Ref. [91].
guishing the two types of astrocytes based on their connexin phenotypes.

### 2.4. Astrocytic syncytial compartments

Based on heterogeneous connexin expression in brain, it has been speculated that regional requirements for GJIC between astrocytes may differ and that brain regions with contrasting connexin levels may possess different capacities for junctional communication [115]. This may create areas of functional compartmentalization within the glial syncytium as previously discussed [63]. Although no evidence for glial compartmentalization was found in studies of astrocyte coupling in the hippocampus [44], this region is far less heterogeneous in Cx43 and Cx30 levels than other brain areas that may serve as better tests for this concept [73,74,115]. In any case, the establishment of putative GJIC compartments must be governed by factors that regulate connexin expression in astrocytes. Considering the metabolic support these cells provide to neural systems, it is likely that such factors include the extracellular milieu generated by active neurons. For example, especially high levels of both Cx30 and Cx43 occur in the globus pallidus [73,74,115] and this area is known to contain an abundance of tonically active neurons. Further, gap junctional coupling efficiency of cultured astrocytes is influenced by interactions with neurons [29] as well as meningeal cells [1], and may ultimately be subject to regulation by interactions with oligodendrocytes.

The notion of GJIC compartment may be considered further in the light of findings that vast numbers of junctions connect not only astrocytic processes of different cells, but also those of the same cell [111,112]. Based on this extensive autacellular (or autologous) coupling, it was suggested that GJIC subserves pathways between astrocytic subcellular compartments as much as it does between different cells. It is noteworthy here that numerous gap junctions typically link layers of astrocytic lamellar processes surrounding synaptic glomeruli, and that global Cx43 distribution in the CNS reflects in part its localization in these lamellae [79,115]. If such lamellar junctions arise from one or a few astrocytes resulting in autologous coupling, then by linking subcellular compartments as proposed [97,111], this coupling could serve as a radially directed gap junctional short-circuit for movement of substances from the glomerular milieu into the lamellar cytoplasm, through their attendant junctions and into the pool of surrounding astrocytes.

### 2.5. Coupling and connexins in cultured astrocytes

Astrocytes in culture as in situ express Cx43 and are coupled by gap junctions [23,31]. However, these cells taken from different brain regions of neonatal rat exhibit markedly different levels of Cx43 expression and dye-coupling [3,51]. While this may simply be due to different developmental rates of coupling, it is noteworthy that levels of Cx43 expression and coupling of cultured astrocytes derived from different brain regions correspond to their levels of Cx43 expression in adult brain [74,115]. Cx30 is also expressed by these cells after a month of plating [47], consistent with the delayed appearance of Cx30 in developing brain. Since Cx43 and Cx30 expression and dye-coupling between astrocytes in neonatal brain is low [8,117], these findings suggest regional and environmental regulation of astrocytic gap junction formation. While all astrocytes appear to express Cx43 in vivo [114,115], Cx43 and dye-coupling occur in type 1, but are absent in type 2 astrocytes in vitro [4]. It is unclear whether this lack of type 2 cell coupling reflects a noncoupled glial cell type in vivo or arises as an artifact of tissue culture. Astrocytes cultured from Cx43 knockout mice lack dye-coupling and display reduced rates of proliferation, suggesting a role of astrocytic GJIC in promoting growth [76]. In organotypic cultures from these mice, the density of astrocytes in central regions of cultures increased enormously, possibly indicating a regulatory role of GJIC in astrocyte migration [106]. Residual GJIC between Cx43 knockout astrocytes may be explained by their expression of Cx30.

### 2.6. Regulation of astrocytic connexins and coupling

Little is known about mechanisms whereby astrocytes regulate levels of connexin expression over long terms, but the uneven distribution of Cx30 and Cx43 in normal CNS clearly indicates that such regulation occurs. An intriguing observation is the localization of basic fibroblast growth factor (FGF-2) specifically at gap junctions between astrocytes in brain and between myocytes in heart [40,113]. These findings suggest a physical association between FGF-2 and gap junctions composed of Cx43 in heart and brain. FGF-2 influences GJIC and Cx43 expression in microvascular endothelial cells [84], but functional relationships between FGF-2 and gap junctions are as yet unclear.

