Neurosci Bull December 1, 2014, 30(6): 956–966. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1437-5

·Review·

The myelin membrane-associated enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase: on a highway to structure and function

Arne Raasakka[1], Petri Kursula[1,2]

[1]*Department of Biochemistry and Biocenter Oulu, University of Oulu, Oulu, Finland*

[2]*Department of Chemistry, University of Hamburg, Hamburg, Germany*

Corresponding author: Petri Kursula. E-mail: petri.kursula@oulu.fi

The membrane-anchored myelin enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) was discovered in the early 1960s and has since then troubled scientists with its peculiar catalytic activity and high expression levels in the central nervous system. Despite decades of research, the actual physiological relevance of CNPase has only recently begun to unravel. In addition to a role in myelination, CNPase is also involved in local adenosine production in traumatic brain injury and possibly has a regulatory function in mitochondrial membrane permeabilization. Although research focusing on the CNPase phosphodiesterase activity has been helpful, several open questions concerning the protein function *in vivo* remain unanswered. This review is focused on past research on CNPase, especially in the fields of structural biology and enzymology, and outlines the current understanding regarding the biochemical and physiological significance of CNPase, providing ideas and directions for future research.

Keywords: 2′,3′-cyclic nucleotide 3′-phosphodiesterase; calmodulin; central nervous system; cytoskeleton; myelin proteins; RNA

Introduction

Myelin is a specialized multilamellar structure, which is wrapped around neuronal axons by oligodendrocytes in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system (PNS). The membrane sheets of myelin are densely packed, resulting in the extrusion of cytosolic elements and in the formation of a lipid-rich, insulating sheath that enables the acceleration of nerve impulses. A mature myelin sheath can be morphologically divided into compact and non-compact myelin, of which the latter has a higher cytosolic content and further contains several subcompartments, such as the abaxonal and adaxonal layers, as well as the paranodal loops[1,2].

Myelin contains a set of unique proteins that generally display strict localization to either compact or non-compact myelin, which is thought to be at least partially driven by size exclusion[3,4]. The myelin environment being low in aqueous content and harboring negatively charged, tightly packed membrane interfaces, myelin proteins typically exhibit attributes such as strong hydrophobicity, peripheral membrane association, transmembrane domains, and a high positive net charge, as well as intrinsic disorder[5].

Several myelin proteins have delicately regulated expression levels and functions. Past research using cell culture and animal models have outlined the importance of various proteins in demyelination – a situation where myelin undergoes morphological changes and loss[6]. Demyelination causes neurological disorders, including multiple sclerosis (MS), Charcot-Marie-Tooth disease, and leukodystrophies, such as Pelizaeus-Merzbacher disease[7-10].

2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) is one of the most abundant proteins in CNS myelin and a possible auto-antigen in MS[4,11]. The high expression levels of CNPase, together with its rather unusual enzymatic

activity, have troubled scientists for years. Still, decades after the initial discovery of CNPase[12], its physiological role remains unclear. This review attempts to outline the current knowledge regarding the structure and activity of CNPase, as well as its putative functions and significance.

The Discovery and Significance of CNPase

Half a Century of Research Defines an Enzyme

In the early 1960s, the observation of nucleoside 2′,3′-cyclic monophosphate (2′,3′-cNMP) hydrolysis to nucleoside 2′-monophosphate (2′-NMP, Fig. 1) in rabbit brain extracts led to the discovery and initial characterization of CNPase, a membrane-anchored enzyme present on the cytosolic side of non-compact myelin[12-14]. In humans, CNPase accounts for 4% of total CNS myelin protein, making it the most abundant protein in non-compact myelin and ranking it as the third-most abundant protein overall in CNS myelin. CNPase is also expressed in the PNS in Schwann cells and olfactory ensheathing cells, although the amount is much less – merely 0.5% of total PNS myelin protein[4,14,15]. Two isoforms of CNPase have been identified, which originate from two alternative promoters and 3′,5′-cAMP-regulated splicing of one of the mRNA variants produced from the *CNP1* gene, in humans encoded in chromosome 17[16-21]. The difference between the variants is the presence of a 20-amino-acid N-terminal mitochondrial targeting sequence (MTS) in the 48-kDa isoform II, which is removed after mitochondrial import to produce a truncated 46-kDa variant that corresponds to the cytosolic isoform I of 400 amino-acids[20,22,23]. In addition to its high expression levels, CNPase has been characterized as a protein of extreme longevity, along with several other myelin proteins[24].

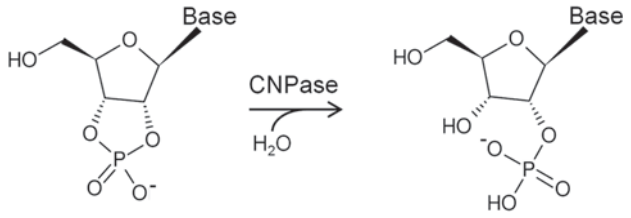

Fig. 1. Hydrolysis of 2′,3′-cNMPs to 2′-NMPs. The hydrolytic cleavage of the 3′-phosphodiester bond of 2′,3′-cNMP is catalyzed by CNPase, as well as by some other cyclic nucleotide phosphodiesterases.

CNPase is composed of two folded domains and a C-terminal extension, which has been characterized to be disordered in solution as a peptide (Fig. 2)[25-28]. The C-terminal domain harbors the catalytic phosphodiesterase activity and is, both reaction- and structure-wise, a member of the 2H phosphoesterase superfamily, harboring a double His-X-Thr-X (X denotes a hydrophobic residue) motif typical for the family[28,29]. With the exception of platypus, CNPase is ubiquitously expressed in vertebrates, as well as in some invertebrates. However, there is some domain variation between species; the conservation of CNPase has recently been outlined *via* bioinformatics[30]. Compared to the catalytic phosphodiesterase domain, the N-terminal domain remains poorly characterized to date. Although its *in vivo* function remains to be determined, it shares some homology with T4 bacteriophage polynucleotide kinase (PNK). This PNK-like domain contains a conserved P-loop structure, which suggests ATP/GTP binding [25].

