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Mini-review

Myelin peroxisomes – Essential organelles for the maintenance of white matter in the nervous system☆

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ABSTRACT

Peroxisomes are cellular compartments primarily associated with lipid metabolism. Most cell types, including nervous system cells, harbor several hundred of these organelles. The importance of peroxisomes for central nervous system white matter is evidenced by a variety of human peroxisomal disorders with neurological impairment frequently involving the white matter. Moreover, the most frequent childhood white matter disease, X-linked adrenoleukodystrophy, is a peroxisomal disorder. During the past decade advances in imaging techniques have enabled the identification of peroxisomes within the myelin sheath, especially close to nodes of Ranvier. Although the function of myelin peroxisomes is not solved yet on molecular level, recently acquired knowledge suggests a central role for these organelles in axo-glial metabolism. This review focuses on the biology of myelin peroxisomes as well as on the pathology of myelin and myelinated axons that is observed as a consequence of partial or complete peroxisomal dysfunction in the brain.

1. Introduction

In the nervous system axons are electrically insulated by lipid-rich myelin sheaths that concentrically enwrap axonal segments. These are formed by oligodendrocyte processes in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system (PNS). Myelinated segments are interrupted by nodes of Ranvier, a region where axonal sodium channels are clustered to allow saltatory impulse propagation, i.e. the fast and energy-saving conduction of action potentials between nodes [1]. In addition accumulating evidence suggests that apart from insulating axons, myelin seems to serve metabolic support functions linked to axonal energy and lipid metabolism [2–5].

Myelinated axon bundles represent the white matter tracts of brain and spinal cord. In certain disease situations regions of CNS white matter are demyelinated and the underlying axons often degenerate leading to permanent neurological disability [6]. The origin of white matter diseases can be multiple and is often directly linked to mutations of myelin proteins, to viral infections, to an immune system failure as in multiple sclerosis, or to metabolic dysfunctions [7]. One example of metabolic impairment is the peroxisomal disorder X-linked adrenoleukodystrophy (X-ALD). This hereditary disease is the most frequent leukodystrophy in children. On the genetic level X-ALD is characterized by loss-of-function mutations of the peroxisomal membrane transporter ABCD1 [8]. Interestingly, many peroxisomal diseases are associated with white matter abnormalities, stressing the role of peroxisomal metabolism for the nervous system and in particular for white matter [9].

Peroxisomes are a divers population of organelles, originally defined by the presence of catalase and oxidases in the peroxisomal lumen [10]. Proteins involved in the biosynthesis of peroxisomes are called peroxins (PEX) [11]. The protein content and function of these organelles can vary between cell types and depends on the overall metabolic demands. Hence, multiple endogenous and exogenous factors such as cell-cycle status and environmental conditions can influence peroxisomal turnover, constitution, and function [12]. Peroxisomes and mitochondria perform β-oxidation of fatty acids. However, substrate-specificity differs between the two organelles. Exclusive substrates of peroxisomal β-oxidation include very long chain fatty acids (VLCFA), pristanic acid, long-chain dicarboxylic acids, certain eicosanoids and polyunsaturated fatty acids (PUFAs) [13,14]. Also biosynthesis of plasmalogens, a group of ether-phospholipids that is highly enriched in myelin membranes, occurs exclusively in peroxisomes [15]. In addition a role for peroxisomes in cholesterol biosynthesis, the major lipid component of myelin [16], is controversially discussed [17,18].

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It is likely that peroxisomes contribute to the enormous lipid metabolism of myelinating cells, whose multi-layered membranes consist of 70% lipids with a characteristic composition [19,20]. Furthermore, it is plausible that such high lipid content makes this tissue especially vulnerable to a defect in lipid metabolism. However, the exact functions of peroxisomes for myelinating glia and the axons of white matter are not well understood. It is thus important to increase the knowledge on peroxisomal biology of myelinating cells and its possible participation in a joined axo-glial metabolism.

2. Peroxisomes in the nervous system

Only in the late 1960's peroxisomes were observed in other tissues than kidney and liver. At that time the organelles were identified in all analyzed nucleated vertebrate cell types. It became clear that peroxisomal morphology and cytochemistry are highly variable between the different tissues [21]. A few years later nervous system peroxisomes were analyzed for the first time in detail by the diaminobenzidine (DAB) cytochemical method for catalase detection on epon-embedded electron microscopy material [22]. Notably, it was revealed that peroxisomal size in the brain is much smaller than in other tissues, especially than in hepatocytes. This has led to the term "microperoxisomes".