There is evidence that factors derived from neurons and/or glial cells modulate astrocytic GJIC via signaling pathways [33]. Conditions where such pathways are involved may provide insight to functions of the astrocytic syncytium. Observations of increased coupling between astrocytes following optic nerve stimulation [58] suggest that strength of connectivity of this syncytium is under dynamic regulation and may be governed by neuronal activity. In addition, coupling between cultured astrocytes is altered by noradrenaline and increased by glutamate and K⁺ [28,32]. Regulatory agents that decrease astrocytic GJIC in vitro includes ATP, endothelin-1, oleic acid, 2-chloroadenosine, nitric oxide or its peroxynitrite products, arachidonic acid and endogenous cannabinoids (anandamide, oleamide) [11,19,27,30,32,33,44,50,64,107]. The functional significance of the many factors that reduce
GJIC between astrocytes is not clear, but has been discussed in relation to K+ spatial buffering and gap junction-mediated metabolite exchange between glial cells [61,67].

At a molecular level, Cx43 is regulated by phosphorylation, and early studies reported the presence of both phosphorylated and nonphosphorylated forms of this protein in rat CNS [74]. Subsequently, it was found that brain contains almost exclusively phosphorylated Cx43 when precautions are taken to prevent artifactual postmortem removal of phosphate moieties by a rapidly acting phosphatase [36]. Rapid dephosphorylation has also been shown with a monoclonal anti-Cx43 antibody that exhibits selective recognition of unphosphorylated Cx43 [55,71]. Cx43 dephosphorylation also occurs after neural injury [55] and at intact gap junctions in spinal cord after mild sciatic nerve stimulation, indicating that this occurs in response to neural activation [70]. Cx43 phosphorylation has been correlated with altered GJIC and appears to contribute to modulation of channel conductance state [14,15,35,49]. While the role of this process in astrocytes and the functions of phosphorylation at specific serine residues in Cx43 remain to be established, it may be relevant that sites in Cx43 serve as substrates for MAP kinase (erk kinase) cascades and that these cascades are activated by agents that influence GJIC between astrocytes [109].

With the discovery that astrocytes in vivo also express Cx30, regulatory responses of this connexin must now be considered. Because astrocytes cultured for a few weeks express very little Cx30 (see Section 3), most studies to date appeared to have examined regulation of astrocytic GJIC mediated largely by Cx43. Thus, current understanding may not reflect the situation in vivo if conductance at homotypic channels (Cx30/Cx30 and Cx43/Cx43) formed by the two connexins in astrocytes is differentially regulated, if these two classes of channels exhibit differential permeability to ions compared with metabolites, or if functional heterotypic Cx30/Cx43 channels exist and allow only unidirectional conductance. These possibilities are raised by evidence that electrical conductance, ionic selectivity and dye permeability can vary widely among channels formed by various connexins [105].

2.7. Astrocytic gap junctions and neural injury

Cx43 has been investigated in various experimental paradigms with a panel of sequence-specific anti-Cx43 antibodies that exhibit differential epitope recognition of Cx43 depending on its cellular location and phosphorylation state. Cx43 levels are elevated in reactive astrocytes surrounding kainic acid (KA) injection sites, although the coupling state of these cells at various stages of their progression from a normal to a reactive state is unclear. Cx43 within these sites undergoes a transition such that some epitopes are hidden, while others are exposed [99,108]. Cx43 epitope masking may be due to molecular interactions or covalent modifications at or near the masked epitope. Despite the presence of Cx43 at such sites, ultrastructurally defined gap junctions are reported not to be present; Cx43 is sequestered intracellularly by internalization of gap junctional membranes, and some Cx43-positive puncta begin to reappear after several weeks survival [38,80].