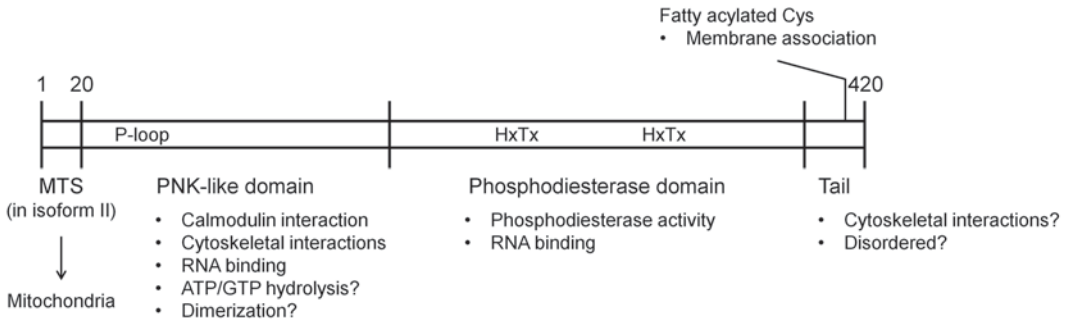

Fig. 2. Domain structure of CNPase. CNPase consists of two folded domains and a C-terminal extension. In addition, isoform II contains an N-terminal mitochondrial targeting sequence (MTS), which is removed after mitochondrial import. The locations of the P-loop and His-X-Thr-X (HxTx) motifs are shown. Known or predicted functions of each domain are also indicated. PNK, polynucleotide kinase.

The function of CNPase has been a mystery ever since its discovery. After decades of research, its putative functions at the molecular level have only recently begun to be unveiled. Advances in structural biology, as well as the discovery of endogenous 2′,3′-cNMPs, have shed some light on its possible functions[31,32]. These matters, as well as the significance of the molecular motifs in CNPase, are addressed in more detail below.

CNPase in Health & Disease

The importance of CNPase at the translational level has been investigated through knockout studies in mice. The absence of CNPase causes premature death through axonal swelling, while maintaining a myelin morphology very similar to wild-type animals. The behavioral phenotype manifests as a loss of motor abilities through muscle weakness and finally death, typically at the age of 6 to 12 months[33]. The overexpression of CNPase results in an altered oligodendrocyte process morphology and diminished myelin compaction at the early stages of myelination, while CNPase is localized throughout myelin, including the compact regions. Despite the cellular phenotype, interestingly, a clear behavioral phenotype is absent[34,35]. An interesting trait is also the accumulation of (poly)ubiquitinated and partially degraded CNPase in myelin lipid rafts, which might contribute to the changes in axonal and myelin morphology upon aging[36].

Cell culture studies have demonstrated the importance of CNPase at the early stages of myelination. CNPase is essential for oligodendrocyte process outgrowth *via* its membrane association, as well as its ability to interact with the cytoskeleton (see below). CNPase is essential in oligodendrocyte process formation and branching, and a C-terminally truncated variant of CNPase, as well as a mutant unable to membrane-associate, affects these processes[37]. The true physiological relevance of this, however, is unclear, since myelination in CNPase-deficient mice seems to proceed normally, and the neuronal aberrations appear months later along with the behavioral phenotype[33]. This suggests that in the absence of CNPase, its possible physiological function in process outgrowth may be complemented by currently unknown factors, and the role of CNPase after active myelination could be related to axonal maintenance[33].

In MS, demyelination occurs in the brain and spinal cord, resulting in a wide array of neurological symptoms. Together with several other CNS myelin proteins, CNPase is an auto-antigen in MS – a chronic autoimmune disease[11,38-40]. Changes in the expression levels of CNPase have been linked to Alzheimer's disease, Down's syndrome, and catatonia-depression syndrome[41,42]. *CNP*1 has also been identified as a susceptibility gene in schizophrenia, although the described findings appear to have a somewhat specific geographical localization and have been challenged by other studies. A single-nucleotide polymorphism that does not alter the amino-acid sequence of CNPase, but rather decreases its expression levels, has been suggested to play a role in schizophrenia[43-54].

Structure and Function of CNPase

The Phosphodiesterase Domain Harbors Catalytic Activity

The catalytic phosphodiesterase domain makes up the C-terminal half of the CNPase polypeptide. Truncation experiments demonstrated that truncations after amino acid 164 in recombinant rat CNPase result in a loss of phosphodiesterase activity[28]. The catalytic domain is composed of ~240 amino acids, is highly conserved in mammals, and is present in all identified CNPases throughout different organisms[30].

The catalytic domain of CNPase catalyzes the hydrolysis of 2′,3′-cNMPs to 2′-NMPs – an activity that was for long considered to be peculiar. Apart from some intermediate states formed in tRNA splicing, endogenous 2′,3′-cNMPs were not observed until recently. Verrier *et al.* discovered a metabolic pathway present in astrocytes, microglia, and most notably, in oligodendrocytes[31,32,55]. The discovery of this pathway linked CNPase to the local production of the neuroprotectant adenosine, where CNPase rapidly depletes adenosine 2′,3′-cyclic monophosphate (2′,3′-cAMP), a compound indentified as a mitochondrial toxin that presumably forms as a by-product of mRNA transphosphorylation[56-59]. The accumulation of 2′,3′-cAMP was found in very young CNPase-deficient mice used to study traumatic brain injury, which developed post-traumatic axonal degeneration similar to that of older CNPase-deficient mice that did not experience brain trauma[31-33]. This further supports the hypothesis of CNPase

playing a role in axonal maintenance after myelination, possibly through neuroprotection arising from a major contribution to the hydrolysis of 2′,3′-cAMP produced within myelinated axons[32,33,56].

In addition, CNPase was earlier thought to be involved in RNA metabolism in a manner similar to several other 2H phosphoesterases[60]. This was supported by CNPase being able to bind RNA molecules and having phosphodiesterase activity towards oligonucleotides with terminal 2′,3′-cyclic monophosphates, which, prior to the discovery of the 2′,3′-cAMP pathway, were the only known endogenous molecules containing such a cyclic phosphodiester linkage[61-63]. Indicating enzymatic functionality *in vivo*, CNPase is capable of rescuing yeast with the cyclic phosphodiesterase (CPDase) domain inactivated from the multifunctional tRNA ligase Trl1p, an essential protein for tRNA maturation[64].

The enzymatic phosphodiesterase activity of CNPase is one of the best characterized features of the protein. The reaction itself was discovered together with the enzyme in the early 1960s, but the catalytically essential residues were identified 40 years later *via* mutagenesis[12,28]. Prior to that, CNPase had already been shown to have substrate stereoselectivity, using nucleoside 2′,3′-cyclic monophosphorothioate analogs[65].