2.1. Subcellular localization

Developmental studies showed numerous microperoxisomes in the CNS during the first two postnatal weeks, which were less frequent or almost entirely absent from some regions at later stages [23]. The increasing abundance of microperoxisomes measured by catalase activity was observed to coincide with the onset and the peak of myelination. Hence, Adamo and colleagues speculated on a relationship between myelination and peroxisomes, in particular regarding plasmalogen biosynthesis [24].

Similar to earlier studies it was confirmed that the vast majority of peroxisomes is localized in the cell soma of nervous system cells [25]. However, peroxisomes were observed frequently in cellular processes of astrocytes, oligodendrocytes, microglia, and Schwann cells ([2,26] and unpublished observations). In astrocytes peroxisomes (200 nm in diameter) were localized in cellular processes including the end-feet [25]. Schwann cells, the myelinating glia of the PNS, contained numerous small peroxisomes (150 nm) [22]. Microperoxisomes were also demonstrated to reside in oligodendroglia, the myelinating cells of the CNS. They were also found in the processes of oligodendrocytes adjacent to the outer cytoplasmic mesaxon of myelin [22]. Recent immunohistochemical work revealed the presence of multiple peroxisomes in myelinated fibers of white and gray matter, which in the majority of cases seemed to reside within myelin, not or only rarely in axons (Fig. 1A) [26]. Peroxisomes were abundant in all cytoplasmic regions of myelin, i.e. the innermost (adaxonal) and outermost (abaxonal) tongue of the sheaths, as well as specialized cytoplasmic channels of peripheral myelin, the Schmidt–Lanterman incisures (Fig. 1B) [26]. They accumulated in the vicinity of nodes of Ranvier, which are regions of close axo-glial interaction.

In neurons the organelles were mainly detected in perikarya, some in dendrites, but rarely within axons shown by use of the DAB catalase staining method [22]. A recent immunohistochemical study by Ahlemeyer and colleagues confirmed this peroxisomal localization pattern in neurons [27]. In accordance with the observed rare localization of peroxisomes in axons, synaptic terminals of the spinal cord were devoid of the organelles [22]. Synapses displayed presence of peroxisomes during early development [22], and presence of axonal peroxisomes was revealed in cultured neurons [28,29].

The *in vivo* lack or low abundance of peroxisomes in CNS axons of adult mice has been described by three independent research groups [22,26,27]. Axonal transport of peroxisomes was demonstrated to be blocked by tau overexpression in a primary neuronal culture system [30]. This microtubule-associated protein is differentially expressed in development [31]. Notably, the level of tau is higher in myelinated than in unmyelinated axons [32]. However, a possible impact of tau expression level on the *in vivo* transport of axonal peroxisomes has not been studied so far.

The distribution of peroxisomes in myelinated fibers leads to speculate on a function of myelin peroxisomes for their myelinated axons. This hypothesis has similarly been phrased already by Holtzman and colleagues in 1973, who proposed an "involvement of the organelles in such thing as the metabolic interrelations of neurons and glial or satellite cells" [22].

2.2. Peroxisomal diversity

Co-localization analysis concentrating on peroxisomal diversity between different nervous system cell types was performed on mouse brain sections and in cultured brain cells. The use of antibodies recognizing peroxisomal membrane proteins and enzymes