In rats subjected to ischemic brain injury, Cx43 was increased after mild to moderate striatal damage, but reduced in regions severely depleted of neurons [37]. Similar results were obtained in a spinal cord trauma model where Cx43 responses differed in severely or mildly damaged regions proximal and distal, respectively, to a compression site [104]. In a rat focal cerebral ischemia model with various ischemia/reperfusion times [55], Cx43 at intact gap junctions underwent reversible dephosphorylation after brief ischemia, while astrocytic gap junction internalization occurred in the ischemic core after longer periods of ischemia. Dephosphorylated Cx43 persisted at intact gap junctions confined to a thin corridor at the ischemic penumbra. These results indicate that different Cx43 regulatory processes including rapid dephosphorylation, membrane dispersal and internalization may be operative in areas with different degrees of neural damage. The state of astrocytic coupling after neural injury in adult CNS is uncertain and may be different from the coupling state of those seen in ischemic immature brain [18] where the levels of Cx30 are minute (compared with adult) and Cx43 is not fully developed [73,117]. Nevertheless, it has been suggested that astrocytic gap junctions may be remodeled after injury in order to redirect flow of ions and metabolites to benefit tissue survival [10,37,38,55].

In other studies, facial nerve lesions caused an increase in Cx43 staining in the facial nucleus and cerebral cortex after 1 to 3 h [48,95,96,111]. In epileptic foci of human brain, Cx43 mRNA, protein or astrocytic GJIC were reported to be either increased [52,75] or unchanged [26]. Elevated levels of Cx43 were observed within βA4-positive amyloid plaques in Alzheimer’s disease brain [69]. This may reflect the invasion of these plaques by reactive astrocytes or an upregulation of Cx43 as is seen in PC12 cells overexpressing a carboxy-terminal fragment of amyloid precursor protein [57,68].

3. Glial connexins during development

The expression patterns of Cx32, Cx43 and Cx30 have been examined by various methods in developing rat brain. Northern and Western blot analysis as well as ISHH indicate that Cx43 appears first at embryonic days 12–18 [5,24,65] and that Cx32 protein and mRNA appear during the first or second postnatal week and increase during development. Both connexins exhibit regional heterogeneity in temporal patterns of expression, and the cells ex-
pressing these connexins during development are assumed
to be similar to those in adult brain.

Immunohistochemical analysis of postnatal rat brain
[117] has shown that Cx43 first appears along radial glial
cells and is most intense along cerebellar Bergmann glial
cells. It then becomes associated with short astrocytic
processes and cell bodies, and finally appears exclusively
as punctate labelling throughout the CNS. These changes
occur at different ages in different brain regions, the adult
pattern emerges at about postnatal day 10 to 15, and Cx43
continues to increase during the first postnatal month.
Likewise, dye-coupling between radial glial cells is absent
in the neocortex of fetal rats [56] and is first seen between
astrocytes at postnatal day 11 [8]. Thus, the earlier appear-
ance of Cx43 in radial glia is curious and may underlie
some form of early coupling that so far has largely gone
undetected. In this regard, it is noteworthy that coupling in
embryonic mouse ventricular zone occurs in clusters of
coupled cells consisting of radial glia and neural precursor
cells [9]. These clusters surround individual radial glial
cells, which were suggested to be organizing centers for
cell migration and propagation of signals via gap junctions
between coupled cells. Analysis of the relationship be-
tween cell cycle and cell coupling indicated that proliferat-
ing cells exhibit greater coupling, and it was suggested that
gap junctions between cells in the ventricular zone may
promote cell cycle synchronization within a cluster [9].

The relatively late emergence of the adult pattern of
Cx43 localization may reflect maturation of various envi-
ronmental factors that influence junctional coupling be-
tween astrocytes. This could include regulation of Cx43
production by neuronal factors in accordance with astro-
cyte support of neuronal activity [74,114]. Since punctate
Cx43 labelling in adult brain reflects Cx43 at astrocytic
gap junctions [74,115], it was suggested that the time of
emergence of this pattern of labelling in particular brain
regions correlates with functional maturity of that region
[117].