Although the domain structure of CNPase had already been known for some time, the C-terminal catalytic domain could be described as a member of the 2H phosphoesterase superfamily only after its initial structure determination in 2003 by NMR spectroscopy[29]. Sakamoto *et al.* postulated a putative binding mode and active site for the enzyme, when they solved the first CNPase crystal structure[66]. A simple mechanistic reaction scheme for CNPase could be then described. Both the NMR and the crystal structure showed the catalytic domain to share high structural homology with 2H phosphoesterases, with a pseudo 2-fold symmetric double His-X-Thr-X motif in the active site (Fig. 3A, B)[28,29,66].

Since its initial structural characterization, CNPase has been extensively studied, and a detailed mechanistic reaction scheme has recently been described, based on protein-ligand complex structures (Fig. 3C, D)[30,67]. In brief, the reaction in mouse CNPase begins with a His309-mediated activation of an adjacent water molecule, which performs a nucleophilic attack on the cyclic phosphate group. A pentavalent phosphane intermediate is formed, which is stabilized by the active-site His and Thr residues, the carbonyl group of the Pro320-Val321 peptide bond in a mobile loop, as well as a panel of ordered water molecules located underneath the phosphate. Then, His230 donates a proton to the leaving group, the 3′-hydroxyl group of the ribose moiety, and the free 2′-phosphate flips under the mobile loop, where the carbonyl group involved in the intermediate state stabilization turns away and the Val321 amide and the N terminus of helix α7 hydrogen bond to the 2′-phosphate[30].

The 2H phosphoesterases form a family of highly sequence-divergent, yet structurally homologous proteins that often function as enzymes. The variety and classification of 2H phosphoesterases have been reviewed[68]. CNPase and its closest homologs, including goldfish RICH and plant CPDases, do not belong to any particular clade of 2H phosphoesterases, although they are related to the YjcG-like group, named after the putative 2′,5′-ligase from *Bacillus subtilis*. Indeed, many 2H phosphoesterases are involved in RNA metabolism as either CPDases or ligases[68]. The active CPDases often target the 2′,3′-cyclic phosphate termini of intermediate oligonucleotides formed during tRNA maturation or ADP-ribose 1″,2″-cyclic phosphate, a by-product formed from oxidized nicotinamide adenine dinucleotide (NAD^+) during the last steps of tRNA maturation[69-71].

One of the most notable differences between CNPase and other 2H phosphoesterases is the presence of an additional α-helix in the proximity of the active site cleft (helix α7, Fig. 3), which apparently is a key factor in determining the substrate stereoselectivity of CNPase. The loop structure before helix α7 is dynamic, and its conformation is linked to the catalytic cycle of the enzyme[30,67]. The only known protein to share this helix with CNPase is the regeneration-induced CNPase homolog, RICH. Both sequence- and structure-wise, the C-terminal domain of RICH is the closest homolog to the catalytic domain of CNPase, and it might also harbor functional similarity, as it has been shown in NMR titration experiments to bind 3′-AMP, an inhibitor of CNPase[72].