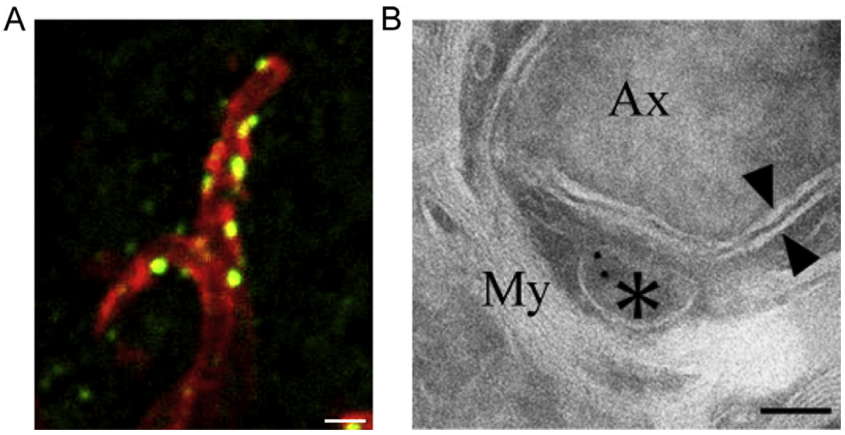

Fig. 1. Peroxisomal localization in myelin of wild-type mice. (A) Two-photon image of cerebral white matter showing the co-localization of peroxisomes stained for peroxisomal membrane protein 70 (PMP70, in green) with cytoplasmic myelin (CNP, in red). (B) Electron micrograph of a callosal fiber after immuno-gold labeling for catalase (indicated by black dots) confirms a peroxisome (asterisk) within the adaxonal mesaxon.

revealed divers peroxisomal populations in the different cell types [27]. This great heterogeneity of peroxisomal proteins possibly reflects a differing cell type-specific spectrum of organelle functions. In oligodendrocytes a much higher content of catalase than in other brain cells was detected [27]. This high peroxisomal catalase content in oligodendrocytes may account for the finding that peroxisomes seemed several times more abundant in oligodendrocytes than in neurons or astrocytes when the catalase based DAB staining method was applied [25].

Recently, it was shown that the peroxisomal protein composition found in myelin-enriched fractions differs biochemically from the constituency of average brain peroxisomes [26]. Histological data by Ahlemeyer and coworkers has demonstrated high expression of peroxin 14 (PEX14) in neurons and astrocytes, but less in oligodendrocytes. Similarly, it was shown that myelin fractions contained only minor amounts of PEX5 and PEX14, the PTS1 receptor and its corresponding docking protein [26]. Instead the myelin fraction contained more PMP70 and was extremely enriched in PEX11b, a peroxin mediating peroxisomal division ([26] and unpublished observations). The prominent presence of this peroxisomal division factor in myelin fractions suggests a high division rate for myelin peroxisomes and is consistent with the finding of extremely small-sized myelin peroxisomes of only 50–100 nm in diameter [26].

3. Peroxisomal dysfunction in humans – CNS and PNS pathology

Peroxisomal disorders can be caused by mutations of peroxins (PEX proteins). Some peroxins are essential for peroxisomal assembly. Therefore loss of such an essential peroxisome biogenesis factor leads to absence of virtually all organelle functions. These disorders are referred to as peroxisomal biogenesis disorders (PBD) and account for the most severe clinical phenotypes of peroxisomal disorders belonging to the Zellweger spectrum [33]. In other cases only a single enzymatic function or a single transporter protein of the peroxisomal membrane are affected. According to Powers and Moser all peroxisomal disorders with neurologic involvement may show abnormalities of white matter in the CNS [9].

X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative peroxisomal disorder caused by mutations of ABCD1, a peroxisomal transporter protein [8]. ABCD1 is involved in the import of very long chain fatty acids (VLCFA) for subsequent peroxisomal degradation [34,35]. Consequently, VLCFA accumulate in tissues and body fluids of X-ALD patients. In the nervous system the greatest excess of VLCFA is found in gangliosides, phosphatidylcholine, myelin-specific proteolipid protein (PLP), and as cholesterylesters [9]. It is a matter of extensive debate, whether or not the level of VLCFA accumulation determines disease severity [36–38].

The neurodegenerative phenotype of X-ALD in most cases manifests as one of two main clinical forms: The cerebral form of X-ALD (CALD) is characterized by white matter abnormalities. White matter of the cerebrum and to a lesser extent of the cerebellum are involved. Onset of demyelination is usually not before four years of age. Approximately in two-third of the cases the lesion appears in the parietooccipital region, progressing symmetrically towards the frontal and temporal lobes. In one-third the lesion starts frontally and progresses in caudal direction [38]. The spinal cord can either remain unaffected or demonstrates bilateral white matter corticospinal tract degeneration. In contrast to the cerebral demyelination, PNS myelin of most cerebral X-ALD patients is only affected to minor extent [9]. This is indicated by electrophysiology measurements of CALD patients. Marked decrease of compound muscle action potentials (CMAP), but only mild reductions in the nerve conduction velocity were found (NCV) [39]. These studies suggest a predominant neuronal or axonal loss in the PNS of cerebral X-ALD rather than demyelination. However, ultra-structural investigation of the peripheral nerves showed a cellular pathology in myelinating cells, i.e. abnormal cytoplasmic inclusions in Schwann cells, but not in neurons [40].