The spatial and temporal developmental profile of Cx30
is somewhat different than that of Cx43. Levels of Cx30
mRNA are very low during the first two postnatal weeks
in mouse brain and reached adult levels only at the end of
the fourth postnatal week [20]. Similarly, Cx30 protein
levels in thalamic and brainstem regions are about 20-fold
lower at postnatal day 15 compared with that in adult brain
[73]. In addition, Cx30 immunolabelling is punctate at the
time of its appearance. The later onset of Cx30 expression
suggests different functional roles of Cx30 and Cx43 at
gap junctions formed by astrocytes. As previously dis-
cussed [73], such roles for Cx30 may include a contribu-
tion to astrocytic coupling with oligodendrocytic elements
during myelination as well as in adult brain, development
of coupling between astrocytic endfeet on blood vessels
during formation of the blood–brain barrier, maturation of
brain energy metabolism which may occur in conjunction
with interglial exchange of metabolites, or compaction of
brain extracellular space which occurs maximally between
P10 and P20 in rat [53] and may require greater homeo-
static processes mediated by astrocytic GJIC.

4. Gap junctional communication and oligodendrocytes

4.1. Homologous, heterologous or autologous coupling

In the CNS, myelin is formed by oligodendrocytes,
which typically extend several branching processes that
myelinate 20–60 internodal segments of a dozen or more
axons throughout a tissue volume of a cubic millimeter or
more [16,85]. Thus, the myelin sheaths of adjacent axons
are often derived from the same oligodendrocyte, resulting
in the possibility of autologous coupling (autologous cou-
pling has also been called homocellular or reflexive cou-
pling because it involves coupling of two or more compo-
nents or processes from the same cell). In addition, axons
ensheathed by several different oligodendrocytes are in-
varily intermixed, thereby providing for direct contact
between oligodendrocyte somata, their processes, and/or
their myelinating segments. Thus, the possibilities of both
homologous and/or autologous oligodendrocyte-to-oligo-
dendrocyte gap junctions must be considered. In addition,
abundant astrocytes and their fine-diameter processes are
intermingled with oligodendrocytes throughout gray and
white matter regions of the CNS, thereby providing for the
possibility of heterologous oligodendrocyte-to-astrocyte
gap junctions. Although the existence of gap junctions in
the plasma membranes of oligodendrocytes is well docu-
mented in vivo and in vitro [21,54,59,63,86,88,98,102],
establishing the cellular identities and coupling patterns of
oligodendrocyte gap junctions has been both difficult and
controversial. Within recent years however, a variety of
biochemical, immunological, ultrastructural, and physio-
logical techniques have been combined and are beginning
to unravel the Gordian knot of glial coupling.

4.2. Ultrastructural investigations

Initial studies by thin-section transmission electron mi-
croscopy (TEM) reported uniformly close membrane appo-
sitions between oligodendrocytes. These were tentatively
identified as gap junctions based on morphological charac-
teristics and conventional staining methods [12]. These
presumptive gap junctions were most commonly found
between paranodal loops of myelin, but because Brightman
and Reese [12] were unable to demonstrate lanthanum-de-
lined hexagonal arrays, they were careful to note that
these appositions ‘appear to be a variety of gap junction’.

In subsequent studies, oligodendrocyte gap junctions were
often found linked to cell processes that could not be
identified because the areas were so small that they lacked
diagnostic cellular markers [21]. Despite the absence of
positive markers in one or both cells, many images were
interpreted as showing abundant homologous oligodendrocyte-to-oligodendrocyte gap junctions.

At about the same time, freeze-fracture electron microscopy (FF) of oligodendrocyte plasma membranes [59,63,98] revealed distinctive patches of random and hexagonally packed intramembrane particles (IMPs) in the protoplasmic leaflet (P-face), similar to the more regular arrays of IMPs in gap junctions of liver [93]. In the apposing extraplasmic leaflet (E-face), equally distinctive complementary impressions were detected as hexagonal arrays of E-face pits [59,63]. Although Sandri et al. [98] concluded that homologous gap junctions occur between paranodal loops of myelin, Mugnaini [63] and Rash et al. [92] detected only heterologous gap junctions in all other locations in oligodendrocytes, with the second cell always an astrocyte.