The PNK-like Domain May Have Several Functions

Our recent bioinformatics study[30] demonstrated that the

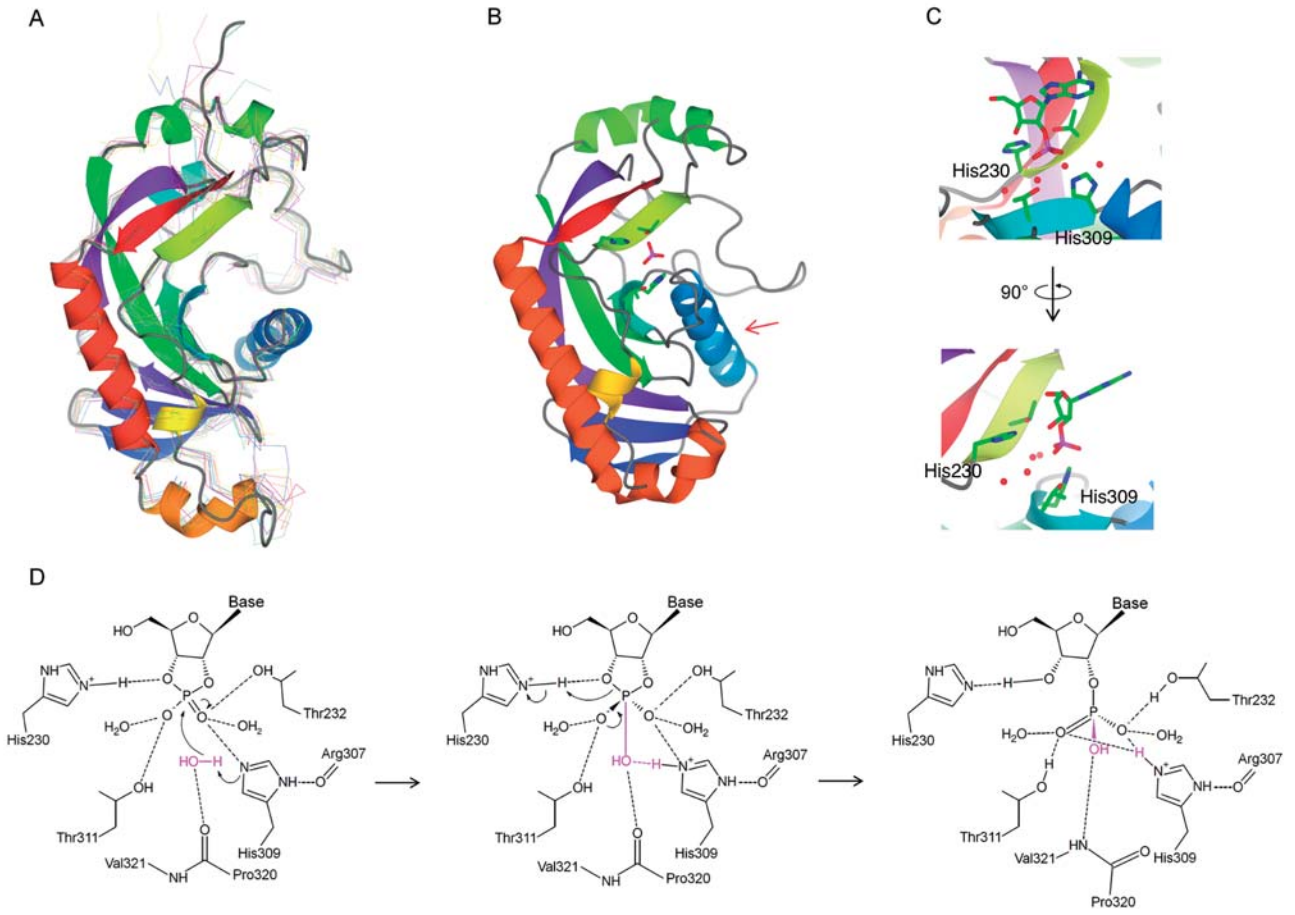

Fig. 3. Structure of the CNPase phosphodiesterase domain and its catalytic mechanism. A: The solution NMR structure of the CNPase phosphodiesterase domain (PDB ID: 2ILX, an ensemble of nine lowest-energy structures are shown in thin bonds and one as ribbons)[29]. B: The first CNPase crystal structure (PDB ID: 1WOJ)[66]. The structure is slightly more compact than the NMR structure, with helix α7 (red arrow) at a more tilted angle towards the active site. Of all 2H phosphoesterases, this helix is only present in CNPase and its nearest homolog, RICH. The catalytic His-X-Thr-X motifs and the bound phosphate ion in the active site are shown as sticks. C: The CNPase active site with bound 2′-AMP (PDB ID: 2YDD)[67]. The catalytic His-X-Thr-X motifs and the conserved active-site water molecules are shown. D: The detailed mechanism of the reaction catalyzed by the CNPase phosphodiesterase domain. The peptide bond between Pro320 and Val321 is part of a mobile loop, which resides at the N terminus of helix α7. The atoms of the catalytic water molecule are in magenta.

PNK-like domain is present in all CNPases throughout vertebrates, except goldfish, zebrafish, and Japanese rice fish. In these three species, the domain has been replaced by a glutamate-rich N-terminal acidic domain. In addition to the PNK-like domain, the acidic domain is also present in salmon and Nile tilapia. On the other hand, the PNK-like domain is present in the six so-far discovered invertebrate CNPase homologs[30].

Compared to the phosphodiesterase domain, the PNK-like domain remains structurally poorly characterized, although models using solution small-angle X-ray scattering and homology modeling based on T4 PNK have been recently described[30,62]. Despite the low overall sequence homology to T4 PNK, a highly conserved P-loop structure, which is usually found in PNKs, is also present in the CNPase PNK-like domain[25,73]. P-loops (phosphate binding loops), or Walker A motifs, are usually ATP-binding sites present in many nucleotide-binding proteins[74,75]. CNPase has been found to bind ATP and GTP as well as to have hydrolytic activity towards them, suggesting putative PNK

activity *in vivo* and a further role in nucleic acid metabolism. CNPase prefers GTP hydrolysis over ATP, and performs faster in the presence of Ca^{2+} than Mg^{2+} [25].

The PNK-like domain interacts with RNA, calmodulin (CaM), and cytoskeletal elements[37,61,62,67,76-78]. Interaction with RNA is a property of many 2H phosphoesterases, and CNPase, indeed, binds RNA and also appears to have some selectivity towards certain RNA sequences[61]. The presence of the catalytic domain alone is enough to mediate RNA binding, but the PNK-like domain was later shown to enhance the interaction[61,67]. Independently of the phosphodiesterase activity, CNPase also inhibits translation *in vitro* as a full-length protein. This was elegantly demonstrated in recent studies, in which CNPase was found to inhibit the replication of hepatitis B virus and HIV-1[61,79,80]. The interaction with RNA also provides a tempting idea to link CNPase to RNA metabolism. RNA processing is a rather typical role amongst other 2H phosphoesterases and, combined with the possible PNK and phosphodiesterase activities outlined above, it has been discussed for years whether CNPase might play a role in RNA metabolism[60]. In addition, the findings that CNPase also interacts with filamentous actin and tubulin, as well as its ability to promote microtubule formation, make CNPase a possible candidate in RNA trafficking[37,61,62,78,81]. In oligodendrocytes, it is known that the mRNAs of several proteins are translocated to various subcellular compartments prior to translation, and CNPase might function in this context[61,82]. It can be speculated that the translocation of compact myelin protein mRNAs for localized translation could be one of the driving forces that result in oligodendrocytic process formation during early myelination. The fact that CNPase binds to microtubules, however, also gives it a possible structural role, taking into account its membrane association. It also prefers to bind heterodimers of α- and β-tubulin and, thus, induces microtubule formation[37,77,78].

CaM has recently been shown to bind directly to the PNK-like domain with a dissociation constant of 100 nmol/L[62,76,83]. The interaction is dependent on Ca^{2+}, and the proposed interaction site is located in the vicinity of the P-loop[62]. While the phosphodiesterase domain does not bind CaM, and its activity is unaffected by the presence of CaM, it remains to be determined whether the binding of CaM has an impact on ATP/GTP binding and/or possible PNK activity. Interestingly, recombinant full-length CNPase only binds CaM *in vitro,* if the very C-terminal extension (the membrane anchor) has been deleted[62].

The C-terminal Extension Mediates Peripheral Membrane Association

The C-terminal tail extension of 13 residues has been characterized *via* circular dichroism spectroscopy to be unfolded as a peptide in membrane-mimicking conditions, although a challenging report was published earlier, and post-translational modifications might contribute to its conformation in the context of the full-length protein[27,84]. While the tail has been shown to be required for tubulin interactions and polymerization, as well as to abolish the binding of CaM to the PNK-like domain *in vitro*, the tail harbors a reactive Cys residue that undergoes fatty acylation *in vivo*[37,62,85,86]. This modification anchors CNPase to the membrane of non-compact myelin and, hence, makes CNPase a peripheral membrane protein. As mentioned above, removal of the tail alters the process formation of cultured oligodendrocytes. Furthermore, mutating the modified Cys residue to Ala produces the same result, indicating the importance of CNPase membrane association in process formation[37,81,87,88].

The C-terminal tail itself is likely to extend from between the two folded domains, and it resides close to the phosphodiesterase domain active site[67]. Hence, when CNPase is membrane-anchored, the active site of the phosphodiesterase domain lies close to the membrane surface and possibly faces the membrane. The tail itself does not influence the catalytic activity of the phosphodiesterase domain, as activity assays have demonstrated that the presence or absence of the C-terminal extension does not significantly affect the kinetic constants[89]. However, especially for possible bulkier substrates, such as RNA, the effect of the tail and the proximity of the membrane remains to be determined.

