

CNP: An Immunological Marker for Oligodendrocytes and Schwann Cells

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The enzyme 2',3'-cyclic nucleotide 3'-phosphohydrolase (EC 3.1.4.37), also known as CNP or CNPase, is strongly associated with myelinated tissues. The availability of specific and sensitive anti-CNP monoclonal antibodies (mAbs) has allowed CNP to be exploited as a specific immunological marker for oligodendrocytes and Schwann cells—two cell types that elaborate and maintain myelin sheaths in the central nervous system (CNS) and peripheral nervous system (PNS), respectively. Notably, CNP immunostaining is virtually absent in neurons and astrocytes with these antibodies. Due to the restricted distribution of CNP and the high specificity of Anti-CNP mAb (Clone 11-5B), this antibody has proven extremely useful in studies of demyelinating diseases and related neuropathies that employ Western analysis, ELISAs and immunohistochemistry.

Introduction

The enzyme CNP appears to be involved in the biogenesis, early growth and differentiation of myelin. Its localization to the cytoplasmic membrane of oligodendrocytes and Schwann cells is consistent with a possible role in membrane synthesis, maintenance and rearrangement as well as signal transduction within these cells.

CNP has been purified from CNS tissues from a number of sources, including human, bovine, rat and other species (1-4). CNP is expressed early and at high levels during development and differentiation of oligodendrocytes and Schwann cells. Temporally, CNP appears prior to myelin basic protein, proteolipid protein, and at or slightly before the rapid synthesis of galactocerebroside and sulfated galactocerebroside (sulfatide). CNP is expressed at very high levels in brain throughout life, and its early developmental appearance parallels myelin biogenesis. The primary structure(s) of the protein and its enzymology, immunological properties and the cDNA sequences coding for the enzyme in several species have been discussed in considerable detail in three reviews (1,5,6).

The 2',3'-cyclic nucleotides of adenosine, guanosine, cytidine and uridine each are hydrolyzed by native CNP to yield the corresponding 2' monophosphate product; a 2' terminal monophosphate product is generated when an oligonucleotide containing a 2',3'-cyclic terminus is used as the substrate.

CNP Properties and Chromosomal Localization

CNP is present as an alternatively spliced protein in all mammalian species examined to date, appearing as a closely spaced doublet at approximately 46kDa (CNP1) and 48kDa (CNP2) by denaturing SDS-PAGE (7-11). Mouse brain, the only exception noted, contains three alternatively spliced forms (12-14). CNP isoforms react with a mouse mAb, allowing immunodetection and staining in a variety of standard immunoassays (15).

The gene encoding human CNP is located on chromosome 17 (16). A polymorphic C → T transition was discovered within intron 3. This information permitted meiotic DNA panel mapping of the human CNP gene within chromosome 17 (17). The latter study clearly showed CNP was sublocalized to 17q21—very near the familial breast cancer gene locus, BRCA1. Moreover, properties of the human CNP gene suggest that it deserves consideration as a candidate proto-oncogene. Some of the more interesting properties of the gene product include phosphorylation by both protein kinase A and C (18), palmitoylation (19), isoprenylation (20,21), partial *ras*-like sequence homology (1), nucleotide binding and association with oligodendrocyte process formation (22,23).

Cellular and Subcellular Distribution

Within the nervous system, CNP is associated almost exclusively with oligodendrocytes and Schwann cells. Heavily myelinated structures, such as the corpus callosum, the optic nerve and the spinal cord exhibit among the highest CNP activities—the levels are somewhat lower in the cerebellum. Not surprisingly, CNP levels are much lower in most areas composed primarily of gray matter.

CNP also is present in the visual system in outer rod segments where extensive membrane reorganization occurs in response to visual input (24,25). In *Xenopus* tadpoles, CNP activity in membrane fractions from the nervous system is even higher than in the adult animal. CNP activity also is present, although at several orders of magnitude lower, in platelets, lymphocytes, erythrocytes, whole wheat germ, cultured cells and many other non-nervous system cells and cell lines (1).