Adrenomyeloneuropathy (AMN) is the most common clinical form of X-ALD with minor cerebral involvement, but degeneration of spinal ascending and descending tracts. Particularly the cervical fasciculus gracilis and the lumbar lateral corticospinal tracts show comparable loss of myelin and axons [9,41]. Results of PNS electrophysiology measurements obtained from AMN patients were similar to findings of CALD patients. In the majority of 23 cases CMAP was reduced but only mild effects on NCV were detected [42,43].

CNS inflammation with massive gliosis in white matter lesions, involvement of perivascular B cells, and invasion of CD4+ and CD8+ T cells is a hallmark of CALD, which is absent in the neurodegeneration of pure AMN patients [44,45]. This has led to speculate on an immune-mediated pathogenesis that is responsible for the severe demyelination in CALD, in contrast to the axonopathy observed in AMN. When peroxisomes are impaired, myelin lipid turnover might trigger the accumulation of lipids, e.g. inflammatory mediators such as eicosanoids, which may actively participate in a self-perpetuating process of demyelination [46]. However, so far all attempts to arrest or prevent CNS demyelination in CALD using immunosuppressive therapies have failed [47,48]. It is necessary to establish in the future, if the invasion of lymphocytes is helpful, harmful, or merely bystander of the disease. Interestingly, bone-marrow transplantation therapy in CALD patients can abrogate disease progression [49,50]. After transplantation healthy donor hematopoietic stem cells can give rise to macrophages that populate the brain in CALD [51,52]. Thus, the successful bone-marrow therapy supports a role for microglial peroxisomes in preventing cerebral demyelination in X-ALD. The importance of microglia in CALD is also stressed by the observation that these cells are lacking in the perilesional zone, which is surrounded by clusters of activated and apoptotic microglia [53]. Compatible with these findings chronic neuroinflammation with early activation of the innate immune system has been shown in mice lacking peroxisome function in the brain [54].

Before it was possible to assign a specific genetic defect to hereditary diseases, additional peroxisomal disorders were named neonatal or pseudoneonatal adrenoleukodystrophy (NALD) according to phenotypic similarities with X-ALD. When scientific progress enabled the identification of underlying genetic defects it became apparent that mutations of several peroxisomal genes can cause NALD: Two enzymes of the peroxisomal β-oxidation pathway were identified, acyl-CoA oxidase (ACOX1) and HSD17B4 (also known as multifunctional protein 2; MFP2 or D-bifunctional protein; DBP), as well as genes coding for peroxisomal biogenesis factors (*Pex1*, *Pex5*, *Pex12*, and *Pex6*, *Pex10*, *Pex13*) [9,55]. Mutations in one of these PEX proteins has dramatic effects on peroxisome biogenesis leading to a generalized dysfunction of peroxisomes and frequently to the most severe phenotype of peroxisomal disorders, the Zellweger Syndrome (ZS). Severely impaired peroxisome biogenesis can also result in a slightly milder clinical phenotype with pronounced adrenal gland insufficiency and white matter abnormalities, such that a close connection to the original X-ALD disease is evident. Vice versa, inhibition of the peroxisomal β-oxidation pathway can have similar effects and even cause a ZS-like phenotype. It is thus tempting to speculate that impairment of peroxisomal β-oxidation, originating from deficiency of either ABCD1, ACOX1, or HSD17B4, results in loss of additional peroxisomal functions resembling a generalized organelle defect as in PBD (see below).