It should be noted that oligodendrocytes also contain other classes of tightly clustered IMPs that resemble gap junctions (Fig. 4C), particularly in early replicas showing evidence for surface alteration by water vapor [see Ref. [89]]. Those IMP clusters, unlike the E-face images of gap junctions, have E-face impression containing a mixture of IMPs and pits, thereby differentiating those IMP arrays from gap junctions. Thus, only when the fracture plane passes from P- to E-face within the margin of an IMP array (Fig. 4B,C,E) was it possible to distinguish gap junctions from qualitatively similar IMP clusters [88]. A second point concerning FF analysis is that only rarely are the types of the two cells contributing to a gap junction positively identifiable based on unambiguous criteria [63,88]. In all situations where only one of the cells contributing to a gap junction was visualized, and in all those where criteria for cell identification were insufficient, the cellular elements with which oligodendrocyte gap junctions were formed remained undeterminable and therefore perhaps incorrectly specified.

4.3. Oligodendrocyte gap junctions

Some studies on oligodendrocytes have produced images in which gap junctions were identified by multiple criteria and where both cells forming gap junctions could also be positively identified [59,63,88]. Of more than 100 gap junctions observed on oligodendrocyte somata, their processes, along outer layers of myelin and at the outermost paranodal loop at nodes of Ranvier, all oligodendrocyte gap junctions were found to be heterologous oligodendrocyte-to-astrocyte gap junctions. Notably, where myelin sheaths caused furrowed impressions in the oligodendrocyte somatic plasma membrane, and autologous or homologous coupling were most likely to occur, no gap junctions were found [88]. Thus, homologous or autologous oligodendrocyte-to-oligodendrocyte gap junctions are very rare, but occasionally have been seen [E. Mugnaini, personal communication; see also Ref. [98]]. On the basis of numerous observations that astrocytic processes make gap junctional contacts with oligodendrocytes [63] and the frequency with which they do [81,88], particularly on oligodendrocyte somata (Fig. 4A–C, 4E,F) and at the outer loops along myelinated fibers (Fig. 3D, Fig. 4D), it was suggested that these junctions connect oligodendrocytes into the extensive gap junctionally coupled astrocytic syncytium.

4.4. Oligodendrocytic connexins

Progress has been made in establishing that oligodendrocytes express Cx32 [see Ref. [68]], and although uncertain, it is doubtful that other CNS cell types contain this connexin. Reports of its presence in neurons [66] require ultrastructural confirmation as discussed above. Moreover, its localization at neuronal gap junctions [100,116] likely involved the use of anti-Cx32 antibody that cross-reacted with then unknown connexins as discussed elsewhere [67]. Recently, Cx45 was identified as a second gap junction protein in oligodendrocytes both in vivo and in vitro [22,46], but has not as yet been thoroughly investigated regarding distribution and ultrastructural localization.

In some regions of adult CNS, Cx32 was found in internodal regions of myelin sheaths and at the paranodal loops of myelin [54,83]. It should be noted, however, that the presence of gap junctions between oligodendrocytic paranodal loops in the CNS [98], and the localization of oligodendrocytic connexins at these loops has not been firmly established. By TEM peroxidase immunocytochemistry, oligodendrocyte gap junctions exhibit both symmetric and asymmetric labelling for Cx32 [54]. Given the rarity of oligodendrocyte-to-oligodendrocyte gap junctions, this symmetric labelling may represent autologous coupling between different processes from the same oligodendrocyte, including coupling between paranodal loops of myelin, at Schmidt–Lanterman incisures, and even between oligodendrocyte processes. Asymmetric Cx32 labelling, on the other hand, was found on the oligodendrocyte side of heterologous gap junctions formed between oligodendrocyte and astrocytes [54], or between the former and unidentified processes that were presumed to be astrocytic (Fig. 4C,D). Asymmetric gap junctional labelling has also been described for Cx43 in cases where oligodendrocytic cell bodies or processes contribute the unstained side to junctional appositions with astrocytes [72,73,79]. This was evident at oligodendrocyte somata and at outermost loop of myelin and is consistent with reports that oligodendrocytes commonly form gap junctions with astrocytes. This is also consistent with findings that oligodendrocytes express Cx32 and Cx45 but not Cx43, whereas astrocytes express Cx43 and Cx30 but not Cx32. Freeze-fracture immunogold labelling (FRIL) [90] has further extended the analysis to demonstrate Cx43 in greater than 90% of P- and E-face images of homologous astrocyte gap junctions (Fig. 3E). A similar level of Cx43 labelling was found beneath all large and most small gap junctions E-face
images of oligodendrocyte gap junctions (Fig. 4F), thereby demonstrating that virtually all oligodendrocyte gap junctions involve heterologous coupling with astrocytes. In contrast, Cx43 was never present in the oligodendrocyte side of the gap junction plaque. Instead, under conditions of mild formaldehyde fixation, Cx32 was demonstrable in a small number of the oligodendrocyte gap junction plaques [91], both on oligodendrocyte somata (Fig. 4C) and the outer tongue of myelin (Fig. 4D).