Isoform II Is the Mitochondrial Variant of CNPase in Non-myelinating Glia

Only a few studies have been published on CNPase isoform II, which, as opposed to isoform I, is expressed only in minor amounts in myelinating glia. The abundance of isoform II is higher in the adult liver and embryonic brain, where isoform I is practically absent. The MTS in

CNPase isoform II is responsible for mitochondrial import, which is regulated *via* phosphorylation by protein kinase C[22]. The mitochondrial, fully-processed isoform II does not drive morphological differentiation in cell cultures as isoform I does in oligodendrocytes[22,37]. Instead, CNPase is localized to the intermembrane space, where it resides as a peripheral membrane protein at the inner mitochondrial membrane[22]. The function of CNPase in mitochondria is not thoroughly understood, but CNPase, together with 2′,3′-cAMP, has been suggested to modulate the mitochondrial permeability transition, a proapoptotic event, in which Ca^{2+} is released from the mitochondrial matrix to the intermembrane space and cytoplasm[59]. This again raises questions regarding the significance of the CNPase-CaM interaction, as it is rather enticing to speculate an interconnection between membrane-bound CNPase, free 2′,3′-cAMP, and Ca^{2+}, as well as the Ca^{2+}-sensor CaM and mitochondrial membrane permeability. Perhaps mitochondrial CNPase functions as a protective enzyme that is involved in toxic 2′,3′-cAMP hydrolysis in a CaM-regulated manner, which then impacts pro-apoptotic membrane permeability.

Summary and Open Questions

The myelin enzyme CNPase was discovered over 50 years ago, and its strong expression profile and peculiar catalytic activity have been known for decades. However, its importance *in vivo* has only recently begun to be unveiled. Its membrane and cytoskeletal association have been shown to be crucial to ensure correct myelination, suggesting a structural role, and the discovery of a 2′,3′-cAMP pathway has linked CNPase enzymatic activity into a clear biological context regarding CNS damage. However, many open questions remain to be answered.

What is the significance of the CNPase catalytic activity outside the context of brain injury? The prominent expression and activity of CNPase make it efficient in 2′,3′-cAMP depletion, and the absence of CNPase in mice becomes evident only after several months of age. In the case that the accumulation of endogenous 2′,3′-cAMP is also related to aging, is CNPase involved in this process? CNPase is possibly one player of many in the axonal support systems provided by myelinating glia[90].

Another important consideration is the function of CNPase in RNA metabolism, and alongside this are its cytoskeletal interactions. As the mRNAs of several oligodendrocytic proteins are known to undergo trafficking, CNPase could be a factor in mRNA translocation from the soma towards the process peripheries during and after myelination. CNPase might also function in a metabolic role when large amounts of myelin protein mRNAs are being degraded. CNPase apparently inhibits viral protein synthesis – is this a general property, and is it related to the possible neuroprotective function of CNPase? The significance of the CNPase-dependent regulatory system for mitochondrial membrane permeabilization in some cell types is also presently unclear. This function could also be related to mitochondrial RNA metabolism, and CNPase might be regulated by CaM and Ca^{2+}.

The biological relevance and molecular properties of CNPase should be studied further, as it appears to be a multifunctional protein. Elucidation of the functional relationships between the diverse binding partners of CNPase in its large interaction network, including cytoskeletal proteins, CaM, oligomerization, RNA, nucleotides, and membrane surfaces, will eventually provide a coherent model, linking all the pieces of the CNPase puzzle together.

ACKNOWLEDGEMENTS

This review was supported by grants from the Department of Biochemistry, University of Oulu, the Sigrid Jusélius Foundation (Finland), the Academy of Finland, and the Hamburg Research and Science Foundation (Germany). We are grateful to Dr. Matti Myllykoski for building up the CNPase project in our laboratory and his deep insight into this enigmatic protein.

Received date: 2013-11-26; Accepted date: 2014-01-23

REFERENCES

[1] Hartline DK. What is myelin? Neuron Glia Biol 2008, 4: 153–163.

[2] Aggarwal S, Yurlova L, Simons M. Central nervous system myelin: Structure, synthesis and assembly. Trends Cell Biol 2011, 21: 585–593.

[3] Aggarwal S, Yurlova L, Snaidero N, Reetz C, Frey S, Zimmermann J, *et al.* A size barrier limits protein diffusion at the cell surface to generate lipid-rich myelin-membrane sheets. Dev Cell 2011, 21: 445–456.

[4] de Monasterio-Schrader P, Jahn O, Tenzer S, Wichert SP, Patzig J, Werner HB. Systematic approaches to central nervous system myelin. Cell Mol Life Sci 2012, 69: 2879–2894.

[5] Kursula P. Structural properties of proteins specific to the myelin sheath. Amino Acids 2008, 34: 175–185.

[6] Quarles R. Myelin sheaths: Glycoproteins involved in their formation, maintenance and degeneration. Cell Mol Life Sci 2002, 59: 1851–1871.

[7] Mirshafiey A, Kianiaslani M. Autoantigens and autoantibodies in multiple sclerosis. Iran J Allergy Asthma Immunol 2013, 12: 292–303.

[8] Li J, Parker B, Martyn C, Natarajan C, Guo J. The PMP22 gene and its related diseases. Mol Neurobiol 2013, 47: 673–698.

[9] Yamamoto T, Shimojima K. Pelizaeus-Merzbacher disease as a chromosomal disorder. Congenit Anom (Kyoto) 2013, 53: 3–8.

[10] Hobson GM, Garbern JY. Pelizaeus-Merzbacher disease, Pelizaeus-Merzbacher-like disease 1, and related hypomyelinating disorders. Semin Neurol 2012, 32: 62–67.

[11] Rösener M, Muraro PA, Riethmüller A, Kalbus M, Sappler G, Thompson RJ, *et al*. 2',3'-cyclic nucleotide 3'-phosphodiesterase: A novel candidate autoantigen in demyelinating diseases. J Neuroimmunol 1997, 75: 28–34.

[12] Drummond GI, Iyer NT, Keith J. Hydrolysis of ribonucleoside 2′,3′-cyclic phosphates by a diesterase from brain. J Biol Chem 1962, 237: 3535–3539.

[13] Drummond GI, Perrott-Yee S. Enzymatic hydrolysis of adenosine 3',5'-phosphoric acid. J Biol Chem 1961, 236: 1126–1129.