4. Dysfunction of myelin peroxisomes – lessons from mouse mutants

To investigate the pathomechanism of X-ALD, mice lacking the homologous *Abcd1* gene were generated in three independent laboratories [56–58]. Initial analysis of these had shown the accumulation of VLCFA, a biochemical hallmark of X-ALD, but no signs of neurodegeneration up to the age of 12 months were detected. In 2002 a late-onset myeloneuropathy in ABCD1-deficient mice was demonstrated. Spinal cord and peripheral nerves were involved starting at 15 months of age [59]. Thus, these mice are a suitable model of AMN. Further analysis of this mouse model revealed involvement of oxidative stress and mitochondrial failure early in the disease pathology (reviewed in Ref. [60]). While these *Abcd1*-deficient mice fail to show cerebral neurodegeneration, conditional knockout mice (referred to as *Cnp-Pex5* mice) lacking peroxisomal function in myelinating cells exhibited a cerebral phenotype. In these mice the essential peroxisomal biogenesis factor *Pex5* was disrupted in a cell type-specific fashion [2]. Despite the lack of peroxisomal function in oligodendroglia, mice displayed normal myelination on the ultra-structural level. However, when myelin was biochemically analyzed, highly elevated VLCFA and severely reduced plasmalogen levels were revealed. Between three and five months of age slight gait abnormalities became apparent. At the same time a demyelinating lesion was illustrated by magnetic resonance imaging (MRI). Brain images displayed hyperintense signals in the frontal subcortical white matter, the genu of corpus callosum. The lesion progressed symmetrically in rostrocaudal direction (Fig. 2A) [2]. On the cellular level microglia were early participants, which massively invaded white matter before obvious demyelination occurred. Perivascular B cells and T lymphocytes mainly of the CD8+ pool were recruited to regions undergoing demyelination. This neuropathology seen in *Cnp-Pex5* mice is in many aspects reminiscent of cerebral X-ALD. It is therefore possible that also in cerebral X-ALD especially myelin peroxisomes acquire additional defects that trigger inflammatory demyelination. Remarkably, *Cnp-Pex5* animals, engineered with a primary defect in oligodendrocytes but normal peroxisomes in other cell types of the brain, display axonal swellings in the CNS before demyelination can be detected. Similar observations were made in the peripheral nervous system of these mice, where paranodal and axonal swellings were found without obvious de- or dysmyelination even in aged animals (Fig. 2B [26]; and unpublished observations). Hence, an axonal pathology can precede myelin abnormalities, although the original metabolic dysfunction is restricted to myelinating cells.

The discrepancy between CNS and PNS seen in *Cnp-Pex5* mice is remarkable, showing a severe central but no apparent peripheral demyelination. This suggests that myelin peroxisomes support myelin maintenance especially in the CNS. In contrast, support of axonal function might be a feature of myelin peroxisomes in both, the central and the peripheral nervous system. Further, since formation of myelin in *Cnp-Pex5* mice is normal, it seems that myelination can progress independent of myelin peroxisomes. However, residual peroxisomal activity in oligodendrocytes of *Cnp-Pex5* mice that is sufficient for myelination cannot be ruled out. Secondly, neighboring cells with intact peroxisomes are likely candidates to provide peroxisomal metabolites for myelin formation. The ability of the brain to adapt to a cell type specific dysfunction by enhancing intercellular transfer of lipids, such as cholesterol, has been demonstrated a few years ago [61].

Horizontal transfer of peroxisomal substrates was also suggested when comparing *Cnp-Pex5* mice with two additional *Pex5* knockout mutants: Mice with astroglial PEX5-deficiency (*Gfap-Pex5* mutants) showed biochemical alterations of myelin [36,62]. CRE-expression driven by the *Nestin*-promoter mediated the ablation of peroxisomal function in neural cells. *Pex5* was targeted in a subset of neurons, in astrocytes, and in oligodendrocytes but not in