4.5. Connexins at heterotypic astro/oligo gap junctions

The expression of Cx30 and Cx43 in astrocytes and Cx32 and Cx45 in oligodendrocytes raises the possible combinations of heterotypic connexin (Cx) pairings at glial gap junctions to include: inter-astrocytic gap junctional channels composed of Cx30/Cx30, Cx43/Cx43 or Cx30/Cx43; inter-oligodendrocytic channels composed of Cx32/Cx32, Cx45/Cx45 or Cx32/Cx45; and heterologous astrocytic–oligodendrocytic channels consisting of four combinations with either or both Cx30 and Cx43 on the astrocytic side and either or both Cx32 or Cx45 on the oligodendrocytic side. The combinations that occur depend on the connexins present in individual junctional plaques and on the pairs of connexins that are able to form functional channels. Since it has been reported that Cx43/Cx32 as well as Cx45/Cx32 pairings are nonpermissive for channel formation [25,110], it seems that oligodendrocytic Cx32 must form channels with astrocytic Cx30, which is permissive [20,110]. Further, since Cx30 and Cx43 are incorporated to various degrees at virtually all astrocytic gap junctions in gray matter [73,80,114,115], it appears that both of these connexins contribute to the formation of not only inter-astrocytic, but also astro-oligodendrocytic junctions in either a homotypic or heterotypic manner. Since astrocytic Cx43 is usually present in astrocyte-to-oligodendrocyte gap junctions (Fig. 4F), and since Cx43 cannot couple with Cx32 of the oligodendrocyte, we conclude that Cx43 couples with a permissive connexin, possibly Cx45. Finally, astrocyte gap junctions in major white matter tracts contain Cx43 but not Cx30 [73], suggesting that oligo-astrocytic gap junctions in white matter arise by pairing astrocytic Cx43 with oligodendrocytic Cx45 [22,46]. A corollary of these conclusions is that in the absence of Cx30 in astrocytes of major fiber tracts, Cx32 in oligodendrocytes at these locations has no currently known functional partner with which it can pair in white matter other than itself. This could suggest that oligodendrocytes in white matter engage only in homologous or autologous gap junctions composed of homotypic Cx32/Cx32 pairings. Alternatively, as previously suggested [79] in the case of astrocytes in the spinal cord, white matter astrocytes may express as yet another unidentified connexin with which Cx32 might form heterologous junctions.

5. Functional considerations

A number of studies have indicated the existence of gap junctional coupling between cultured oligodendrocytes [41–43,59,60,89,94]. However, inter-oligodendrocytic gap junctions were few in vitro, and coupling was often found to be weak. These reports have been widely cited as