[14] Trapp BD, Bernier L, Andrews SB, Colman DR. Cellular and subcellular distribution of 2',3'-cyclic nucleotide 3'-phosphodiesterase and its mRNA in the rat central nervous system. J Neurochem 1988, 51: 859–868.

[15] Radtke C, Sasaki M, Lankford KL, Gallo V, Kocsis JD. CNPase expression in olfactory ensheathing cells. J Biomed Biotechnol 2011, 2011: 608496.

[16] Monoh K, Kurihara T, Takahashi Y, Ichikawa T, Kumanishi T, Hayashi S, *et al.* Structure, expression and chromosomal localization of the gene encoding human 2',3'-cyclic-nucleotide 3'-phosphodiesterase. Gene 1993, 129: 297–301.

[17] Kurihara T, Monoh K, Sakimura K, Takahashi Y. Alternative splicing of mouse-brain 2',3'-cyclic-nucleotide 3'-phosphodiesterase messenger-RNA. Biochem Biophys Res Commun 1990, 170: 1074–1081.

[18] Kurihara T, Tohyama Y, Yamamoto J, Kanamatsu T, Watanabe R, Kitajima S. Origin of brain 2',3'-cyclic-nucleotide 3'-phosphodiesterase doublet. Neurosci Lett 1992, 138: 49–52.

[19] Douglas AJ, Fox MF, Abbott CM, Hinks LJ, Sharpe G, Povey S, *et al.* Structure and chromosomal localization of the human 2',3'-cyclic nucleotide 3'-phosphodiesterase gene. Ann Hum Genet 1992, 56: 243–254.

[20] O'Neill R, Minuk J, Cox M, Braun P, Gravel M. CNP2 mRNA directs synthesis of both CNP1 and CNP2 polypeptides. J Neurosci Res 1997, 50: 248–257.

[21] Gravel M, Gao E, Hervouet-Zeiber C, Parsons V, Braun P. Transcriptional regulation of 2',3'-cyclic nucleotide 3'-phosphodiesterase gene expression by cyclic AMP in C6 cells. J Neurochem 2000, 75: 1940–1950.

[22] Lee J, O'Neill R, Park M, Gravel M, Braun P. Mitochondrial localization of CNP2 is regulated by phosphorylation of the N-terminal targeting signal by PKC: Implications of a mitochondrial function for CNP2 in glial and non-glial cells. Mol Cell Neurosci 2006, 31: 446–462.

[23] Gravel M, DeAngelis D, Braun PE. Molecular-cloning and characterization of rat-brain 2',3'-cyclic nucleotide 3'-phosphodiesterase isoform-2. J Neurosci Res 1994, 38: 243–247.

[24] Toyama BH, Savas JN, Park SK, Harris MS, Ingolia NT, Yates JR 3rd, *et al.* Identification of long-lived proteins reveals exceptional stability of essential cellular structures. Cell 2013, 154: 971–982.

[25] Stingo S, Masullo M, Polverini E, Laezza C, Ruggiero I, Arcone R, *et al.* The N-terminal domain of 2',3'-cyclic nucleotide 3'-phosphodiesterase harbors a GTP/ATP binding site. Chem Biol Drug Des 2007, 70: 502–510.

[26] Kursula P. The current status of structural studies on proteins of the myelin sheath (review). Int J Mol Med 2001, 8: 475–479.

[27] Myllykoski M, Baumgärtel P, Kursula P. Conformations of peptides derived from myelin-specific proteins in membrane-mimetic conditions probed by synchrotron radiation CD spectroscopy. Amino Acids 2012, 42: 1467–1474.

[28] Lee J, Gravel M, Gao E, O'Neill R, Braun P. Identification of essential residues in 2',3'-cyclic nucleotide 3'-phosphodiesterase - chemical modification and site-directed mutagenesis to investigate the role of cysteine and histidine residues in enzymatic activity. J Biol Chem 2001, 276: 14804–14813.

[29] Kozlov G, Lee J, Elias D, Gravel M, Gutierrez P, Ekiel I, *et al.* Structural evidence that brain cyclic nucleotide phosphodiesterase is a member of the 2H phosphodiesterase superfamily. J Biol Chem 2003, 278: 46021–46028.

[30] Myllykoski M, Raasakka A, Lehtimäki M, Han H, Kursula I, Kursula P. Crystallographic analysis of the reaction cycle of 2′,3′-cyclic nucleotide 3′-phosphodiesterase, a unique member of the 2H phosphoesterase family. J Mol Biol 2013, 425: 4307–4322.

[31] Verrier JD, Jackson TC, Bansal R, Kochanek PM, Puccio AM, Okonkwo DO, *et al.* The brain *in vivo* expresses the 2',3'-cAMP-adenosine pathway. J Neurochem 2012, 122: 115–125.

[32] Verrier J, Jackson T, Bansal R, Kochanek PM, Jackson E. Oligodendrocyte 2', 3'-cyclic nucleotide 3'-phosphodiesterase participates in localized adenosine production: Possible role in traumatic brain injury. J Neurotrauma 2012, 29: A168–A169.

[33] Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, *et al*. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet 2003, 33: 366–374.

[34] Gravel M, Peterson J, Yong VW, Kottis V, Trapp B, Braun PE. Overexpression of 2',3'-cyclic nucleotide 3'-phosphodiesterase in transgenic mice alters oligodendrocyte development and produces aberrant myelination. Mol Cell Neurosci 1996, 7: 453–466.

[35] Yin X, Peterson J, Gravel M, Braun P, Trapp B. CNP overexpression induces aberrant oligodendrocyte membranes and inhibits MBP accumulation and myelin compaction. J Neurosci Res 1997, 50: 238–247.

[36] Hinman JD, Chen C, Oh S, Hollander W, Abraham CR. Age-dependent accumulation of ubiquitinated 2',3'-cyclic nucleotide 3'-phosphodiesterase in myelin lipid rafts. Glia 2008, 56: 118–133.

[37] Lee J, Gravel M, Zhang R, Thibault P, Braun P. Process outgrowth in oligodendrocytes is mediated by CNP, a novel microtubule assembly myelin protein. J Cell Biol 2005, 170: 661–673.

[38] Walsh MJ, Murray JM. Dual implication of 2',3'-cyclic nucleotide 3' phosphodiesterase as major autoantigen and C3 complement-binding protein in the the pathogenesis of multiple sclerosis. J Clin Invest 1998, 101: 1923–1931.