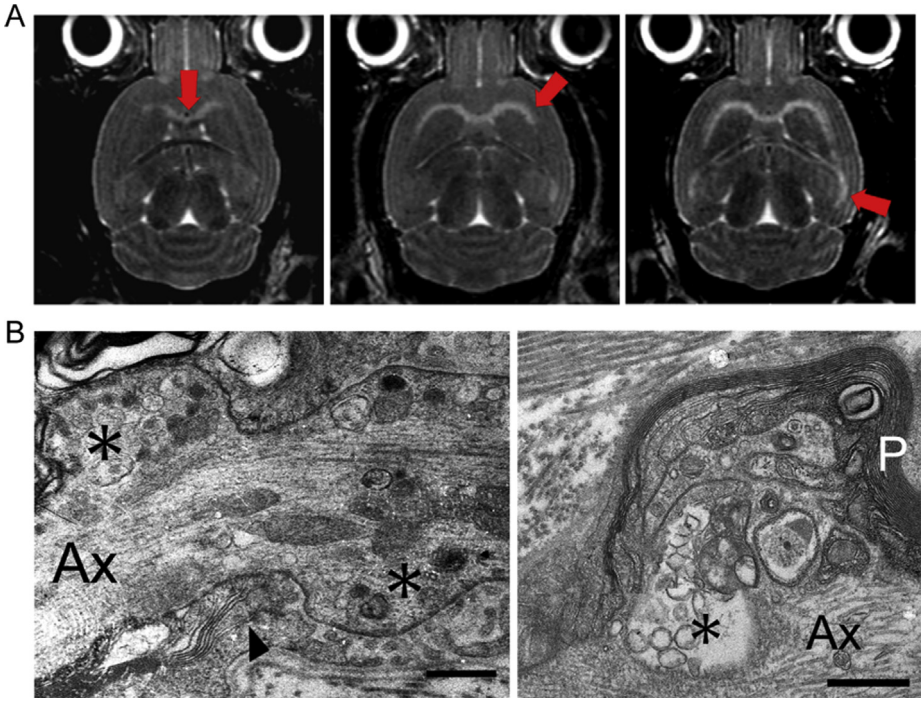

Fig. 2. CNS and PNS pathology caused by dysfunction of myelin peroxisomes. (A) Magnetic resonance images of a mouse brain display the progression of hyperintense signals (red arrows) in rostrocaudal direction. The same *Cnp-Pex5* mutant mouse was monitored at 4 (left), 6 (middle), and 8 (right) months. (B) Electron micrographs show axonal (left) and paranodal (right) swellings in the PNS of *Cnp-Pex5* mice.

microglia [63]. In contrast to *Cnp-Pex5* mice, *Nes-Pex5* mice displayed neurodevelopmental abnormalities, developed motor impairments already at 3 weeks of age, and died before 6 months. Remarkably, in this mouse mutant the axonal pathology was observed on other axonal segments than demyelination. In accordance with findings of *Cnp-Pex5* mice, the authors concluded that peroxisomes might have a dual function in the nervous system, a direct role for axons and one for myelin [36,63].

Additional mouse models of human peroxisomal diseases, including neonatal adrenoleukodystrophy (NALD), have been generated. The cause of NALD is frequently a mutation in one of several peroxins. In addition impaired peroxisomal β-oxidation by mutations of peroxisomal enzymes ACOX1 or HSD17B4 can elicit an NALD phenoytpe. Astonishingly, signs of neurodegeneration were absent from ACOX1 knockout mice up to the age of approximately 6 months, when the animals died prematurely likely due to a liver failure [64]. Later also the *Hsd17b4* gene was ablated in mice, which caused a severe brain pathology [36,65]. These mice displayed severe coordination problems and massive neuroinflammation, which was less severe but still present in mice lacking HSD17B4 specifically in neural cells (*Nestin-Hsd17b4* mice) [66]. Together, these findings may suggest that peroxisomal β-oxidation is the major function of peroxisomes in the nervous system. Alternatively, impairment of peroxisomal β-oxidation may induce a secondary defect in peroxisomes, i.e. an additional loss of peroxisomal functions. Thus, peroxisomal biogenesis disorder phenotypes can be mirrored in mice and men when peroxisomal β-oxidation is damaged, and vice versa.

5. Axonal support by myelin peroxisomes – central players in axo-glial metabolism?

The nature of an axonal support function that could be provided by myelin peroxisomes remains elusive. Pieces of information are available on the distribution of peroxisomes in the nervous system, on general metabolic functions of peroxisomes, and on pathological features seen in conditional mouse mutants. This knowledge on peroxisomal biology prompts to speculate on possible subcellular mechanisms that may be involved in axonal support. Myelinated axons themselves harbor peroxisomes only rarely. However, since peroxisomes are abundant within adaxonal myelin and accumulate close to nodes of Ranvier, they are ideally positioned to support axonal metabolism. Such a supportive role is likely associated with lipid turnover and with axonal energy metabolism. In the following, three possible functions will be illustrated.