Immunostaining studies of young oligodendrocytes have shown CNP to occupy primarily a perinuclear location; CNP also is detected on, or near, the cytoplasmic membrane (26). As oligodendrocytes send out processes, CNP immunostaining becomes more peripheral and diffuse within the soma. Extensive staining then is observed in paranodal loops, inner and outer loop and tongue structures, incisure-like membranes in the larger CNS axons and in periaxonal regions (26). Most earlier accounts characterized CNP as an enzymatic marker for compact, multilamellar myelin, but we now know the enzyme has a very asymmetric distribution in myelin—a distribution quite distinct from that of myelin basic protein and proteolipid protein. CNP is primarily confined to the cytoplasmic compartment and in more “loose” single and double membrane wraps of myelin. The loss of CNP in the earlier stages of demyelinating disorders in humans and in animal models (e.g., experimental autoimmune encephalitis; EAE) is clearly consistent with a cytoplasmic distribution of the enzyme.

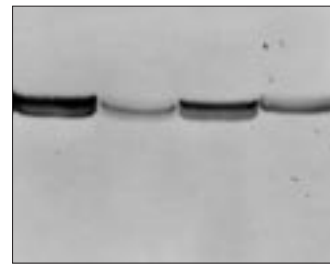
Available Immunological Reagents

Mouse monoclonal antibodies (7,15,27) to purified human CNP produced in this laboratory cross-react among various species, including human, bovine, rat, mouse, guinea pig, pig, dog, rabbit, goat, sheep and others. These antibodies are highly specific and react with CNP under a variety of conditions (Table 1) (7-11). Immunoreactivity to CNP is particularly useful in applications involving oligodendrocyte and Schwann cell identification and differentiation of specific cell types in cell culture and in tissue sections.

Table 1.

CNP-CONTAINING MATERIAL AND CELLS THAT REACT WITH ANTI-CNP mAb.
Wolfgram protein fractions
Isolated CNS enzymes
CNS and PNS myelin
Cultured oligodendrocytes and Schwann cells
Bulk-isolated oligodendrocytes
Oligodendrocytes in fresh frozen and fixed tissue sections
Human oligodendrogliomas

Western Analysis Using Anti-CNP mAb (Clone 11-5B)



Human Mouse Rabbit Rat

◀ Figure 1.

Detection of CNP from various mammalian species by Western analysis using Anti-CNP mAb (Clone 11-5B). Fifty micrograms of human, mouse, rabbit and rat delipidated whole brain protein were separated by denaturing SDS-PAGE and detected by Western analysis according to the protocol outlined below.

Protocol for the Immunodetection of CNP by Western Analysis*

- Soak nitrocellulose membrane in 50-100ml of TBST for 10 minutes. Decant.
- Add at least 10ml of BLOTTO and incubate for 10 minutes. Decant.
- Add Anti-CNP mAb (Clone 5B-11) at the proper dilution (1:500-1:5000) in BLOTTO. Incubate at room temperature for 60-90 minutes.
- Wash the membrane 2 times in 50-100ml of TBST for 10 minutes with gentle shaking. Decant after each wash.
- Add 20ml of BLOTTO per membrane.
- Add (goat anti-mouse) HRP-conjugated secondary antibody at the appropriate dilution (1:1000-1:2000) in BLOTTO. Incubate at room temperature for 60-90 minutes.
- Wash the membrane 2 times in 50-100ml of TBST for 10 minutes with gentle shaking. Decant after each wash.
- Wash the membrane 2 times in 50-100ml of TBST (without TWEEN®) for 10 minutes with gentle shaking. Decant after each wash.
- Perform the HRP reaction by adding 60mg of HRP Color Reagent (BioRad) in 20ml of cold methanol and 100ml of TBS. Add 60µl of 30% H₂O₂, mix and add to the nitrocellulose membrane immediately.
- Stop the reaction before it has run to completion using extensive water rinses.


* This immunoblot procedure is used after electrophoretic transfer of separated proteins onto a nitrocellulose membrane.

Applications of Mouse Anti-CNP Monoclonal Antibody (Clone 11-5B)