[39] Muraro PA, Kalbus M, Afshar G, McFarland HF, Martin R. T cell response to 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) in multiple sclerosis patients. J Neuroimmunol 2002, 130: 233–242.

[40] Lovato L, Cianti R, Gini B, Marconi S, Bianchi L, Armini A, *et al*. Transketolase and 2',3'-cyclic-nucleotide 3'-phosphodiesterase type I isoforms are specifically recognized by IgG autoantibodies in multiple sclerosis patients. Mol Cell Proteomics 2008, 7: 2337–2349.

[41] Vlkolinsky R, Cairns N, Fountoulakis M, Lubec G. Decreased brain levels of 2',3'-cyclic nucleotide-3'-phosphodiesterase in Down syndrome and Alzheimer's disease. Neurobiol Aging 2001, 22: 547–553.

[42] Hagemeyer N, Goebbels S, Papiol S, Kästner A, Hofer S, Begemann M, *et al.* A myelin gene causative of a catatonia-depression syndrome upon aging. EMBO Mol Med 2012, 4: 528–539.

[43] Peirce TR, Bray NJ, Williams NM, Haroutunian V, Buxbaum J, Buckland P, *et al*. Convergent functional genomics, association and linkage analysis suggests 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) as a potential susceptibility gene for schizophrenia. Am J Med Genet, B Neuropsychiatr Genet 2004, 130B: 81.

[44] Peirce TR, Bray NJ, Williams NM, Norton N, Moskvina V, Preece A, *et al*. Convergent evidence for 2',3'-cyclic nucleotide 3'-phosphodiesterase as a possible susceptibility gene for schizophrenia. Arch Gen Psychiatry 2006, 63: 18–24.

[45] Georgieva L, Moskvina V, Peirce T, Norton N, Bray NJ, Jones L, *et al.* Convergent evidence that oligodendrocyte lineage transcription factor 2 (OLIG2) and interacting genes influence susceptibility to schizophrenia. Proc Natl Acad Sci U S A 2006, 103: 12469–12474.

[46] Dracheva S, Davis K, Chin B, Woo D, Schmeidler J, Haroutunian V. Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. Neurobiol Dis 2006, 21: 531–540.

[47] Barley K, Dracheva S, Byne W. Subcortical oligodendrocyte- and astrocyte-associated gene expression in subjects with schizophrenia, major depression and bipolar disorder. Schizophr Res 2009, 112: 54–64.

[48] Usui H, Takahashi N, Saito S, Ishihara R, Aoyama N, Ikeda M, *et al.* The 2',3'-cyclic nucleotide 3'-phosphodiesterase and oligodendrocyte lineage transcription factor 2 genes do not appear to be associated with schizophrenia in the Japanese population. Schizophr Res 2006, 88: 245–250.

[49] Donohoe G, Morris D, Allot E, Clarke S, Quinn E, Robertson I, *et al.* Olig-2 and CNP are associated with schizophrenia risk and variance in general cognition and memory function in an Irish sample. Biol Psychiatry 2007, 61(8): 189S.

[50] Le-Niculescu H, Balaraman Y, Patel S, Tan J, Sidhu K, Jerome RE, *et al.* Towards understanding the schizophrenia code: An expanded convergent functional genomics approach. Am J Med Genet B Neuropsychiatr Genet 2007, 144B: 129–158.

[51] Tang F, Qu M, Wang L, Ruan Y, Lu T, Zhang H, *et al.* Case-control association study of the 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) gene and schizophrenia in the Han Chinese population. Neurosci Lett 2007, 416: 113–116.

[52] Voineskos AN, de Luca V, Bulgin NL, van Adrichem Q, Shaikh S, Lang DJ, *et al.* A family-based association study of the myelin-associated glycoprotein and 2',3'-cyclic nucleotide 3'-phosphodiesterase genes with schizophrenia. Psychiatr Genet 2008, 18: 143–146.

[53] Voineskos AN, Bulgin N, von Adrichem Q, Wong A, Lang D,

Honer W *et al.* MAG gene but not CNP gene associated with schizophrenia. Biol Psychiatry 2007, 61: S209.

[54] Mitkus SN, Hyde TM, Vakkalanka R, Kolachana B, Weinberger DR, Kleinman JE, *et al*. Expression of oligodendrocyte-associated genes in dorsolateral prefrontal cortex of patients with schizophrenia. Schizophr Res 2008, 98: 129–138.

[55] Verrier JD, Exo JL, Jackson TC, Ren J, Gillespie DG, Dubey RK, *et al.* Expression of the 2',3'-cAMP-adenosine pathway in astrocytes and microglia. J Neurochem 2011, 118: 979–987.

[56] Verrier JD, Jackson TC, Gillespie DG, Janesko-Feldman K, Bansal R, Goebbels S, *et al*. Role of CNPase in the oligodendrocytic extracellular 2′,3′-cAMP-adenosine pathway. Glia 2013, 61: 1595–1606.

[57] Jackson EK. The 2′,3′-cAMP-adenosine pathway. Am J Physiol Renal Physiol 2011, 301: 1160–1167.

[58] Thompson JE, Venegas FD, Raines RT. Energetics of catalysis by ribonucleases: Fate of the 2',3'-cyclic phosphodiester intermediate. Biochemistry 1994, 33: 7408–7414.

[59] Azarashvili T, Krestinina O, Galvita A, Grachev D, Baburina Y, Stricker R, *et al.* Ca2+-dependent permeability transition regulation in rat brain mitochondria by 2',3'-cyclic nucleotides and 2',3'-cyclic nucleotide 3'-phosphodiesterase. Am J Physiol Cell Physiol 2009, 296: C1428–C1439.

[60] Popow J, Schleiffer A, Martinez J. Diversity and roles of (t) RNA ligases. Cell Mol Life Sci 2012, 69: 2657–2670.

[61] Gravel M, Robert F, Kottis V, Gallouzi I, Pelletier J, Braun PE. 2',3'-cyclic nucleotide 3'-phosphodiesterase: A novel RNA-binding protein that inhibits protein synthesis. J Neurosci Res 2009, 87: 1069–1079.

[62] Myllykoski M, Itoh K, Kangas SM, Heape AM, Kang SU, Lubec G, *et al.* The N-terminal domain of the myelin enzyme 2',3'-cyclic nucleotide 3'-phosphodiesterase: Direct molecular interaction with the calcium sensor calmodulin. J Neurochem 2012, 123: 515–524.