Fig. 5. Immunofluorescence localization of Cx32 in oligodendrocytes of mouse brain. (A,B) Micrographs of the cerebellar granule cell layer showing cells double-labelled for Cx32 (A) and the oligodendrocyte marker cyclic nucleotide 3’-phosphodiesterase (CNPase) (B). Corresponding arrows indicate Cx32-positive cells that are also CNPase-positive. (C) Cx32-positive cells extending several processes (arrows) that contact labelled fibers. Magnifications: (A), (B), ×560; (C), ×500. Reprinted from Li et al. (Ref. [54], p. 571), with permission from Wiley-Liss.
showing that homologous gap junctions can occur between adjacent oligodendrocytes, but that these cells have minimal coupling relative to that of astrocytes. However, another possibility is that oligodendrocyte coupling is minimal in pure culture in the absence of astrocyte intermediaries, or that cultures lack other cell types that may contribute to maturation of coupling [59]. Analyses conducted in situ have indicated some coupling between these cells in gray matter, but little or none between those in white matter [6, 83]. Thus, in a manner consistent with regional heterogeneity in their levels of Cx32 expression [54], oligodendrocytes appear to exhibit regional difference in gap junctional coupling, which could reflect different phenotypic populations or, alternatively, regulation of their connexin expression by factors that ultimately determine their functional needs for GJIC with neighbors at various locations in the CNS.

Of interest in view of the above is the reported localization of Cx32 along subpopulations of myelinated fibers [54], as in the case of the cerebellar cortex where it appears to be associated only with myelin sheaths of axons arising from Purkinje cells (Figs. 5 and 6). Further, Cx32 was sparse in some white matter regions, but concentrated along individual fibers or small bundles passing through gray matter. On the basis of this, it was suggested that presumptive oligodendrocyte subtypes expressing high Cx32 levels may myelinate particular classes of axons or, alternatively, oligodendrocytes as a uniform population may be induced to express different levels of Cx32 depending on the axons they myelinate [54]. In either case, the presence of oligodendrocytic Cx32 along myelinated fibers is consistent with evidence for many small gap junctions between astrocytic processes and the outermost layer of myelin [88]. These junctions may be important for maintaining ion homeostasis during axonal activity. For example, ions accumulating in paranodal loops at nodes of Ranvier may collect in oligodendrocytic cytoplasm at the outer surface of myelin near internodes and then move into astrocytic processes via gap junctions. A contribution of Cx32 to such communication pathways might explain its presence along particular fiber types having potentially greater demands for ion redistribution.

More generally, based on recent freeze-fracture analyses showing that oligodendrocytes are nearly exclusively coupled to astrocytes allowing the creation of a panglial syncytium [88], it might be speculated that extensive as-

Fig. 6. Pairs of immunofluorescence micrographs of mouse brain sections showing double-labelling for Cx32 (A,B) and CNPase (B,D) in the cerebellar granule cell layer. Individual fibers are labelled for both Cx32 (A) and CNPase (B) (corresponding arrows), but not all CNPase-positive fibers (D) appear to immunopositive for Cx32 (C). ML, molecular layer; Pcl, Purkinje cell layer, gcl, granule cell layer. Magnifications: (A), (B), ×410; (C), (D), ×140. Reprinted with permission as in Fig. 5.
tro/oligo coupling may enable astrocytes to provide oligodendrocytes with metabolic constituents that may be needed to support the huge territorial expanse of their processes. The importance of this coupling in the CNS is indicated by observations that individuals with Charcot–Marie–Tooth type X1 (CMTX) syndrome, which is a peripheral neuropathy caused by mutations in the Cx32 gene [7], have CNS symptoms of this disease [2]. How disruption of CNS function occurs in CMTX and how glial gap junctions contribute to normal function presumably depends on the nature of these junctions at specific cellular loci, which ultimately has important implications for the formation of communication compartments within the glial syncytium. Thus, depending on selectivity of ion and metabolite passage through glial connexin channels, capability of various glial connexins to form functional heterotypic channels, and heterogeneity of connexin expression even by a particular glial cell type, it is likely that different operationally defined cellular and subcellular compartments may emerge in neural tissue.

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References

[28] M.O.K. Enkvist, K.D. McCarthy, Astroglial gap junction communication is increased by treatment with either glutamate or high K⁺ concentration, J. Neurochem. 62 (1994) 489–495.


H. Kettenmann, B.R. Ransom, Electrical coupling between astrocytes and between oligodendrocytes studied in mammalian cell cultures, Glia 1 (1988) 64–73.


[104] L. Venance, D. Piomelli, J. Glowinski, C. Giaume, Inhibition by