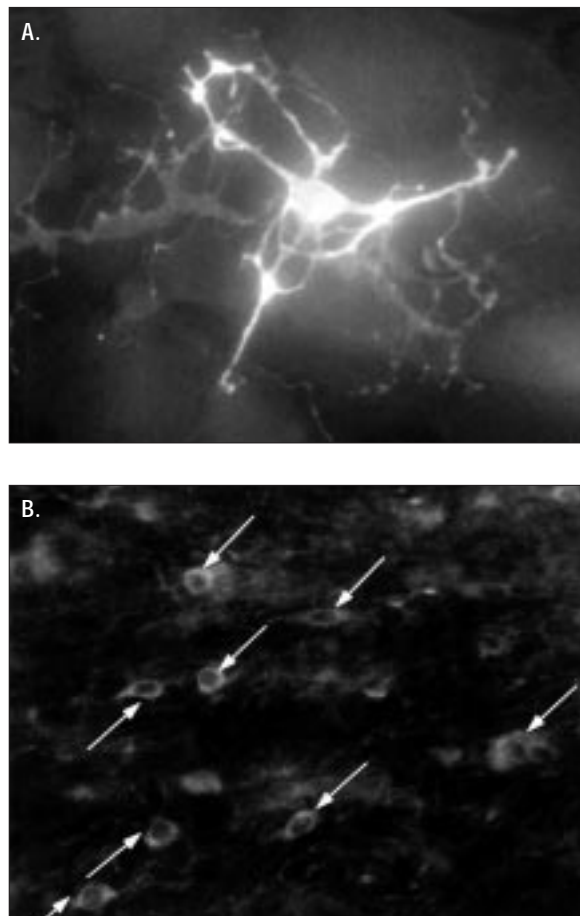
Both CNP1 and CNP2 react with a mouse IgG₁ mAb (Clone 11-5B), allowing Western analysis (Figure 1) and immunostaining of both isoforms in cultured oligodendrocytes (Figure 2A) and brain sections (Figure 2B), as well as in peripheral nerve sections and membrane fractions (15). Anti-CNP mAb (Clone 11-5B) also detects CNP quite well on immunoblots of delipidated (acetone) powders of whole brain (data not shown). With respect to the use of Anti-CNP mAb (Clone 11-5B) in immunostaining, reactivity is strong, highly specific, and extends out into the oligodendrocyte processes. Unlike some lipid antigens, there is virtually no rearrangement or redistribution of immunostained CNP upon treatment with organic solvents and commonly used fixatives.

Anti-CNP mAb (Clone 11-5B) has a variety of applications in studies of diseases in which central or peripheral myelin, or both, are affected. Some of these diseases include subacute sclerosing panencephalitis (SSPE), multiple sclerosis (MS), acquired immunodeficiency syndrome (AIDS) with CNS involvement and a number of peripheral neuropathies. In addition, a variety of experimental animals exist which demonstrate hypomyelination, demyelination and other aberrations in normal CNS and PNS myelin formation. It is expected that antibodies to CNP will be useful in studies involving these animals in experimental models of myelin-related diseases.

Summary

While the biological role of CNP in myelin biogenesis and myelin-related disease requires further investigation, the restricted distribution of CNP allows the enzyme to be used as a specific immunological marker for oligodendrocytes and Schwann cells. Moreover, Mouse Anti-CNP mAb (Clone 11-5B) has proven a useful immunological reagent for identifying CNP in Western analysis and dot blots, ELISA assays, as well as in cultured cells such as oligodendrocytes and C6 rat glioma cells, human oligodendroglioma and mixed glioma cells, and in various myelin and membrane fractions. In addition, the mouse Anti-CNP mAb (Clone 11-5B) has broad application for immunocytochemistry and the study of demyelinating diseases. 

Immunostaining Using Anti-CNP mAb (Clone 11-5B)




▲ **Figure 2.**

Anti-CNP immunostaining of rat brain oligodendrocytes (A) and fresh frozen sections of adult rat brain (B). Panel A: Details on oligodendrocyte isolation, culture conditions and staining can be found in reference 29. Panel B: Fresh frozen sections of adult rat brain were treated with Anti-CNP mAb (Clone 5B-11) diluted 1:10 in growth medium containing serum for 2 hours. FITC-conjugated goat anti-mouse IgG diluted 1:10 was applied to the section for 1 hour at room temperature. No fluorescent structures were observed using a control IgG instead of Anti-CNP mAb (Clone 5B-11). Photomicrograph kindly provided by Dr. Wendy Cammer, Albert Einstein College of Medicine, Department of Neurology.

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Anti-CNP mAb (Clone 11-5B)

As described in the preceding minireview, in conjunction with Anti-CNP mAb (Clone 11-5B), the enzyme CNP can be used as a specific immunological marker for oligodendrocytes and Schwann cells. Because of CNP's close association with these myelin-producing cell types, Anti-CNP mAb (Clone 11-5B) can be used in the study of demyelinating disease and related neuropathies. The antibody can be used in Western analysis and immunohistochemistry applications in a variety of mammalian species. 

Product	Size	Cat.#
Anti-CNP mAb (Clone 11-5B)	100µg	G3461