[63] Olafson R, Drummond G, Lee J. Studies on 2',3'-cyclic nucleotide-3'-phosphohydrolase from brain. Can J Biochem 1969, 47: 961–966.

[64] Schwer B, Aronova A, Ramirez A, Braun P, Shuman S. Mammalian 2',3' cyclic nucleotide phosphodiesterase (CNP) can function as a tRNA splicing enzyme *in vivo*. RNA 2008, 14: 204–210.

[65] Heaton P, Eckstein F. Diastereomeric specificity of 2',3'-cyclic nucleotide 3'-phosphodiesterase. Nucleic Acids Res 1996, 24: 850–853.

[66] Sakamoto Y, Tanaka N, Ichimiya T, Kurihara T, Nakamura K. Crystal structure of the catalytic fragment of human brain 2',3'-cyclic-nucleotide 3'-phosphodiesterase. J Mol Biol 2005, 346: 789–800.

[67] Myllykoski M, Raasakka A, Han H, Kursula P. Myelin 2',3'-cyclic nucleotide 3'-phosphodiesterase: Active-site ligand binding and molecular conformation. PLoS One 2012, 7: e32336.

[68] Mazumder R, Iyer L, Vasudevan S, Aravind L. Detection of novel members, structure-function analysis and evolutionary classification of the 2H phosphoesterase superfamily. Nucleic Acids Res 2002, 30: 5229–5243.

[69] Nasr F, Filipowicz W. Characterization of the saccharomyces cerevisiae cyclic nucleotide phosphodiesterase involved in the metabolism of ADP-ribose 1'',2''-cyclic phosphate. Nucleic Acids Res 2000, 28: 1676–1683.

[70] Greer CL, Peebles CL, Gegenheimer P, Abelson J. Mechanism of action of a yeast RNA ligase in tRNA splicing. Cell 1983, 32: 537–546.

[71] Xu Q, Teplow D, Lee TD, Abelson J. Domain structure in yeast tRNA ligase. Biochemistry 1990, 29: 6132–6138.

[72] Kozlov G, Denisov AY, Pomerantseva E, Gravel M, Braun PE, Gehring K. Solution structure of the catalytic domain of RICH protein from goldfish. FEBS J 2007, 274: 1600–1609.

[73] Koonin EV, Gorbalenya AE. Related domains in yeast tRNA ligase, bacteriophage T4 polynucleotide kinase and RNA ligase, and mammalian myelin 2',3'-cyclic nucleotide phosphohydrolase revealed by amino acid sequence comparison. FEBS Lett 1990, 268: 231–234.

[74] Hanson PI, Whiteheart SW. AAA+ proteins: Have engine, will work. Nat Rev Mol Cell Biol 2005, 6: 519–529.

[75] Walker JE, Saraste M, Runswick MJ, Gay NJ. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J 1982, 1: 945–951.

[76] Berggård T, Arrigoni G, Olsson O, Fex M, Linse S, James P. 140 mouse brain proteins identified by Ca^{2+}-calmodulin affinity chromatography and tandem mass spectrometry. J Proteome Res 2006, 5: 669–687.

[77] Braun PE, Bambrick LL, Edwards AM, Bernier L. 2',3'-cyclic nucleotide 3'-phosphodiesterase has characteristics of cytoskeletal proteins - a hypothesis for its function. Ann NY Acad Sci 1990, 605: 55–65.

[78] Bifulco M, Laezza C, Stingo S, Wolff J. 2',3'-cyclic nucleotide 3'-phosphodiesterase: A membrane-bound, microtubule-associated protein and membrane anchor for tubulin. Proc Natl Acad Sci U S A 2002, 99: 1807–1812.

[79] Wilson SJ, Schoggins JW, Zang T, Kutluay SB, Jouvenet N, Alim MA, *et al*. Inhibition of HIV-1 particle assembly by 2',3'-cyclic nucleotide 3'-phosphodiesterase. Cell Host Microbe 2012, 12: 585–597.

[80] Ma H, Zhao XL, Wang XY, Xie XW, Han JC, Guan W, *et al*. 2',3'-cyclic nucleotide 3'-phosphodiesterases inhibit hepatitis B virus replication. PLoS One 2013, 8: e80769.

[81] De Angelis DA, Braun PE. 2',3'-cyclic nucleotide 3'-phosphodiesterase binds to actin-based cytoskeletal elements in an isoprenylation-independent manner. J Neurochem 1996, 67: 943–951.

[82] Carson JH, Barbarese E. Systems analysis of RNA trafficking in neural cells. Biol Cell 2005, 97: 51–62.

[83] Zhang Z, Ottens AK, Golden EC, Hayes RL, Wang KKW. Using calmodulin-affinity capture to study the rat brain calmodulin binding proteome and its vulnerability to calpain and caspase proteolysis. Calcium Bind Proteins 2006, 2: 125–134.

[84] Esposito C, Scrima M, Carotenuto A, Tedeschi A, Rovero P, D'Errico G, *et al*. Structures and micelle locations of the nonlipidated and lipidated C-terminal membrane anchor of 2',3'-cyclic nucleotide-3'-phosphodiesterase. Biochemistry 2008, 47: 308–319.

[85] De Angelis DA, Braun PE. Isoprenylation of brain 2',3'-cyclic nucleotide 3'-phosphodiesterase modulates cell morphology. J Neurosci Res 1994, 39: 386–397.

[86] Agrawal HC, Sprinkle TJ, Agrawal D. 2',3'-cyclic nucleotide-3'-phosphodiesterase in the central-nervous-system is fatty-acylated by thioester linkage. J Biol Chem 1990, 265: 11849–11853.

[87] De Angelis DA, Braun PE. Binding of 2',3'-cyclic nucleotide 3'-phosphodiesterase to myelin: An *in vitro* study. J Neurochem 1996, 66: 2523–2531.

[88] Braun PE, De Angelis DA, Shtybel WW, Bernier L. Isoprenoid modification permits 2',3'-cyclic nucleotide 3'-phosphodiesterase to bind to membranes. J Neurosci Res 1991, 30(3): 540–544.

[89] Myllykoski M, Kursula P. Expression, purification, and initial characterization of different domains of recombinant mouse 2',3'-cyclic nucleotide 3'-phosphodiesterase, an enigmatic enzyme from the myelin sheath. BMC Res Notes 2010, 3: 1–7.

[90] Bankston AN, Mandler MD, Feng Y. Oligodendroglia and neurotrophic factors in neurodegeneration. Neurosci Bull 2013, 29: 216–228.