5.1. Turnover of axonal membrane constituents

Membranes contain lipid components that require peroxisomes for degradation. Other membrane lipids are exclusively synthesized in peroxisomes, like for example docosahexaenoic acid (C22:6), which constitutes a major component of excitable membranes [67]. Thus, a reasonable question arises: How do axons accomplish the turnover of exclusive peroxisomal metabolites, if peroxisomes are absent? In this case it would be beneficial for the organism to transfer molecules between axonal compartment and myelin peroxisomes. Otherwise, movement of peroxisomal metabolites would require energy dependent retrograde and anterograde transport between synapses and neuronal perikarya, where neuronal peroxisomes are located. Considering the lengths of axons, the shortcut between axons and myelin peroxisomes would likely be an energy saving solution to the problem.

Experiments showing the localization of axon-derived molecules in Schwann cells have been performed several years ago [68]. After injecting fluorescent tracers, axonal transport and sequestration thereof by Schwann cells were observed. Uptake occurred specifically in paranodal areas, far away from the injection sites. A

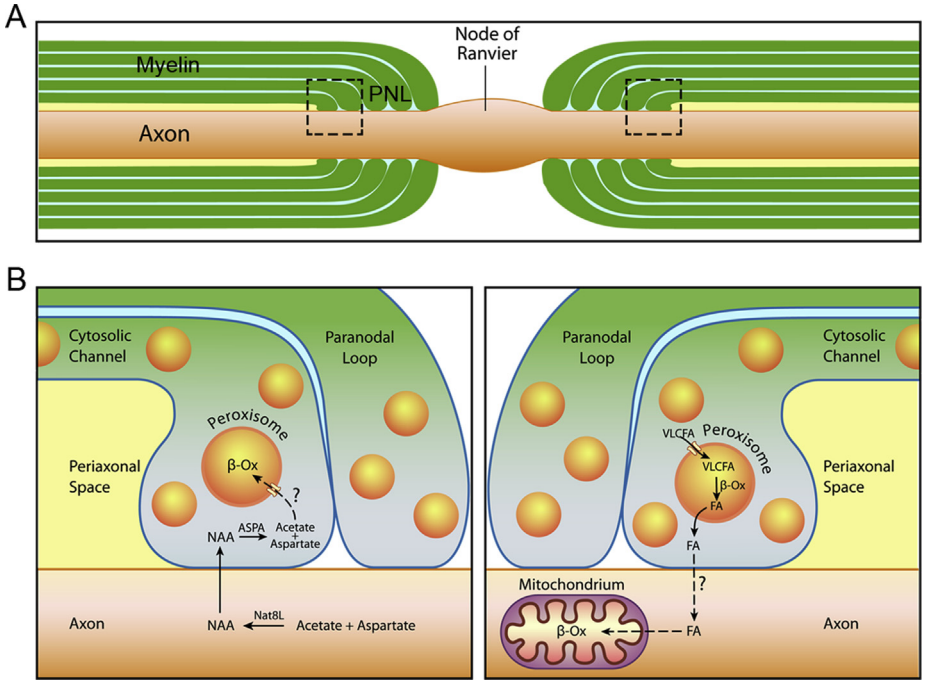

Fig. 3. Hypothetical model of axonal support by myelin peroxisomes. (A) Schematic view of two adjacent myelinated axonal segments, separated by a node of Ranvier. The node is flanked by paranodal regions, which are defined by the presence of paranodal loops (PNL). (B) Two possible axonal support functions of myelin peroxisomes (orange) are illustrated by enlarged view of the axon and two paranodal loops (inset from A). Left: Neuronal N-acetyltransferase 8-like (NAT8L) produces N-acetylaspartate (NAA), which is transferred to the glial compartment. Oligodendroglial aspartoacylase (ASPA) mediates the reverse reaction producing acetate and -aspartate. It is hypothesized (indicated by a question mark) that peroxisomes may take up acetate moieties derived from axonal NAA. Right: Peroxisomal β-oxidation might modify fatty acids thereby producing suitable substrates for mitochondrial β-oxidation. Very long chain fatty acids (VLCFA) may be chain shortened by peroxisomal metabolism. The resulting fatty acids may be transported to axonal mitochondria by a carnitine dependent mechanism.

similar intercellular transport route of molecules might also be responsible for the exchange of peroxisomal metabolites between myelin and axons. In addition, such a shuttling mechanism between axons and myelin would also be consistent with the accumulation of vesicles in the paranodal region of *Cnp-Pex5* mutants and in other dying-back axonopathies, as suggested earlier [26,69]. The intriguing question remains about possible advantages of such a complex shuttling mechanism over axons possessing own peroxisomes.

5.2. Synthesis of myelin lipids

Although myelin with reduced plasmalogen and elevated VLCFA levels can initially be formed in the CNS of mutants lacking myelin peroxisomes, myelin cannot be maintained in brains of adult mice [2]. Hence, an important issue is to clarify whether peroxisomes could contribute to myelin turnover by additional, yet unknown lipid synthesis pathways. Several years ago, experiments with ^{14}C-labeled N-acetylaspartate (NAA) suggested the incorporation of acetate derived from neuronal NAA into myelin lipids [70]. NAA is a major amino acid derivate of the mammalian brain. This molecule is generated by the neuronal enzyme N-acetyltransferase 8-like (NAT8L) and delivered to oligodendrocytes. Aspartoacylase (ASPA), which is an exclusive oligodendroglial enzyme, degrades NAA into its acetate and aspartate moieties [71]. A peroxisome dependent acetate metabolism for lipid synthesis has been considered earlier as a result of experiments in plants [72]. Also, other experiments in fungi supported a peroxisomal function in acetate metabolism [73]. Remarkably, if comatose, the Arabidopsis homolog of the *Abcd1* gene is lacking, the seedlings' acetate metabolism is compromised [74]. This obliges to ask, whether mammalian ABCD1 could have a function in acetate metabolism as well (Fig. 3B, left).

5.3. Support of axonal energy metabolism

Absence of functional peroxisomes from myelin forming glia leads to APP+ swellings [2,26], a pathology that is interpreted as lack of energy for axonal transport [75]. Peroxisomes are capable of performing β-oxidation of fatty acids that is probably involved in cellular energy production, e.g. by mobilization of storage lipids [76–78]. Hence, myelin peroxisomes might support axons by delivery of energy-rich metabolites. Fatty acids with chain lengths that are suitable for mitochondrial β-oxidation could be interesting candidates. Such fatty acids could for example derive from peroxisomal β-oxidation of VLCFA that require chain shortening to be accepted as substrates by mitochondrial β-oxidation. Subsequent binding to L-carnitine could possibly enable the transport from myelin peroxisomes to axonal mitochondria through high affinity carnitine-transporters. These transporters seem to be present in all membranes that have to be passed to allow such a transport route [79–81]. The observed accumulation of neuronal L-carnitine, which cannot be explained so far, would be consistent with this hypothesis [82]. In addition, a cluster of genes that is tied to lipid energy metabolism is highly expressed in the adult mouse sciatic nerves [83]. It is thus tempting to imagine myelin lipids as an energy storage reservoir for the brain that can be mobilized by myelin peroxisomes (Fig. 3B, right).

6. Conclusions

Shortly after peroxisomes were observed in the nervous system by electron microscopy, it was speculated that they play an important role in white matter. This conclusion is supported by the identification of human peroxisomal disorders that are frequently associated with white matter abnormalities. Furthermore, genetic manipulation and analysis of mutant mice have confirmed the importance of peroxisomes for myelin maintenance and for the integrity of myelinated axons: Analysis of *Cnp-Pex5* mice has demonstrated that myelin peroxisomes seem to serve a direct axonal support function, in both CNS and PNS. In contrast, myelin peroxisomes are important for myelin maintenance primarily in the CNS, which exhibits severe demyelination, while PNS myelin is spared when myelin peroxisomes are impaired. On the molecular level peroxisomal functions that are required to prevent neurodegeneration remain elusive. Therefore, one major future task is to better characterize the cell type specific heterogeneity of the peroxisomal populations in the nervous system.

The term "adrenoleukodystrophy" encompasses a heterogeneous group of peroxisomal disorders. ALD might originate from a defect in peroxisomal biogenesis that affects all peroxisomal functions. Alternatively, ALD can be caused by defects in a single protein (i.e. ABCD1, ACOX1, or HSD17B4) leading to impairment of peroxisomal β-oxidation. Thus, interfering with peroxisomal β-oxidation can lead to clinical symptoms that phenocopy the complete loss of peroxisomal functions, and vice versa. This is further illustrated by the CALD-like phenotype in mice with complete loss of peroxisomal function in myelinating glia.

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